Universitat de València

Departamento de Psicobiología

DOCTORADO DE NEUROCIENCIA

DESARROLLO DE UN PARADIGMA DE ENRIQUECIMIENTO AMBIENTAL Y SU POTENCIACIÓN MEDIANTE AGONISTAS NICOTÍNICOS: EFECTOS CONDUCTUALES EN RATONES

Tesis Doctoral

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Valencia, 2014

A mis padres

A Quique

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CAPÍTULO 1

El Paradigma de Enriquecimiento Ambiental

Parte de este capítulo ha sido publicado en (véase ANEXO 9.1, 9.2, 9.3):

Redolat, R., & Mesa-Gresa, P. (2012). Potential benefits and limitations of enriched environments and cognitive activity on age-related behavioural decline. In Behavioral Neurobiology of Aging (pp. 293-316). Springer Berlin Heidelberg.

Mesa-Gresa, P., Ramos-Campos, M., & Redolat, R. (2013). Enriched Environments for Rodents and their Interaction with Nicotine Administration. Current Drug Abuse Reviews, 6(3), 191-200.

Mesa-Gresa, P., Pérez-Martínez, A., & Redolat-Iborra, R. (2012). Nicotina y modelos animales: ¿Qué nos aporta el paradigma de enriquecimiento ambiental? Adicciones, 24(2).

1. INTRODUCCIÓN

"Yo creo que estamos entrando en el siglo que pasaremos del genoma al ambioma. Pasaremos de lo mucho que conocemos de los genes, a conocer algo de lo que no conocemos nada, que es el medio ambiente" (Francisco Mora, 2010).

Se han realizado numerosos estudios con el objetivo de comprender mejor los factores tanto genéticos como farmacológicos que contribuyen al funcionamiento cerebral. Sin embargo, la exploración del posible papel que desempeñan los factores ambientales ha sido comparativamente mucho menor. Aunque algunos estudios clásicos, como los de Hebb (1947) o Rosenzweig (1966), ya sugerían que los animales que se desarrollan en un ambiente complejo y estimulante muestran cambios químicos y neuroanatómicos, así como mejoras a nivel cognitivo, la interacción entre factores ambientales y farmacológicos requiere mayor investigación (Burrows & Hannan, 2013; Mesa-Gresa et al., 2012, 2014; Simpson & Kelly, 2012). Una de las estrategias más populares para evaluar el papel del ambiente en la conducta animal es el paradigma denominado "enriquecimiento ambiental".

Diversas investigaciones en roedores ponen de manifiesto que distintos tipos de alojamiento inducen efectos diferenciados a nivel físico, neurológico y conductual (Pizzorusso et al., 2007; Redolat & Mesa-Gresa, 2012). Los tipos de alojamiento más estudiados han sido el *aislamiento social* o situación de *empobrecimiento ambiental*, en el que el animal permanece alojado de manera individual en una caja de pequeñas dimensiones (López et al., 2010); la situación estándar o de *enriquecimiento social,* en la que se alojan de 4 a 6 sujetos en cajas de dimensiones estándar (diferentes según el laboratorio) y, por último, el *enriquecimiento ambiental*, que consiste en el alojamiento de un mayor número de animales en cajas más grandes con distintos tipos de objetos que favorecen la estimulación de los animales. Por tanto, el paradigma del enriquecimiento ambiental (*environmental enrichment* o EE) se constituye como un modelo de alojamiento caracterizado por la estimulación de los animales (generalmente ratas o ratones) a nivel físico, cognitivo y sensorial mediante diversos tipos de objetos inanimados e interacción social (Laviola et al., 2008, Nithianantharajah & Hannan, 2006; van Praag et al., 2000; van Praag, 2009) (Véase Figura 1).

Este modelo fue ya descrito en la década de 1940 por Donald Hebb, quien observó diferencias a nivel conductual entre las ratas que había mantenido en su casa como mascotas durante un tiempo y las que habían permanecido en su laboratorio, estableciendo la novedad y complejidad del ambiente como las características principales del EE (Hebb, 1947; 1949). En los años 60, Rosenzweig y sus colaboradores introdujeron el EE como un protocolo diseñado específicamente para investigar la influencia del ambiente sobre el cerebro y la conducta. Sus experimentos demostraron que modificaciones en la intensidad y calidad de la estimulación ambiental pueden inducir cambios marcados en la morfología, química y fisiología del cerebro (Rosenzweig, 1966). Desde entonces, numerosos estudios realizados en base a este paradigma muestran que el EE induce importantes efectos y cambios sobre el cerebro de los animales sometidos a estas condiciones de alojamiento (Sale et al., 2009; Sale et al., 2014; van Praag et al., 2000).

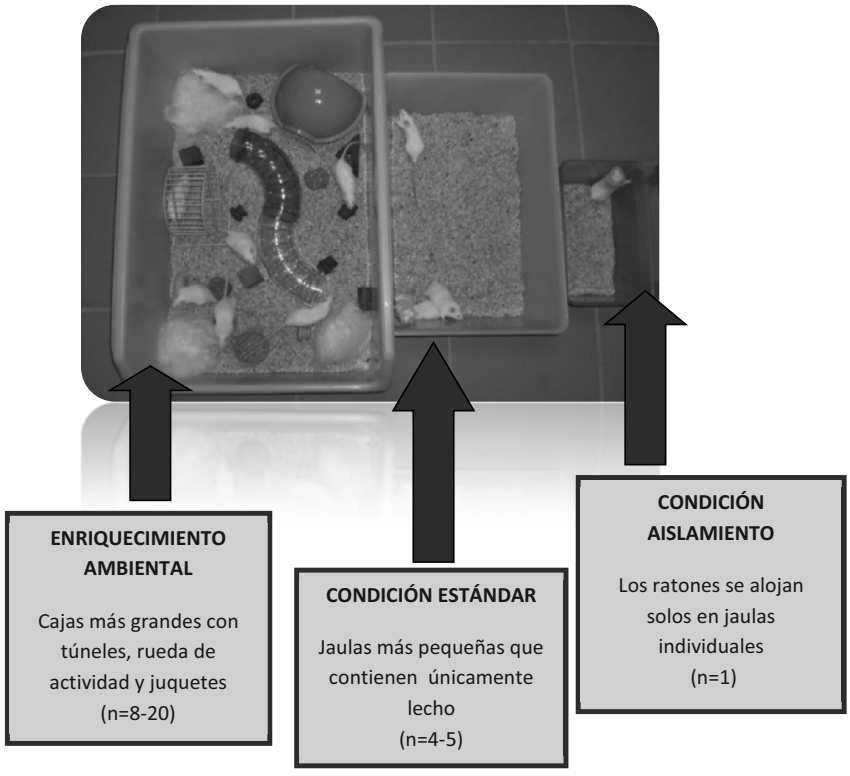

Figura 1. Comparación del paradigma de enriquecimiento ambiental con otros tipos de alojamiento utilizados en el laboratorio (Adaptada de Redolat y Mesa-Gresa, 2012).

2. EL PARADIGMA DE ENRIQUECIMIENTO AMBIENTAL

El EE se conceptualiza como toda aquella situación de alojamiento en la que se produce una combinación de estimulación cognitiva, física y/o social distinta a la que el animal recibe en condiciones de alojamiento estándar, con el objetivo de incrementar las funciones cognitivas, motoras y sensoriales (Bennett et al., 2006; Rosenzweig, 1966). Mediante este modelo se intenta proporcionar a los animales las condiciones óptimas que favorezcan la exploración, la actividad cognitiva, la interacción social y el ejercicio físico, tratándose en última instancia de "*enriquecer el ambiente para potenciar el cerebro*" (Sale et al., 2009).

Las condiciones de EE generalmente se constituyen mediante grandes cajas en las que se alojan entre 5 y 12 animales con varios juguetes entre los que destacan los destinados tanto al ejercicio físico (como por ejemplo ruedas para correr, túneles, plataformas, etc.), como a la estimulación cognitiva y sensorial mediante objetos inanimados (casitas, juguetes de distintas formas, texturas y colores, materiales para hacer nidos y campanas, entre otros) (Lazarov et al., 2005), así como a la interacción social (dependiendo de los estudios realizados, el número de animales por caja puede llegar a los 20). Esta complejidad ofrece estimulación en los animales a nivel visual, somatosensorial e incluso olfatorio (Nithianantharajah & Hannan, 2006; Redolat & Mesa-Gresa, 2012). Estos objetos (y en ocasiones incluso la localización de la comida) se suelen cambiar de posición y renovarse entre una y tres veces por semana dependiendo del paradigma experimental utilizado (Zhu et al., 2007) con el fin de estimular la curiosidad y la exploración de los animales (van Praag et al., 2000). Muchos investigadores mantienen permanentemente algunos objetos como las casitas, la rueda de correr y los túneles, y cambian el resto de juguetes semanalmente (Bennett et al., 2006; Laviola et al., 2008; Peña et al., 2006; Solinas et al., 2008). El constante cambio de objetos y de la posición de los mismos aporta también estimulación cognitiva adicional, principalmente en lo que respecta a la formación de mapas espaciales (Nithianantharajah & Hannan, 2006) así como las actividades de juego y curiosidad, incrementando de este modo las conductas motivadas de los animales. En definitiva, en el modelo de EE se complementan mediante estimulación,

novedad y complejidad las condiciones que los animales reciben cuando están alojados en cajas de ambiente estándar (Redolat & Mesa-Gresa, 2012).

El periodo durante el cual se mantienen los animales en estas condiciones y la edad de los mismos varía en función de los objetivos del estudio, de los análisis y pruebas que se realicen y de las hipótesis que guíen la investigación. Diferentes protocolos de EE han sido aplicados en modelos preclínicos a diferentes edades y etapas obteniéndose resultados positivos (Freret et al., 2012). La duración del periodo mínimo necesario para observar cambios a nivel neurobiológico y conductual se ha relacionado con las tareas a analizar (Simpson & Kelly, 2011) o con la especie o cepa de los animales evaluados (Abramov et al., 2008). De hecho, estudios previos han indicado que 3-4 semanas de enriquecimiento pueden ser suficientes para observar cambios neurobiológicos y conductuales en los animales (Brenes et al., 2008), aunque para algunas tareas, o para incrementar la duración de los cambios, el tiempo necesario de exposición puede ser más prolongado (Brenes et al., 2009). A nivel neurofisiológico, se observó que la exposición a un ambiente enriquecido durante 30 días en ratas dio lugar a un aumento del grosor del córtex dorsal, frontal, parietal y occipital (Diamond, 2001). Por otra parte, la edad a la que se inicia la exposición al EE parece tener gran relevancia sobre los efectos producidos (Freret et al., 2012). Diferentes estudios indican resultados más evidentes cuando el alojamiento en ambientes enriquecidos comienza a edades tempranas como la adolescencia (Pietropaolo et al., 2008) o la adultez (Madroñal et al., 2010; Ramírez-Rodríguez et al., 2013), sugiriéndose incluso que los efectos producidos sobre la plasticidad cerebral pueden mantenerse en edades avanzadas (Mora, 2013; Paban et al., 2009; Sale et al., 2014; Sampedro-Piquero et al., 2013a).

La principal dificultad para estudiar los beneficios y/o efectos producidos por el EE es la gran disparidad de paradigmas usados, ya que, a pesar de que la base es similar, se pueden observar notables diferencias en cuanto al tamaño de las cajas, la composición, la duración del enriquecimiento, el número de sujetos, la complejidad de los objetos estimulantes y la frecuencia de cambio de los mismos (Bennett et al., 2006; Fares et al., 2012; Mesa-Gresa et al., 2013a; Solinas et al., 2008; van Praag et al., 2000; Xie et al., 2013). Un modo de evitar estas discrepancias entre modelos de EE es

mediante la estandarización del paradigma, cuestión que ha sido propuesta por estudios previos (Sztainberg & Chen, 2010). Recientemente, un tipo de cajas denominadas Marlau™ han sido planteadas como modelo de alojamiento más complejo compuesto por escaleras, rampas, ruedas de correr y laberintos intercambiables, considerándose que esta complejidad incluida en el modelo permite a los roedores desarrollar las conductas específicas de cada especie (Fares et al., 2012; 2013) (Véase Figura 2).

Aun teniendo en cuenta estas diferencias, existe evidencia de que la estimulación ambiental puede producir cambios notables en distintas funciones cerebrales (van Praag et al., 2000), efectos duraderos en los sistemas neuroconductuales relacionados con la memoria y el aprendizaje (Bennett et al., 2006; Chen et al., 2010; Peña et al., 2006; Pizzorusso et al., 2007) así como prevenir y/o revertir diferentes funciones patológicas como las derivadas de lesiones cerebrales (Laviola et al., 2008; Pizzorusso et al., 2007). En roedores, la manipulación del ambiente social temprano puede inducir consecuencias a largo plazo sobre las funciones cerebrales y la conducta (Branchi et al., 2006). Entre los principales efectos y modificaciones causados por el EE en estudios con roedores, destacan los cambios a nivel físico, neurobiológico, cognitivo y emocional (Sale et al., 2014). Por otra parte, diferentes investigaciones recientes han puesto de manifiesto la necesidad de realizar más estudios preclínicos con el fin de establecer las principales consecuencias de la exposición a ambientes enriquecidos en interacción con diferentes fármacos y drogas (Burrows & Hannan, 2013; Mesa-Gresa et al., 2014; Simpson & Kelly, 2012).

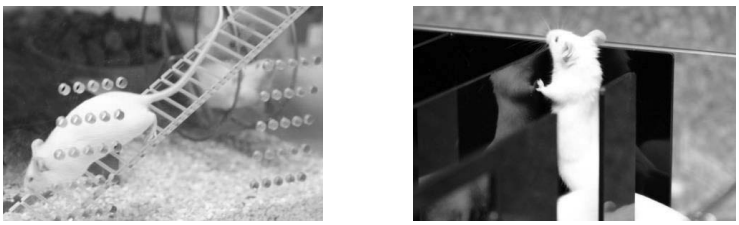

Figura 2. Detalle de rampa y laberinto como tipo de estimulación física y cognitiva incluidos en la caja Marlau™.

2.1. Cambios inducidos por la exposición a ambientes enriquecidos

a) Cambios físicos

Las condiciones de EE pueden dar lugar a efectos muy diferentes sobre los distintos parámetros fisiológicos en los animales de laboratorio, que podrían a su vez modular los cambios a nivel conductual. Por ello, el control y evaluación de los parámetros físicos es muy importante para determinar el impacto de la exposición a ambientes enriquecidos (Van de Weerd et al., 1997). Desde las primeras investigaciones realizadas en la década de los sesenta se han documentado cambios físicos en roedores como resultado de modificaciones en su ambiente (Rosenzweig, 1966). En estos estudios iniciales se constató un aumento significativo en el peso de los ratones que eran manipulados y cogidos diariamente frente a los no manipulados (Bayne, 2005). Este tipo de observaciones se ha extendido para evaluar directamente la influencia del EE sobre el peso corporal, confirmando los hallazgos anteriores. Así, diversos estudios indican que se produce mayor aumento de peso en los animales alojados en ambientes enriquecidos frente a los no enriquecidos, aunque en ocasiones no se encuentran diferencias significativas en la cantidad de comida consumida por ambos grupos (Bayne, 2005). Por el contrario, otras investigaciones muestran un menor peso en roedores alojadas en condiciones de EE (Mesa-Gresa et al., 2013a). Estas diferencias pueden atribuirse a un incremento de la actividad conductual de los animales enriquecidos debido a la mayor dimensión de las cajas de alojamiento y a la mayor oportunidad de realizar actividad física (Redolat y Mesa-Gresa, 2012). Se ha sugerido que el paso del tiempo puede ser un factor importante, y se han confirmado también diferencias entre machos y hembras, siendo más significativo el aumento de peso en los machos (Van de Weerd et al., 1997).

b) Cambios neurobiológicos.

Los cambios a nivel cerebral son los efectos más ampliamente documentados del EE. Se ha demostrado que esta situación de alojamiento da lugar a modificaciones estructurales y funcionales en áreas neurales relacionadas, entre otras, con mejoras en las capacidades de aprendizaje y memoria (Bayne, 2005; Bennett et al., 2006; Chen et al., 2010; Peña et al., 2006; Pizzorusso et al., 2007; Sampedro-Piquero et al., 2013a).

Tanto los primeros estudios realizados en los años 60, como investigaciones posteriores, muestran que el EE induce un incremento en el tamaño del cerebro y el grosor cortical, la densidad de los contactos sinápticos y de la ramificación dendrítica, el número de células gliales, el tamaño de los núcleos neuronales así como la cantidad de conexiones dendríticas (Diamond, 2001; Eckert & Abraham, 2012; Madroñal et al., 2010; Peña et al., 2006; Simpson & Kelly, 2011; van Praag et al., 2000). También se ha observado un incremento de la concentración de serotonina y de la actividad monoaminérgica y colinérgica central en animales expuestos a ambientes enriquecidos (Brenes et al., 2009; Simpson & Kelly, 2011). Estudios más recientes sugieren que el EE amplía las representaciones en el córtex somatosensorial, mejora el procesamiento de la corteza auditiva y visual, incrementa la plasticidad neuronal y los niveles de proteínas sinápticas, promueve cambios en los astrocitos, aumenta la neurogénesis en el giro dentado del hipocampo y la supervivencia de las nuevas neuronas, además de aumentar los niveles del factor neurotrófico derivado del cerebro (BDNF) y favorecer la reserva cognitiva (Artola et al., 2006; Diniz et al., 2010; Kempermann et al., 2010; Nithianantharajah & Hannan, 2011; Peña et al., 2006; Petrosini et al., 2009; Segovia et al., 2010; Viola et al., 2010). Sin embargo, no se conocen completamente los mecanismos neurobiológicos relacionados con algunos de estos cambios cerebrales inducidos por la estimulación ambiental (Sale et al., 2009; Nithianantharajah & Hannan, 2009).

El enriquecimiento también afecta a la expresión de diversos genes que regulan las estructuras neuronales, las señales sinápticas y la plasticidad cerebral (Sale et al., 2014), de modo que gran parte del interés en esta área se ha dirigido a estudiar las interacciones entre genes y ambiente que podrían mediar la plasticidad dependiente de la experiencia en modelos animales de enfermedades neurodegenerativas (Laviola et al., 2008), observándose efectos positivos a nivel cognitivo en modelos preclínicos de la enfermedad de Alzheimer (Barak et al., 2013; Beauquis et al., 2013; Freret et al., 2012), la enfermedad de Parkinson (Yuan et al., 2009) y otras enfermedades neurodegenerativas como la enfermedad de Huntington (Hannan, 2014). Estudios previos indican que la exposición a ambientes enriquecidos podría considerarse como una estrategia no farmacológica de neuroprotección frente a estas enfermedades

neurodegenerativas así como frente al declive cognitivo asociado a la edad (Mora, 2013; Patel, 2012; Redolat & Mesa-Gresa, 2012) y otras psicopatologías como el trastorno por déficit de atención con hiperactividad (Pamplona et al., 2009), la depresión (Richter et al., 2013) o algunas de las consecuencias de la exposición a situaciones de estrés crónico (Garrido et al., 2013; Hutchinson et al., 2012a; McQuaid et al., 2013). Los datos obtenidos con modelos animales sobre la enfermedad de Huntington muestran que los animales alojados en EE presentan retraso en la aparición de los síntomas motores y de los déficits cognitivos característicos de esta enfermedad, observándose un aumento considerable en la proliferación y en la maduración neuronal como base de estos efectos beneficiosos. Los principales hallazgos obtenidos sobre la enfermedad de Parkinson muestran que los animales alojados en condiciones de EE son un 200% más resistentes ante diversas toxinas que favorecen la neurodegeneración comparados con los animales control. Además, mientras los animales alojados en situaciones estándar presentan un 75% de pérdida de neuronas dopaminérgicas, los animales enriquecidos muestran una pérdida únicamente del 40%, dato que estaría también relacionado con la regulación a la baja en el transporte de dopamina (DA), cambio que como se verá posteriormente es importante para los modelos de abuso de drogas basados en el paradigma del EE. En modelos animales de la enfermedad de Alzheimer realizados con ratones transgénicos se ha observado que el EE altera los aspectos moleculares, conductuales y celulares relacionados con la patogénesis de esta enfermedad. Los animales alojados en condiciones de EE muestran también mayor protección frente al deterioro cognitivo característico de esta enfermedad, así como una disminución en los depósitos cerebrales de β-amiloide, y un incremento en la inmunoreactividad sináptica hipocampal. Del mismo modo, también se ha observado que el EE influye sobre los niveles cerebrales de neurotrofinas, especialmente en el BDNF (Laviola et al., 2008).

Los efectos del EE también han sido evaluados en otros parámetros a nivel neurobiológico. Se han descrito mayores niveles de testosterona y de inmunoglobulinas G en los animales alojados en EE frente a los controles, aunque existen diferencias en función de las cepas evaluadas (Larsson et al., 2002). También se han observado niveles de triglicéridos más bajos en animales aislados y un menor nivel

de colesterol en animales enriquecidos (Bayne, 2005). Otros estudios realizados en roedores han demostrado que el alojamiento en EE puede afectar a la liberación de las hormonas del estrés, reduciendo la activación del eje hipotalámico-pituitario-adrenal (HPA), incluso en investigaciones realizadas con estrés prenatal (Bayne, 2005; Chen et al., 2010; Cymerblit-Sabba et al., 2013). Por lo que respecta a los datos publicados sobre los efectos de la exposición a ambientes complejos sobre los niveles de corticosterona, los resultados no son concluyentes, habiéndose observado tanto incremento (Emack & Matthews, 2011; Hutchinson et al., 2012a; McQuaid et al., 2013) como disminución (Garrido et al., 2013; Hutchinson et al., 2012b) en los niveles analizados. En un estudio reciente realizado por McQuaid y colaboradores (2013) se observó un incremento de los niveles de corticosterona en animales alojados en condiciones de EE en comparación con los estándares, sugiriéndose que la exposición a este tipo de ambientes enriquecidos puede llegar a ser estresante para los animales debido al incremento en la conducta territorial inducido por el acceso a los juguetes y recursos incluidos en las cajas así como la necesidad de establecer la jerarquía social constantemente. Por el contrario, otros estudios han atribuido algunos de los efectos positivos relacionados con el alojamiento en condiciones de EE, como por ejemplo mejores capacidades de afrontamiento en situaciones estresantes, a niveles más bajos de corticosterona obtenidos en estos animales (Garrido et al., 2013). Otra posible explicación a los efectos beneficiosos del EE sobre la respuesta de ansiedad o el rendimiento cognitivo ha sido el incremento de receptores de glucocorticoides en el hipocampo (Sampedro-Piquero et al., 2014)

Numerosos estudios han analizado el efecto del EE sobre la recuperación de un cambio físico inducido experimentalmente en animales, como puede ser una lesión cerebral (Hoffman et al., 2008). Estudios previos muestran que el alojamiento en condiciones de EE puede atenuar los cambios estructurales y funcionales inducidos por lesiones cerebrales (Mandolesi et al., 2008) o accidentes cerebro vasculares (Saucier et al., 2010), sugiriéndose como estrategia neuroprotectora contra los efectos del daño cerebral adquirido en modelos preclínicos (Frasca et al., 2013; Johnson et al., 2013). Otras investigaciones han mostrado también efectos positivos en la recuperación de

hipoxia-isquemia neonatal (Rojas et al. 2013) o ambliopía (Baroncelli et al., 2012; Sale et al., 2014).

c) Cambios conductuales.

Los cambios observados a nivel físico y neurobiológico muestran correlatos a nivel conductual. Diversos estudios han mostrado que la exposición a ambientes enriquecidos induce disminución en la actividad motora basal (Bowling et al., 1993; Mesa-Gresa et al., 2013a; Varty et al., 2000), en las conductas estereotipadas (Gross et al., 2012), en la sensibilidad al dolor observada mediante el test de *hot-plate* (Abramov et al., 2008) y en la conducta de exploración y/o "búsqueda de novedad" (Brenes et al., 2008; Mesa-Gresa et al., 2013a), así como una habituación más rápida a los ambientes nuevos (Renner & Rosenzweig, 1986; Vedovelli et al., 2011).

Uno de los resultados conductuales más destacados de la exposición a EE es la mejora en el rendimiento de tareas de aprendizaje y memoria, especialmente en tareas de memoria dependientes de hipocampo (Harati et al., 2011; Simpson & Kelly, 2011; van Praag et al., 2000). Estos resultados han sido observados tanto en roedores machos (Kazlauckas et al., 2011; Mesa-Gresa et al., 2013a) como en hembras (Kulesskaya et al., 2011) expuestos a ambientes enriquecidos, aunque algunas diferencias han sido observadas en función del paradigma experimental de EE utilizado en el estudio (Mesa-Gresa et al., 2012) o de la tarea de aprendizaje utilizada (García-Capdevila et al., 2009). Estudios realizados en el laberinto de agua de Morris sugieren que tanto ratas como ratones enriquecidos presentan mejor memoria espacial que los controles ya que nadan distancias más cortas y muestran menor latencia para llegar a la plataforma (Laviola et al., 2008; Peña et al., 2006). En la tarea del laberinto radial se observa que el EE mejora la memoria espacial en ratas de distintas edades (Sampedro-Piquero et al., 2013a), así como la memoria a largo plazo en el test de reconocimiento de objetos nuevos, e induce una habituación más rápida de la actividad motora (Peña et al., 2006). En las tareas de reconocimiento social los datos son más contradictorios (Peña et al., 2006); observándose también efectos positivos en la tarea de evitación inhibitoria (Mesa-Gresa et al., 2013a). El test de campo abierto confirma la disminución de la actividad motora de los animales alojados con EE, mostrándose

además una elevada habituación ante esta tarea. La magnitud y persistencia de los efectos conductuales en ratones dependen de la duración del EE, de modo que debe darse un periodo mínimo de enriquecimiento para poder observar efectos significativos (Amaral et al., 2008). Dichos cambios persisten, al menos parcialmente, durante varios meses después de la exposición inicial al EE, y el grado de persistencia parece estar relacionado de forma positiva con periodos de exposición más largos (Amaral et al., 2008). Por lo que respecta al test de reconocimiento de objetos, tarea utilizada para evaluar la memoria de trabajo en roedores (Dere et al., 2007; Ennaceur et al., 2010; Heyser & Chemero, 2012; Kenney et al., 2011), se han observado también resultados positivos tras la exposición tanto a EE como a paradigmas basados en la estimulación física (Bechara & Kelly, 2013; Kazlauckas et al., 2011; Mesa-Gresa et al., 2013b; Viola et al., 2010).

Otros estudios han indicado cambios respecto a la respuesta emocional de los animales expuestos a ambientes enriquecidos, aunque se han registrado resultados contradictorios. En estudios realizados mediante el test del laberinto elevado o *plus-maze*, se ha indicado que los animales alojados en EE muestran menor respuesta de ansiedad que los alojados en condiciones estándar, observándose fundamentalmente un aumento del porcentaje de tiempo y entradas en brazos abiertos (Hughes & Otto, 2013; Mesa-Gresa et al., 2013a, 2014; Simpson & Kelly, 2011; Sztainberg & Chen, 2010), mientras otras investigaciones muestran resultados opuestos (Brenes et al., 2009; Peña et al., 2006; Zhu et al., 2009). Trabajos posteriores demostraron que los ratones macho alojados en EE realizaban mayor número de entradas totales en los brazos del laberinto elevado que los controles, efecto que no fue observado en las hembras, sugiriendo que el EE podía inducir efectos diferenciales en machos y en hembras a nivel emocional (Peña et al., 2006). Estudios recientes han concluido además que la exposición a ambientes enriquecidos puede incrementar la consecución de la homeostasis (Skwara et al., 2012) e influir sobre los factores que aumentan la resiliencia frente a las situaciones estresantes en roedores (Lehmann et al., 2012; Segovia et al., 2008; Schloesser et al., 2010; Skwara et al., 2012; Solinas et al., 2010).

Por lo que respecta a los resultados obtenidos en la conducta de interacción social, la agresión y la sumisión/dominancia, los resultados no son del todo concluyentes,

aunque sí que se observa que la exposición a EE induce cambios en estas respuestas (Hutchinson et al., 2012b). Distintos estudios muestran que la presencia de objetos novedosos en las cajas de EE, la complejidad de las mismas, el mayor número de sujetos por caja y el aumento de la proximidad entre ellos, hacen que se incremente la conducta agonística y la competición por los recursos disponibles (Abou-Ismail, 2011; Haemisch & Gartner, 1997; Haemisch et al., 1994; McQuaid et al., 2013). De hecho, se ha observado que la proximidad induce un aumento la conducta territorial entre ratones macho, mostrada principalmente por daños más severos en sus colas (Abou-Ismail, 2011; Haemisch & Gartner, 1997; Haemisch et al., 1994). También se ha reportado un incremento en las conductas de interacción social y en la conducta de juego de los animales evaluados (Mesa-Gresa et al., 2013b; Morley-Fletcher et al., 2003). Sin embargo, otras investigaciones muestran que la exposición a ambientes enriquecidos produce una disminución de la conducta agresiva en comparación con los animales expuestos a situaciones estándar (Abou-Ismail et al., 2010; Abramov et al., 2008; Chamove, 1989; Pietropaolo et al., 2004) o de aislamiento (Workman et al., 2011).

Tal y como se ha podido observar, existen discrepancias en cuanto a los efectos del EE, que podrían estar basadas en las variaciones en cuanto a la metodología utilizada, el tipo de estudios realizados, las variables de estudio y las diferencias individuales, así como la edad y el sexo de la muestra utilizada. Un tema de los que mayor interés ha despertado en los últimos años ha sido la investigación acerca de cómo las condiciones ambientales pueden influir sobre las respuestas emocionales y conductuales a diferentes drogas de abuso (Solinas et al., 2008; Mesa-Gresa et al., 2013c).

2.2. Componentes del Enriquecimiento Ambiental

Los estudios experimentales indican que los animales expuestos a ambientes enriquecidos o complejos suelen ser estimulados a distintos niveles. Se han establecido como principales componentes del modelo de EE aquellos relacionados con la estimulación a nivel físico, cognitivo, social y somatosensorial, y como factores

principales de estimulación la novedad y complejidad que ofrece la exposición a sus distintos factores (Nithianantharajah & Hannan, 2009; Sale et al., 2014). Los principales componentes incluidos en el modelo se caracterizan por: 1) *Estimulación física:* El mayor espacio que tienen las cajas utilizadas en EE estimula a los animales a mostrar mayor actividad y exploración. Además, los diferentes objetos incluidos en las cajas (ruedas de actividad, túneles, cuerdas, pelotas, plataformas, juguetes, casas, escaleras, etc.) hacen que la actividad física sea mayor en estos sujetos; 2) *Estimulación cognitiva:* Las oportunidades de exploración y aprendizaje constante ofrecidas por los ambientes enriquecidos se basan en los objetos que contienen así como el cambio constante de los mismos, y también en algunos materiales de nido; 3) *Estimulación social:* Un mayor número de animales por caja (entre 4 y 20 según el modelo) hace que aumente la interacción social; y 4) *Estimulación somatosensorial:* Diferentes estímulos y objetos contenidos en las cajas, con distintas formas, colores y texturas, ofrecen mayor estimulación somatosensorial y visual a los animales alojados en estas condiciones. Teniendo en cuenta la variabilidad de modelos de EE existentes, actualmente existe falta de acuerdo sobre cómo actúa cada uno de sus componentes en los efectos observados (Bechara & Kelly, 2013; Mesa-Gresa et al., 2013c; Pang & Hannan, 2013; Rojas et al., 2013), o bien si se produce sinergia entre los mismos. Si nos basamos en los estudios realizados en humanos observamos la importancia de mantener un estilo de vida activo a todos los niveles (social, intelectual y físico), con el objetivo de conservar las funciones cognitivas intactas y poder retrasar el declive cognitivo asociado a la edad (Daffner, 2010; Mora et al., 2013) (Véase Figura 3).

Con el fin de dilucidar esta cuestión, analizaremos los principales componentes incluidos en el paradigma de EE mediante investigaciones realizadas al respecto, tratando de entender el modo en que cada uno de ellos, sólo o en combinación con los otros, pueden estar implicados en los cambios inducidos por la exposición a este tipo de **ambiente.**

a) Actividad física.

Los estudios en humanos sugieren que el ejercicio físico puede mejorar la cognición a corto plazo y reducir el riesgo de demencia y deterioro cognitivo a largo plazo (Denkinger et al., 2012; Kempermann et al., 2010; Ravenelle et al., 2013). En

modelos preclínicos realizados en roedores, tal y como ya se ha descrito, el mayor tamaño de las jaulas y los objetos que contienen hacen que se incremente el movimiento y la actividad física voluntaria que muestran los animales.

Estudios recientes indican que los efectos inducidos en el cerebro por el EE y por los modelos basados en actividad física son muy similares. Ambos tipos de intervenciones aumentan la plasticidad del cerebro, la neurogénesis del hipocampo y los niveles de factores neurotróficos (principalmente de BDNF) (Chourbaji et al., 2012; Hotting & Roder, 2013; Kannangara et al., 2011). Estos cambios neurobiológicos inducidos por el ejercicio físico producen mejoras a nivel de aprendizaje y memoria similares a las observadas tras la exposición a EE, especialmente con respecto al reconocimiento de patrones y la coordinación motora (Creer et al., 2010; Madroñal et al., 2010). Otros resultados obtenidos muestran, sin embargo, que los efectos producidos por el ejercicio físico difieren de los de la exposición a largo plazo a los ambientes enriquecidos más complejos (Pang & Hannan, 2013). Estudios recientes sugieren que el principal componente neurogénico de EE es la actividad física (Kobilo et al., 2011; Mustroph et al., 2012), aunque la exposición a ambientes más complejos de EE puede tener algunos beneficios, tanto a nivel biológico como conductual, incluso en ausencia de ejercicio físico (Kobilo et al., 2011).

Tras la exposición a EE puede resultar complejo distinguir entre las consecuencias producidas por la estimulación cognitiva y social, y aquellos cambios inducidos por el ejercicio físico ya que, por ejemplo, la rueda de correr es un elemento que suele estar presente en la mayoría de los modelos de los entornos enriquecidos. En un estudio realizado por O'Callaghan y colaboradores (2009) se observó una mejora en la potenciación a largo plazo (LTP) y el aprendizaje espacial en ratas de edad avanzada que recibieron estimulación ambiental (exposición a una cinta de correr de la adultez en adelante), a pesar de que no se detectó ningún beneficio adicional proporcionado por la actividad física. En otras investigaciones se ha mostrado que la realización de ejercicio físico voluntario en una rueda de correr durante dos semanas mejoró la función hipocampal, el aprendizaje y la consolidación del condicionamiento de miedo, y disminuyó la respuesta de estrés y ansiedad mostrada por los ratones (Falls et al., 2010; Salam et al., 2009). Más recientemente, Creer y colaboradores (2010)

informaron de que la actividad realizada en la rueda de actividad indujo mejoras en la discriminación de estímulos similares mediante el test *touch-screen* en ratones de la cepa C57BL/6, sugiriéndose que la neurogénesis inducida por el ejercicio físico podría mejorar la respuesta de separación y discriminación de estímulos situados en posiciones similares.

Los mecanismos de la plasticidad cerebral responsables de los beneficios de la actividad física no están claramente establecidos, pero se han propuesto variables tales como el aumento de la neurogénesis y la expresión de BDNF en la formación hipocampal o la activación del eje HPA (Fuss et al., 2010; Hotting & Roder, 2013). A pesar de que los resultados de la actividad física han sido evaluados en diferentes trabajos publicados, pocos estudios los han comparado con los efectos de la exposición a ambientes enriquecidos, siendo discrepantes los pocos resultados obtenidos hasta la fecha.

b) Estimulación cognitiva.

Diversos paradigmas de estimulación cognitiva han sido aplicados en los estudios preclínicos. En el caso de la exposición a ambientes enriquecidos, las oportunidades de aprendizaje son ofrecidas por la diversidad de los objetos incluidos en las cajas y por el material de nido, que permiten la estimulación de la exploración y la manipulación del ambiente en los animales expuestos a estas condiciones. Además, estos objetos se cambian o reordenan de forma rutinaria, lo cual favorece aún más la novedad, manipulación, complejidad y exploración en los roedores. Es importante tener en cuenta que, para obtener estos efectos, los sujetos deben poder interactuar físicamente con su entorno y manipular todos y cada uno de los objetos que contiene. Algunos autores han propuesto que la exposición a EE puede inducir en los animales diferentes tipos de experiencias relativas al aprendizaje y la memoria, como son el aprendizaje motor, espacial e inhibitorio basado en objetos nuevos o redistribuidos (Will et al., 2004; Sale et al., 2014).

Los resultados obtenidos con estudios preclínicos sugieren que la actividad cognitiva a edades tempranas puede tener efectos a largo plazo (Vicens et al., 1999; 2003). Investigaciones más recientes han mostrado que los beneficios de la

estimulación cognitiva son independientes de los producidos por otros componentes del ambiente enriquecido (Codita et al., 2012; Wood et al., 2011); sugiriéndose incluso que los efectos de la exposición al EE sobre la memoria y el aprendizaje se deben principalmente a la estimulación cognitiva y la novedad proporcionados por este ambiente (Kumar et al., 2012). De hecho, la evidencia experimental obtenida sugiere que los beneficios obtenidos por la exposición a modelos de actividad física podrían ser limitados si no se acompañan de estimulación de tipo cognitivo.

c) Interacción social

En estudios realizados con humanos, se ha observado que la interacción y el apoyo social parece ser un factor importante en la prevención de la demencia (Middleton & Yaffe, 2010; Qiu et al., 2010), además de contribuir a la formación de la reserva cognitiva (Mangialasche et al., 2012; Marioni et al., 2012) y la prevención del abuso de drogas (Neisewander et al., 2012). La interacción social ha demostrado tener efectos neurobiológicos a nivel estructural y funcional, de especial relevancia en el desarrollo cerebral y conductual a edades tempranas (Mesa-Gresa y Moya-Albiol, 2011). En los estudios de EE se ha observado que la estimulación social puede inducir mejoras en situaciones estresantes, aunque en ocasiones también se ha relacionado con un aumento de las conductas agonísticas en cepas dóciles de roedores (Mesa-Gresa et al., 2013b).

La exposición a ambientes enriquecidos ha mostrado un aumento de la interacción social a diferencia de la observada en las cajas de alojamiento estándar, que obviamente puede estar inducida por el mayor número de sujetos alojados por caja en condiciones de EE. Algunos investigadores establecen diferencias entre el EE y el denominado “enriquecimiento social”, aunque esta distinción es difícil de realizar puesto que una de las características propias del ambiente enriquecido está basada en el alojamiento en grupo de los animales (Fares et al., 2012). En un estudio realizado por Kannangara y colaboradores (2011) no observaron efectos significativos del “enriquecimiento social” sobre los niveles de proliferación celular, sugiriendo que, al menos en animales envejecidos, este tipo de estimulación (sin estimulación física y/o cognitiva), no produce los mismos efectos que el EE. Otros estudios han mostrado que

la exposición a estimulación social aumenta significativamente la proliferación de células y neurogénesis en el hipocampo y muestra beneficios para la actividad y coordinación motora, especialmente en animales jóvenes; pero no parece ser un factor crucial para la mejora del aprendizaje y la memoria (Madroñal et al., 2010). Otras investigaciones, como la realizada por Goldberg et al. (2012) han mostrado sin embargo que la estimulación social bloquea la degeneración nigroestriatal en modelos animales de la enfermedad de Parkinson.

Si tenemos en cuenta los resultados obtenidos sobre el aislamiento en roedores, se aprecia cierto consenso sobre sus efectos perjudiciales (Fone y Porkess, 2008; Miyazaki et al., 2012). Por ejemplo, en un estudio se evaluó la proliferación celular en el hipocampo en ratas alojadas en grupo en comparación con otras aisladas pero con posibilidad de realizar ejercicio físico voluntario, observándose que el aislamiento social anula los efectos beneficiosos de la actividad física sobre este parámetro (Leasure & Decker, 2009).

Figura 3. Principales componentes incluidos en el paradigma de enriquecimiento ambiental (Adaptada de Redolat y Mesa-Gresa, 2012).

2.3. Factores que modulan el enriquecimiento ambiental.

Si se comparan los distintos estudios en los que se ha aplicado el paradigma de EE se puede observar que existen diferentes variables que parecen tener influencia en los efectos conductuales y neurobiológicos observados (Hu et al., 2010). Algunas de las principales variables evaluadas y que pueden modular los efectos observados serían: el tipo de modelo de EE utilizado, la edad de los animales, el periodo de tiempo en el que son expuestos a los ambientes enriquecidos así como el sexo de los sujetos experimentales.

a) Tipo de modelo de enriquecimiento ambiental utilizado.

Como ya se ha comentado previamente, la gran disparidad de modelos utilizados como método de EE puede inducir ciertas dificultades a la hora de generalizar y/o definir sus efectos principales. Algunos autores han usado modelos simplificados de EE (basados en material de nido y papel o cuerdas), mientras que otros han creado ambientes mucho más complejos, incluso con cajas diseñadas específicamente a tal efecto como las citadas cajas Marlau^{TM} (Marques et al., 2009; Fares et al., 2012; 2013). La variabilidad de modelos y diseños es muy amplia, lo cual puede llegar a hacer complicado la generalización de los resultados obtenidos. Algunos grupos de investigación han utilizado diferentes objetos para enriquecer el ambiente, que son cambiados de lugar constantemente (Mesa-Gresa et al., 2013a; 2013b; Sampedro-Piquero et al., 2013a; 2013b), mientras que otros autores han basado su modelo de EE en la exposición continua a distintos tests de evaluación conductual como método de estimulación de los animales (O'Callaghan et al., 2009). Otras variaciones han sido realizadas en función del tipo de exposición a los ambientes estimulantes. Por ejemplo, en un estudio realizado por Bennett y colaboradores (2006) compararon ratones expuestos a manipulación o *handling* diario, enriquecimiento diario (en que los animales fueron expuestos a cajas grandes durante 3h/día), y enriquecimiento continuo (con alojamiento las 24h en cajas grandes con objetos y ruedas de correr). Los resultados de este estudio indicaron que cada uno de los tipos de EE indicados tuvo efectos diferentes a nivel cognitivo en ratones macho de edades avanzadas, siendo los beneficios a nivel de memoria espacial más pronunciados en los animales

que habían recibido EE continuo que en aquellos que habían sido expuestos diariamente. Sin embargo, otros estudios han desarrollado ambientes enriquecidos muy complejos tratando de ofrecer a los roedores una estimulación multisensorial completa, pero no realizando cambios constantes en sus objetos. Los resultados obtenidos en este estudio no muestran diferencias significativas en los resultados obtenidos entre animales expuestos a EE y los alojados en situación estándar en los tests de aprendizaje evaluados (Pamplona et al., 2009).

b) Edad en que se inicia la exposición al ambiente enriquecido.

La edad es una de las variables más relevantes a la hora de definir los efectos del enriquecimiento, fundamentalmente cuando el inicio de la exposición se realiza en edades críticas para el desarrollo del sujeto (exposición prenatal, pre adolescencia o adolescencia) (Pietropaolo et al., 2008). Existen diferentes estudios preclínicos llevados a cabo en ratones en los que el inicio de la exposición al ambiente enriquecido varía entre la adolescencia (Solinas et al., 2009); la adultez (Madroñal et al., 2010) o en edades avanzadas (Diamond, 2001), habiendo incluso estudios donde la exposición es prenatal o en el momento del destete (Sparling et al., 2010). En estos estudios se ha observado que los efectos de la exposición postnatal a edades tempranas está mediada por el cuidado de la madre, observándose que las crías expuestas a EE reciben mayor estimulación y contacto físico de sus madres (Marouka et al., 2009). Distintos estudios han sugerido la posibilidad de que haya una edad crítica para beneficiarse de los efectos positivos del EE, fundamentalmente en edades avanzadas, aunque estudios recientes han mostrado efectos positivos del EE en tareas de aprendizaje tanto en animales jóvenes como en viejos (Sampedro-Piquero et al., 2013a).

c) Periodo de tiempo total en el que los animales son expuestos a enriquecimiento ambiental.

El tiempo total que los animales están expuestos al EE varía en función de los estudios analizados. En algunas investigaciones, los animales permanecen expuestos a estos ambientes durante largos periodos (que pueden variar de semanas a meses, e incluso años), mientras que en otros estudios la exposición se limita a cortos periodos

de tiempo (que oscilan entre unas horas al día o exposiciones repetidas durante diversos días). Algunos autores han sugerido que la exposición de los animales a ambientes enriquecidos durante al menos un mes es suficiente para inducir efectos significativos (Brenes et al., 2008; Mesa-Gresa et al., 2013a, 2013b), aunque exposiciones más duraderas producirán efectos más robustos. Los efectos observados tras distintos periodos de exposición también pueden relacionarse con la tarea de evaluación utilizada. En el test de campo abierto los cambios conductuales han sido observados tras una semana de exposición a EE, mientras que en tareas más complejas, como el test de natación forzada o el laberinto de agua de Morris, periodos más largos de exposición son necesarios para producir efectos significativos (Brenes & Fornaguera, 2008; Leggio et al., 2005). En un estudio realizado por Kobayashi et al. (2002) se observó que el alojamiento en EE tenía efectos beneficiosos tanto en ratas adultas (11 meses de edad) como en las envejecidas (22 meses), pero el tiempo de exposición que era requerido para observar dichos efectos variaba en función de la edad de los animales. En las ratas adultas, los efectos obtenidos tras una exposición a corto plazo (3 meses) o a largo plazo (24 meses) fueron similares, mientras que en las ratas envejecidas se observó que los efectos obtenidos tras una exposición a largo plazo fueron mucho más pronunciados, observándose incluso plasticidad cerebral en sus funciones cognitivas. Futuros estudios podrían ayudar a determinar si el estilo de vida activo debería ser mantenido durante toda la vida con el fin de obtener y conservar los beneficios cognitivos observados, o bien si las exposiciones a estimulación y a ambientes enriquecidos realizadas únicamente en periodos críticos son suficientes para prevenir o retrasar determinados trastornos y enfermedades (Van Dellen et al., 2008; Redolat & Mesa-Gresa, 2012).

d) Sexo de la muestra.

Pocas investigaciones han tratado de dilucidar los posibles efectos diferenciales del EE en machos y en hembras. A nivel neurobiológico, se ha obtenido un incremento en la densidad dendrítica del córtex occipital en machos, observándose este incremento a nivel de córtex somatosensorial en hembras (Diamond, 2001). En cuanto a efectos conductuales, los machos muestran un incremento de la actividad motora en

el test de campo abierto, mientras que en hembras se ha registrado descenso (Elliott & Grunberg, 2005). En animales con daño cerebral y expuestos a EE se ha observado que los machos muestran mejoras en tareas de aprendizaje espacial, no obtenidas en las hembras (Peña et al., 2006). Estudios relativos a los efectos del EE sobre la respuesta emocional en roedores aportan datos indicativos de un efecto ansiolítico en machos y ansiogénico en hembras (Lin et al., 2011). Dado que los resultados son poco concluyentes, son necesarios más estudios con el fin de evaluar las posibles diferencias existentes en función del sexo de la muestra entre los efectos conductuales y neurobiológicos producidos por el EE.

2.4. Una cuestión a tener en cuenta: ¿El modelo de enriquecimiento ambiental como práctica obligatoria en el laboratorio?

Los esfuerzos realizados por los distintos estudios e investigaciones científicas durante décadas han ido dirigidos a minimizar las diferencias entre procedimientos y protocolos experimentales (Hutchinson et al., 2012a; 2012b). Las nuevas normativas sobre el mantenimiento y cuidado de los animales de laboratorio (2010/63/EU) indican que, con el fin de mejorar las condiciones de alojamiento y el bienestar de los animales, se deberán aplicar técnicas de EE en todos los laboratorios (Transposición de la normativa en España: Real Decreto 53/2013, de 1 de Febrero). Como se ha podido observar, existen multitud de efectos y variaciones a tener en cuenta a la hora de aplicar estos paradigmas, de modo que puede generarse cierta controversia al respecto (Hutchinson et al., 2012a; Macri et al., 2013). Estos estudios basan su argumentación en el hecho de que los datos base obtenidos hasta la fecha pueden ser completamente modificados teniendo en cuenta el cambio en las condiciones de alojamiento de los animales, basándose en la máxima de que el fenotipo de los animales evaluados es el resultado de la interacción entre la base genética (o genotipo) y el ambiente (Macri et al., 2013). Considerando los efectos observados en modelos animales de distintas enfermedades y patologías, como por ejemplo la enfermedad de Alzheimer o la adicción (Laviola et al., 2008), y teniendo como grupo de referencia la comparación con alojamiento estándar de laboratorio, se hace

complicado establecer efectos de los nuevos tratamientos si en aplicación a la normativa el grupo control es un grupo alojado y probablemente beneficiado y/o influido por los efectos del EE (Macri et al., 2013). Además, cabe tener en cuenta que, en contra de los planteamientos iniciales en los que se trataba de buscar un modelo de EE estandarizado (Sztainberg & Chen, 2010; Fares et al., 2012; 2013), si se atiende a la nueva normativa se observa que lo que se indica específicamente es que *"el enriquecimiento ambiental debe ser adaptado a las necesidades individuales y de la especie de los animales implicados"* (Normativa 2010/63/EU, Anexo III). Esto puede dar a lugar a múltiples tipos de modelos de EE, a un aumento de los animales necesarios para llevar a cabo la investigación, a inconsistencias en los resultados obtenidos así como a dificultar la reproductibilidad de los resultados alcanzados en la investigación (Hutchinson et al., 2012a; Macri et al., 2013). Esta cuestión plantea la necesidad de realizar más estudios acerca de la comparación entre los efectos producidos por distintos tipos de EE, así como una descripción sistemática y detallada en cada uno de los estudios publicados de los detalles y características del modelo de EE utilizado (Macri et al., 2013); a lo que cabría añadir la diferenciación entre los efectos de sus diferentes componentes y/o variaciones (edad de inicio, periodo de exposición, sexo de la muestra, incluso especie y cepa de los animales) y la estimación de los resultados atribuidos al tratamiento o condiciones experimentales analizadas, o bien los efectos atribuidos al tipo y características de EE utilizado.

3. ENRIQUECIMIENTO AMBIENTAL Y ADICCIÓN A DROGAS DE ABUSO

A pesar de que muchas personas experimentan puntualmente los efectos de las drogas psicoactivas, tan sólo un pequeño porcentaje desarrolla conductas de abuso, dependencia y adicción (Green et al., 2010; Solinas et al., 2008). Actualmente, la adicción se considera una enfermedad crónica que da lugar a cambios duraderos en el funcionamiento cerebral, que a su vez, interactúan con factores del ambiente (Puhl et al., 2012). A la base de tales diferencias en la adquisición de las drogodependencias se

encuentran aspectos como el genotipo del sujeto o su carga genética y su fenotipo (conformado por las características personales determinadas por la historia del sujeto y por las variables ambientales en las que se ha desarrollado) (Laviola et al., 2008; Macri et al., 2013). Diversos estudios recientes otorgan un papel central a estos factores ambientales como determinantes de la sensibilidad a los efectos reforzantes de las drogas y, por tanto, de la vulnerabilidad a desarrollar adicción (El Rawas et al., 2011; Solinas et al., 2010; Thiel et al., 2010a).

Existe una amplia evidencia experimental que indica que la exposición a experiencias negativas durante los primeros años de vida, como el maltrato infantil, producen una serie de cambios estructurales y funcionales a nivel cerebral que favorecen el consumo de alcohol y otras sustancias psicoactivas tanto durante la adolescencia como en la adultez (Mesa-Gresa & Moya-Albiol, 2011). La asociación entre adicción a drogas y la exposición a otras experiencias adversas como el estrés de combate, abuso sexual o estrés laboral también ha sido documentada (Wong et al., 2011). Sin embargo, se ha observado que las relaciones familiares positivas parecen disminuir las posibilidades de consumo y podrían prevenir frente al abuso y adicción a drogas; por lo que se podría hipotetizar que un ambiente positivo, especialmente durante los periodos críticos de desarrollo, podría tener ciertos efectos protectores y/o curativos frente al consumo de drogas (Laviola et al., 2008; Mesa-Gresa & Moya-Albiol, 2011; Solinas et al., 2010; Wong et al., 2011). La principal dificultad de las investigaciones en sujetos humanos se plantea porque en los estudios retrospectivos resulta difícil aislar el “ambiente enriquecido” de otras variables que pueden afectar al sujeto a lo largo de su vida (Nithianantharajah & Hannan, 2009, 2011; Mesa-Gresa et al., 2013c). Por ello, se sugiere que la utilización de paradigmas como el EE en roedores podría proporcionar un modelo adecuado para investigar posibles efectos “protectores” de un estilo de vida positivo y activo frente a la adicción (Laviola et al., 2008). Teniendo en cuenta esta limitación, se han llevado a cabo diversos estudios preclínicos en primates y roedores acerca de los efectos negativos de la exposición a estrés a edades tempranas, sugiriendo que ante esta situación se producen una serie de modificaciones a nivel fisiológico, neurobiológico y hormonal, que son resultado de interrupciones y cambios en la regulación de las vías neurobiológicas del refuerzo en el

cerebro y de los sistemas de respuesta al estrés que podrían llevar a una mayor propensión al consumo de sustancias adictivas (Laviola et al., 2008; Schloesser et al., 2010; Thiriet et al., 2008).

Tal y como se ha analizado en puntos anteriores, la estimulación y enriquecimiento del ambiente da lugar a cambios neuroquímicos, celulares y moleculares que podrían desempeñar una importancia fundamental en los procesos de adicción (Laviola et al., 2008; Solinas et al., 2008; Macri et al., 2013) así como en el estudio de los efectos del ambiente sobre la misma (Carrol et al., 2009; Chauvet et al., 2009; Thiel et al., 2010b). En esta línea de estudio, el modelo de EE ha mostrado ser válido ya que la exposición a un ambiente enriquecido en etapas críticas del desarrollo parece disminuir la propensión a la adicción (El Rawas et al., 2009; Green et al., 2010; Solinas et al., 2010).

Recientemente, se han ido proponiendo aplicaciones del EE que abren nuevas posibilidades en la investigación neuroconductual basadas en la idea de que un mejor conocimiento del papel que desempeñan las condiciones ambientales en la susceptibilidad individual a desarrollar adicción podría resultar crucial para mejorar las estrategias de prevención e intervención de diferentes drogas de abuso (Green et al., 2003; Solinas et al., 2010; Wong et al., 2011). Investigaciones realizadas por los equipos de Marcello Solinas (en la Universidad de Poitiers, Francia) y Henriette Van Praag (en el National Institue of Aging, USA) confirman esta hipótesis, mostrando que el alojamiento en un ambiente enriquecido puede prevenir el desarrollo de la adicción a drogas o, en última instancia, convertirse en un tratamiento potencial.

Como ya se ha indicado previamente, la exposición a ambientes enriquecidos induce mejoras en el aprendizaje y la memoria, facilita la recuperación tras lesiones cerebrales y protege del desarrollo de enfermedades neurodegenerativas, por lo que diversos autores sugieren que este tipo de alojamiento representa un ambiente más saludable que las condiciones estándar de laboratorio (Pang & Hannan, 2013; Solinas et al., 2010; Sale et al., 2014). Además, el EE también da lugar a diversos cambios a nivel celular y molecular, ya que se observa que los ratones enriquecidos poseen menos neuronas dopaminérgicas en la sustancia negra, menores niveles de transporte

de DA, que es la vía molecular de sustancias como la cocaína o la nicotina, así como un mayor nivel BDNF en áreas como el estriado (Laviola et al., 2008; Solinas et al., 2008). En base a estos datos se sugiere que el EE podría ser crucial para determinar la resistencia ante algunas drogas de abuso como la cocaína y las anfetaminas (Bardo et al., 2001; Laviola et al., 2008; Magalhaes et al., 2007; Solinas et al., 2008; 2010; Thiriet et al., 2011), la heroína (El Rawas et al., 2009), el alcohol (Rueda et al., 2011) y la nicotina (Mesa-Gresa et al., 2012; Zhu et al., 2009). Se ha suferido que el objetivo principal de las futuras investigaciones realizadas al respecto podría ser el de estudiar el papel protector del EE ante el fenómeno de la adicción a las drogas, de modo que permita obtener información sobre los factores ambientales que influyen en la vulnerabilidad ante ciertas sustancias adictivas (Laviola et al., 2008).

Los estudios más amplios sobre la relación entre EE y vulnerabilidad a la adicción se han realizado fundamentalmente con cocaína. En investigaciones iniciales realizadas por el grupo de Bezard y colaboradores se demostró que las ratas enriquecidas son menos responsivas a esta droga que las mantenidas en ambientes estándar. Además, los ratones enriquecidos muestran un patrón diferente de expresión de la proteína *c-fos,* incremento en la expresión de factores neurotróficos (especialmente BDNF) y regulación a la baja en la expresión del transportador de DA, principal diana de los psicoestimulantes (Bezard et al., 2003; Solinas et al., 2008), así como disminución de la reactividad de las neuronas estriatales a los efectos de la cocaína y otras drogas dopaminérgicas (Solinas et al., 2009; Thiriet et al., 2010). En un estudio realizado por Solinas y colaboradores (2010) se evaluó el efecto de la exposición a ambientes enriquecidos en roedores que previamente habían desarrollado adicción a la cocaína utilizando para ello diferentes modelos animales (sensibilización conductual, condicionamiento de preferencia de lugar y reinstauración inducida por la droga de una preferencia de lugar ya extinguida) en base a los cuales sugieren que los efectos del EE pueden no ser solo preventivos sino también “curativos” frente a una adicción ya establecida (Smith et al., 2009; Solinas et al., 2010; Thiriet et al., 2010), conclusiones interesantes que abren nuevas vías de investigación.

En estudios previos se ha demostrado que el EE disminuye la auto-administración de anfetaminas (Green et al., 2002). Bowling y Bardo (1994) observaron que las ratas

criadas en ambientes enriquecidos mostraban un incremento en el efecto estimulante a nivel locomotor y de preferencia de lugar inducido por anfetaminas en comparación con las criadas en aislamiento o en condición social. Posteriormente, Bardo y colaboradores confirmaron que el EE disminuye la sensibilización conductual inducida por anfetaminas (Bardo et al., 1995). Sin embargo, Thiriet y colaboradores (2010) evaluaron los posibles efectos beneficiosos del EE en la adicción a metanfetamina, sugiriendo que la exposición a un ambiente enriquecido no disminuye sus efectos reforzantes y activadores y, por tanto, el EE no parece tener efectos directos sobre la vulnerabilidad al desarrollo de la adicción a esta sustancia (Thiriet et al., 2010).

Green y colaboradores (2010) han planteado recientemente la hipótesis de que la activación repetida de las vías de la recompensa mediante la novedad, el ejercicio y el contacto social llevan a que las ratas criadas en ambientes enriquecidos sean menos vulnerables a la auto-administración compulsiva de diferentes drogas de abuso. En un estudio experimental, estos autores demostraron que las ratas criadas en EE, en comparación con las "empobrecidas", muestran una disminución en la auto-administración de cocaína pero mayor condicionamiento de preferencia de lugar inducido por esta droga, reducción de las conductas relacionadas con depresión pero incremento de la ansiedad y una menor actividad de la proteína CREB (*cAMP response element binding protein*) en el Núcleo Accumbens (NAcc) (que se asocia con expresión reducida del BDNF). Por tanto, numerosos resultados experimentales apoyan la hipótesis de que el EE podría tener efectos protectores frente a la adicción a la cocaína, aunque no se conocen totalmente los mecanismos subyacentes. También se ha evaluado si los efectos beneficiosos del EE que se habían demostrado previamente en relación con la cocaína se observaban en el caso de la heroína, utilizando el paradigma del condicionamiento de preferencia de lugar (El Rawas et al., 2009).

En relación con el alcohol, diversos estudios también han demostrado la utilidad de las manipulaciones ambientales en modelos animales para estudiar posibilidades de intervención en la adicción a esta sustancia (Rueda et al., 2011). Se ha hipotetizado que los niños con Síndrome Alcohólico Fetal presentan mayores alteraciones en su desarrollo debido a que son criados en ambientes de "riesgo", exacerbando el impacto de la exposición prenatal al alcohol. De ahí la necesidad de que estos niños sean

criados en ambientes adecuados, con condiciones ambientales estructuradas y enriquecidas. Utilizando modelos animales, se han realizado diferentes intentos para evaluar cómo las manipulaciones experimentales del ambiente postnatal pueden utilizarse para "tratar" los efectos de la exposición fetal al alcohol. Estas aproximaciones incluyen el "*handling*" (manoseo) postnatal, el EE y el entrenamiento basado en la rehabilitación motora (Hannigan et al., 2007). En general, se observa que cuando los roedores son criados en ambientes enriquecidos (a nivel motor, sensorial y social) después de haber estado expuestos al alcohol durante el periodo prenatal, el EE puede mitigar los efectos del alcohol sobre la conducta, confirmando la existencia de plasticidad cerebral.

Aunque todavía no se comprenden bien los cambios moleculares que subyacen a los efectos del EE sobre la adicción (Burrows et al., 2010; Stairs & Bardo, 2009), se sugiere que la exposición a este ambiente podría proporcionar a los sujetos mayor capacidad de discriminar la presencia de recompensa (Grimm et al., 2008). En estudios previos, las ratas enriquecidas muestran un incremento en el condicionamiento tanto de la preferencia como de la aversión a un lugar (Bardo et al., 1995; Smith et al., 2005), así como extinción acelerada del condicionamiento del miedo (Pietropaolo et al., 2006). Ello indicaría que los animales criados en ambientes enriquecidos muestran mayor capacidad de aprendizaje acerca del significado de los estímulos asociados a la recompensa y el castigo, y de distinguir entre "disponibilidad" y "no disponibilidad" del refuerzo. Durante el proceso de extinción, también parecen aprender más rápidamente que sus acciones no van a ser recompensadas y, por ello, dejan de responder más pronto que los criadas en ambientes empobrecidos (Grimm et al., 2008), aunque la exposición al EE parece disminuir su impulsividad (Perry et al., 2008). Estos cambios podrían ir asociados a una disminución de la vulnerabilidad a las conductas de recaída (Grimm et al., 2008). Son necesarios más estudios para establecer las bases neurobiológicas de los efectos que la exposición a un ambiente enriquecido induce sobre los sistemas de refuerzo, aunque las vías dopaminérgica, mesolímbica y mesocortical, así como el glutamato, han sido implicadas (Segovia et al., 2010).

3.1. Enriquecimiento ambiental y agonistas colinérgicos

Existen numerosos estudios realizados en animales en los que se investigan los efectos de la nicotina, a través distintas modalidades de tratamiento y combinaciones experimentales y en función de diversas tareas de laboratorio, pero se han publicado relativamente pocos trabajos en los que se tengan en cuenta las características del alojamiento de los sujetos. La mayoría de las investigaciones han tratado de dilucidar los factores genéticos y moleculares implicados en la adicción a esta sustancia, pero es poco conocido cómo los factores ambientales asociados a la plasticidad dependiente de la experiencia pueden influir en la modulación y progreso de la adicción a la nicotina, así como en el cese de la misma o en la recaída tras un periodo de abstinencia (Coolon & Cain, 2009; Solinas et al., 2010). Existen numerosas investigaciones, realizadas tanto en modelos clínicos como preclínicos, que muestran los efectos y cambios estructurales y moleculares inducidos por la nicotina, pero no se han investigado de modo tan detallado los cambios mediados por el ambiente en el que se desarrolla el sujeto (Coolon & Cain, 2009).

Al igual que la cocaína, la anfetamina o la heroína, la nicotina presente en el tabaco induce un patrón de conducta adictiva (De Biasi & Dani, 2011), estimula la liberación de DA en las áreas mesolímbicas, en el cuerpo estriado y en el córtex prefrontal, siendo estos efectos de especial importancia en las neuronas dopaminérgicas del área tegmental ventral que liberan, como efecto inmediato, DA en el NAcc. Esta vía mesolímbica resulta esencial en el refuerzo asociado a diferentes drogas de abuso (nicotina, cocaína, opiáceos, alcohol...) (Benowitz, 2008; Koob & Le Moal, 2006; Rose, 2007). Los efectos globales de esta sustancia en cuanto a la liberación de DA son dependientes de la interacción entre los efectos directos de la nicotina y los efectos modulados por el glutamato y el GABA (Benowitz, 2008). Los receptores colinérgicos nicotínicos neuronales, principalmente los α4β2, desempeñan un papel crucial en la liberación de DA y otros neurotransmisores relacionados con el refuerzo inducido por drogas, así como en los mecanismos implicados en la mejora de las funciones cognitivas como la memoria y el aprendizaje. La exposición crónica a la nicotina induce un efecto de neuroadaptación o desarrollo de la tolerancia, una

regulación a la alza de los receptores nicotínicos ("*up-regulation*") (Benowitz, 2008) y cambios en la expresión génica y en la síntesis de proteínas, lo cual da lugar a la generación de nuevas conexiones sinápticas, análogas a otras formas de aprendizaje (Benowitz, 2008). Por su parte, diferentes subtipos de receptores nicotínicos (especialmente los α7 y los α4β2) parecen mediar los efectos neuroprotectores de este agonista colinérgico (Gotti et al., 2007).

A nivel neurobiológico, una de las investigaciones más importantes sobre la relación entre EE y adicción a la nicotina fue la realizada por el grupo de Zhu y colaboradores acerca de la influencia del EE en la liberación de DA inducida por nicotina en áreas del córtex prefrontal medial y del estriado. En ratas macho de la cepa Sprague-Dawley de 21 días de edad obtuvieron medidas electroquímicas *in vivo* tras la administración de nicotina y salino. Sus resultados indicaron que las condiciones de EE eliminan la respuesta de transporte de DA en el córtex prefrontal medial en el grupo control y potencian el incremento inducido por la nicotina en el transporte de DA en esta área pero no en la zona del estriado (Zhu et al., 2007). Otro estudio realizado más recientemente por este grupo de investigación (Zhu et al., 2012) ha mostrado los efectos de la exposición a un ambiente enriquecido sobre la liberación de DA en las regiones de la corteza (*shell*) y el núcleo (*core*) del NAcc en ratas macho. En los animales del grupo control (tratados con salino) y alojados en condiciones de EE, en comparación con aquellos alojados en aislamiento, se observó una mayor liberación de DA en la corteza y menor liberación de DA en la zona central del NAcc. Por el contrario, tras la administración aguda de nicotina, observaron el efecto contrario en las ratas alojadas en ambiente enriquecido. En este estudio se relacionan los resultados obtenidos con una disminución en las concentraciones de DA a nivel extracelular, que podría estar asociada con los efectos observados a nivel psicomotor en los animales alojados en EE y sometidos a tratamiento agudo con nicotina. Hamilton y Kolb (2005) analizaron los efectos que la exposición a la nicotina y a determinadas experiencias ambientales tenían sobre la plasticidad estructural. Se compararon ratas hembra de la cepa Long-Evans alojadas en condiciones estándar con ratas alojadas en EE. En la primera parte del experimento, las ratas pasaron por la situación de tratamiento y posteriormente fueron alojadas en distintas situaciones experimentales, observándose

que la exposición continuada a la nicotina bloquea el incremento de ramificaciones dendríticas, longitud y densidad así como el total de espinas dendríticas en el NAcc relacionadas con las condiciones de EE. Durante el segundo experimento, se siguió el procedimiento contrario, esto es, se mantuvo a la muestra en las dos condiciones de alojamiento (estándar y EE) y posteriormente se las sometió al tratamiento con nicotina. En este procedimiento no se observó que las condiciones de alojamiento bloquearan los efectos de la nicotina, concluyendo que las condiciones de EE no bloquearon la plasticidad estructural producida por la nicotina. Estos resultados demuestran que existen importantes diferencias en la capacidad de las drogas y de la experiencia con respecto los cambios inducidos en las estructuras dendríticas (Hamilton & Kolb, 2005). Otro estudio llevado a cabo recientemente por Skwara y colaboradores (2012) indicó que la exposición a EE induce una disminución en la liberación de hormonas del eje HPA en respuesta a la administración de nicotina, siendo las hembras más sensibles a los efectos del EE que los machos. Teniendo en cuenta la variable sexo, en este estudio también se observó que las ratas hembra alojadas en condiciones de EE se mostraron menos sensibles a los efectos inhibitorios del tratamiento agudo con mecamilamina y nicotina sobre la liberación de corticosterona que a los efectos del tratamiento con mecamilamina y salino. Una de las últimas investigaciones llevadas a cabo en esta área es la de Gómez y colaboradores (2012), en la que se han evaluado los mecanismos moleculares inducidos por la exposición a ambientes enriquecidos y relacionados con los efectos del tratamiento con nicotina. En este estudio se observó que las ratas alojadas en EE muestran menor nivel de pDARPP-32 Thr34 (fosfoproteína 32 reguladora de DA y cAMP) en la corteza prefrontal y NAcc y de la proteína CREB que las ratas alojadas en condiciones estándar o aisladas. También observaron que las ratas del grupo EE mostraron mayor sensibilización a los efectos motores de la nicotina. Como se puede observar, los datos obtenidos de los estudios neurobiológicos sobre los efectos y posible interacción entre EE y nicotina muestran que la exposición de los animales a ambiente enriquecidos puede modificar la repuesta molecular, neuroquímica y hormonal a esta sustancia, y que estos resultados dependen de distintos factores como son el sexo, la especie o la cepa evaluada, así como la complejidad de los

elementos que componen el ambiente enriquecido y la edad y periodo de exposición al mismo (Mesa-Gresa et al., 2013c).

Además de las investigaciones llevadas a cabo para esclarecer los efectos neurobiológicos de la interacción entre EE y nicotina, otros estudios han tratado de dilucidar los principales efectos conductuales de dicha interacción. En una investigación ya clásica del grupo de Green y colaboradores (2003), analizaron el efecto del EE sobre la actividad motora en ratas Sprague-Dawley tras la administración repetida de nicotina (Green et al., 2003), sugiriendo que EE podría reducir los efectos estimulantes de esta droga. En la primera parte de este estudio se evaluaron diferencias en cuanto a actividad motora inducida por nicotina comparando grupos de EE frente a grupos de ratas aisladas, mientras que en la segunda parte la comparación se realizó entre grupos de EE y grupos de "enriquecimiento social". En ambos experimentos se observó que las ratas del grupo de EE mostraron menor sensibilidad a los efectos de hiperactividad inducidos por la nicotina que las ratas aisladas o enriquecidas socialmente. Estos resultados sugieren que el EE podría reducir los efectos estimulantes de la nicotina (Green et al., 2003), aunque es importante destacar que este estudio no examinó los efectos ambientales sobre la hiperactividad condicionada inducida por nicotina (Coolon & Cain, 2009). Otro de los estudios publicados al respecto se corresponde con el experimento realizado por Coolon y Cain (2009) en el que se han analizado los cambios producidos por el ambiente de crianza sobre los efectos conductuales de la nicotina. Para ello se examinó el efecto sobre la actividad motora en tres grupos de ratas Sprague-Dawley: ratas criadas en EE, en "enriquecimiento social" y en ambiente empobrecido o aisladas. Además, se evaluó el efecto de la mecamilamina, antagonista nicotínico, sobre la expresión de la hiperactividad y sensibilización a la nicotina. Se observó una disminución de la sensibilización a los efectos hiperactivos locomotores de la nicotina en las ratas alojadas en EE frente a las de los grupos de enriquecimiento social y aislamiento, reportándose además que las ratas alojadas en ambiente empobrecido se mostraron más sensibles a las claves contextuales asociadas a la nicotina. Por lo que respecta a los efectos del pre-tratamiento con mecamilamina, se observó que esta sustancia bloqueó la hiperactividad condicionada a la nicotina en ratas de los grupos de EE y de

enriquecimiento social, pero no en las ratas del grupo empobrecido. Estos resultados sugieren que la hiperactividad condicionada a la nicotina está en parte mediada por los receptores colinérgicos y que las condiciones de alojamiento del EE pueden alterar estos receptores, confirmándose la hipótesis de que el ambiente es uno de los factores que contribuye a las diferencias individuales en la adicción, cese y recaída de la nicotina (Coolon & Cain, 2009).

Teniendo en cuenta los principales resultados obtenidos en los estudios descritos previamente, parece obvio deducir que la exposición a ambientes enriquecidos produce efectos fisiológicos y conductuales que claramente interaccionan con los efectos de la nicotina, fundamentalmente en lo relativo a la variación del sistema dopaminérgico y a la hiperactividad motora. De hecho, un estudio reciente evidenció esta interacción, observando que el EE produce un efecto protector tanto en la sensibilización a la nicotina así como a la sensibilización cruzada ante sustancias como la D-anfetamina (Adams et al., 2013). Sin embargo, otros estudios no han mostrado una evidencia concluyente de esta posible interacción y la escasez de estudios al respecto no permite determinar con exactitud el efecto de las manipulaciones ambientales en la respuesta conductual a esta droga. Futuros estudios deberían investigar si variables experimentales como las diferencias en edad y/o cepa, variación en condiciones ambientales o periodo de exposición al EE, así como tipo de tratamiento y dosis de la nicotina pueden afectar a los posibles efectos neuroprotectores de esta droga de abuso.

Son escasos los estudios realizados sobre la posible interacción entre las condiciones de EE y la administración de otros agonistas colinérgicos (Adams et al., 2013; Mesa-Gresa et al., 2012; 2014; Solinas et al., 2010; Zhu et al., 2013), siendo aún menos frecuente la evaluación de los efectos conductuales obtenidos (Mesa-Gresa et al., 2012; 2013a). Los estudios analizados previamente en los que se muestra interacción entre las condiciones ambientales y la administración de agonistas nicotínicos, así como la falta de evidencia concluyente acerca de sus efectos conductuales, hace necesaria nueva investigación al respecto (Mesa-Gresa et al., 2014). Estudios en humanos y animales han mostrado el importante rol del sistema colinérgico en la atención, memoria y aprendizaje (Levin, 2012). De hecho, existe

evidencia experimental que indica la relación existente entre el envejecimiento cerebral y la pérdida de receptores colinérgicos (lo cuáles están distribuidos principalmente en el córtex cerebral, en la región del hipocampo y en las áreas límbicas del cerebro), además de evidencias sobre la neurodegeneración colinérgica y su asociación con el declive cognitivo e incluso con la aparición de enfermedades neurodegenerativas (McLean et al., 2011). Más específicamente, los receptores α7 han demostrado tener ciertas propiedades neuroprotectoras que parecen estar relacionadas con los efectos a nivel cognitivo, motor y sensorial inducidos por la nicotina (Hunter et al., 2012; Lendvai et al., 2012; Welch et al., 2013), aunque sus efectos a nivel biológico y conductual no se conocen totalmente (Maloku et al., 2011; Vicens et al., 2011; 2013a). El hecho de que determinadas sustancias puedan mostrar una afinidad más selectiva hacia determinados tipos de receptores colinérgicos justifica la creciente investigación hacia este tipo de sustancias farmacológicas (Graef et al., 2011; Vicens et al., 2013a; 2013b). Entre los fármacos más estudiados encontramos la citisina, con probadas propiedades neuroprotectoras y de reducción de los síntomas de abstinencia a la nicotina (Abin-Carriquiry et al., 2008; Cahill et al., 2010). Otros agonistas colinérgicos, aún en fase experimental como SSR591813, dianicline o PNU-282987, están siendo actualmente investigados, especialmente en cuanto a sus posibles efectos neuroprotectores y potenciadores a nivel cognitivo (Mesa-Gresa et al., 2014; Vicens et al., 2011; 2013b). La acción de los agonistas parciales de la nicotina es importante puesto que modulan su acción en base a la cantidad basal de neurotransmisor en el sistema, de modo que podrían actuar como tratamiento de diversos trastornos por dependencia a sustancias de abuso al modular la disponibilidad de receptores dopaminérgicos (Crunelle et al., 2010). Otros estudios han investigado la posible aplicación de los agonistas nicotínicos en el tratamiento de trastornos como la esquizofrenia, el trastorno por déficit de atención con hiperactividad, y de enfermedades neurodegenerativas como Parkinson y Azheimer (Levin et al., 2012; Pandya & Yekel, 2013; Vicens et al., 2013a; 2013b). Específicamente, en el presente trabajo nos centraremos en el agonista selectivo de los receptores α7 denominado PNU-282987. Teniendo en cuenta que este agonista colinérgico ha demostrado tener claros efectos a nivel cognitivo en roedores (Vicens et al., 2013b) además de mostrar sus propiedades neuroprotectoras, se plantea que

podría tener efectos sumativos a los ya descritos del EE en las funciones cognitivas relacionadas con tareas de aprendizaje y memoria.

3.2. Perspectivas de futuro en el estudio de Enriquecimiento Ambiental y adicción

Aunque los resultados de los estudios que han evaluado la interacción entre exposición a ambientes enriquecidos y desarrollo de la adicción a drogas de abuso resulta de enorme interés, debemos reconocer que todavía presentan evidentes limitaciones que deben ser superadas en futuras investigaciones. La mayoría de trabajos han utilizado periodos cortos de enriquecimiento y han comparado las condiciones de ambiente enriquecido no con ambientes estándar, sino con situaciones de aislamiento o empobrecimiento, lo que puede confundir los resultados y limitar su generalización (Laviola et al., 2008; Whitaker et al., 2009). Tradicionalmente el ambiente enriquecido se ha establecido durante la adolescencia y únicamente en estudios recientes se ha evaluado el efecto del EE iniciado en la edad adulta en ratas adictas a diferentes sustancias de abuso (Puhl et al., 2012). Además, con frecuencia se obtienen resultados contradictorios que podrían explicarse por la utilización de diferentes especies, cepas, dosis o rutas de administración, así como por el uso de diversos procedimientos de EE. La principal dificultad para estudiar los beneficios y/o efectos producidos por el EE es la gran disparidad de paradigmas usados ya que, a pesar de que la base es similar, se pueden observar notables diferencias en cuanto al tamaño de las cajas, la composición, el número de sujetos, la complejidad de los objetos estimulantes y la frecuencia de cambio de los mismos (Bennett et al., 2006; Whitaker et al., 2009). Una de las variables que más parecen influir en los resultados obtenidos y que resulta relevante en los estudios sobre adicción es la edad a la que se inicia la exposición al ambiente enriquecido (especialmente si se inicia en la adolescencia, etapa también critica en el establecimiento de la adicción) y durante cuánto tiempo se mantiene a los roedores en esa situación.

A pesar de estas limitaciones, los resultados disponibles hasta el momento confirman la hipótesis de que la adicción a diferentes drogas parece estar influida en gran medida por las condiciones ambientales. En este sentido, los datos obtenidos en diferentes modelos animales apoyan la idea de que con el objetivo de prevenir la adicción sería muy importante proporcionar ambientes positivos y ampliamente enriquecidos, especialmente en etapas críticas del desarrollo (Solinas et al., 2010). La evidencia experimental reciente confirma que la exposición a ambientes complejos y enriquecidos podría resultar crucial para determinar el papel de las variables ambientales en la vulnerabilidad a la adicción y el desarrollo de la resistencia ante algunas drogas de abuso como la cocaína (Laviola et al., 2008; Magalhaes et al., 2007), la heroína (El Rawas et al., 2009), el alcohol (Rueda et al., 2011) o la nicotina (Green et al., 2003; Hamilton & Kolb, 2005; Zhu et al., 2009).

Aunque los mecanismos neurobiológicos que subyacen a esta protección no se conocen totalmente, se están comenzando a proponer interesantes modelos teóricos. Los posibles efectos protectores del EE frente a la adicción podrían atribuirse a cambios hormonales, neurales, celulares y/o moleculares, por lo que la investigación de estas posibles alteraciones queda abierta a próximos estudios (Puhl et al., 2012). Parece estar claro que el EE ejerce importantes efectos sobre el cerebro a nivel estructural y funcional, centrándose la mayoría de los datos publicados en la memoria y el aprendizaje (Laviola et al, 2008). Son necesarios más estudios para evaluar con mayor detalle otras funciones conductuales y determinar cómo el ambiente enriquecido puede inducir cambios adaptativos cuando el cerebro se enfrenta a retos como la degeneración asociada el envejecimiento (Segovia et al., 2010), las alteraciones provocadas por el estrés crónico (Chen et al., 2010) o la exposición a sustancias de abuso como la nicotina (Redolat et al., 2009). También resultaría de gran interés diferenciar si los efectos beneficiosos del ambiente complejo se observan únicamente en comparación con las situaciones de “empobrecimiento ambiental” en las que en muchas ocasiones se mantiene a los animales en el laboratorio.

Las investigaciones preclínicas pueden contribuir a establecer más claramente qué factores individuales y ambientales contribuyen a la vulnerabilidad y/o resistencia a la adicción a determinadas sustancias. Estos estudios experimentales plantean

importantes implicaciones prácticas y terapéuticas ya que pueden ayudarnos a entender qué factores explicarían la observación frecuente de que no todos los sujetos que experimentan con la nicotina u otras drogas se conviertan en adictos. Un mejor conocimiento de aspectos relacionados con la plasticidad (teniendo en cuenta cambios epigenéticos, cambios a nivel molecular y alteraciones en el circuito de recompensa) podría resultar útil para abordar nuevas aproximaciones al tratamiento (Foscarin et al., 2012).

4. ENRIQUECIMIENTO AMBIENTAL Y ESTRÉS

La respuesta de estrés se produce cuando las demandas personales y ambientales superan las capacidades y competencias del organismo (Malter Cohen et al., 2013); alterando el equilibrio físico y psicológico del individuo (Franklin et al., 2012). De hecho, las situaciones cuyas características principales son la incontrolabilidad e impredecibilidad suelen ser altamente estresantes (Koolhaas et al., 2011). Habitualmente, la respuesta de estrés ha sido considerada como necesaria y adaptativa en situaciones diarias. Sin embargo, algunas dimensiones de la situación de estrés como son la frecuencia, la duración o la intensidad de la misma pueden variar las consecuencias de la exposición así como sus posibles implicaciones, incluso pudiéndose convertir en un indicio de vulnerabilidad para desarrollar determinadas enfermedades (Malter Cohen et al., 2013; McEwen et al., 2012). De hecho, la exposición crónica a situaciones estresantes o durante largos periodos de tiempo ha sido considerada como un factor de riesgo para la aparición de diversos trastornos cardiovasculares, metabólicos y mentales (Franklin et al., 2012; Goh & Agius, 2010; McEwen & Gianaros, 2011; Pardon & Rattay, 2008), así como en el desarrollo de adicción a drogas de abuso (Briand et al., 2010; De Biasi & Salas, 2008; Solinas et al., 2010). Investigaciones recientes han comprobado que la exposición a situaciones de estrés crónico puede inducir alteraciones en el sistema nervioso (principalmente a nivel de córtex prefrontal, hipocampo y amígdala) y modificaciones en los sistemas

endocrino e inmune, e incluso cambios a nivel conductual (Arranz et al., 2010; Franklin et al., 2012; Malter Cohen et al., 2013; McEwen et al., 2012; McQuaid et al., 2013), aunque los datos sobre los efectos a largo plazo son limitados (Garrido et al., 2013). Se ha observado también que la exposición a situaciones estresantes está relacionada con alteraciones en el eje HPA, relacionado con la reactividad emocional y con la posible aparición de trastornos de ansiedad (Almela et al., 2011; Marter Cohen et al., 2013; Pérez-Tejada et al., 2013; Wang et al., 2012). Las alteraciones en tareas de memoria y el deterioro a nivel cognitivo como consecuencia del estrés también han sido ampliamente estudiadas (Hidalgo et al., 2012; 2014; Solinas et al., 2008). Diversas investigaciones han mostrado un gran interés por determinar las bases neurobiológicas que establecen la percepción individual ante situaciones estresantes, así como la resiliencia o vulnerabilidad individual que pueden mostrar los sujetos y su posible relación con la aparición de trastornos psicológicos (De Miguel et al., 2011; Franklin et al., 2012; Goh & Agius, 2010; Razzoli et al., 2011a; 2011b).

Los efectos negativos del estrés crónico también han sido evaluados en investigaciones preclínicas, observándose que los animales expuestos a situaciones estresantes muestran un incremento en los niveles de serotonina y norepinefrina en córtex prefrontal e hipocampo, aumento de los niveles de DA y disminución incluso en el BDNF (McQuaid et al., 2013). También se ha observado afectación del eje HPA que correlaciona con incremento en la secreción de corticosterona en roedores (Franklin et al., 2012; McQuiad et al., 2013; Pérez-Tejada et al., 2013; Sterlemann et al., 2008). Diferentes estudios han tratado de identificar variables moduladoras de la respuesta a estrés en animales, como son las diferencias individuales en la conducta de afrontamiento al estrés (De Miguel et al., 2011), o bien mediante tratamientos de tipo farmacológico (Pérez-Tejada et al., 2013; Scharf et al., 2013) o la exposición a distintas condiciones ambientales (Garrido et al., 2013; McQuaid et al., 2013; Sale et al., 2014). En este ámbito, se ha observado que el alojamiento en EE puede reducir el impacto negativo del estrés, observándose incluso efectos opuestos ante la exposición a ambientes enriquecidos o a situaciones estresantes (McQuaid et al., 2013; Schloesser et al., 2010). El EE parece revertir algunos de los efectos neurobiológicos del estrés (Garrido et al., 2013; Segovia et al., 2009), como son los efectos sobre la plasticidad

sináptica y los sistemas de recompensa cerebral (Mitra & Sapolsky, 2009); mostrando beneficios sobre la respuesta emocional y las estrategias de afrontamiento en animales estresados (Schloesser et al., 2010), así como en la ejecución en tareas de aprendizaje y memoria (Chen et al., 2010; Cymerblit-Sabba et al., 2013). El modelo de EE ha sido considerado como un paradigma válido para incrementar la resiliencia frente a situaciones de estrés social crónico (Lehmann & Henkermann, 2011; Schloesser et al., 2010), sin embargo, pocos estudios han evaluado si la exposición a condiciones de enriquecimiento puede contrarrestar los efectos del estrés social utilizando modelos de alta validez predictiva. Además, los mecanismos mediante los cuales el ambiente enriquecido disminuiría los efectos adversos del estrés no son aún bien conocidos, habiéndose hipotetizado desde variables neurobiológicas hasta procesos cognitivo-conductuales (McQuaid et al., 2013; Sale et al., 2014; Segovia et al., 2008). En un estudio previo realizado por Schloesser y colaboradores (2010), se observó que el EE disminuía las conductas de sumisión y depresión mostradas por los animales sometidos a estrés psicosocial, hipotetizando que la exposición a ambientes enriquecidos y complejos podía aislar al individuo de los efectos producidos por la incontrolabilidad de las situaciones de estrés, disminuyendo la reactividad emocional y mejorando la resiliencia al estrés y las estrategias de afrontamiento. En un estudio más reciente se observó que el EE podía atenuar los efectos estructurales y funcionales del estrés crónico sobre el hipocampo, incluso cuando la exposición a estrés comenzó dos semanas antes que el alojamiento en EE, mostrándose la idea de que ambos tipos de exposiciones deben activar mecanismos paralelos en esta área cerebral (Hutchinson et al., 2012b). Similares resultados se han obtenido respecto a los niveles de corticosterona inducidos por el estrés en áreas como el córtex prefrontal, mostrándose una disminución en ratas expuestas a EE (Garrido et al., 2013).

En general, los resultados obtenidos muestran efectos positivos del EE sobre la respuesta de estrés y ansiedad en los animales, aunque algunas investigaciones indican que, en los casos en los que se utiliza este modelo con roedores macho pueden aparecer conductas territoriales y de competición por los recursos del ambiente que disminuyan estos efectos (McQuaid et al., 2013). Son pocos los estudios que han analizado los efectos neurobiológicos y conductuales de la exposición a situaciones de

estrés crónico en interacción con distintos tipos de alojamiento, lo que plantea nuevas perspectivas en la investigación con diversas estrategias terapéuticas frente al estrés basadas en técnicas de intervención conductual (Schloesser et al., 2010).

5. OBJETIVOS E HIPÓTESIS

En la literatura revisada en apartados anteriores se han descrito los principales resultados obtenidos en los diversos estudios preclínicos que muestran los efectos de la exposición a ambientes enriquecidos tanto a nivel neurobiológico como conductual. No obstante, la multiplicidad de modelos utilizados así como la gran diversidad de técnicas de evaluación, entre otros factores, hace que algunas de las conclusiones alcanzadas en los mismos puedan resultar contradictorias. Además, pocos estudios han evaluado la combinación del alojamiento en ambientes enriquecidos junto con otros factores como la administración de fármacos o la exposición a situaciones de estrés. El trabajo que aquí se presenta se enmarca en un proyecto de investigación más amplio cuyo *objetivo general* es el desarrollo de un modelo de enriquecimiento ambiental y la evaluación de sus efectos conductuales y fisiológicos en ratones con el fin de valorar si dicha manipulación contribuye a prevenir y/o revertir algunos de los efectos adversos que se observan asociados a factores como el envejecimiento y la exposición a situaciones de estrés social crónico.

Dentro de este marco, los estudios incluidos en la presente Tesis Doctoral tienen como principales *objetivos específicos*:

- Estandarizar un modelo de enriquecimiento ambiental (a utilizar en el resto de estudios realizados), definiendo de manera detallada los principales efectos fisiológicos y conductuales en ratones macho NMRI, valorando peso corporal e ingesta así como respuesta locomotora y conducta exploratoria en situaciones nuevas, conducta agonística, respuesta emocional y aprendizaje/memoria.

- Determinar si la administración de nicotina u otros agonistas nicotínicos, como el PNU-282987, puede potenciar y/o modular el posible efecto protector del enriquecimiento ambiental.

- Analizar los efectos conductuales de la exposición a un ambiente enriquecido complejo estandarizado (basado en cajas Marlau^{TM}) y su posible aplicación como método de enriquecimiento ambiental, así como comparar sus principales efectos con los obtenidos con el modelo desarrollado en nuestro laboratorio.

- Definir las consecuencias a corto y a largo plazo de la exposición a ambientes enriquecidos en ratones de distintas edades (adolescencia temprana, adolescencia tardía, edad adulta), valorando el impacto diferencial que puede tener la edad y/o el periodo de tiempo de exposición.

- Evaluar en qué medida una situación de estrés social crónico (inducido por un modelo de ambiente social inestable) puede provocar efectos fisiológicos (peso e ingesta), neurobiológicos (niveles de corticosterona en sangre) y conductuales (actividad motora y exploratoria, ansiedad y aprendizaje) en ratones macho de la cepa NMRI.

- Valorar si el enriquecimiento ambiental puede contrarrestar los efectos adversos del estrés social iniciado a edades tempranas y si la exposición a un ambiente enriquecido durante la adolescencia podría prevenir los cambios inducidos por un estresor aplicado de forma simultánea a estas condiciones de alojamiento.

Por lo que respecta a las *hipótesis respecto a los principales efectos del enriquecimiento ambiental*, cabe destacar que no existen apenas estudios sobre los efectos del enriquecimiento ambiental en ratones de la cepa NMRI, por lo que en base a los estudios previos realizados en ratas y en otras cepas de ratones se hipotetiza que la exposición a ambientes enriquecidos inducirá cambios en variables fisiológicas como el peso de los animales o la ingesta (Hutchinson et al., 2012b; Mainardi et al., 2010) y en la respuesta conductual de los animales. Específicamente, en base a resultados de estudios previos, se hipotetiza que se producirá disminución de la conducta exploratoria (Brenes et al., 2008; Elizalde et al., 2008) y motora (Gross et al., 2012; Zhu

et al., 2009) y modificaciones en la conducta de interacción social y agonística, a pesar de que los datos no son concluyentes (Abou-Ismail et al., 2010; McQuaid et al., 2013; Van Praag et al., 2000). También se espera observar cambios en la respuesta emocional, habiéndose descrito principalmente una disminución significativa de la ansiedad en animales alojados en ambientes enriquecidos (Hughes & Otto, 2013; Simpson & Kelly, 2011); así como mejoras en las capacidades cognitivas de aprendizaje y memoria (Bechara & Kelly, 2013; Kazlauckas et al., 2011; Leger et al., 2012). En cuanto a las consecuencias a corto y largo plazo de la exposición a ambientes enriquecidos, se hipotetiza que los beneficios de dicha exposición variarán en función de la edad a la que se inicie y el periodo de tiempo durante el cual se mantenga (Bouet et al., 2011; Freret et al., 2012; Patel, 2012; Sampedro-Piquero et al., 2013a).

Respecto a la posible *interacción entre la exposición a ambientes enriquecidos y la manipulación farmacológica con agonistas nicotínicos*, se hipotetiza que dichos fármacos podrían potenciar algunos de los efectos del enriquecimiento ambiental, especialmente en tareas de aprendizaje y memoria debido a las propiedades activadoras de estas sustancias (Carroll et al., 2010; Levin, 2012). Específicamente, dado que la administración de fármacos como el agonista nicotínico PNU-282987 con acciones específicas sobre el receptor α7 ha mostrado efectos positivos en tareas cognitivas (Vicens et al., 2011; 2013b) se espera que la administración de este agoniza nicotínico potenciará los efectos positivos del ambiente enriquecido, especialmente en tareas cognitivas. Además, se hipotetiza que la respuesta conductual a la administración de fármacos puede diferir entre animales mantenidos en condiciones ambientales diferentes (Bardo et al., 2013; Burrows et al., 2010).

En lo referente a los efectos de la *exposición a estrés social crónico y a su posible interacción con el enriquecimiento ambiental*, se hipotetiza que el alojamiento en ambientes complejos podría modular algunos de los efectos provocados por el estrés, como el incremento en los niveles de corticosterona en los animales sometidos a estrés (Schloesser et al., 2010), y optimizar las estrategias cognitivas de los animales para enfrentarse a la situación estresante (Garrido et al., 2013, Malter Cohen et al., 2013; Sale et al., 2014).

REFERENCIAS

Abin-Carriquiry, J. A., Costa, G., Urbanavicius, J., Cassels, B. K., Rebolledo-Fuentes, M., Wonnacott, S., & Dajas, F. (2008). In vivo modulation of dopaminergic nigrostriatal pathways by cytisine derivatives: Implications for parkinson's disease. *European Journal of Pharmacology,* 589, 80-84.

Abou-Ismail, U. A. (2011). The effects of cage enrichment on agonistic behaviour and dominance in male laboratory rats (rattus norvegicus). *Research in Veterinary Science,* 90, 346-51.

Abou-Ismail, U. A., Burman, O. H., Nicol, C. J., & Mendl, M. (2010). The effects of enhancing cage complexity on the behaviour and welfare of laboratory rats. *Behavioural Processes,* 85, 172-80.

Abramov, U., Puussaar, T., Raud, S., Kurrikoff, K., & Vasar, E. (2008). Behavioural differences between C57BL/6 and 129S6/SvEv strains are reinforced by environmental enrichment. *Neuroscience Letters,* 443, 223-27.

Adams, E., Klug, J., Quast, M., & Stairs, D. J. (2013). Effects of environmental enrichment on nicotine-induced sensitization and cross-sensitization to D-amphetamine in rats. *Drug and Alcohol Dependence*, 129, 247-53.

Almela, M., Hidalgo, V., Villada, C., Espín, L., Gómez-Amor, J., & Salvador, A. (2011). The impact of cortisol reactivity to acute stress on memory: Sex differences in middle-aged people. *Stress,* 14, 117-27.

Amaral, O. B., Vargas, R. S., Hansel, G., Izquierdo, I., & Souza, D. O. (2008). Duration of environmental enrichment influences the magnitude and persistence of its behavioral effects on mice. *Physiology & Behavior,*93, 388-94.

Arranz, L., De Castro, N. M., Baeza, I., Maté, I., Viveros, M. P., & De la Fuente, M. (2010). Environmental enrichment improves age-related immune system impairment: Long-term exposure since adulthood increases life span in mice. *Rejuvenation Research,* 13, 415-28.

Artola, A., von Frijtag, J. C., Fermont, P. C., Gispen, W. H., Schrama, L. H., Kamal, A., & Spruijt, B. M. (2006). Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. *The European Journal of Neuroscience,* 23, 261-72.

Barak, B., Shvarts-Serebro, I., Modai, S., Gilam, A., Okun, E., Michaelson, D., … Ashery, U. (2013). Opposing actions of environmental enrichment and Alzheimer's disease on the expression of hippocampal microRNAs in mouse models. *Translational Psychiatry,* 3, e304.

Bardo, M. T., Bowling, S. L., Rowlett, J. K., Manderscheid, P., Buxton, S. T., & Dwoskin, L. P. (1995). Environmental enrichment attenuates locomotor sensitization, but not in vitro

dopamine release, induced by amphetamine. *Pharmacology, Biochemistry, and Behavior,* 51, 397-405.

Bardo, M. T., Klebaur, J. E., Valone, J. M., & Deaton, C. (2001). Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats. *Psychopharmacology,* 155, 278-84.

Bardo, M. T., Dwoskin, L. P., & Rebec, G. V. (2013). S. 9.2-neurobehavioural mechanisms underlying the protective effects of environmental enrichment on drug abuse. *Behavioural Pharmacology, 24*, e11.

Baroncelli, L., Bonaccorsi, J., Milanese, M., Bonifacino, T., Giribaldi, F., Manno, I., … Sale, A. (2012). Enriched experience and recovery from amblyopia in adult rats: Impact of motor, social and sensory components. *Neuropharmacology,* 62, 2388-97.

Bayne, K. (2005). Potential for unintended consequences of environmental enrichment for laboratory animals and research results. *ILAR Journal / National Research Council, Institute of Laboratory Animal Resources,* 46, 129-39.

Beauquis, J., Pavia, P., Pomilio, C., Vinuesa, A., Podlutskaya, N., Galvan, V., & Saravia, F. (2013). Environmental enrichment prevents astroglial pathological changes in the hippocampus of APP transgenic mice, model of Alzheimer's disease. *Experimental Neurology,* 239, 28-37.

Bechara, R. G., & Kelly, A. M. (2013). Exercise improves object recognition memory and induces BDNF expression and cell proliferation in cognitively enriched rats. *Behavioural Brain Research,* 245, 96-100.

Bennett, D. A., Schneider, J. A., Tang, Y., Arnold, S. E., & Wilson, R. S. (2006). The effect of social networks on the relation between alzheimer's disease pathology and level of cognitive function in old people: A longitudinal cohort study. *Lancet Neurology,* 5, 406-12.

Benowitz, N. L. (2008). Neurobiology of nicotine addiction: Implications for smoking cessation treatment. *The American Journal of Medicine,* 121, S3-10.

Bezard, E., Dovero, S., Belin, D., Duconger, S., Jackson-Lewis, V., Przedborski, S., … Jaber, M. (2003). Enriched environment confers resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and cocaine: Involvement of dopamine transporter and trophic factors. *The Journal of Neuroscience,* 23, 10999-1007.

Bouet, V., Freret, T., Dutar, P., Billard, J., & Boulouard, M. (2011). Continuous enriched environment improves learning and memory in adult NMRI mice through theta burst-related-LTP independent mechanisms but is not efficient in advanced aged animals. *Mechanisms of Ageing and Development, 132*, 240-48.

Bowling, S. L., & Bardo, M. T. (1994). Locomotor and rewarding effects of amphetamine in enriched, social, and isolate reared rats. *Pharmacology, Biochemistry, and Behavior,* 48, 459-64.

Bowling, S. L., Rowlett, J. K., & Bardo, M. T. (1993). The effect of environmental enrichment on amphetamine-stimulated locomotor activity, dopamine synthesis and dopamine release. *Neuropharmacology,* 32, 885-93.

Branchi, I., D'Andrea, I., Sietzema, J., Fiore, M., Di Fausto, V., Aloe, L., & Alleva, E. (2006). Early social enrichment augments adult hippocampal BDNF levels and survival of BrdU-positive cells while increasing anxiety-and "depression"-like behavior. *Journal of Neuroscience Research, 83*, 965-73.

Brenes, J. C., & Fornaguera, J. (2008). Effects of environmental enrichment and social isolation on sucrose consumption and preference: Associations with depressive-like behavior and ventral striatum dopamine. *Neuroscience Letters,* 436, 278-82.

Brenes, J. C., Padilla, M., & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behavioural Brain Research,* 197, 125-37.

Brenes, J. C., Rodriguez, O., & Fornaguera, J. (2008). Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. *Pharmacology, Biochemistry, and Behavior,* 89, 85-93.

Burrows, E. L., McOmish, C. E., & Hannan, A. J. (2010). Gene-environment interactions and construct validity in preclinical models of psychiatric disorders. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 35, 1376-82.

Burrows, E. L., McOmish, C. E., & Hannan, A. J. (2011). Gene-environment interactions and construct validity in preclinical models of psychiatric disorders. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 35, 1376-82.

Burrows, E. L., & Hannan, A. J. (2013). Towards environmental construct validity in animal models of CNS disorders: Optimizing translation of preclinical studies. *CNS & Neurological Disorders Drug Targets,* 12, 597-82.

Cahill, K., Stead, L. F., & Lancaster, T. (2010). Nicotine receptor partial agonists for smoking cessation. *The Cochrane Database of Systematic Reviews, (12):CD006103.*

Carroll, M. E., Anker, J. J., & Perry, J. L. (2009). Modeling risk factors for nicotine and other drug abuse in the preclinical laboratory. *Drug and Alcohol Dependence,* 104 Suppl 1, S70-8.

Chamove, A. S. (1989). Cage design reduces emotionality in mice. *Laboratory Animals,* 23, 215-19.

Chauvet, C., Lardeux, V., Jaber, M., & Solinas, M. (2011). Brain regions associated with the reversal of cocaine conditioned place preference by environmental enrichment. *Neuroscience,* 184, 88-96.

Chen, Y., Mao, Y., Zhou, D., Hu, X., Wang, J., & Ma, Y. (2010). Environmental enrichment and chronic restraint stress in ICR mice: Effects on prepulse inhibition of startle and Y-maze spatial recognition memory. *Behavioural Brain Research,* 212, 49-55.

Chourbaji, S., Hortnagl, H., Molteni, R., Riva, M. A., Gass, P., & Hellweg, R. (2012). The impact of environmental enrichment on sex-specific neurochemical circuitries - effects on brain-derived neurotrophic factor and the serotonergic system. *Neuroscience,* 220, 267-76.

Codita, A., Mohammed, A. H., Willuweit, A., Reichelt, A., Alleva, E., Branchi, I., …, Krackow, S. (2012). Effects of spatial and cognitive enrichment on activity pattern and learning performance in three strains of mice in the IntelliMaze. *Behavior Genetics*, 42, 449-60.

Coolon, R. A., & Cain, M. E. (2009). Effects of mecamylamine on nicotine-induced conditioned hyperactivity and sensitization in differentially reared rats. *Pharmacology, Biochemistry, and Behavior,* 93, 59-66.

Creer, D. J., Romberg, C., Saksida, L. M., van Praag, H., & Bussey, T. J. (2010). Running enhances spatial pattern separation in mice. *Proceedings of the National Academy of Sciences of the United States of America*,107, 2367-72.

Crunelle, C. L., Miller, M. L., Booij, J., & Van den Brink, W. (2010). The nicotinic acetylcholine receptor partial agonist varenicline and the treatment of drug dependence: A review. *European Neuropsychopharmacology,* 20, 69-79.

Cymerblit-Sabba, A., Lasri, T., Gruper, M., Aga-Mizrachi, S., Zubedat, S., & Avital, A. (2013). Prenatal enriched environment improves emotional and attentional reactivity to adulthood stress. *Behavioural Brain Research, 241*, 185-190.

Daffner, K. R. (2010). Promoting successful cognitive aging: A comprehensive review. *Journal of Alzheimer's Disease,* 19, 1101-22.

De Biasi, M., & Dani, J. A. (2011). Reward, addiction, withdrawal to nicotine. *Annual Review of Neuroscience, 34*, 105-130.

De Biasi, M., & Salas, R. (2008). Influence of neuronal nicotinic receptors over nicotine addiction and withdrawal. *Experimental Biology and Medicine, 233*, 917-29.

De Miguel, Z., Vegas, O., Garmendia, L., Arregi, A., Beitia, G., & Azpiroz, A. (2011). Behavioral coping strategies in response to social stress are associated with distinct neuroendocrine, monoaminergic and immune response profiles in mice. *Behavioural Brain Research, 225*, 554-61.

De Witt, B. W., Ehrenberg, K. M., McAloon, R. L., Panos, A. H., Shaw, K. E., Raghavan, P. V., … Kline, A. E. (2011). Abbreviated environmental enrichment enhances neurobehavioral recovery comparably to continuous exposure after traumatic brain injury. *Neurorehabilitation and Neural Repair,* 25, 343-50.

Denkinger, M. D., Nikolaus, T., Denkinger, C., & Lukas, A. (2012). Physical activity for the prevention of cognitive decline: Current evidence from observational and controlled studies. *Zeitschrift Fur Gerontologie Und Geriatrie,* 45, 11-16.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience and Biobehavioral Reviews,* 31, 673-704.

Diamond, M. C. (2001). Response of the brain to enrichment. *Anais Da Academia Brasileira De Ciencias,* 73, 211-20.

Diniz, D. G., Foro, C. A., Rego, C. M., Gloria, D. A., de Oliveira, F. R., Paes, J. M., … Diniz, C. W. (2010). Environmental impoverishment and aging alter object recognition, spatial learning, and dentate gyrus astrocytes. *The European Journal of Neuroscience,* 32, 509-19.

Eckert, M. J., & Abraham, W. C. (2012). Effects of environmental enrichment exposure on synaptic transmission and plasticity in the hippocampus. *Current Topics in Behavioral Neurosciences, in press.*

Elizalde, N., Gil-Bea, F. J., Ramirez, M. J., Aisa, B., Lasheras, B., Del Rio, J., & Tordera, R. M. (2008). Long-lasting behavioral effects and recognition memory deficit induced by chronic mild stress in mice: Effect of antidepressant treatment. *Psychopharmacology, 199*, 1-14.

El Rawas, R., Thiriet, N., Lardeux, V., Jaber, M., & Solinas, M. (2009). Environmental enrichment decreases the rewarding but not the activating effects of heroin. *Psychopharmacology,* 203, 561-70.

El Rawas, R., Thiriet, N., Nader, J., Lardeux, V., Jaber, M., & Solinas, M. (2011). Early exposure to environmental enrichment alters the expression of genes of the endocannabinoid system. *Brain Research,* 1390, 80-89.

Elliott, B. M., & Grunberg, N. E. (2005). Effects of social and physical enrichment on open field activity differ in male and female sprague-dawley rats. *Behavioural Brain Research,* 165, 187-96.

Emack, J., & Matthews, S. G. (2011). Effects of chronic maternal stress on hypothalamo–pituitary–adrenal (HPA) function and behavior: No reversal by environmental enrichment. *Hormones and Behavior, 60*, 589-98.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research,* 215, 244-54.

Falls, W. A., Fox, J. H., & MacAulay, C. M. (2010). Voluntary exercise improves both learning and consolidation of cued conditioned fear in C57 mice. *Behavioural Brain Research,* 207, 321-31.

Fares, R. F., Kouchi, H., & Bezin, L. (2012). Standardized environmental enrichment for rodents in marlau cage. *Protocol Exchange,* in press.

Fares, R. P., Belmeguenai, A., Sanchez, P. E., Kouchi, H. Y., Bodennec, J., Morales, A., … Bezin, L. (2013). Standardized environmental enrichment supports enhanced brain plasticity in healthy rats and prevents cognitive impairment in epileptic rats. *PloS One,* 8, e53888.

Fone, K. C., & Porkess, M. V. (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. *Neuroscience and Biobehavioral Reviews,* 32, 1087-102.

Foscarin, S., Rossi, F., & Carulli, D. (2012). Influence of the environment on adult CNS plasticity and repair. *Cell and Tissue Research, 349*, 161-167.

Fox, C., Merali, Z., & Harrison, C. (2006). Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress.*Behavioural Brain Research,*175,1-8.

Franklin, T. B., Saab, B. J., & Mansuy, I. M. (2012). Neural mechanisms of stress resilience and vulnerability. *Neuron,* 75, 747-61.

Frasca, D., Tomaszczyk, J., McFadyen, B. J., & Green, R. E. (2013). Traumatic brain injury and post-acute decline: What role does environmental enrichment play? A scoping review. *Frontiers in Human Neuroscience,* 7, 31.

Freret, T., Billard, J. M., Schumann-Bard, P., Dutar, P., Dauphin, F., Boulouard, M., & Bouet, V. (2012). Rescue of cognitive aging by long-lasting environmental enrichment exposure initiated before median lifespan. *Neurobiology of Aging,* 33, 1005.e1-1005.10.

Fuss, J., Ben Abdallah, N. M., Vogt, M. A., Touma, C., Pacifici, P. G., Palme, R., … Gass, P. (2010). Voluntary exercise induces anxiety-like behavior in adult C57BL/6J mice correlating with hippocampal neurogenesis. *Hippocampus,* 20, 364-76.

Garcia-Capdevila, S., Portell-Cortes, I., Torras-Garcia, M., Coll-Andreu, M., & Costa-Miserachs, D. (2009). Effects of long-term voluntary exercise on learning and memory processes: Dependency of the task and level of exercise. *Behavioural Brain Research,* 202, 162-70.

Garrido, P., De Blas, M., Ronzoni, G., Cordero, I., Anton, M., Gine, E., … Mora, F. (2013). Differential effects of environmental enrichment and isolation housing on the hormonal and neurochemical responses to stress in the prefrontal cortex of the adult rat: Relationship to working and emotional memories. *Journal of Neural Transmission,* 120, 829-43.

Goldberg, N. R., Fields, V., Pflibsen, L., Salvatore, M. F., & Meshul, C. K. (2012). Social enrichment attenuates nigrostriatal lesioning and reverses motor impairment in a progressive 1-methyl-2-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *Neurobiology of Disease,* 45, 1051-67.

Gomez, A. M., Midde, N. M., Mactutus, C. F., Booze, R. M., & Zhu, J. (2012). Environmental enrichment alters nicotine-mediated locomotor sensitization and phosphorylation of DARPP-32 and CREB in rat prefrontal cortex. *PloS One,* 7, e44149.

Goh, C., & Agius, M. (2010). The stress-vulnerability model how does stress impact on mental illness at the level of the brain and what are the consequences? *Psychiatria Danubina, 22*, 198-202.

Gotti, C., Moretti, M., Gaimarri, A., Zanardi, A., Clementi, F., & Zoli, M. (2007). Heterogeneity and complexity of native brain nicotinic receptors. *Biochemical Pharmacology, 74*, 1102-11.

Graef, S., Schönknecht, P., Sabri, O., & Hegerl, U. (2011). Cholinergic receptor subtypes and their role in cognition, emotion, and vigilance control: An overview of preclinical and clinical findings.*Psychopharmacology, 215*, 205-29.

Green, T., Gehrke, B., & Bardo, M. (2002). Environmental enrichment decreases intravenous amphetamine self-administration in rats: Dose-response functions for fixed-and progressive-ratio schedules.*Psychopharmacology, 162*, 373-78.

Green, T. A., Cain, M. E., Thompson, M., & Bardo, M. T. (2003). Environmental enrichment decreases nicotine-induced hyperactivity in rats. *Psychopharmacology,* 170, 235-41.

Green, T. A., Alibhai, I. N., Roybal, C. N., Winstanley, C. A., Theobald, D. E., Birnbaum, S. G., … Nestler, E. J. (2010). Environmental enrichment produces a behavioral phenotype mediated by low cyclic adenosine monophosphate response element binding (CREB) activity in the nucleus accumbens. *Biological Psychiatry*, 67, 28-35.

Grimm, J. W., Osincup, D., Wells, B., Manaois, M., Fyall, A., Buse, C., & Harkness, J. H. (2008). Environmental enrichment attenuates cue-induced reinstatement of sucrose seeking in rats. *Behavioural Pharmacology,*19, 777-85.

Gross, A. N., Richter, S. H., Engel, A. K., & Wurbel, H. (2012). Cage-induced stereotypies, perseveration and the effects of environmental enrichment in laboratory mice. *Behavioural Brain Research,* 234, 61-68.

Haemisch, A., & Gartner, K. (1997). Effects of cage enrichment on territorial aggression and stress physiology in male laboratory mice. *Acta Physiologica Scandinavica,* 640, 73-76.

Haemisch, A., Voss, T., & Gartner, K. (1994). Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J mice. *Physiology & Behavior,* 56, 1041-48.

Hamilton, D. A., & Kolb, B. (2005). Differential effects of nicotine and complex housing on subsequent experience-dependent structural plasticity in the nucleus accumbens. *Behavioral Neuroscience,* 119, 355-65.

Hannan, A. J. (2014). Environmental enrichment and brain repair: Harnessing the therapeutic effects of cognitive stimulation and physical activity to enhance experience-dependent plasticity. *Neuropathology and Applied Neurobiology, 40*, 13-25.

Hannigan, J. H., O'leary-Moore, S. K., & Berman, R. F. (2007). Postnatal environmental or experiential amelioration of neurobehavioral effects of perinatal alcohol exposure in rats. *Neuroscience and Biobehavioral Reviews, 31*, 202-11.

Harati, H., Majchrzak, M., Cosquer, B., Galani, R., Kelche, C., Cassel, J. C., & Barbelivien, A. (2011). Attention and memory in aged rats: Impact of lifelong environmental enrichment. *Neurobiology of Aging,* 32, 718-36.

Hebb, D.O. (1947). The effects of early experience on problem solving at maturity. *Am Psychol*, *2,* 306-307.

Hebb, D.O. (1949). The organization of behaviour (Wiley, New York).

Heyser, C. J., & Chemero, A. (2012). Novel object exploration in mice: Not all objects are created equal. *Behavioural Processes*, 89, 232-38.

Hidalgo, V., Villada, C., Almela, M., Espín, L., Gómez-Amor, J., & Salvador, A. (2012). Enhancing effects of acute psychosocial stress on priming of non-declarative memory in healthy young adults. *Stress, 15*, 329-38.

Hidalgo, V., Villada, C., Pulopulos, M., Almela, M., & Salvador, A. (2014). Estrés y cambios cognitivos asociados al envejecimiento. *Informacio Psicologica,* 105, 14-28.

Hoffman, A. N., Malena, R. R., Westergom, B. P., Luthra, P., Cheng, J. P., Aslam, H. A., … Kline, A. E. (2008). Environmental enrichment-mediated functional improvement after experimental traumatic brain injury is contingent on task-specific neurobehavioral experience. *Neuroscience Letters,* 431, 226-30.

Hotting, K., & Roder, B. (2013). Beneficial effects of physical exercise on neuroplasticity and cognition. *Neuroscience and Biobehavioral Reviews,* 37, 2243-57.

Hu, Y. S., Pigino, G., Brady, S. T., Larson, J., & Lazarov, O (2010). Complex environment experience rescues impaired neurogenesis, enhances synaptic plasticity and attenuates neuropathology in familial alxheimer's disease linked APPswe/PS1Delta E9 mice. FASEB J, 24, 1667-81.

Hughes, R. N., & Collins, M. A. (2010). Enhanced habituation and decreased anxiety by environmental enrichment and possible attenuation of these effects by chronic alpha-tocopherol (vitamin E) in aging male and female rats. *Pharmacology, Biochemistry, and Behavior,* 94, 534-42.

Hughes, R. N., & Otto, M. T. (2013). Anxiolytic effects of environmental enrichment attenuate sex-related anxiogenic effects of scopolamine in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 40, 252-59.

Hunter, R. G. (2012). Stress and the alpha7 nicotinic acetylcholine receptor. *Current Drug Targets*, 13, 607-12.

Hutchinson, E. K., Avery, A. C., & Vandewoude, S. (2012a). Environmental enrichment during rearing alters corticosterone levels, thymocyte numbers, and aggression in female BALB/c mice. *Journal of the American Association for Laboratory Animal Science, 51*, 18-24.

Hutchinson, K. M., McLaughlin, K. J., Wright, R. L., Bryce Ortiz, J., Anouti, D. P., Mika, A., … Conrad, C. D. (2012b). Environmental enrichment protects against the effects of chronic stress on cognitive and morphological measures of hippocampal integrity. *Neurobiology of Learning and Memory, 97*, 250-60.

Johnson, E. M., Traver, K. L., Hoffman, S. W., Harrison, C. R., & Herman, J. P. (2013). Environmental enrichment protects against functional deficits caused by traumatic brain injury. *Frontiers in Behavioral Neuroscience,* 7, 44.

Kannangara, T. S., Lucero, M. J., Gil-Mohapel, J., Drapala, R. J., Simpson, J. M., Christie, B. R., & van Praag, H. (2011). Running reduces stress and enhances cell genesis in aged mice. *Neurobiology of Aging*, 32, 2279-86.

Kazlauckas, V., Pagnussat, N., Mioranzza, S., Kalinine, E., Nunes, F., Pettenuzzo, L., … Lara, D. R. (2011). Enriched environment effects on behavior, memory and BDNF in low and high exploratory mice. *Physiology & Behavior,* 102, 475-80.

Kempermann, G., Fabel, K., Ehninger, D., Babu, H., Leal-Galicia, P., Garthe, A., & Wolf, S. A. (2010). Why and how physical activity promotes experience-induced brain plasticity. *Frontiers in Neuroscience,* 4, 189.

Kenney, J. W., Adoff, M. D., Wilkinson, D. S., & Gould, T. J. (2011). The effects of acute, chronic, and withdrawal from chronic nicotine on novel and spatial object recognition in male C57BL/6J mice. *Psychopharmacology,* 217, 353-65.

Kobayashi, S., Ohashi, Y., & Ando, S. (2002). Effects of enriched environments with different durations and starting times on learning capacity during aging in rats assessed by a refined procedure of the hebb-williams maze task. *Journal of Neuroscience Research, 70*, 340-46.

Kobilo, T., Liu, Q. R., Gandhi, K., Mughal, M., Shaham, Y., & van Praag, H. (2011). Running is the neurogenic and neurotrophic stimulus in environmental enrichment. *Learning & Memory,*18, 605-9.

Koob, G. F., & Le Moal, M. (2008). Addiction and the brain antireward system. *Annu.Rev.Psychol., 59*, 29-53.

Kulesskaya, N., Rauvala, H., & Voikar, V. (2011). Evaluation of social and physical enrichment in modulation of behavioural phenotype in C57BL/6J female mice. *PloS One,* 6, e24755.

Kumar, A., Rani, A., Tchigranova, O., Lee, W. H., & Foster, T. C. (2012). Influence of late-life exposure to environmental enrichment or exercise on hippocampal function and CA1 senescent physiology. *Neurobiology of Aging,* 33, 828.e1-828.17.

Larsson, F., Winblad, B., & Mohammed, A. H. (2002). Psychological stress and environmental adaptation in enriched vs. impoverished housed rats. *Pharmacology, Biochemistry, and Behavior,* 73, 193-207.

Laviola, G., Hannan, A. J., Macri, S., Solinas, M., & Jaber, M. (2008). Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. *Neurobiology of Disease,* 31, 159-68.

Lazarov, O., Robinson, J., Tang, Y. P., Hairston, I. S., Korade-Mirnics, Z., Lee, V. M., … Sisodia, S. S. (2005). Environmental enrichment reduces abeta levels and amyloid deposition in transgenic mice. *Cell,* 120, 701-13.

Leasure, J. L., & Decker, L. (2009). Social isolation prevents exercise-induced proliferation of hippocampal progenitor cells in female rats. *Hippocampus,* 19, 907-12.

Lee, V., & Hoaken, P. N. (2007). Cognition, emotion, and neurobiological development: Mediating the relation between maltreatment and aggression. *Child Maltreatment,* 12, 281-98.

Leger, M., Bouet, V., Freret, T., Darmaillacq, A. S., Dacher, M., Dauphin, F., … Schumann-Bard, P. (2012). Environmental enrichment improves recent but not remote memory in association with a modified brain metabolic activation profile in adult mice. *Behavioural Brain Research,* 228, 22-29.

Leggio, M. G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research,* 163, 78-90.

Lehmann, M. L., & Herkenham, M. (2011). Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway. *The Journal of Neuroscience, 31*, 6159-73.

Lehmann, M. L., Mustafa, T., Eiden, A. M., Herkenham, M., & Eiden, L. E. (2012). PACAP-deficient mice show attenuated corticosterone secretion and fail to develop depressive behavior during chronic social defeat stress. *Psychoneuroendocrinology,* in press.

Lendvai, B., Kassai, F., Szajli, A., & Nemethy, Z. (2012). Alpha7 nicotinic acetylcholine receptors and their role in cognition. *Brain Research Bulletin,* 93, 86-96.

Levin, E. D. (2012). Alpha7-nicotinic receptors and cognition. Current Drug Targets, 13, 602-6.

Lin EJ, Choi E, Liu X, Martin A, During MJ. 2011. Environmental enrichment exerts sex-specific effects on emotionality in C57BL/6J mice. *Behavioural Brain Research*, 216, 349-57

Lopez, M. F., Doremus-Fitzwater, T. L., & Becker, H. C. (2010). Chronic social isolation and chronic variable stress during early development induce later elevated ethanol intake in adult C57BL/6J mice. *Alcohol,* 45, 355-64.

Macri, S., Ceci, C., Altabella, L., Canese, R., & Laviola, G. (2013). The directive 2010/63/EU on animal experimentation may skew the conclusions of pharmacological and behavioural studies. *Scientific Reports*, 3, 2380.

Madroñal, N., Lopez-Aracil, C., Rangel, A., del Rio, J. A., Delgado-Garcia, J. M., & Gruart, A. (2010). Effects of enriched physical and social environments on motor performance, associative learning, and hippocampal neurogenesis in mice. *PloS One*, 5, e11130.

Magalhaes, A., Summavielle, T., Tavares, M. A., & de Sousa, L. (2007). Postnatal exposure to cocaine in rats housed in an enriched environment: Effects on social interactions. *Human & Experimental Toxicology,*26, 303-9.

Mainardi, M., Landi, S., Gianfranceschi, L., Baldini, S., De Pasquale, R., Berardi, N., … Caleo, M. (2010). Environmental enrichment potentiates thalamocortical transmission and plasticity in the adult rat visual cortex. *Journal of Neuroscience Research, 88*, 3048-59.

Maloku, E., Kadriu, B., Zhubi, A., Dong, E., Pibiri, F., Satta, R., & Guidotti, A. (2011). Selective alpha4beta2 nicotinic acetylcholine receptor agonists target epigenetic mechanisms in cortical GABA neurons. *Neuropsychopharmacology,* 36, 1366-74.

Malter Cohen, M., Tottenham, N., & Casey, B. (2013). Translational developmental studies of stress on brain and behavior: Implications for adolescent mental health and illness? *Neuroscience,* 249, 53-62.

Mandolesi, L., De Bartolo, P., Foti, F., Gelfo, F., Federico, F., Leggio, M. G., & Petrosini, L. (2008). Environmental enrichment provides a cognitive reserve to be spent in the case of brain lesion. *Journal of Alzheimer's Disease,* 15, 11-28.

Mangialasche, F., Kivipelto, M., Solomon, A., & Fratiglioni, L. (2012). Dementia prevention: Current epidemiological evidence and future perspective. *Alzheimer's Research & Therapy,* 4, 6.

Marioni, R. E., van den Hout, A., Valenzuela, M. J., Brayne, C., Matthews, F. E., & MRC Cognitive Function and Ageing Study. (2012). Active cognitive lifestyle associates with cognitive recovery and a reduced risk of cognitive decline. *Journal of Alzheimer's Disease,* 28, 223-30.

Marques, J. M., Alonso, I., Santos, C., Silveira, I., & Olsson, I. A. (2009). The spatial learning phenotype of heterozygous leaner mice is robust to systematic variation of the housing environment. *Comparative Medicine, 59*, 129-38.

McEwen, B.S., Eiland, L., Hunter, R.G., & Miller, M.M. (2012). Stress and anxiety: Structural plasticity and epigenetic regulation as a consequence of stress. *Neuropharmacology, 62*, 3-12.

McEwen B.S., & Gianaros P.J. (2011). S*tress- and allostasis-induced brain plasticity*. *Annual Review of Medicine,* 62, 431-45.

McLean, S. L., Grayson, B., Idris, N. F., Lesage, A. S., Pemberton, D. J., Mackie, C., & Neill, J. C. (2011). Activation of alpha7 nicotinic receptors improves phencyclidine-induced deficits in cognitive tasks in rats: Implications for therapy of cognitive dysfunction in schizophrenia. *European Neuropsychopharmacology,* 21, 333-43.

McQuaid, R. J., Audet, M., Jacobson-Pick, S., & Anisman, H. (2013). Environmental enrichment *influences brain cytokine variations elicited by social defeat in mice. Psychoneuroendocrinology,* 38, 987-96.

Mesa-Gresa, P., & Moya-Albiol, L. (2011). Neurobiologia del maltrato infantil: el 'ciclo de la violencia. *Revista De Neurologia,* 52, 489-503.

Mesa-Gresa, P., Perez-Martinez, A., & Redolat-Iborra, R. (2012). Nicotina y modelos animales: ¿qué nos aporta el paradigma de enriquecimiento ambiental? *Adicciones,* 24, 87-94.

Mesa-Gresa, P., Perez-Martinez, A., & Redolat, R. (2013a). Behavioral effects of combined environmental enrichment and chronic nicotine administration in male NMRI mice. *Physiology & Behavior,* 114-115, 65-76.

Mesa-Gresa, P., Perez-Martinez, A., & Redolat, R. (2013b). Environmental enrichment improves novel object recognition and enhances agonistic behavior in male mice. *Aggressive Behavior,* 39, 269-79.

Mesa-Gresa, P., Ramos-Campos, M., & Redolat, R. (2013c). Enriched environment for rodents and their interaction with nicotine administration. *Current Drug Abuse Reviews,* 6, 191-200.

Mesa-Gresa, P., Ramos-Campos, M., & Redolat, R. (2014). Behavioral effects of different enriched environments in mice treated with the cholinergic agonist PNU-282987. *Behavioural Processes,* 103, 117-24.

Middleton, L. E., & Yaffe, K. (2010). Targets for the prevention of dementia. *Journal of Alzheimer's Disease,* 20, 915-24.

Mitra, R., & Sapolsky, R. M. (2009). Effects of enrichment predominate over those of chronic stress on fear-related behavior in male rats. *Stress: The International Journal on the Biology of Stress, 12*, 305-12.

Miyazaki, T., Takase, K., Nakajima, W., Tada, H., Ohya, D., Sano, A., … Takahashi, T. (2012). Disrupted cortical function underlies behavior dysfunction due to social isolation. *The Journal of Clinical Investigation,*122, 2690-701.

Mora, F. (2010). "Nada ocurre ni existe en el mundo humano, que no haya sido filtrado y elaborado por el cerebro". *IMSERSO,* 285, 14-17.

Mora, F. (2013). Successful brain aging: Plasticity, environmental enrichment, and lifestyle. *Dialogues in Clinical Neuroscience,* 15, 45-52.

Morley-Fletcher, S., Rea, M., Maccari, S., & Laviola, G. (2003). Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. *The European Journal of Neuroscience,* 18, 3367-74.

Mustroph, M. L., Chen, S., Desai, S. C., Cay, E. B., Deyoung, E. K., & Rhodes, J. S. (2012). Aerobic exercise is the critical variable in an enriched environment that increases hippocampal neurogenesis and water maze learning in male C57BL/6J mice. *Neuroscience,* 219, 62-71

Neisewander, J. L., Peartree, N. A., & Pentkowski, N. S. (2012). Emotional valence and context of social influences on drug abuse-related behavior in animal models of social stress and prosocial interaction. *Psychopharmacology,* 224, 33-56.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews.Neuroscience,* 7, 697-709.

Nithianantharajah, J., & Hannan, A. J. (2009). The neurobiology of brain and cognitive reserve: Mental and physical activity as modulators of brain disorders. *Progress in Neurobiology,* 89, 369-82.

Nithianantharajah, J., & Hannan, A. J. (2011). Mechanisms mediating brain and cognitive reserve: Experience-dependent neuroprotection and functional compensation in animal models of neurodegenerative diseases. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 35, 331-39.

O'Callaghan, R. M., Griffin, E. W., & Kelly, A. M. (2009). Long-term treadmill exposure protects against age-related neurodegenerative change in the rat hippocampus*. Hippocampus,* 19, 1019-29.

Paban, V., Chambon, C., Manrique, C., Touzet, C., & Alescio-Lautier, B. (2009). Neurotrophic signaling molecules associated with cholinergic damage in young and aged rats environmental enrichment as potential therapeutic agent. *Neurobiology of Aging,* 32, 470-85.

Pamplona, F. A., Pandolfo, P., Savoldi, R., Prediger, R. D., & Takahashi, R. N. (2009). Environmental enrichment improves cognitive deficits in spontaneously hypertensive rats:

Relevance for attention Deficit/Hyperactivity disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 33, 1153-60.

Pandya, A. A., & Yakel, J. L. (2013). Activation of the alpha7 nicotinic ACh receptor induces anxiogenic effects in rats which is blocked by a 5-HT(1a) receptor antagonist. *Neuropharmacology,* 70C, 35-42.

Pang, T. Y., & Hannan, A. J. (2013). Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. *Neuropharmacology,* 64, 515-28.

Pardon, M. C., & Rattray, I. (2008). What do we know about the long-term consequences of stress on ageing and the progression of age-related neurodegenerative disorders? *Neuroscience & Biobehavioral Reviews,* 32, 1103-20.

Patel, T. R. (2012). Environmental enrichment: Aging and memory. *The Yale Journal of Biology and Medicine,* 85, 491-500.

Peña, Y., Prunell, M., Dimitsantos, V., Nadal, R., & Escorihuela, R. M. (2006). Environmental enrichment effects in social investigation in rats are gender dependent. *Behavioural Brain Research,* 174, 181-87.

Perez-Tejada, J., Arregi, A., Gomez-Lazaro, E., Vegas, O., Azpiroz, A., & Garmendia, L. (2013). Coping with chronic social stress in mice: Hypothalamic-pituitary-adrenal/ sympathetic-adrenal-medullary axis activity, behavioral changes and effects of antalarmin treatment: Implications for the study of stress-related psychopathologies. *Neuroendocrinology, 98*, 73-88.

Perry, J. L., Stairs, D. J., & Bardo, M. T. (2008). Impulsive choice and environmental enrichment: Effects of d-amphetamine and methylphenidate. *Behavioural Brain Research,* 193, 48-54.

Petrosini, L., De Bartolo, P., Foti, F., Gelfo, F., Cutuli, D., Leggio, M. G., & Mandolesi, L. (2009). On whether the environmental enrichment may provide cognitive and brain reserves. *Brain Research Reviews,* 61, 221-39.

Pietropaolo, S., Branchi, I., Cirulli, F., Chiarotti, F., Aloe, L., & Alleva, E. (2004). Long-term effects of the periadolescent environment on exploratory activity and aggressive behaviour in mice: Social versus physical enrichment. *Physiology & Behavior,* 81, 443-53.

Pietropaolo, S., Feldon, J., Alleva, E., Cirulli, F., & Yee, B. K. (2006). The role of voluntary exercise in enriched rearing: A behavioral analysis. *Behavioral Neuroscience,* 120, 787-03.

Pietropaolo, S., Sun, Y., Li, R., Brana, C., Feldon, J., & Yee, B. K. (2008). The impact of voluntary exercise on mental health in rodents: A neuroplasticity perspective. *Behavioural Brain Research,* 192, 42-60.

Pizzorusso, T., Berardi, N., & Maffei, L. (2007). A richness that cures. *Neuron,* 54, 508-10.

Puhl, M. D., Blum, J. S., Acosta-Torres, S., & Grigson, P. S. (2012). Environmental enrichment protects against the acquisition of cocaine self-administration in adult male rats, but does not eliminate avoidance of a drug-associated saccharin cue. *Behavioural Pharmacology,* 23, 43-53.

Qiu, C., Xu, W., & Fratiglioni, L. (2010). Vascular and psychosocial factors in alzheimer's disease: Epidemiological evidence toward intervention. *Journal of Alzheimer's Disease,* 20, 689-97.

Ramírez-Rodríguez, G., Ocaña-Fernández, M., Vega-Rivera, N., Torres-Pérez, O., Gómez-Sánchez, A., Estrada-Camarena, E., & Ortiz-López, L. (2014). Environmental enrichment induces neuroplastic changes in middle age female BalbC mice and increases the hippocampal levels of BDNF, p-akt and p-MAPK1/2*. Neuroscience,* 260, 158-70.

Ravenelle, R., Byrnes, E. M., Byrnes, J. J., McInnis, C., Park, J. H., & Donaldson, S. T. (2013). Environmental enrichment effects on the neurobehavioral profile of selective outbred trait anxiety rats. *Behavioural Brain Research,* 252C, 49-57.

Razzoli, M., Domenici, E., Carboni, L., Rantamaki, T., Lindholm, J., Castrén, E., & Arban, R. (2011a). A role for BDNF/TrkB signaling in behavioral and physiological consequences of social defeat stress. *Genes, Brain and Behavior, 10*, 424-33.

Razzoli, M., Carboni, L., Andreoli, M., Ballottari, A., & Arban, R. (2011b). Different susceptibility to social defeat stress of BalbC and C57BL6/J mice. *Behavioural Brain Research, 216*, 100-8.

Redolat, R., & Mesa-Gresa, P. (2012). Potential benefits and limitations of enriched environments and cognitive activity on age-related behavioural decline. *Current Topics in Behavioral Neurosciences,* 10, 293-316.

Redolat, R., Perez-Martinez, A., Carrasco, M. C., & Mesa, P. (2009). Individual differences in novelty-seeking and behavioral responses to nicotine: A review of animal studies. *Current Drug Abuse Reviews,* 2, 230-42.

Renner, M. J., & Rosenzweig, M. R. (1986). Social interactions among rats housed in grouped and enriched conditions. *Developmental Psychobiology,* 19, 303-13.

Richter, S. H., Zeuch, B., Riva, M. A., Gass, P., & Vollmayr, B. (2013). Environmental enrichment ameliorates depressive-like symptoms in young rats bred for learned helplessness. *Behavioural Brain Research,* 252, 287-92.

Rojas, J. J., Deniz, B. F., Miguel, P. M., Diaz, R., Hermel Edo, E., Achaval, M., … Pereira, L. O. (2013). Effects of daily environmental enrichment on behavior and dendritic spine density in hippocampus following neonatal hypoxia-ischemia in the rat. *Experimental Neurology,* 241, 25-33.

Rose, J. E. (2007). Multiple brain pathways and receptors underlying tobacco addiction. *Biochemical Pharmacology, 74*, 1263-70.

Rosenzweig, M. R. (1966). Environmental complexity, cerebral change, and behavior. *The American Psychologist,* 21, 321-32

Rueda, A. V., Teixeira, A. M., Yonamine, M., & Camarini, R. (2012). Environmental enrichment blocks ethanol-induced locomotor sensitization and decreases BDNF levels in the prefrontal cortex in mice. *Addiction Biology, 17*, 736-45.

Salam, J. N., Fox, J. H., Detroy, E. M., Guignon, M. H., Wohl, D. F., & Falls, W. A. (2009). Voluntary exercise in C57 mice is anxiolytic across several measures of anxiety. *Behavioural Brain Research,* 197, 31-40.

Sale, A., Berardi, N., & Maffei, L. (2009). Enrich the environment to empower the brain. *Trends in Neurosciences,* 32, 233-39.

Sale, A., Berardi, N., & Maffei, L. (2014). Environment and brain plasticity: Towards an endogenous pharmacotherapy. *Physiological Reviews,* 94, 189-234.

Sampedro-Piquero, P., Begega, A., Zancada-Menendez, C., Cuesta, M., & Arias, J.L. (2013a). Age-dependent effects of environmental enrichment on brain networks ans spatial memory in rats. *Neuroscience,* 248, 43-53.

Sampedro-Piquero, P., Zancada-Menendez, C., Begega, A., Rubio, S., & Arias, J.L. (2013b). Effects of environmental enrichment on anxiety responses, spatial memory and cytochrome c oxidase activity in adult rats. *Brain Research Bulletin*, 98, 1-9.

Sampedro-Piquero, P., Begega, A., & Arias, J. (2014). Increase of glucocorticoid receptor expression after environmental enrichment: Relations to spatial memory, exploration and anxiety-related behaviors.*Physiology & Behavior, 129*, 118-129.

Saucier, D. M., Yager, J. Y., & Armstrong, E. A. (2010). Housing environment and sex affect behavioral recovery from ischemic brain damage. *Behavioural Brain Research,* 214, 48-54.

Scharf, S. H., Sterlemann, V., Liebl, C., Müller, M. B., & Schmidt, M. V. (2013). Chronic social stress during adolescence: Interplay of paroxetine treatment and ageing. *Neuropharmacology, 72*, 38-46.

Schloesser, R. J., Lehmann, M., Martinowich, K., Manji, H. K., & Herkenham, M. (2010). Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. *Molecular Psychiatry,*15, 1152-63.

Segovia, G., Del Arco, A., Garrido, P., de Blas, M., & Mora, F. (2008). Environmental enrichment reduces the response to stress of the cholinergic system in the prefrontal cortex during aging. *Neurochemistry International*, 52, 1198-203.

Segovia, G., Del Arco, A., & Mora, F. (2009). Environmental enrichment, prefrontal cortex, stress, and aging of the brain. *Journal of Neural Transmission, 116*, 1007-16.

Segovia, G., Del Arco, A., De Blas, M., Garrido, P., & Mora, F. (2010). Environmental enrichment increases the in vivo extracellular concentration of dopamine in the nucleus accumbens: A microdialysis study. *Journal of Neural, 117*, 1123-30.

Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats--behavioural and neurochemical aspects. *Behavioural Brain Research,* 222, 246-64.

Simpson, J., & Kelly, J. P. (2012). The effects of isolated and enriched housing conditions on baseline and drug-induced behavioural responses in the male rat. *Behavioural Brain Research,* 234, 175-83.

Skwara, A. J., Karwoski, T. E., Czambel, R. K., Rubin, R. T., & Rhodes, M. E. (2012). Influence of environmental enrichment on hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine by osmotic mini-pumps, and nicotine withdrawal by mecamylamine in male and female rats. *Behavioural Brain Research,* 234, 1-10.

Smith, M. A., Chisholm, K. A., Bryant, P. A., Greene, J. L., McClean, J. M., Stoops, W. W., & Yancey, D. L. (2005). Social and environmental influences on opioid sensitivity in rats: Importance of an opioid's relative efficacy at the mu-receptor. *Psychopharmacology,* 181, 27-37.

Smith, M. A., Iordanou, J. C., Cohen, M. B., Cole, K. T., Gergans, S. R., Lyle, M. A., & Schmidt, K. T. (2009). Effects of environmental enrichment on sensitivity to cocaine in female rats: Importance of control rates of behavior. *Behavioural Pharmacology,* 20, 312-21.

Solinas, M., Chauvet, C., Thiriet, N., El Rawas, R., & Jaber, M. (2008). Reversal of cocaine addiction by environmental enrichment. *Proceedings of the National Academy of Sciences of the United States of America,* 105, 17145-50.

Solinas, M., Thiriet, N., Chauvet, C., & Jaber, M. (2010). Prevention and treatment of drug addiction by environmental enrichment. *Progress in Neurobiology,* 92, 572-92.

Solinas, M., Thiriet, N., El Rawas, R., Lardeux, V., & Jaber, M. (2009). Environmental enrichment during early stages of life reduces the behavioral, neurochemical, and molecular effects of cocaine. *Neuropsychopharmacology,* 34, 1102-11.

Sparling, J. E., Mahoney, M., Baker, S., & Bielajew, C. (2010). The effects of gestational and postpartum environmental enrichment on the mother rat: A preliminary investigation. *Behavioural Brain Research,208*, 213-23.

Stairs, D. J., & Bardo, M. T. (2009). Neurobehavioral effects of environmental enrichment and drug abuse vulnerability. *Pharmacology, Biochemistry, and Behavior,* 92, 377-82.

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., … Schmidt, M. V. (2008). Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: Implications for stress-related disorders. *Hormones and Behavior, 53*, 386-94.

Sztainberg, Y., & Chen, A. (2010). An environmental enrichment model for mice. *Nature Protocols,* 5, 1535-39.

Thiel, K. J., Pentkowski, N. S., Peartree, N. A., Painter, M. R., & Neisewander, J. L. (2010a). Environmental living conditions introduced during forced abstinence alter cocaine-seeking behavior and fos protein expression. *Neuroscience, 171*, 1187-96.

Thiel, K. J., Engelhardt, B., Hood, L. E., Peartree, N. A., & Neisewander, J. L. (2011) The interactive effects of environmental enrichment and extinction interventions in attenuating cue-elicited cocaine-seeking behavior in rats. *Pharmacology, Biochemistry & Behavior,* 97, 595-602.

Thiriet, N., Amar, L., Toussay, X., Lardeux, V., Ladenheim, B., Becker, K. G., … Jaber, M. (2008). Environmental enrichment during adolescence regulates gene expression in the striatum of mice. *Brain Research,*1222, 31-41.

Van de Weerd, H. A., Van Loo, P. L., Van Zutphen, L. F., Koolhaas, J. M., & Baumans, V. (1997). Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. *Physiology & Behavior,* 62, 1019-28.

van Dellen, A., Cordery, P. M., Spires, T. L., Blakemore, C., & Hannan, A. J. (2008). Wheel running from a juvenile age delays onset of specific motor deficits but does not alter protein aggregate density in a mouse model of Huntington's disease. *Neuroscience,* 9, 34.

van Praag, H. (2009). Exercise and the brain: Something to chew on. *Trends in Neurosciences,* 32, 283-90.

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews.Neuroscience,* 1, 191-98.

Varty, G. B., Paulus, M. P., Braff, D. L., & Geyer, M. A. (2000). Environmental enrichment and isolation rearing in the rat: Effects on locomotor behavior and startle response plasticity. *Biological Psychiatry,* 47, 864-73.

Vedovelli, K., Silveira, E., Velho, E., Stertz, L., Kapczinski, F., Schroder, N., & Bromberg, E. (2011). Effects of increased opportunity for physical exercise and learning experiences on recognition memory and brain-derived neurotrophic factor levels in brain and serum of rats. *Neuroscience,* 199, 284-91.

Vicens, P., Bernal, M. C., Carrasco, M. C., & Redolat, R. (1999). Previous training in the water maze: Differential effects in NMRI and C57BL mice. *Physiology & Behavior, 67*, 197-203.

Vicens, P., Carrasco, M. C., & Redolat, R. (2003). Effects of early training and nicotine treatment on the performance of male NMRI mice in the water maze. *Neural Plasticity,* 10, 303-17.

Vicens, P., Ribes, D., Heredia, L., Torrente, M., & Domingo, J. L. (2013a). Motor and anxiety effects of PNU-282987, an alpha7 nicotinic receptor agonist, and stress in an animal model of Alzheimer's disease. *Current Alzheimer Research,* 10, 516-23.

Vicens, P., Ribes, D., Heredia, L., Torrente, M., & Domingo, J. L. (2013b). Effects of an alpha7 nicotinic receptor agonist and stress on spatial memory in an animal model of Alzheimer's disease. *BioMed Research International,* 2013, 952719.

Vicens, P., Ribes, D., Torrente, M., & Domingo, J. L. (2011). Behavioral effects of PNU-282987, an alpha7 nicotinic receptor agonist, in mice. *Behavioural Brain Research,* 216, 341-48.

Viola, G. G., Botton, P. H., Moreira, J. D., Ardais, A. P., Oses, J. P., & Souza, D. O. (2010). Influence of environmental enrichment on an object recognition task in CF1 mice. *Physiology & Behavior,* 99, 17-21.

Welch, K. D., Pfister, J. A., Lima, F. G., Green, B. T., & Gardner, D. R. (2013). Effect of alpha(7) nicotinic acetylcholine receptor agonists and antagonists on motor function in mice. *Toxicology and Applied Pharmacology,* 266, 366-74.

Whitaker, J., Moy, S. S., Godfrey, V., Nielsen, J., Bellinger, D., & Bradfield, J. (2009). Effects of cage size and enrichment on reproductive performance and behavior in C57BL/6Tac mice. *Lab Animal,* 38, 24-34.

Will, B., Galani, R., Kelche, C., & Rosenzweig, M. R. (2004). Recovery from brain injury in animals: Relative efficacy of environmental enrichment, physical exercise or formal training (1990-2002). *Progress in Neurobiology,* 72, 167-82.

Wong, C. C., Mill, J., & Fernandes, C. (2011). Drugs and addiction: An introduction to epigenetics. *Addiction,* 106, 480-89.

Wood, N. I., Glynn, D., & Morton, A. J. (2011). "Brain training" improves cognitive performance and survival in a transgenic mouse model of Huntington's disease. *Neurobiology of Disease,* 42, 427-37.

Workman, J. L., Fonken, L. K., Gusfa, J., Kassouf, K. M., & Nelson, R. J. (2011). Post-weaning environmental enrichment alters affective responses and interacts with behavioral testing to alter nNOS immunoreactivity. *Pharmacology, Biochemistry, and Behavior,* 100, 25-32.

Xie, H., Wu, Y., Jia, J., Liu, G., Zhang, Q., Yu, K., … Hu, R. (2013). Enrichment-induced exercise to quantify the effect of different housing conditions: A tool to standardize enriched environment protocols. *Behavioural Brain Research,* 249, 81-89.

Yuan, Z. Y., Gu, P., Liu, L., Wang, Y. Y., Liu, J., Cui, D. S., … Wang, M. W. (2009). Neuroprotective effects of enriched environment in MPTP-treated SAMP8 mice. *Neuroscience Letters,* 454, 6-10.

Zhu, J., Bardo, M. T., & Dwoskin, L. P. (2013). Distinct effects of enriched environment on dopamine clearance in nucleus accumbens shell and core following systemic nicotine administration. Synapse, 67, 57-67.

Zhu, J., Bardo, M. T., Green, T. A., Wedlund, P. J., & Dwoskin, L. P. (2007). Nicotine increases dopamine clearance in medial prefrontal cortex in rats raised in an enriched environment. *Journal of Neurochemistry,*103, 2575-88.

Zhu, S. W., Codita, A., Bogdanovic, N., Hjerling-Leffler, J., Ernfors, P., Winblad, B., … Mohammed, A. H. (2009). Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice. *Behavioural Brain Research,* 197, 339-46.

CAPÍTULO 2

ESTUDIO 1

Desarrollo de un modelo de Enriquecimiento Ambiental: efectos sobre aprendizaje e interacción social

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Publicado en (véase ANEXO 9.4):

Mesa-Gresa P, Pérez-Martinez A, Redolat R. Environmental enrichment improves novel object recognition and enhances agonistic behavior in male mice. *Aggressive Behavior*; 39(4):269-79.

ENVIRONMENTAL ENRICHMENT IMPROVES NOVEL OBJECT RECOGNITION AND ENHANCES AGONISTIC BEHAVIOR IN MALE MICE

Abstract

Environmental enrichment (EE) is an experimental paradigm in which rodents are housed in complex environments containing objects that provide stimulation, the effects of which are expected to improve the welfare of these subjects. EE has been shown to considerably improve learning and memory in rodents. However, knowledge about the effects of EE on social interaction is generally limited and rather controversial. Thus, our aim was to evaluate both novel object recognition and agonistic behavior in NMRI mice receiving EE, hypothesizing enhanced cognition and slightly enhanced agonistic interaction upon EE rearing. During a 4-week period half the mice (n=16) were exposed to EE and the other half (n=16) remained in a standard environment (SE). On PND 56-57, animals performed the object recognition test, in which recognition memory was measured using a discrimination index. The social interaction test consisted of an encounter between an experimental animal and a standard opponent. Results indicated that EE mice explored the new object for longer periods than SE animals ($p<0.05$). During social encounters, EE mice devoted more time to sociability and agonistic behavior ($p<0.05$) than their non-EE counterparts. In conclusion, EE has been shown to improve object recognition and increase agonistic behavior in adolescent/early adulthood mice. In the future we intend to extend this study on a longitudinal basis in order to assess in more depth the effect of EE and the consistency of the above mentioned observations in NMRI mice.

1. INTRODUCTION

Environmental Enrichment (EE) is an experimental paradigm in which rodents are housed in large groups in complex environments consisting of cages containing toys, houses, nesting materials, tunnels and running wheels (Nithianantharajah & Hannan, 2009; Redolat & Mesa-Gresa, 2012; Rosenzweig & Bennett, 1996). The complexity of enriched environments provides animals with physical activity (Harburger, Nzerem, & Frick, 2007) and cognitive, visual and somatosensory stimulation (Nithianantharajah & Hannan, 2009; Rosenzweig & Bennett, 1996; Yuan, Gu, Liu, Wang, Liu, Cui, et al., 2009), and offers more opportunities for social stimulation and interaction (van Praag, Kempermann, & Gage, 2000) than standard housing conditions. Introducing animals into this complex environment induces neurochemical, physiological and behavioral changes to name a few. At a neurochemical level, it has been reported that EE augments the concentration of serotonin and central monoaminergic and cholinergic activity (Brenes, Padilla, & Fornaguera, 2009; Simpson & Kelly, 2011; van Praag et al., 2000). This type of enriched accommodation also induces structural changes in the brain, such as an increase in dendritic branching, hippocampal neurogenesis (Madroñal, Lopez-Aracil, Rangel, del Rio, Delgado-Garcia, & Gruart, 2010; Simpson & Kelly, 2011) and neuroplasticity (Artola, von Frijtag, Fermont, Gispen, Schrama, Kamal, et al., 2006; Petrosini, De Bartolo, Foti, Gelfo, Cutuli, Leggio, et al., 2009), promoting cognitive reserve (Nithianantharajah & Hannan, 2011; Petrosini et al., 2009) which is related to the lack of a direct relationship between age-related neuropathological changes in the brain and the clinical symptoms of cognitive decline (Stern, 2012).

At a behavioral level, it has been reported that EE improves performance in learning and memory tasks (Nithianantharajah & Hannan, 2006; Renner & Rosenzweig, 1986; Vedovelli, Silveira, Velho, Stertz, Kapczinski, Schroder, et al., 2011), alleviates anxiety and stress-related disorders (Benaroya-Milshtein, Hollander, Apter, Kukulansky, Raz, Wilf, et al., 2004; Fernandez-Teruel, Escorihuela, Castellano, Gonzalez, & Tobena, 1997), reduces exploratory behavior (Brenes, Rodriguez, & Fornaguera, 2008), and promotes social interaction (van Praag et al., 2000) and grooming (Konkle, Kentner, Baker, Stewart, & Bielajew, 2010). An improvement in a

variety of learning and memory tasks after exposure of EE conditions has been demonstrated by different studies in both mice and rats (Kulesskaya, Rauyala, & Voikar, 2011; Renner & Rosenzweig, 1986; Vedovelli et al., 2011; Winters, Saksida, & Bussey, 2008). This improvement can be applied to hippocampal-dependent memory tasks (Branchi, D'Andrea, Fiore, Di Fausto, Aloe, & Alleva, 2006; Gresack, Kerr, & Frick, 2007; Harati, Majehrzak, Cosquer, Galani, Kelche, Cassel, et al., 2011; Mirochnic, Wolf, Staufenbiel, & Kempermann, 2009), the ability to learn spatial and non-spatial activities (Diniz, Foro, Rego, Gloria, de Oliveira, Paes, et al. 2010; Hansalik, Skalicky, & Viidik, 2006; Leggio, Mandolesi, Federico, Spirito, Ricci, Gelfo, et al., 2005; Viola, Botton, Moreira, Ardais, Oses, & Souza, 2010) and habituation and adaptation to novel environments (Brenes et al., 2008; Hughes & Collins, 2010; Zimmermann, Stauffacher, Langhans, & Wurbel, 2001). These results have been obtained in different paradigms in both male (Bennett, McRae, Levy, & Frick, 2006; Harburger et al., 2007; Kazlauckas, Pagnussat, Mioranzza, Kalinine, Nues, Pettenuzzo, et al., 2011) and female mice (Kulesskaya et al., 2011; Lambert, Fernandez, & Frick, 2005), although differences have been reported depending on the type of enrichment paradigm applied (Lambert et al., 2005; Mesa-Gresa, Pérez-Martínez, & Redolat, 2012) or the learning task under evaluation (Garcia-Capdevila, Portell-Cortes, Torras-Garcia, Coll-Andreu, & Costa-Miserachs, 2009). Both EE and physical exercise have been shown to improve object recognition (Kazlauckas et al., 2011; Viola et al., 2010), whereas aging (Malin, Lee, Goyarzu, Chang, Ennis, Beckett, et al., 2011) and stressful situations usually impair it (Silvers, Harrod, Mactutus, & Booze, 2007). The one-trial novel object recognition test is a working memory task used towards evaluation of behavioral effects of different pharmacological/environmental interventions in rodents (Dere, Huston, & De Souza Silva, 2007; Ennaceur, 2010; Heyser & Chemero, 2012; Kenney, Adoff, Wilkinson, & Gould, 2011). This test is based on the tendency of rodents to spend more time exploring novel objects than familiar ones and to realize when the position of an object has been changed. Thus, from the above observations, it could be anticipated an enhanced cognitive performance in rodents upon environmental enrichment in general.

Apart from learning and memory, given its involvement with large groups of animals (usually 8-10 and sometimes up to 20 mice) being housed together in the same cage for several weeks or months (Redolat & Mesa-Gresa, 2012; Swetter, Karpiak, & Cannon, 2011), EE have also been shown to induce changes in social interactions among the conspecifics although only inconsistently. Some studies have shown that exposure to EE diminishes agonistic interaction among rodents when compared to those reared in standard cages (Abou-Ismail, Burman, Nicol, & Mendl, 2010; Abramov, Puussaar, Raud, Kurrikoff, & Vasar, 2008; Chamove, 1989; Pietropaolo, Banchi, Ciruli, Chiarotti, Aloe, & Alleva 2004) or in isolation (Workman, Fonken, Gusfa, Kassouf, & Nelson, 2011). For example, Abramov et al. (2008) reported that 129S6/SvEv mice housed in standard conditions carried out significantly more attacks on intruders than C57BL/6 mice, but did not observe these strain-related differences when the same mice were housed in enriched conditions. On the contrary, an increase in aggression has been reported in CS mice, but not in ABG docile mice, following exposure to an enriched environment (Marashi, Barnekow, & Sachser, 2004). More recently, McQuaid, Audet, Jacobson-Pick & Anisman (2012) reported an increase of aggressive behavior among CD-1 mice after 2 weeks in an enriched environment, whereas this increase was not confirmed in more docile strains such as BALB (McQuaid et al., 2012). This enhanced aggression has been suggested in different studies (Haemisch & Gartner, 1997; Haemisch, Voss, & Gartner, 1994; Van Loo, Mol, Koolhaas, Van Zutphen, & Baumans, 2001) and related to the complexity of the enriched cages and the presence of new objects in the cage, such as houses, wheels or toys, which increase the proximity and competition for resources between animals. Proximity has been shown to enhance territorial behavior between male mice, as seen from damages to the tail (Abou-Ismail, 2011; Haemisch & Gartner, 1997; Haemisch et al., 1994). However, some studies have found no changes in aggressive behavior between male mice exposed to enriched environments.

Despite the lack of a definitive pattern, we could get some information from studies including the ones done on wild-type Groningen mice that were genetically selected for high and low aggression. While the high aggressive violent SAL mice display invariant species-atypical aggression along with impaired cognitive

performance, the low aggressive LAL mice display considerable flexibility regardless of social/cognitive contexts, suggestive of the likely links between cognitive performance and agonistic interactions (Benus, Bohus, Koolhaas, & Van Oortmerssen, 1991; Caramaschi, de Boer, de Vries, & Koolhaas, 2008; Coppens, de Boer, & Koolhaas, 2010; Koolhaas, de Boer, Buwalda, & van Reenen 2007; Koolhaas, de Boer, Coppens, & Buwalda, 2010; Natarajan, de Boer, & Koolhaas, 2009a). It may be also noted how the above findings were actually made under standard housing conditions and therefore any hypothesis regarding effects of EE suggested in this direction may be subject to speculation.

Considering these hypotheses, the main aim of the present study was to evaluate both cognitive performance (through a novel object recognition test) and social and aggressive behavior (using a social interaction test) in NMRI mice housed in EE conditions at early age. Given the low aggressive docile phenotype of this strain, we thus anticipate greater cognitive and social flexibility with respect to enhanced cognition and, to some extent, enhanced agonistic interaction upon EE rearing.

2. MATERIALS AND METHODS

2.1. Subjects

Sixty four male NMRI mice (Charles River, Barcelona, Spain) were included in the study. The NMRI strain was used in the current study since it is a docile strain which behavioral profile is well known (Gómez, Carrasco & Redolat, 2008; Moragrega, Carrasco & Redolat, 2005; Vihavainen, Piltonen, Tuominen, Korpi & Ahtee, 2008) although few studies have been carried out with the EE paradigm (Bouet, Freret, Dutar, Billard & Boulouard, 2011; Freret, Billard, Schumann-Bard, Dutar, Dauphin, Boulouard, et al., 2011). The mice arrived at our laboratory at 21 days of age (mean weight: 10-12 gr.) and were housed in groups of 4 in a controlled environment (lights on at 20:00 hr) at a constant temperature (21ºC) in order for them to adapt. At 28 days of age, half of the mice (n=32) were allocated to environmental enrichment (n=16), in which mice

were housed in groups of 8 in large cages with a house, a running wheel, a tunnel and five different toys that were changed twice a week, or standard rearing conditions in groups of 4 in each cage (n=16), while the other half (n=32) were maintained in the same standard conditions in order to be used as opponents during social interaction tests. All tests were video-recorded (Panasonic HDC SD10) and analysed by a researcher who was blind to the experimental condition of each animal. All procedures complied with national and international guidelines (Real Decreto 1201/2005; Decreto 13/2007; European Community's Council Directive of November 24, 1986 -86/609/EEC; 2007/526/CE; 2010/63/EU) for the care and treatment of animals.

2.2. Procedure

Mice underwent a battery of behavioral tests from post natal day (PND) 56 to PND 58. The novel object recognition test took place on day 56-57 and the social interaction test took place on day 58.

2.2.1. Novel object recognition test

The novel object recognition test was carried out in a neutral cage (60x33x30 cm). Following the procedure reported by Greco, Bryan, Sarkar, Zhu, Smith, Ashford, et al. (2010), the test protocol consisted of three phases: 1) **Habituation**, by which mice were habituated to exploring an open field for 5 min one day prior to testing; 2) **Training**, by which two identical novel objects (two cups or small bottles) were placed in the arena and mice were allowed to explore them for 10 min; and 3) **Test** (1 hour after training), by which mice explored a novel object and a familiar one previously explored for 5 min, after which the total time spent exploring the new object with respect to total exploration time was calculated. Objects and cage were cleaned after each test with a solution of ethanol and water. Both objects and object location were counterbalanced across experimental conditions in order to avoid object and location preference. A video camera was used to record the behaviors displayed by each mouse during the test. An object was considered to have been explored when the head of the animal was ½ cm from the object or when it touched the object. In contrast, exploration was considered not to have taken place when an animal climbed onto an

object or used it as a base to explore the environment (Ennaceur, 2010; Greco et al., 2010). Grooming and rearing behaviors were also evaluated, as indicated in the procedure reported by Greco et al., 2010. In order to measure recognition memory during the test phase, a discrimination index (total time spent with new object/total time devoted to exploration of objects) was calculated for each experimental group.

2.2.2. Social interaction test

Social interaction encounters took place in a neutral cage made of clear Plexiglas (60x33x30 cm). In these encounters EE or SE mice were confronted with a standard opponent (group-housed mice) of similar age and weight (32-35 gr). Standard opponents were marked with fur dye in order to identify them in the video recordings. Before the social encounter took place, both mice were allowed 1 min to adapt to the neutral cage while remaining separated by a white plastic barrier. Following the procedure reported by Chistyakov, Patkina, Tammimaki, Talka, Salminen, Belozertseva, et al. (2010), the total duration of agonistic encounters was limited to four minutes in order to avoid serious injuries. The encounters were video-recorded. After each test, the cage was cleaned with a solution of ethanol and water and the sawdust bedding was replaced.

The behavior displayed by mice during their social encounters was analysed by a blind researcher using “Raton-time” software, a programme which allows ethological analysis of ten behavioral categories (Brain, McAllister, & Walmsley, 1989; Redolat, Brain, & Simon, 1991). The following behavioral categories were evaluated:

- Body care (self-grooming, washing, shaking, scratching),
- Digging (dig, kick dig, push dig),
- Non-social exploration (explore, rear, supported rear, scan),
- Exploration from a distance (approach, attend, circle, head orient, stretched attention),
- Social exploration (crawl over, crawl under, follow, groom, head groom, investigate, nose sniff, sniff, push past, walk around),
- Threat (aggressive groom, sideways offensive, upright offensive, tail rattle)

- Attack (charge, lunge, attack, chase),
- Avoidance flee (evade, flinch, retreat, ricochet, wheel, startle, jump, leave, wall, clutch),
- Defence/submission (upright defensive, upright submissive, sideways defensive),
- Immobility (squat, cringe).

Additionally, in order to explore in more depth the changes induced by EE in terms of aggression, the behavior displayed by mice during social encounters was evaluated according to the main categories set out by Chistyakov et al. (2010), which include "Social behavior" and "Individual behavior". The category of "Social behavior" includes sociability (social investigation and social contact) and agonistic behavior (aggressive behavior, ambivalent behavior and defensive behavior). The category of "Individual behavior" includes the parameters of motor activity, static behavior and self-directed behavior. The third behavioral category is "Submissive behavior", which is the sum of defensive and static behaviors (for more details see Chistyakov et al., 2010).

2.3. Statistical analysis

All statistical analyses were performed using SPSS (Version 19) for Windows. During social encounters, the time allocated to each behavioral category and the number of times that mice displayed each behavior were analysed by means of an analysis of variance (ANOVA), using "Housing" as a factor. Recognition memory in the test phase of the object recognition test was measured using a discrimination index (total time spent exploring the new object/total exploration time). Differences between EE and SE groups were measured by means of ANOVA. Pearson's correlation coefficient was used to evaluate the relationship between the data obtained in the novel object recognition task (discrimination index) and the time accumulated in each one of the behavioral categories evaluated in the social interaction test.

All data are presented as mean ± standard error of the mean (SEM). In all cases, significance levels were set at $p<0.05$.

3. RESULTS

3.1. Effects of environmental enrichment in the novel object recognition test

In relation to the results of the novel object recognition test, the ANOVA indicated significant differences between the discrimination index of EE and SE groups [$F(1,30)= 4.55$, $p<0.05$]. Animals reared in enriched cages spent more time exploring the new object during the test phase than those housed in standard cages; this was evident in the discrimination index of >50%, which indicated a preference for exploring novel objects (Siopi, Llufriu-Daben, Fanucchi, Plotkine, Marchand-Leroux, & Jafarian-Tehrani, 2012). Furthermore, the enriched group spent more time engaged in grooming behavior (64.16 ± 11.43) and less in lower rearing behavior (15.12±2.13) than their standard-housed counterparts (41.71 ± 11.47 and 19.50±2.81, respectively) (Figure 1).

3.2. Effects of environmental enrichment on social interaction and aggressive behaviour

a) Analysis following the behavioral categories set out by Brain et al. (1989):

If we consider behaviors displayed by mice according to the ethogram reported by Brain et al. (1989), our results show statistical differences between the time dedicated to the behavioral categories of non-social exploration [$F(1,31)=19.916$, $p<0.0001$], social exploration [$F(1,31)=10.651$, $p<0.001$] and threat [$F(1,31)=6.993$, $p<0.05$]. EE animals spent less time engaged in non-social exploration ($p<0.0001$) and more time engaged in social exploration ($p<0.001$) and threat ($p<0.05$) than standard-housed mice. The results for frequency of each of the aforementioned behaviors showed statistical differences between digging [$F(1,31)=4.751$, $p<0.05$], non-social exploration [$F(1,31)=8.750$, $p<0.01$] and threat episodes [$F(1,31)=6.827$, $p<0.05$]. Mice assigned to EE conditions engaged in a lower number of digging ($p<0.05$) and non-social exploration ($p<0.01$) episodes and a higher number of threat episodes ($p<0.05$) than those reared in SE conditions.

In standard-housed mice, a significant negative correlation was obtained between time allocated to non-social exploration during social encounters and the discrimination index in the object recognition task ($r=-0.526$, $p<0.05$). No significant correlation was observed between this learning index and time engaged in other behavioral categories in either SE or EE mice (Figures 2A and 2B and Table 1).

b) Analysis following the behavioral categories set out by Chystiakov et al. (2010):

The video recordings of the social encounters were analysed according to the behavioral categorization of Chistyakov et al. (2010) in order to confirm variations in the time allocated to offensive behavior. The recordings revealed significant effects of housing conditions on the categories of "sociability" [$F(1,31)=10.299$, $p<0.05$], "agonistic behavior" [$F(1,31)=4.762$, $p<0.05$] and "individual behavior" [$F(1,31)= 18.387$, $p<0.0001$]. Mice housed in enriched conditions spent more time engaged in behaviors classified within "sociability" and "agonistic behavior" categories ($p<0.05$) and less time engaged in "individual behavior" (e.g., grooming ($p<0.001$)) than standard-housed animals. Taking into account the frequency of each behavioral category, statistical differences were obtained in "social behavior" [$F(1,31)=5.903$, $p<0.05$]. EE mice displayed postures considered to represent social behavior more frequently ($p<0.05$) than their SE counterparts. No differences were detected between SE and EE groups in the category of "submissive behavior".

Time allocated to "agonistic behavior" during social encounters revealed a significant positive correlation with the discrimination index obtained in the novel object recognition test ($r=0.618$, $p<0.05$) among standard-housed mice. No significant correlation was observed between this learning index and time in other behavioral categories in either SE or EE mice.

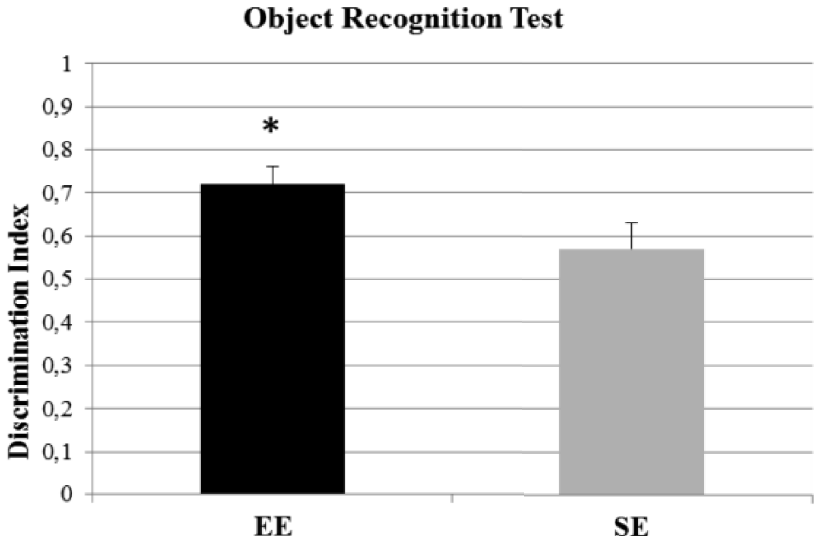

Figure 1. Discrimination Index obtained in the novel object recognition task (total time spent with new object / total time of object exploration) by the two experimental groups: Environmental Enrichment (EE) and Standard Environment (SE).
(*) $p<0.05$ EE vs SE

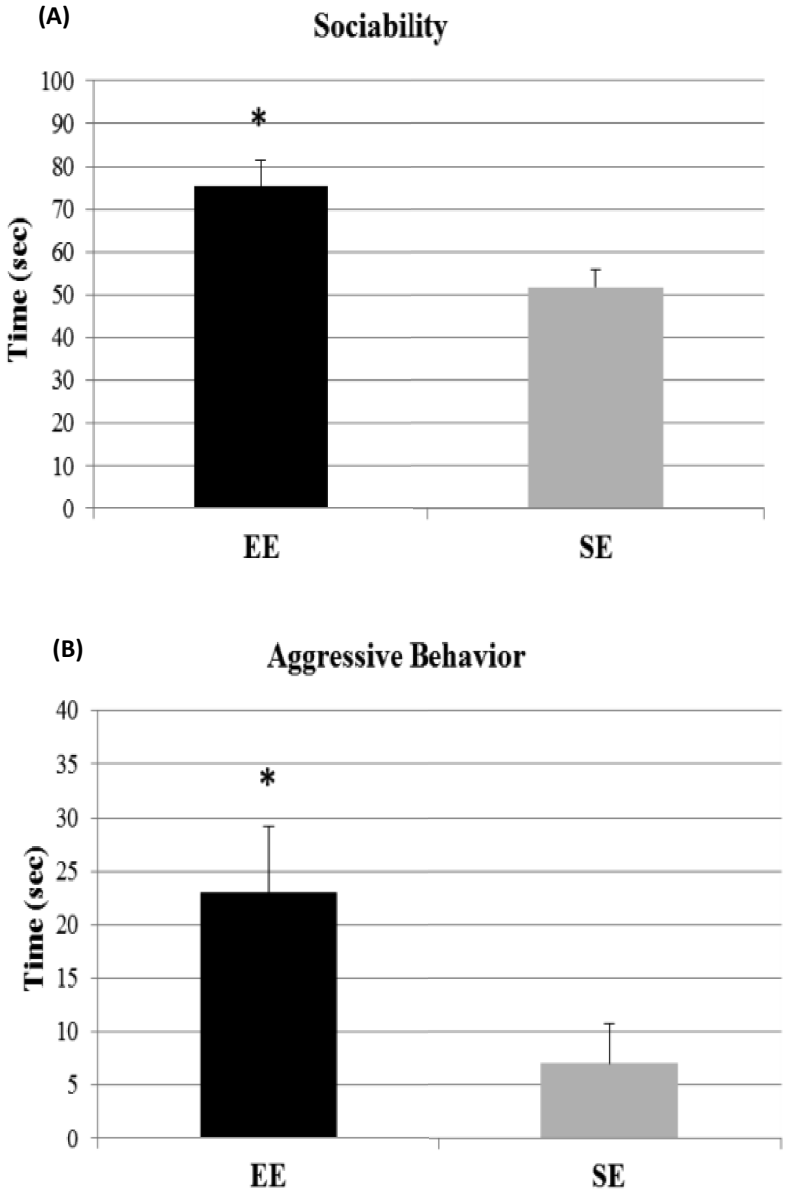

Figure 2. **(A)** Total time (seconds) allocated in the behavioral categories of Sociability and **(B)** Aggressive behavior during social encounters by the two experimental groups: Environmental Enrichment (EE) and Standard Environment (SE).
(*) $p<0.05$ EE vs SE

Table 1. Effects of exposure to an enriched (EE) or standard environment (SE) displayed by male NMRI male mice during a social encounter with a conspecific. Median time (in seconds) with ranges allocated to broad behavioral categories is shown.

Behavioral categories	ENRICHED ENVIRONMENT (EE)	STANDARD ENVIRONMENT (SE)
Self-grooming	10.50 (0-57)	6.19 (1.10-22.70)
Digging	6.48 (0-36.9)	11.07 (0.9-31.2)
Non-social exploration#	110.29 (67.8-156.4)	149.16 (106.9-192)
Explore from a distance	3.57 (1-11)	2.77 (0-17.7)
Social investigation##	71.63 (23.8-121.8)	48.94 (26.3-75.4)
Threat###	13.59 (0-42.5)	16 (0-18.5)
Attack	5.91 (0-26.8)	2.45 (0-27.9)
Avoidance/flee	0 (0-0)	0.12 (0-1.1)
Defensive/submissive	3.49 (0-45.9)	2.25 (0-32.10)
Immobility	0.25 (0-3)	0 (0-0)

(#) $p<0.0001$
(##) $p<0.001$
(###) $p<0.05$

4. DISCUSSION

In the current study we have assessed social behavior in NMRI mice reared in enriched versus standard environments and have analysed differences between the two groups in their performance of a learning task. The EE paradigm improved object recognition and increased agonistic behavior during social interactions in this strain of mice. To our knowledge, this is one of the first studies to have evaluated both cognitive performance as well as social interactions in NMRI mice exposed to EE. Until now, no study has addressed the role of EE in both social interaction and learning tasks, which have been evaluated separately, although recent evidence suggests that emotional and cognitive changes can be observed in the same group of animals exposed to EE conditions (Akillioglu, Babar Melik, Melik, & Kocahan, 2012).

Our results for the novel object recognition test indicate that enriched mice display better recognition memory than standard-housed mice, which suggests that learning is enhanced by exposure to a more challenging environment. These results are in accordance with those of previous studies that have found an enriched environment to improve the performance of animals in different memory and learning tasks (Branchi et al., 2006; Kulesskaya et al., 2011; Gresack et al., 2007; Vedovelli et al., 2011). Moreover, our data suggest that, in the case of NMRI mice, a test protocol for the novel object recognition task including a 1 hr interval between training and test and a final observation period of 5 min is necessary in order to observe the beneficial effects induced by an enriched environment on working memory measured in the novel object recognition task. Other studies have demonstrated the same beneficial effects of EE on the cognitive performance of animals after 24 hours exposure to the novel object recognition task (Vedovelli et al., 2011). In order to interpret the results obtained in the current study, we must take into account that the novel object recognition task is extremely sensitive to different variables and modifications in its application (apparatus, kind of objects, light conditions, etc.) (Antunes & Biala, 2012). Furthermore, different factors, such as cognitive processes, sensory information or locomotion, inter-trial interval between sample and test trials, duration of the sample trial or observation interval may influence the performance of animals in this test

(Coutellier & Wurbel, 2009; van Goethem, Rutten, van der Staay, Jans, Akkerman, Steinbusch, et al., 2012). In a previous study carried out using the EE paradigm, García-Capdevila et al. (2009) observed that mice with lower levels of locomotor activity obtained better results in the object recognition test, whereas Kazlauckas et al. (2011) only detected a significant improvement in the inhibitory avoidance task in mice with a low basal level of exploratory behavior. Recent research in NMRI mice has confirmed that three weeks of EE improves object recognition in a Y-maze, and that this improvement is accompanied by a reorganization of the neural network and alterations in hippocampal activation (Leger, Bouet, Freret, Darmaillacq, Dacher, Dauphin, et al., 2012).

The results obtained in the social interaction test showed that animals reared under EE conditions engaged more frequently and for longer periods of time in social exploration behaviors (or "sociability") and threat episodes (or "agonistic behavior") and spent less time engaged in non-social behaviors than standard-housed mice. An increase in sociability during social encounters has been interpreted as a sign of diminished anxiety (File & Seth, 2003; Navarro, Ibañez, & Luna, 2004). Our results are in accordance with those of previous studies carried out in other anxiety paradigms, such as the elevated plus-maze, in which mice reared in enriched environments have been seen to display lower levels of anxiety (Abramov et al., 2008; Zhu, Yee, Nyffeler, Winblad, Feldon, & Mohammed, 2006). Our data revealing increased agonistic behavior are in line with those of prior studies reporting an increase of agonistic behavior under EE conditions, particularly in laboratory mice (Akre, Bakken, Hovland, Palme, & Mason, 2011; Kaliste, Mering & Huuskonen, 2006; Van Loo et al., 2001). Explanations proposed for the increase aggression include an increment of territorial behavior (Haemisch & Gartner, 1997; Marashi et al., 2004), the elimination of odor marking necessary for territory formation (Kaliste et al., 2006), or the inability to control the environment and the objects included in the cages (Van Loo et al., 2001). In order to evaluate in more depth variations in aggressive behavior, future studies should take into account the bodily areas that are most frequently attacked by opponents. Extensive attacks primarily aimed at ventral regions or massive attacks directed at both dorsal and ventral regions may indicate atypical aggression (de Boer,

Caramaschi, Natarajan, & Koolhaas, 2009; Natarajan, de Vries, de Boer & Koolhaas, 2009b). This type of aggression is likely to be accompanied by cognitive deficits, such as those generally observed in SAL mice. In contrast, if the increase of aggression observed in NMRI mice is mainly of a territorial basis, the improvement of cognitive functions generally observed after EE exposure will be evident. Our observations during cage changes indicate that NMRI mice usually display damage to their tails, suggesting increased territorial agonistic behavior and a normal adaptive response of the animals. These responses seem to differ from those usually displayed by rats or mice of strains used for the study of pathological aggression (de Boer et al., 2009).

Moreover, some authors have proposed that this increase of agonistic behavior in mice exposed to EE could mask some of the potential beneficial effects of this type of housing, such as cognitive enhancement, thus diminishing the main aim of the paradigm and resulting in physical injury to animals (Abou-Ismail, 2011). In contrast, other authors suggest that animals housed in enriched environments with more resources are less likely to display agonistic behavior (Akre et al., 2011). It is important to evaluate if these changes are related to the display of atypical aggression or to a significant rise of species-typical aggression. In NMRI mice, we can confirm that even short periods of EE can lead to improvement in learning tasks and to a significant increase in agonistic behaviors after exposure to these housing conditions. Despite the rise in agonistic interaction and aggression observed in the current study, the NMRI mice seem no to display atypical aggression followed by cognitive impairment. Thus, one may observe the likely reciprocal links between the nature of aggression displayed during the social interaction test and cognition.

We have also observed that mice reared in an enriched environment engage in more self-grooming than standard-reared animals. Grooming is thought to be a part of the habituation process and is more pronounced in animals reared in an enriched environment (Konkle et al., 2010). This increase has been attributed to the combination of social and physical stimuli to which enriched mice are exposed. According to these previous observations, this more pronounced behavior is accompanied by a reduction in locomotion and exploratory behaviors (Brenes et al., 2009). Decreased non-social investigation in enriched mice could be related to the

faster habituation to new environments usually observed among these mice, or an increase in sociability. Previous studies in our laboratory have confirmed a decrease in spontaneous locomotor activity in NMRI mice reared in enriched environments (Mesa-Gresa, Pérez-Martínez, & Redolat, 2011). Other authors have associated the stimulation provided by EE conditions with a significant decrease in motor activity and a more rapid habituation to novel environments (Zhu, Codita, Bogdanovic, Hjerling-Leffler, Ernfors, Winblad, et al., 2009; Zimmermann et al., 2001). In some strains (e.g. C57), however, it has been reported that an enriched environment decreases self-grooming, thus reducing the probability of alopecia/baldness (Bechard, Meagher, & Mason, 2011). A more detailed analysis of this behavior may also help to differentiate whether or not behavioral changes in EE mice are related to habituation to the environment (which can be accompanied by an increase in self-grooming) or increased sociability (with increased grooming of conspecifics). Future studies should explore a possible relation between lower levels of self-grooming and increased allo-grooming (Pobbe, Pearson, Defensor, Bolivar, Blanchard, & Blanchard, 2010).

Discrepancies between the results of studies of the effects of EE on aggression could be due to variations in experimental design (Abou-Ismail et al., 2010; Marashi, Barnekow, Ossendorf, & Sachser, 2003; Marashi et al., 2004; Redolat, Pérez-Martínez, Carrasco, & Mesa, 2009), objective measurements of aggression (Van Loo et al., 2001), group and cage size (enriched cages are bigger than standard ones and can contain between 4 and 20 mice or more, which increases competition for resources such as food or running wheels) (Akre et al., 2011), type and duration of enrichment (Akre et al., 2011; Pietropaolo et al., 2004) or the animal model applied (Totht, Kregel, Leon, and Musch (2011). The age at which EE is initiated in mice is also a relevant variable. In general, effects are more evident when exposure takes place during early adolescence, although significant effects have also been observed in aged mice and in transgenic mice with Alzheimer's disease (Redolat & Mesa-Gresa, 2012). For example, in the present study only one age group was assessed (adolescence). Therefore, it would be of interest in future studies to evaluate the effects of exposure during different periods and beginning at different ages and to compare the effect of EE on agonistic behavior displayed by male mice of different strains. Differences have also been attributed to

the species or strain used, since enriched environments can differentially influence the social behavior of mice strains (Abou-Ismail, 2011; Kaliste et al., 2006; Marashi et al., 2004). Previous research carried out in rats has shown that animals assigned to enriched environments display less aggressive behavior than those housed in standard cages (Abou-Ismail, 2011), whereas results in mice, though contradictory, point to heightened aggression after enrichment exposure (McQuaid, et al., 2012). This difference between species in the behavioral response to EE may be due to the fact that male mice are more territorial and less socially tolerant than rats (Abou-Ismail, 2011; Rich & Hurst, 1998). For that reason, it would also be of relevance to evaluate the changes that take place in the cage hierarchy while animals are housed in the enrichment cage and to analyse group dynamics during enrichment and the test (Konkle et al., 2010).

As different authors have recently pointed out, there is a need for more research concerning EE in animals in order to clarify several aspects (van Praag et al., 2000), such as the social stress this paradigm can produce (Kaliste et al., 2006). A neuroethological approach would be useful for this purpose (Oliva, Salcedo, Hellier, Ly, Koka, Tollin, et al., 2010). This paradigm is currently being applied in order to prevent or counteract the physiological and behavioral effects of exposure to chronic social stress situations (Redolat & Mesa-Gresa, 2012; Schloesser, Lehmann, Martinowich, Manji, & Herkenham, 2010). Future studies are needed to assess in more depth changes in stress reactivity in animals housed in enriched conditions vs. those maintained in standard rearing conditions. Experiments need to be designed carefully in order to create the most adequate environment for each experiment (Toth et al., 2011) and to evaluate long-term effects of enriched environments at different time-points and changes due to habituation or increased sociability after exposure to EE.

REFERENCES

Abou-Ismail, U. A. (2011). The effects of cage enrichment on agonistic behaviour and dominance in male laboratory rats (rattus norvegicus). *Research in Veterinary Science, 90*, 346-51.

Abou-Ismail, U. A., Burman, O. H., Nicol, C. J., & Mendl, M. (2010). The effects of enhancing cage complexity on the behaviour and welfare of laboratory rats. *Behavioural Processes, 85*, 172-80.

Abramov, U., Puussaar, T., Raud, S., Kurrikoff, K., & Vasar, E. (2008). Behavioural differences between C57BL/6 and 129S6/SvEv strains are reinforced by environmental enrichment. *Neuroscience Letters, 443*, 223-27.

Akillioglu, K., Babar Melik, E., Melik, E., & Kocahan, S. (2012). The investigation of neonatal MK-801 administration and physical environmental enrichment on emotional and cognitive functions in adult Balb/c mice. *Pharmacology, Biochemistry, and Behavior, 102*, 407-14.

Akre, A.K., Bakken, M., Hovland, A.L., Palme, R., & Mason, G. (2011). Clustered environmental enrichments induce more aggression and stereotypic behaviour than do dispersed enrichments in female mice. *Applied Animal Behaviour Science,* 131, 145-52.

Antunes, M., & Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cognitive Processing, 13*, 93-110.

Artola, A., von Frijtag, J. C., Fermont, P. C., Gispen, W. H., Schrama, L. H., Kamal, A., & Spruijt, B. M. (2006). Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. *The European Journal of Neuroscience, 23*, 261-72.

Bechard, A., Meagher, R., & Mason, G. (2011). Environmental enrichment reduces the likelihood of alopecia in adult C57BL/6J mice. *Journal of the American Association for Laboratory Animal Science, 50*, 171-74.

Benaroya-Milshtein, N., Hollander, N., Apter, A., Kukulansky, T., Raz, N., Wilf, A., … Pick, C. G. (2004). Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. *The European Journal of Neuroscience, 20*, 1341-47.

Bennett, J. C., McRae, P. A., Levy, L. J., & Frick, K. M. (2006). Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. *Neurobiology of Learning and Memory, 85*, 139-52.

Benus, R. F., Bohus, B., Koolhaas, J. M., & van Oortmerssen, G. A. (1991). Heritable variation for aggression as a reflection of individual coping strategies. *Experientia, 47*, 1008-19.

Bouet, V., Freret, T., Dutar, P., Billard, J. M., & Boulouard, M. (2011). Continuous enriched environment improves learning and memory in adult NMRI mice through theta burst-related-LTP independent mechanisms but is not efficient in advanced aged animals. *Mechanisms of Ageing and Development,132*, 240-48.

Brain, P.F., McAllister, K.H., & Walmsley, S. (1989). Drugs effects on social behavior. In A.A. Boulton, G.B. Baker, A.J. Greenshaw (Eds.), Methods in Ethopharmacology, Neuromethods, Vol. 13, Humana Press, Clifton, NJ (1989), pp. 687–739 Psychopharmacology.

Branchi, I., D'Andrea, I., Fiore, M., Di Fausto, V., Aloe, L., & Alleva, E. (2006). Early social enrichment shapes social behavior and nerve growth factor and brain-derived neurotrophic factor levels in the adult mouse brain. *Biological Psychiatry, 60*, 690-96.

Brenes, J. C., Padilla, M., & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behavioural Brain Research, 197*, 125-37.

Brenes, J. C., Rodriguez, O., & Fornaguera, J. (2008). Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. *Pharmacology, Biochemistry, and Behavior, 89*, 85-93.

Caramaschi, D., de Boer, S. F., de Vries, H., & Koolhaas, J. M. (2008). Development of violence in mice through repeated victory along with changes in prefrontal cortex neurochemistry. *Behavioural Brain Research, 189*, 263-72.

Chamove, A. S. (1989). Cage design reduces emotionality in mice. *Laboratory Animals, 23*, 215-19.

Chistyakov, V., Patkina, N., Tammimaki, A., Talka, R., Salminen, O., Belozertseva, I., … Zvartau, E. (2010). Nicotine exposure throughout early development promotes nicotine self-administration in adolescent mice and induces long-lasting behavioural changes. *European Journal of Pharmacology,640*, 87-93.

Coppens, C. M., de Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: Towards underlying mechanisms. *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences, 365*, 4021-28.

Coutellier, L., & Wurbel, H. (2009). Early environmental cues affect object recognition memory in adult female but not male C57BL/6 mice. *Behavioural Brain Research, 203*, 312-15.

de Boer, S. F., Caramaschi, D., Natarajan, D., & Koolhaas, J. M. (2009). The vicious cycle towards violence: Focus on the negative feedback mechanisms of brain serotonin neurotransmission. *Frontiers in Behavioral Neuroscience, 3*, 52.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience and Biobehavioral Reviews, 31*, 673-704.

Diniz, D. G., Foro, C. A., Rego, C. M., Gloria, D. A., de Oliveira, F. R., Paes, J. M… Diniz, C. W. (2010). Environmental impoverishment and aging alter object recognition, spatial learning, and dentate gyrus astrocytes. *The European Journal of Neuroscience, 32*, 509-19.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research, 215*, 244-54.

Fernandez-Teruel, A., Escorihuela, R. M., Castellano, B., Gonzalez, B., & Tobena, A. (1997). Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: Focus on the roman rat lines. *Behavior Genetics,27*, 513-26.

File, S. E., & Seth, P. (2003). A review of 25 years of the social interaction test. *European Journal of Pharmacology, 463*, 35-53.

Freret, T., Billard, J. M., Schumann-Bard, P., Dutar, P., Dauphin, F., Boulouard, M., & Bouet, V. (2012). Rescue of cognitive aging by long-lasting environmental enrichment exposure initiated before median lifespan. *Neurobiology of Aging, 33*, 1005.e1-1005.10.

Garcia-Capdevila, S., Portell-Cortes, I., Torras-Garcia, M., Coll-Andreu, M., & Costa-Miserachs, D. (2009). Effects of long-term voluntary exercise on learning and memory processes: Dependency of the task and level of exercise. *Behavioural Brain Research, 202*, 162-70.

Gomez, C., Carrasco, C., & Redolat, R. (2008). Effects of bupropion, alone or coadministered with nicotine, on social behavior in mice. *Addiction Biology, 13*, 301-9.

Greco, S. J., Bryan, K. J., Sarkar, S., Zhu, X., Smith, M. A., Ashford, J. W… Casadesus, G. (2010). Leptin reduces pathology and improves memory in a transgenic mouse model of alzheimer's disease. *Journal of Alzheimer's Disease, 19*, 1155-67.

Gresack, J. E., Kerr, K. M., & Frick, K. M. (2007). Life-long environmental enrichment differentially affects the mnemonic response to estrogen in young, middle-aged, and aged female mice. *Neurobiology of Learning and Memory, 88*, 393-408.

Haemisch, A., & Gartner, K. (1997). Effects of cage enrichment on territorial aggression and stress physiology in male laboratory mice. *Acta Physiologica Scandinavica, 640*, 73-76.

Haemisch, A., Voss, T., & Gartner, K. (1994). Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J mice. Physiology & Behavior, 56, 1041-48.

Hansalik, M., Skalicky, M., & Viidik, A. (2006). Impairment of water maze behaviour with ageing is counteracted by maze learning earlier in life but not by physical exercise, food restriction or housing conditions. *Experimental Gerontology, 41*, 169-74.

Harati, H., Majchrzak, M., Cosquer, B., Galani, R., Kelche, C., Cassel, J. C., & Barbelivien, A. (2011). Attention and memory in aged rats: Impact of lifelong environmental enrichment. *Neurobiology of Aging, 32*, 718-36.

Harburger, L. L., Nzerem, C. K., & Frick, K. M. (2007). Single enrichment variables differentially reduce age-related memory decline in female mice. *Behavioral Neuroscience, 121*, 679-88.

Heyser, C. J., & Chemero, A. (2012). Novel object exploration in mice: Not all objects are created equal. *Behavioural Processes, 89*, 232-38.

Hughes, R. N., & Collins, M. A. (2010). Enhanced habituation and decreased anxiety by environmental enrichment and possible attenuation of these effects by chronic alpha-tocopherol (vitamin E) in aging male and female rats. *Pharmacology, Biochemistry, and Behavior, 94*, 534-42.

Kaliste, E. K., Mering, S. M., & Huuskonen, H. K. (2006). Environmental modification and agonistic behavior in NIH/S male mice: Nesting material enhances fighting but shelters prevent it. *Comparative Medicine, 56*, 202-8.

Kazlauckas, V., Pagnussat, N., Mioranzza, S., Kalinine, E., Nunes, F., Pettenuzzo, L., … Lara, D. R. (2011). Enriched environment effects on behavior, memory and BDNF in low and high exploratory mice. *Physiology & Behavior, 102*, 475-80.

Kenney, J. W., Adoff, M. D., Wilkinson, D. S., & Gould, T. J. (2011). The effects of acute, chronic, and withdrawal from chronic nicotine on novel and spatial object recognition in male C57BL/6J mice. *Psychopharmacology, 217*, 353-65.

Konkle, A. T., Kentner, A. C., Baker, S. L., Stewart, A., & Bielajew, C. (2010). Environmental-enrichment-related variations in behavioral, biochemical, and physiologic responses of sprague-dawley and long evans rats. *Journal of the American Association for Laboratory Animal Science, 49*, 427-36.

Koolhaas, J. M., de Boer, S. F., Buwalda, B., & van Reenen, K. (2007). Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. *Brain, Behavior and Evolution, 70*, 218-26.

Koolhaas, J. M., de Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology, 31*, 307-21.

Kulesskaya, N., Rauvala, H., & Voikar, V. (2011). Evaluation of social and physical enrichment in modulation of behavioural phenotype in C57BL/6J female mice. *PloS One, 6*, e24755.

Lambert, T. J., Fernandez, S. M., & Frick, K. M. (2005). Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. *Neurobiology of Learning and Memory, 83*, 206-16.

Leger, M., Bouet, V., Freret, T., Darmaillacq, A. S., Dacher, M., Dauphin, F., … Schumann-Bard, P. (2012). Environmental enrichment improves recent but not remote memory in association with a modified brain metabolic activation profile in adult mice. *Behavioural Brain Research, 228*, 22-29.

Leggio, M. G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research, 163*, 78-90.

Madronal, N., Lopez-Aracil, C., Rangel, A., del Rio, J. A., Delgado-Garcia, J. M., & Gruart, A. (2010). Effects of enriched physical and social environments on motor performance, associative learning, and hippocampal neurogenesis in mice. *PloS One, 5*, e11130.

Malin, D. H., Lee, D. R., Goyarzu, P., Chang, Y. H., Ennis, L. J., Beckett, E., … Joseph, J. A. (2011). Short-term blueberry-enriched diet prevents and reverses object recognition memory loss in aging rats. *Nutrition (Burbank, Los Angeles County, Calif.), 27*(3), 338-342.

Marashi, V., Barnekow, A., Ossendorf, E., & Sachser, N. (2003). Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Hormones and Behavior, 43*, 281-92.

Marashi, V., Barnekow, A., & Sachser, N. (2004). Effects of environmental enrichment on males of a docile inbred strain of mice. *Physiology & Behavior, 82*, 765-76.

McQuaid, R. J., Audet, M. C., Jacobson-Pick, S., & Anisman, H. (2012). The differential impact of social defeat on mice living in isolation or groups in an enriched environment: Plasma corticosterone and monoamine variations. *The International Journal of Neuropsychopharmacology*, 1-13.

Mesa-Gresa, P., Pérez-Martínez, M.A., & Redolat, R. (2011). Decreased locomotor activity in enriched mice is independent of the duration of the enrichment period: a longitudinal study

in NMRI mice. Poster presented at *8th IBRO World Conference of Neuroscience*, Florence (Italy).

Mesa-Gresa, P., Perez-Martinez, A., & Redolat-Iborra, R. (2012). Nicotine and animal models: What does the environmental enrichment paradigm tell us? *Adicciones, 24*, 87-94.

Mirochnic, S., Wolf, S., Staufenbiel, M., & Kempermann, G. (2009). Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of alzheimer disease. *Hippocampus, 19*, 1008-18.

Moragrega, I., Carrasco, M.C., & Redolat, R. (2005). Effects of housing and nicotine on shuttle-box avoidance in male NMRI mice. *Behavioural Brain Research, 164*, 178-87.

Natarajan, D., de Boer, S. F., & Koolhaas, J. M. (2009a). Lack of differential serotonin biosynthesis capacity in genetically selected low and high aggressive mice. *Physiology & Behavior, 98*, 411-15.

Natarajan, D., de Vries, H., de Boer, S. F., & Koolhaas, J. M. (2009b). Violent phenotype in SAL mice is inflexible and fixed in adulthood. *Aggressive Behavior, 35*, 430-36.

Navarro, J. F., Ibanez, M., & Luna, G. (2004). Behavioral profile of SB 269970, a selective 5-HT(7) serotonin receptor antagonist, in social encounters between male mice. *Methods and Findings in Experimental and Clinical Pharmacology, 26*, 515-18.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews Neuroscience,* , 697-709.

Nithianantharajah, J., & Hannan, A. J. (2009). The neurobiology of brain and cognitive reserve: Mental and physical activity as modulators of brain disorders. *Progress in Neurobiology, 89*, 369-82.

Nithianantharajah, J., & Hannan, A. J. (2011). Mechanisms mediating brain and cognitive reserve: Experience-dependent neuroprotection and functional compensation in animal models of neurodegenerative diseases. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 35*, 331-39.

Oliva, A. M., Salcedo, E., Hellier, J. L., Ly, X., Koka, K., Tollin, D. J., & Restrepo, D. (2010). Toward a mouse neuroethology in the laboratory environment. *PloS One, 5*, e11359.

Petrosini, L., De Bartolo, P., Foti, F., Gelfo, F., Cutuli, D., Leggio, M. G., & Mandolesi, L. (2009). On whether the environmental enrichment may provide cognitive and brain reserves. *Brain Research Reviews, 61*, 221-39.

Pietropaolo, S., Branchi, I., Cirulli, F., Chiarotti, F., Aloe, L., & Alleva, E. (2004). Long-term effects of the periadolescent environment on exploratory activity and aggressive behaviour in mice: Social versus physical enrichment. *Physiology & Behavior, 81*, 443-53.

Pobbe, R. L., Pearson, B. L., Defensor, E. B., Bolivar, V. J., Blanchard, D. C., & Blanchard, R. J. (2010). Expression of social behaviors of C57BL/6J versus BTBR inbred mouse strains in the visible burrow system. *Behavioural Brain Research, 214*, 443-49.

Redolat, R., Brain, P. F., & Simon, V. M. (1991). Sulpiride has an antiaggressive effect in mice without markedly depressing motor activity. *Neuropharmacology, 30*, 41-46.

Redolat, R., & Mesa-Gresa, P. (2012). Potential benefits and limitations of enriched environments and cognitive activity on age-related behavioural decline. *Current Topics in Behavioral Neurosciences, 10*, 293-316.

Redolat, R., Perez-Martinez, A., Carrasco, M. C., & Mesa, P. (2009). Individual differences in novelty-seeking and behavioral responses to nicotine: A review of animal studies. *Current Drug Abuse Reviews, 2*, 230-42.

Renner, M. J., & Rosenzweig, M. R. (1986). Social interactions among rats housed in grouped and enriched conditions. *Developmental Psychobiology, 19*, 303-13.

Rich, T.J., & Hurst, J.L. (1998). Scent marks as reliable signals of the competitive ability of mates. *Animal Behaviour, 56*, 727-35.

Rosenzweig, M. R., & Bennett, E. L. (1996). Psychobiology of plasticity: Effects of training and experience on brain and behavior. *Behavioural Brain Research, 78*, 57-65.

Schloesser, R. J., Lehmann, M., Martinowich, K., Manji, H. K., & Herkenham, M. (2010). Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. *Molecular Psychiatry, 15*, 1152-63.

Silvers, J. M., Harrod, S. B., Mactutus, C. F., & Booze, R. M. (2007). Automation of the novel object recognition task for use in adolescent rats. *Journal of Neuroscience Methods, 166*, 99-103.

Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats--behavioural and neurochemical aspects. *Behavioural Brain Research, 222*, 246-64.

Siopi, E., Llufriu-Daben, G., Fanucchi, F., Plotkine, M., Marchand-Leroux, C., & Jafarian-Tehrani, M. (2012). Evaluation of late cognitive impairment and anxiety states following traumatic brain injury in mice: The effect of minocycline. *Neuroscience Letters, 511*, 110-15.

Stern, Y. (2012). Cognitive reserve in ageing and alzheimer's disease. *Lancet Neurology, 11*, 1006-12.

Swetter, B. J., Karpiak, C. P., & Cannon, J. T. (2011). Separating the effects of shelter from additional cage enhancements for group-housed BALB/cJ mice. *Neuroscience Letters, 495*, 205-9.

Toth, L. A., Kregel, K., Leon, L., & Musch, T. I. (2011). Environmental enrichment of3 laboratory rodents: The answer depends on the question. *Comparative Medicine, 61*, 314-21.

van Goethem, N. P., Rutten, K., van der Staay, F. J., Jans, L. A., Akkerman, S., Steinbusch, H. W., . . . Prickaerts, J. (2012). Object recognition testing: Rodent species, strains, housing conditions, and estrous cycle. *Behavioural Brain Research, 232*, 323-34.

Van Loo, P. L., Mol, J. A., Koolhaas, J. M., Van Zutphen, B. F., & Baumans, V. (2001). Modulation of aggression in male mice: Influence of group size and cage size. *Physiology & Behavior, 72*, 675-83.

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews. Neuroscience, 1*, 191-98.

Vedovelli, K., Silveira, E., Velho, E., Stertz, L., Kapczinski, F., Schroder, N., & Bromberg, E. (2011). Effects of increased opportunity for physical exercise and learning experiences on

recognition memory and brain-derived neurotrophic factor levels in brain and serum of rats. *Neuroscience, 199*, 284-91.

Vihavainen, T., Piltonen, M., Tuominen, R. K., Korpi, E. R., & Ahtee, L. (2008). Morphine-nicotine interaction in conditioned place preference in mice after chronic nicotine exposure. *European Journal of Pharmacology, 587*, 169-74.

Viola, G. G., Botton, P. H., Moreira, J. D., Ardais, A. P., Oses, J. P., & Souza, D. O. (2010). Influence of environmental enrichment on an object recognition task in CF1 mice. *Physiology & Behavior, 99*, 17-21.

Winters, B. D., Saksida, L. M., & Bussey, T. J. (2008). Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. *Neuroscience and Biobehavioral Reviews, 32*, 1055-70.

Workman, J. L., Fonken, L. K., Gusfa, J., Kassouf, K. M., & Nelson, R. J. (2011). Post-weaning environmental enrichment alters affective responses and interacts with behavioral testing to alter nNOS immunoreactivity. *Pharmacology, Biochemistry, and Behavior, 100*, 25-32.

Yuan, Z. Y., Gu, P., Liu, L., Wang, Y. Y., Liu, J., Cui, D. S., … Wang, M. W. (2009). Neuroprotective effects of enriched environment in MPTP-treated SAMP8 mice. *Neuroscience Letters, 454*, 6-10.

Zhu, S. W., Codita, A., Bogdanovic, N., Hjerling-Leffler, J., Ernfors, P., Winblad, B., … Mohammed, A. H. (2009). Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice. *Behavioural Brain Research, 197*, 339-46.

Zhu, S. W., Yee, B. K., Nyffeler, M., Winblad, B., Feldon, J., & Mohammed, A. H. (2006). Influence of differential housing on emotional behaviour and neurotrophin levels in mice. *Behavioural Brain Research, 169*, 10-20.

Zimmermann, A., Stauffacher, M., Langhans, W., & Wurbel, H. (2001). Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. *Behavioural Brain Research, 121*, 11-20.

CAPÍTULO 3

ESTUDIO 2

Efectos conductuales de la combinación de enriquecimiento ambiental y administración crónica de nicotina

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Publicado en (véase ANEXO 9.5):

Mesa-Gresa, P., Pérez-Martínez, A., & Redolat, R. (2013). Behavioral effects of combined environmental enrichment and chronic nicotine administration in male NMRI mice. Physiology & Behavior, 114, 65-76.

BEHAVIORAL EFFECTS OF COMBINED ENVIRONMENTAL ENRICHMENT AND CHRONIC NICOTINE ADMINISTRATION IN MALE NMRI MICE

Abstract

Environmental enrichment (EE) is an experimental paradigm which provides sensory, social, physical and cognitive stimulation for rodents. Experimental evidence indicates that this type of housing induces different neurobiological and behavioral changes in rodents. However, few studies have evaluated the consequences of combined exposure to an enriched environment and nicotine administration during a critical period of development such as adolescence. Taking into account previous studies, it can be hypothesized that a chronic treatment with nicotine would modulate the effects of rearing animals in enriched environments. In the current study, our main aim was to evaluate the effects of EE and chronic nicotine administration on physiological parameters (weight, fluid intake and cotinine levels), motor activity, exploratory behavior, anxiety and learning in male NMRI mice. Half of the mice (n=32) were exposed to an enriched environment (EE) and the other half (n=32) were housed in standard environments (SE) with or without oral nicotine administration (100 µg/ml). After 3 weeks, mice were evaluated in a behavioral battery that included an elevated plus-maze, a hole board, an actimeter and an inhibitory avoidance task. Blood cotinine levels were measured in an additional group of 32 mice in order to confirm nicotine intake. Results indicated that mice reared in an enriched environment gained less body weight and displayed higher fluid intake than those maintained in a standard environment. EE reduced motor activity, exploratory behavior and anxiety, whereas it enhanced inhibitory avoidance learning. In relation to the effects of chronic nicotine treatment, the data reflected a lower increase in body weight and a reduced fluid intake in nicotine-treated mice. In the elevated plus-maze, nicotine induced a reduction of total arm entries and rearings. Cotinine levels were higher in mice that received oral nicotine than in the control group. We conclude that the EE paradigm applied in this study induces physiological and behavioral changes in NMRI mice. Chronic nicotine treatment diminished motor activity displayed by mice in the elevated plus-maze but did not have significant effects on inhibitory avoidance learning. Future studies should explore in greater depth the interaction between environmental factors and nicotine administration using longer periods of EE, a wider range of doses and/or other cholinergic agonists, acute drug administration, and sequential exposure to nicotine and EE.

1. INTRODUCTION

In enriched environments, animals are provided with different stimulating objects (houses, tunnels, platforms, ropes, nesting materials, assorted colourful toys…) which are frequently changed during the experiment [1-3]. In some cases, running wheels for voluntary exercise are also introduced into the cages [4]. These objects provide visual and somatosensory stimulation, thus increasing the probability of exploratory behavior, stimulation and activity [1,2,5]. In addition, the number of animals housed in these environments is larger than that kept in standard cages, which offers animals more opportunities for social stimulation and interaction [1,3,5].

Different neurobiological and behavioral changes have been reported as a consequence of exposure to enriched environments. Regarding neurobiological changes, environmental enrichment (EE) promotes synaptic plasticity [6] and cognitive reserve [7], stimulates neurogenesis in the dentate gyrus of the hippocampus [3,8] and dendritic branching [9], and elevates the levels of brain-derived neurotrophic factor (BDNF) and other neurotrophic factors [10]. EE also produces variations in the shape [11] and number [12] of astrocytes. In addition, experimental evidence in animal models suggests that enriched environments offer a non-pharmacological intervention for protecting against the onset of Parkinson's [5], Alzheimer's [13,14], Huntington's disease [1] and other conditions such as Attention Deficit/Hyperactivity Disorder [15] or depression [13]. EE also attenuates the structural and functional effects of different brain lesions [16,17] and strokes [18].

In the context of behavioral changes, it has been reported that rearing rats in an enriched environment reduces anxiety levels and improves emotional stability [19], accelerates habituation to new environments [20,21], enhances social play behavior [22], increases hot-plate latencies and reduces aggression [23], although contradictory results do exist. Exposure to an EE may also aid recovery from different stressors [24]. An improvement in a variety of hippocampal-dependent memory tasks after exposure to EE conditions has been confirmed in numerous studies in both young [25,26] and old rodents [27,28]. This improvement has been related to the sensory, physical and

cognitive stimulation provided by enriched environments and the increased social interaction that this encourages [26,28].

Recently, the need has been stressed for further research regarding the interaction between EE and pharmacological manipulation [15,29]. The role of environment in the regulation of behavior and in the effects of different drugs has also been underscored lately [30-32] as a result of the hypothesis which holds that EE plays a protective role against the development of addiction to different drugs of abuse [13,29,31,33-35]. Few studies, however, have evaluated the consequences of combined exposure to an enriched environment and nicotine administration. Nicotine is the primary substance involved in tobacco addiction [36], and its behavioral and physiological effects have been widely evaluated in animals and humans. The results of such studies suggest that nicotine improves cognitive function [37] and that EE modulates some motor and neurochemical effects of nicotine in rodents [38], although these effects seems to depend on factors such as age and sex [39-41], or dosage and route of administration [42-44]. Rats housed in an enriched environment have been reported to show less sensitivity to the stimulant effects of nicotine [45] and to exhibit alterations in cholinergic receptors associated with the hyperactivity produced by this drug [38]. Nicotine also blocks the increase in dendritic branching in the nucleus accumbens related to EE [46]. Additionally, Zhu et al. [47] observed an increased release of dopamine in the medial prefrontal cortex area in animals housed in an enriched environment and treated with nicotine. Many studies have attempted to shed light on the genetic and molecular factors involved in nicotine addiction, but very little work has been carried out in order to clarify the influence of environmental factors associated with the progression of addiction to this substance [13,29,34]. A deeper understanding of the role of environmental factors in vulnerability to addiction is critical if strategies for prevention and treatment are to be effective [29,34,48]. The current lack of data concerning the combined use of nicotine and EE is an obstacle to determining the effects of environmental manipulation on behavioral responses to this drug.

Different studies have revealed that negative and stressful experiences during early life can induce structural and functional changes in the brain that encourage the

consumption of drugs of abuse during adolescence and adulthood [49,50]. Previous data show that exposure to enriched environments during critical stages of development, such as adolescence, enhances the effects of EE and can reduce an individual's susceptibility to developing neurological diseases and mental disorders [13,51] as well as addiction [29,50]. In the light of previous studies of the behavioral effects of enriched environments and the relevance of being reared in this type of environment during early life, and taking into account previous research about the emotional and cognitive effects of nicotine [37], especially during adolescence, it can be hypothesized nicotine would modulate the effects of EE in the performance of different behavioral tests. The present study was conducted in order to evaluate whether or not the effects of EE are modulated by exposure to chronic oral nicotine during a critical period of development. We set out to explore the effects of EE combined with chronic treatment with nicotine on locomotor activity, exploration, anxiety and learning.

2. MATERIALS AND METHODS

2.1. Animals

Ninety six male mice of the outbred stock Crl:NMRI (Han) were used in the current study. Mice were obtained from Charles River (Barcelona, Spain) at 21 days of age and weighing between 10-12 gr. and were housed in controlled facilities. Sixty-four of these animals were used in order to assess behavioral and physiological parameters and the other thirty two were employed to obtain blood samples. After an eight-day period of adaptation to the laboratory, half of the mice were exposed to an enriched environment (EE) and the other half remained in standard cages (SE) until the behavioral tasks were performed. Since NMRI mice are nocturnal rodents and their level of activity increases during the dark phase of their circadian rhythm [52], standardized conditions with a reversed 12 hr light/dark cycle (lights on: 19:30 hr) were maintained in a room with constant temperature (20-24ºC) and humidity

(55±10%). This procedure has been applied in other studies employing enriched environments [53-55]. All animals were allowed free access to food (Tekald Global Rodent Diet, supplied by Harlan) and water.

Experimental procedures were approved by the local ethical committee (University of Valencia) and complied with national (Real Decreto 1201/2005, de 10 de Octubre) and international guidelines (European Community's Council Directive of November 24, 1986 -86/609/EEC) for the care and handling of animals.

2.2. Procedures and apparatuses

2.2.1. Housing conditions

At 28 days of age, mice were randomly assigned to one of two housing conditions (Enriched or Standard). On this day, baseline hole board activity was measured and data indicated that there were no significant differences between groups in the total number of head-dips. This factor was taken into account in order to avoid individual differences in basal exploratory behavior [60], which may influence preference or use of the objects present in the enriched environments. Both groups also had a comparable mean body weight at the beginning of the study [61,62].

Mice were maintained in enriched or standard conditions for 3 weeks before behavioral testing began. In the standard environment, mice were housed in groups of 4 in standard cages (42x26x14 cm) containing only sawdust. In the enriched environment, mice were housed in groups of 8 in larger cages (55x36x19 cm) that contained fixed objects (a running wheel, a coloured plastic tunnel and an igloo), and an assortment of changeable toys (five per cage) and sawdust. Handling procedures were similar for both enriched and control mice. All cages were cleaned once per week and mice were weighed during the cleaning process. In the enriched cages, toys and objects were changed twice per week in order to encourage exploration and enhance the novelty of the environment [5]: once when the sawdust was changed, and a second time during which care was taken to minimize disturbance. In each change, fixed objects were repositioned and variable toys were substituted by other different items.

In each housing condition, mice were divided randomly into one of two treatment sub-groups: a Nicotine-treated group (NIC) and a Saccharin-treated group (SAC). Mice were maintained in the same housing and treatment conditions throughout the experiment. Nicotine was replaced by a water solution 1 hour before tests in order to rule out any acute effects of the drug [63].

2.2.2. General procedure

Sixty-four mice underwent a baseline test of exploratory behavior in the hole board (day 28) and a battery of behavioral tests from post natal day (PND) 50 to PND 53. The test schedule was as follows: elevated plus-maze (day 50), hole board (day 50), spontaneous motor activity (day 51) and training-test in the inhibitory avoidance learning task (days 52-53). All tests were performed between the second and fifth hours of the dark period under dim red light [52]. This schedule was established based on battery tests performed in previous EE studies and taking into account the sensitivity of the elevated plus-maze and hole board test to factors such as the prior manipulation of animals [56-59]. Test apparatuses were cleaned before the first test began and between animals with a solution containing ethanol. During behavioral testing, a researcher remained in the room outside the scope of the animals' vision in order to avoid interferences in their behavior. In a group of thirty two animals, blood samples were obtained on PND 50.

2.2.3. Body weight and fluid intake

Animal body weight and fluid intake were the physiological parameters assessed in this study. The former was measured once a week and the average fluid consumption (ml/mouse/day) was calculated as the mean of fluid consumption measurements, each calculated as the total fluid consumption divided by the number of mice in each cage [61,62]. Previous studies have shown that similar doses and levels of fluid consumption are associated with a significant rise in plasma nicotine and cotinine levels [42,64-66].

2.2.4. Analysis of cotinine levels

Serum cotinine levels were analysed in order to validate oral nicotine exposure during the experiment [42]. On PND 50, mice in the complementary group were anaesthetized with halothane and their blood was collected via inferior vena cava so as to determine blood concentrations of cotinine (10-12 a.m.). The blood samples were centrifuged in heparin-containing tubes (Inmulite 2000: Nicotine Metabolite), and the plasma was frozen at -20ºC until assays were carried out. Cotinine levels were measured using chemiluminescence techniques. All the samples were tested in duplicate, and the average value of the two tests was employed in the statistical analyses. The inferior limit of sensitivity was 10 ng/ml and the intra- and interassay coefficients of variation were 7.42% and 9.37% respectively.

We performed these additional measurements to confirm that levels of cotinine in mice in the oral nicotine group were significant and to compare them with those of the control group.

2.2.5. Elevated plus-maze

The elevated plus-maze (EPM) is a task that is widely used in the study of anxiety-like behavior in mice and rats [67]. The maze is constructed of Plexiglas and is situated at a height of 45 cm from the floor. The apparatus has two closed arms (enclosed by transparent walls 15 cm high) opposite to two open arms surrounded by an additional border 0.5 cm high. The arms, which extend outwards from the central platform (5x5 cm), are 30 cm long and 5 cm wide. Thirty min before each behavioral test, mice were transported to the testing room in order to become habituated to it. At the initiation of each session, mice were placed on the central platform facing an open arm and were allowed to explore the maze freely for 5 min. Behavior was videotaped under red lighting (60W230V). Recordings were subsequently analyzed by a blind observer using a computerized program in order to evaluate both classic and ethological measures. The following behaviors were scored: 1) Classical parameters: number of entries into open and closed arms and into the central platform, time spent in open and closed arms, percentage of open entries and percentage of time spent in open arms and in central platform; 2) Ethological measures: rearing, head-dipping (exploratory

movement of the head and neck in the direction of the bottom side of the maze), stretched attend posture (SAP) (exploratory behavior, in which the animal stretches its body forward and then returns to its original position without any forward locomotion), peeping-out (exploratory behavior in which the animal stretches its head into the central platform while standing on a closed arm), and end-arm maze (number of times the mice reaches the end of the open arms). Ethological analysis was carried out according to that described by other authors for assessing risk assessment behavior [68,69].

Percentage of entries and percentage of time spent in the open arms are used as measures of anxiety-like behavior [70,71]. Number of entries into the closed arms is generally considered to be a measure of motor activity [64]. Entries into the open and closed arms were confirmed when mice placed all four paws on the arm [71].

2.2.6. Hole board

The hole board was first introduced by Boissier and colleagues [72,73] and has been widely used to assess unconditioned and exploratory behavior , emotionality, anxiety-like behavior and/or response to stress in animals [74-76]. The head-dipping behavior displayed by rodents is thought to reflect exploration and is distinct from locomotor activity [77,78]. Some authors have argued that this test also reflects the anxiolytic and/or anxiogenic effects of different manipulations [79,80], although it has been suggested that the hole board is not strictly a test of anxiety [75].

An acrylic black board (31.5x31.5x20.5 cm) with 16 holes, which automatically records the number of head-dips performed by animals, was employed for the hole board test (Cibertec, Barcelona, Spain). The distance between the holes was 5 cm, the hole diameter was 2 cm and the hole sensors were situated at a depth of 1 cm. At the beginning of each session, mice were placed in the central area of the hole board and allowed to explore it freely for 5 min. The parameters analysed were: 1) latency to the first head-dip (measured by the experimenter); and 2) total number of head-dips.

2.2.7. Locomotor activity in a novel cage

An *Actimet* (Cibertec, Madrid, Spain) was employed to assess locomotor activity over a 30 min period (divided into six 5-min periods). The actimeter consists of eight individual novel cages (30x14x12 cm) attached to a continuous recording system that detects the horizontal movements of the animals using an infrared photocell system.

2.2.8. Inhibitory avoidance test

A step-through inhibitory avoidance apparatus for mice (Ugo-Basile, Comerio Basere, Italy), located within an isolation box, was employed for this experiment. The apparatus was divided into two compartments (15x9x16.5 cm): a white "safe" compartment continuously illuminated by fluorescent light (24 V, 10 W) and a black "shock" compartment maintained in darkness. The two compartments were divided by a partition with an automatically-operated sliding door. The floor consisted of stainless steel bars (0.7 mm in diameter and 8 mm apart). Training and testing began with an adaptation period (90 sec) in the safe compartment before the door was opened. During training, if the mouse crossed to the other compartment, a low intensity shock was delivered (0.3 mA shock, 5 sec duration). During the test session, the mouse was placed in the safe chamber and the procedure used in the training phase (but without shock administration) was reproduced. During both training and testing sessions (separated 24 hr), the apparatus automatically registered crossing latencies. In both sessions, a cut-off time of 300 sec was established. Mice underwent the training procedure in the inhibitory avoidance apparatus on PND 52 and performed the test on PND 53.

2.3. Drugs

Mice were exposed to chronic oral nicotine treatment from PND 28 until the end of the experiment. Oral nicotine was prepared with (-)- Nicotine hydrogen tartrate salt (Sigma-Aldrich. Madrid) diluted in water [81]. The dose used was 100 µg/ml with 2% Saccharin sodium salt hydrate (Sigma-Aldrich, Madrid). Saccharin was added to the animals' drinking water to improve the palatability of the nicotine solutions. Control mice received tap water with 2% saccharin. Each drinking bottle was checked five times

a week to determine the volume that had been consumed [66,82]. Nicotine solutions were substituted for tap water 1 hr before behavioral tests and cotinine measurement.

The dose of nicotine was chosen based on experiments which have indicated that it is not aversive to mice [83,84]. It is in the range of nicotine doses usually employed in oral nicotine treatments [42,43,64,65,85-87] and is thought to induce significant behavioral changes in mice [42,64] and detectable serum levels of cotinine [42]. Due to the group housing required by the EE paradigm, individual fluid intake for each mouse could not be calculated. However, it should be pointed out that the nicotine solution was the sole source of fluid available to the nicotine-treated mice throughout the experiment.

2.4. Statistical analysis

All statistical analysis was performed using IBM® SPSS® Statistics 19.0 (IBM corp, NY, USA) for Windows. Fluid intake and body weight data were analyzed using a repeated measures analysis of variance (ANOVA) and post-hoc Tukey tests. Behavioral data obtained in the EPM and in the hole board tests were analyzed by ANOVA using "Housing" and "Drug Treatment" as between-subject factors.

Locomotor activity was analyzed with a repeated measures ANOVA, considering "Time period" as the within subject factor and "Housing" and "Drug Treatment" as between-subject factors.

Following the procedure reported by Monleón et al. [88] and Snedecor and Cochran [89], the inhibitory avoidance data were transformed first into proportion values ($p=x/300$) and then into arc sin values (arc sin $\sqrt{p}$). Data from the training and test sessions were analyzed separately. For each experimental group, crossing latencies for training and test sessions were compared using the Student's t test for dependent samples. Due to technical difficulties during behavioral tests two animals were eliminated from the statistical analysis of ethological measures of EPM and one from the inhibitory avoidance test. Significance levels were set at $p<0.05$ and assumptions

for ANOVA were taken into account for parametric testing of data obtained. All data are presented as mean± standard error of the mean (SEM).

3. RESULTS

3.1. Body weight and fluid intake

Weight gain during the experiment for EE and SE groups is shown in Figure 1. The ANOVA for repeated measures indicated that the factor Housing reached statistical significance for body weight gain [$F(1, 60)=18.20$, $p<0.0001$]. Mice housed in enriched environments gained less body weight during the three weeks of treatment than standard reared mice ($p<0.0001$). A more detailed ANOVA showed that the factor Housing induced significant differences in body weight gain during week 1 [$F(1,60)=7.16$, $p<0.05$], week 2 [$F(1,60)=4.62$, $p<0.05$] and week 3 of the study [$F(1,60)=25.17$, $p<0.0001$]. The mean body weight gain (obtained by calculating the difference between the beginning of the study and its end) was 12.59±0.56 in enriched mice and 14.78±0.38 in standard mice. The main factor (nicotine treatment) did not reach statistical significance [$F(1,60)=1.96$, *ns*], whereas the interaction of Housing conditions x Treatment did [$F(1,60)=10.39$, $p<0.001$]. Further study of this interaction indicated significant differences between the groups during the first week of treatment. Tukey's post-hoc comparisons of the mean body weight at this week indicated that enriched mice treated with nicotine were significantly lighter (25.43±1.12) than enriched saccharin-treated mice (30.69±0.78) ($p<0.0001$), standard-housed mice receiving nicotine (31.24±0.35) ($p<0.0001$) and standard saccharine-treated mice (29.08±0.69) ($p<0.001$).

Repeated measure analysis for the data of fluid intake throughout the experiment showed significant differences between Housing conditions [$F(1,60)=8.16$, $p<0.01$]. Enriched mice displayed a higher fluid intake (7.19±0.13 ml/day per animal) than those housed in standard conditions (6.36±0.31). The effect of Treatment was also significant

[F(1,60)=25.19, p<0.0001], reflecting a lower fluid consumption in mice exposed to nicotine through their drinking water than in those receiving saccharin (See Figure 2).

3.2. Plasma cotinine levels

The cotinine analyses of the additional group of animals indicated differences between the nicotine and control groups [F(1,31)=99.88, p<0.0001]. Mice exposed to oral nicotine intake showed significantly higher plasma levels of cotinine (509.64 ± 36.45) than those receiving the saccharine solution (10 ± 34.22). There was also a significant interaction between Housing and Treatment [F(1,14)=15.19, p<0.01]. In the nicotine-treated group, animals assigned to EE conditions presented higher cotinine levels (718.14 ± 72.27 ng/ml) than those reared in SE conditions (301.14 ± 77.46 ng/ml) (See Figure 3).

3.3. Behavioral battery

3.3.1. Elevated Plus-Maze:

In relation to the classic measures, ANOVA revealed significant effects of Housing conditions on closed-arm entries [F(1,60)=17.77, p<0.001], central platform entries [F(1,60)=4.69, p<0.05] and percentage of open-arm entries [F(1,60)=11.11, p<0.001]. Animals reared in enriched cages displayed a lower number of entries into the closed arms (p<0.001) and central platform (0.05) and a significantly higher percentage of entries into the open arms (p<0.001) than those housed in standard cages. A significant main effect for Treatment was observed for closed-arm [F(1,60)=9.91, p<0.01], central platform [F(1,60)=8.08, p<0.01] and total entries [F(1,60)=7.71, p<0.01]. Mice treated with nicotine displayed less entries into the closed arms and the central platform, and a lower number of total entries (p<0.01) than those treated with saccharin. Data are summarized in Figure 4 and Table 1.

Results obtained for ethological measures indicated that Housing conditions had significant effects on the total number of head-dips [F(1,58)=9.82, p<0.01], percentage of protected SAP [F(1,58)=3.79, p<0.05] and peeping-out behavior [F(1,58)=14.19,

p<0.001] (see Table 1). Mice housed under enriched conditions displayed a higher total number of head-dips (p<0.01) and a lower percentage of protected SAP (p<0.05) and peeping-out behavior (p<0.001) than standard-housed animals. Nicotine treatment had significant effects on the total number of rearings [F(1,58)=4.26, p<0.05]. Mice treated with nicotine performed a lower total number of rearings than the saccharin-treated animals (p<0.05). The interaction between Housing conditions and Treatment did not reach statistical significance.

3.3.2. Hole board

Neither Housing conditions nor Treatment had significant effects on "Latency to the first head-dip". When the variable "Total number of head-dips" was considered, ANOVA revealed that the effect of Housing conditions was significant [F(1,60)= 4.02, p<0.05)] (See Figure 5). Enriched mice performed less head-dips than standard-housed animals (p<0.05). Nicotine treatment [F(1,60)=0.02, *ns*] and the interaction between Housing conditions and Treatment [F(1,60)=0.01, *ns*] were not significant.

3.3.3. Locomotor activity in a novel cage

Analysis of activity counts during 5-min periods displayed by each group in the actimeter indicated a significant effect of Housing conditions [F(1,60)=44.66, p<0.0001]. Motor activity of enriched mice was lower than that of mice reared in standard cages across the different time periods evaluated (p<0.0001). Neither the factor Treatment [F(1,60)=3.57, *ns*] nor the interaction Housing conditions X Treatment [F(1,60)=2.79, *ns*] were statistically significant (See Figure 6).

3.3.4. Inhibitory avoidance test

Training: No statistically significant differences were detected in this phase of the inhibitory avoidance test between mice housed under different Housing conditions [F(1,59)=0.18, *ns*] or receiving a different Treatment [F(1,59)=0.09, *ns*]. Nor was the interaction effect of Housing conditions x Treatment [F(1,59)=0.17, *ns*] significant.

Test: In the test phase, there was a statistical significant difference between mice from different Housing conditions [F(1,59)=10.75, p<0.01]. The analysis showed that

subjects housed in enriched conditions exhibited longer latencies from the light compartment to the dark compartment than subjects housed in standard environments ($p<0.01$). Treatment [$F(1,59)=0.06$, *ns*] and interaction of Housing conditions x Treatment [$F(1,59)=0.06$, *ns*] were not significant (see Figure 7).

Training vs Test: Inhibitory avoidance learning (longer latencies in test phase than training phase) was confirmed in the groups EE+NIC [$t(15)=-3.37$, $p<0.01$]; EE+SAC [$t(14)=-2.61$, $p<0.05$] and SE+NIC [$t(15)=-2.29$, $p<0.05$] (see Figure 6). In the SE+SAC group a trend towards significance was observed in the crossing latencies between training and test phases [$t=1.93(15)$, $p=0.072$].

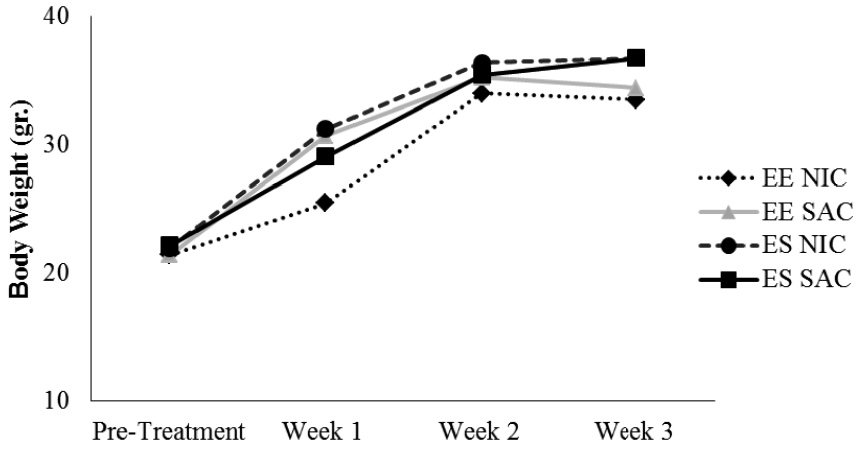

Figure 1. Evolution of bodyweight (g) in the four experimental groups throughout the study: Environmental Enrichment + Nicotine (EE + NIC), Environmental Enrichment + Saccharin (EE + SAC), Standard Environment + Nicotine (SE + NIC) and Standard Environment + Saccharin (SE + SAC). Data are presented as mean ± SEM.
(+) $p < 0.05$ EE vs. SE; NIC vs. SAC.
(*) $p < 0.05$ EE vs. SE.
(**) $p < 0.0001$ EE vs. SE.

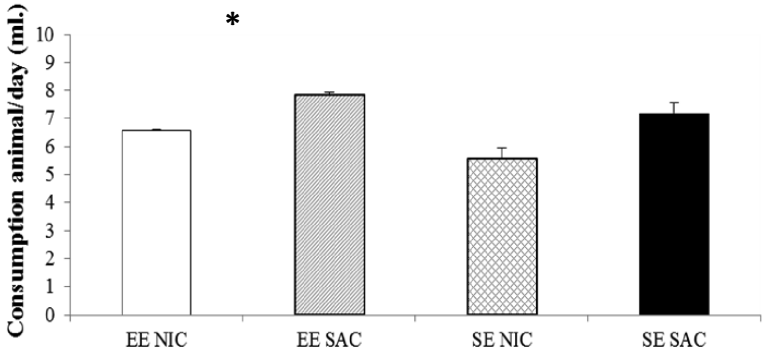

Figure 2. Average fluid intake (ml) shown by each experimental group throughout the study: Environmental Enrichment + Nicotine (EE + NIC), Environmental Enrichment + Saccharin (EE + SAC), Standard Environment + Nicotine (SE + NIC) and Standard Environment + Saccharin (SE + SAC). Data are presented as mean ± SEM.
(*) $p < 0.01$ EE vs. SE.
(+) $p < 0.0001$ NIC vs. SAC.

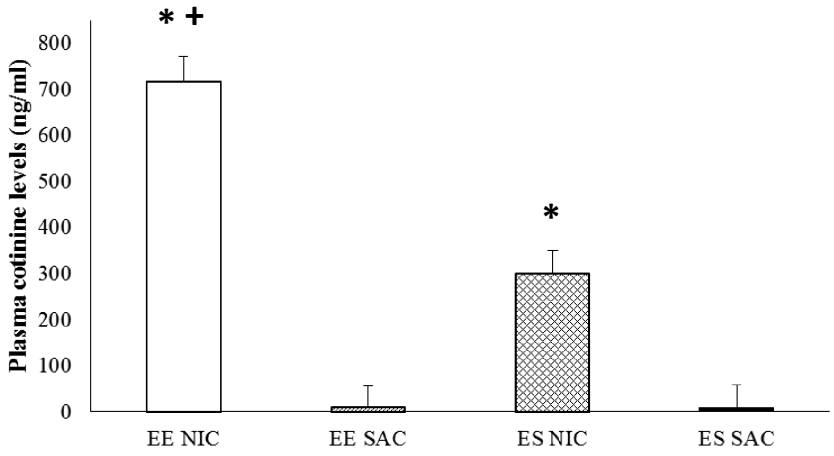

Figure 3. Plasma cotinine levels in the four experimental groups: Environmental Enrichment + Nicotine (EE + NIC), Environmental Enrichment + Saccharin (EE + SAC), Standard Environment + Nicotine (SE + NIC) and Standard Environment + Saccharin (SE + SAC).
Data are presented as mean ± SEM.
(*) $p < 0.0001$ NIC vs. SAC.
(+) $p < 0.01$ EE + NIC vs. ES + NIC.

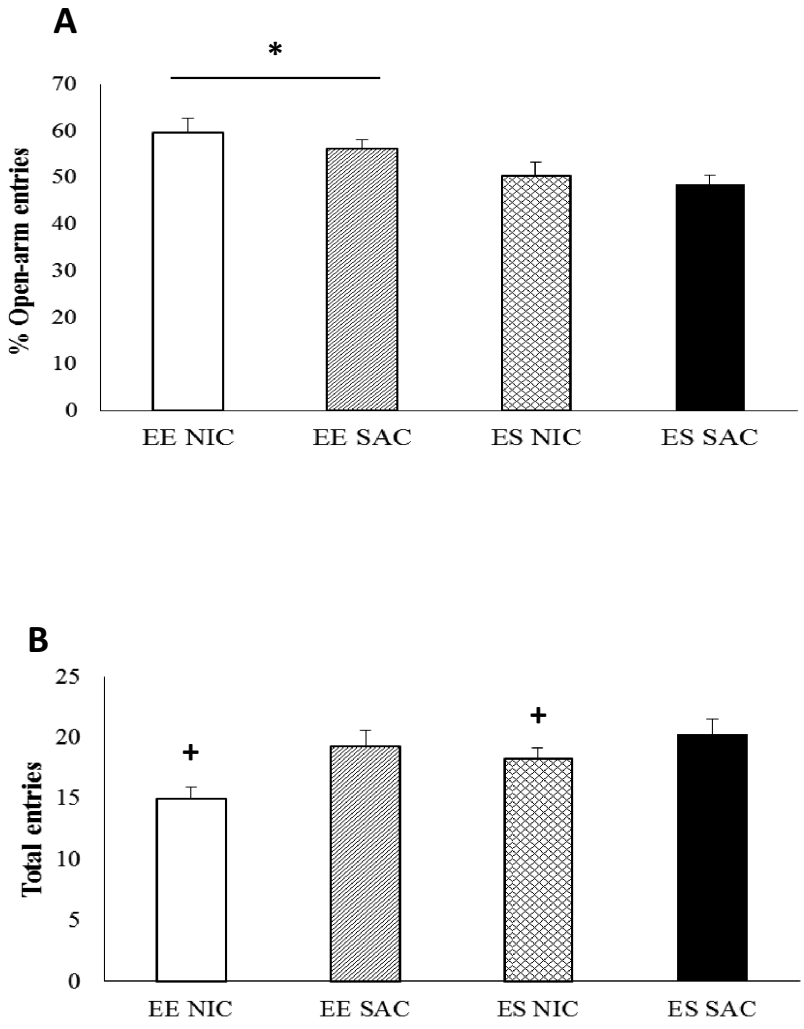

Figure 4. **(A)** Percentage of entries into the open arms and **(B)** Total entries (open + closed arms) in the elevated plus maze test in the four experimental groups: Environmental Enrichment + Nicotine (EE + NIC), Environmental Enrichment + Saccharin (EE + SAC), Standard Environment + Nicotine (SE + NIC) and Standard Environment + Saccharin (SE + SAC). Data are presented as mean ± SEM.

(*) $p < 0.001$ EE vs. SE.

(+) $p < 0.01$ NIC vs. SAC.

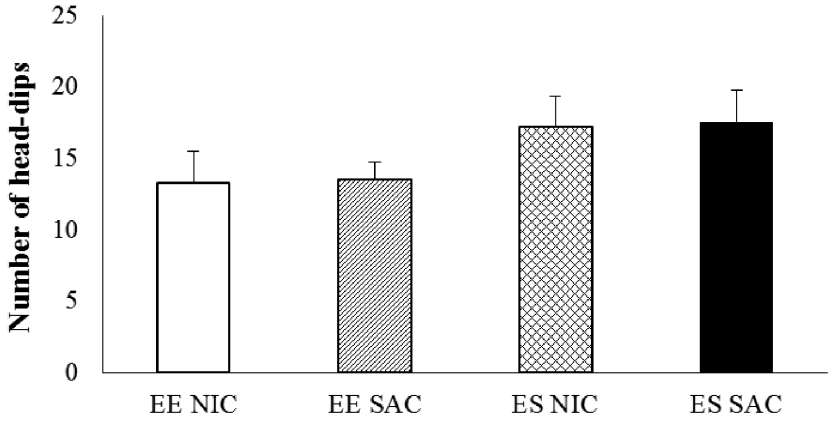

Figure 5. Total number of head-dips displayed in the hole board test by the four experimental groups: Environmental Enrichment + Nicotine (EE + NIC), Environmental Enrichment + Saccharin (EE + SAC), Standard Environment + Nicotine (SE + NIC) and Standard Environment + Saccharin (SE + SAC). Data are presented as mean ± SEM.
(*) $p < 0.05$ EE vs. SE.

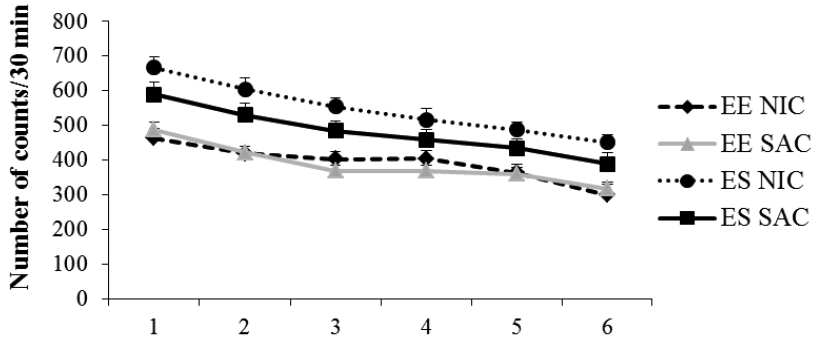

Figure 6. Motor activity counts for each 5-min time period evaluated over 30 min for the four experimental groups, as displayed by the actimeter: Environmental Enrichment + Nicotine (EE + NIC), Environmental Enrichment + Saccharin (EE + SAC), Standard Environment + Nicotine (SE + NIC) and Standard Environment + Saccharin (SE + SAC). Data are presented as mean ± SEM.
(*) $p < 0.0001$ EE vs. SE.

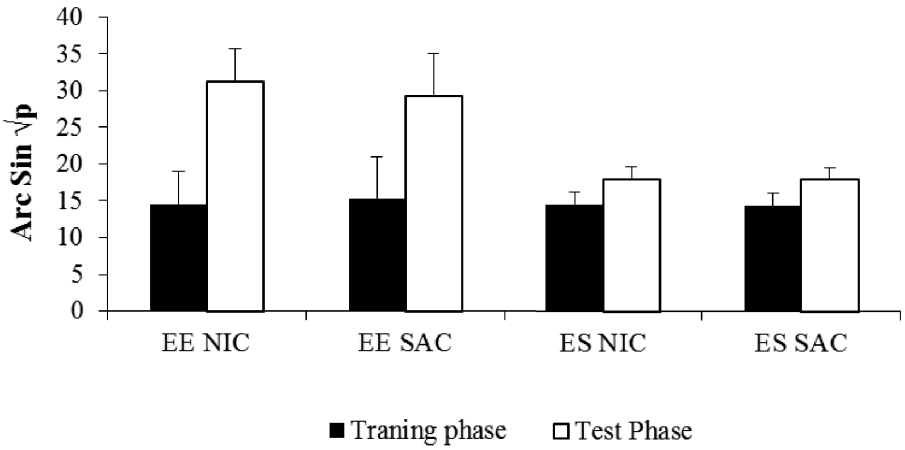

Figure 7. Comparison between the training and test crossing latencies in the inhibitory avoidance task displayed by each of the study groups: Environmental Enrichment + Nicotine (EE + NIC), Environmental Enrichment + Saccharin (EE + SAC), Standard Environment + Nicotine (SE + NIC) and Standard Environment + Saccharin (SE + SAC).

Data are presented as mean ± SEM of square root of proportions (p = x / 300) transformed to arc sin.

(*) $p < 0.05$ Training phase vs. test phase in groups EE + SAC and SE + NIC.

(**) $p < 0.01$ Training phase vs. test phase in group EE + NIC.

Table 1. Effects of housing in enriched environment (EE) or standard environment (ES) on the behavior displayed in the elevated plus-maze test by male NMRI mice under chronic treatment with oral nicotine (NIC) (100µg/ml nicotine +2% saccharin) or saccharin (SAC) (2% saccharin).

	EE		ES	
BEHAVIORAL CATEGORIES	**NIC**	**SAC**	**NIC**	**SAC**
TOTAL ENTRIES#	14.94±1.04	19.25±1.32	18.25±0.88	20.25±1.25
OPEN ENTRIES	8.94±0.76	10.88±0.97	9.25±0.67	9.94±0.91
CLOSED ENTRIES*,#	6.00±0.59	8.38±0.55	9.00±0.62	10.31±0.58
% OPEN ENTRIES*	59.60±3.05	56.10±1.96	50.35±2.89	48.45±2.06
% OPEN TIME	42.44±4.94	35.11±2.12	32.07±2.29	37.16±3.28
% CENTER TIME	34.97±3.75	37.75±2.06	40.32±2.15	33.53±2.51
TOTAL HD**	35.50±4.51	27.29±3.02	17.94±2.77	23.31±3.03
% p HD	36.09±5.84	37.23±3.79	41.14±4.59	38.60±5.65
TOTAL SAP	12.88±1.68	14.29±1.01	14.94±1.30	15.31±1.62
% p SAP***	32.31±3.89	40.74±2.51	46.63±4.38	41.06±3.70
TOTAL REARS##	12.44±1.72	17.57±2.25	11.31±1.52	13.31±1.43
PEEPING-OUT*	3.94±0.49	5.43±0.55	7.19±0.65	6.75±0.69
ACTIVITY END MAZE	7.31±1.08	7.36±0.56	7.19±0.74	7.75±0.71

Data are presented as mean values ± SEM.
Abbreviations: HD: head-dip; SAP: stretched attend posture; % p: percentage protected.

(*) $p < 0.0001$ EE vs. SE.
(**) $p < 0.01$ EE vs. SE.
(***) $p < 0.05$ EE vs. SE.
(#) $p < 0.01$ NIC vs. SAC.
(##) $p < 0.05$ NIC vs. SAC.

Table 2. Time in seconds obtained in the avoidance learning task (training and test crossing latencies) in NMRI male mice housed in enriched environment (EE) or standard environment (SE) under chronic treatment with oral nicotine (NIC) (100 µg/ml nicotine + 2% saccharin) or saccharin (SAC) (2% saccharin).

	EE		**ES**	
	NIC	**SAC**	**NIC**	**SAC**
Training Phase	20.48±2.90	21.95±2.47	20.21±2.91	20.68±3.54
Test Phase	8.12±20.0	78.51±22.71	31.49±5.85	31.18±5.31

Data are presented as mean values ± SEM.

4. DISCUSSION

In the present work, we have evaluated the effects of exposure to an EE combined with chronic oral nicotine administration on physiological parameters and behavioral performance in mice. Hereafter, we will discuss in detail the results obtained for the different variables evaluated.

4.1. Effects of enriched environment combined with chronic oral nicotine on body weight, fluid intake and cotinine levels

Our results indicate that changes in body weight and fluid intake are induced by different housing conditions: mice housed in EE gained less weight along the three weeks of the study and exhibited higher fluid intake than those housed in SE. Previous studies examining changes in body weight have yielded controversial results as greater gain of body weight in enriched environments [62], lower increase [55,88,89] and absence of significant changes [70,90]. These discrepancies could be related to differences between the strains or species used in each study [91]. Some authors have attributed the lower increase of body weight in EE mice to the complexity of this environment and larger dimensions of the cages [26], the presence of more conspecifics with which to interact [92], or to changes in food intake and reduction in the energy necessary to maintain body heat [62]. Our data also reflect a lower increase in body weight and a reduced fluid intake in mice receiving a nicotine solution. Previous results regarding the effects of chronic nicotine exposure are contradictory. Age at initiation of exposure, dose administered and other variables, such as the sex or strain, could explain discrepancies. Abreu-Villaca et al. [56] observed a reduction in fluid intake in C57BL/6J mice consuming nicotine when compared to saccharin-treated mice, an aversive effect related to the bitter taste of nicotine and to a possible compensation for the antidiuretic actions of the drug. In the current study, the increase in body weight was lower in mice treated with oral nicotine during the first week of treatment, especially in mice reared in EE conditions, than for mice of the other experimental groups. Our data are in accordance with those obtained by Grabus et al.

[93], but contrast with those of Adriani et al. [85] and Klein et al. [42]. Our results also suggest a higher fluid intake in NMRI mice housed in EE, although other studies have yielded contrary data in other strains of mice [94] or an absence of differences [61]. This higher fluid intake could be related to the bigger groups and larger cages employed in EE, although differences of strain [95] and/or lower levels of anxiety displayed by enriched mice [96] may also be involved. A higher number of animals in each cage is necessary in order to potentiate the social component of the EE paradigm, although it could difficult the assessment of some physiological measures as food or fluid intake. These differences may also influence total nicotine consumption in each group, although we found such variations to be slight. A higher water and ethanol intake have also been observed in rats reared in EE [97,98], although there are studies which did not confirm this increase [99]. This suggests the relevance of drug dose and time of administration of the drug.

With regard to the validation of nicotine exposure during the experiment, the analysis of plasma cotinine levels indicated that the group which had been exposed to nicotine had significantly higher cotinine levels than controls. In fact, similar results were obtained in previous studies in which the same nicotine dosage was employed [42]. This increase in cotinine levels was especially significant in EE- nicotine treated mice when compared with those reared in SE, which is in accordance with previous evidence that EE housing increases fluid intake.

4.2. Behavioral effects of enriched environment combined with nicotine

4.2.1. Anxiety

The EPM is a widely used task that is sensitive to pharmacological and behavioral manipulations that can influence anxiety response [68,100]. The main index of anxiety-like behavior is related to space and time measures of open arm avoidances rather than thigmotaxis (i.e., an emotional response related with the fear of crossing open areas) [101]; whereas locomotor activity is evaluated according to the frequency of closed arm entries [77] or total number of entries [102]. Results regarding the effects

of EE on the EPM are inconsistent [21], and data obtained with this test are frequently difficult to interpret. If we take into account both classic parameters and ethological measures, a more complete picture is formed [71,79,101]. In the current study, effects of EE on classical measures can be interpreted as anxiolytic, since an increase in the percentage of open-arm entries and a decrease in the number of central platform and closed-arm entries was observed. Ethological measures confirmed the anxiolytic-like effects of EE, since enriched mice displayed a significant decrease in the percentage of protected SAP and in peeping out behavior and an increase in the total number of head-dips. These measures are indicative of the anxiolytic effects of different drugs and behavioral manipulations [68,71].

Some authors suggest that EE induces anxiolytic effects in the EPM in mice [19,88,103]. In a standardized model of EE established by Sztainberg and Chen [103] the EPM test revealed lower anxiety-like behaviors and a decrease of basal corticosterone levels in mice. However, other authors have observed different consequences after exposure to enriched environments such as an increase of entries in closed arms of the EPM, which could be interpreted as an anxiogenic effect of EE although it could be also reflecting a different strategy for obtaining environmental information [76,104]. Brenes and co-workers [20] did not observe significant effects of EE on plus-maze behavior in rats after 4 weeks (with the exception of closed arm entries, which were significantly higher in enriched rats). This discrepancy could be attributable to methodological differences (for example, in the level of illumination) or type of EE applied during the experiments [20,105]. Our study confirms that exposure to an enriched environment modifies the emotional behavior of NMRI male mice. Future studies should explore whether or not these changes are modulated by the sex of the subjects, since recent studies in other mice strains, such as C57BL/6J, have confirmed that exposure to EE during adolescence induces anxiolytic effects in male mice and anxiogenic effects in female mice, while locomotor effects are similar in both sexes [106].

The results obtained in the current study also show a reduction of motor activity in the EPM in mice receiving chronic nicotine treatment, reflected by a decrease in total arm entries. With regard to ethological measures, nicotine induced a significant

decrease in the total number of rearings (both protected and non-protected), a behavior associated with psychomotor reactivity and response to novelty [107]. Caldarone et al. [64] found that a similar dose to that used in the current study was anxiogenic in C57BL/6J female mice but had no significant effects on their male counterparts. For that reason, it would be of interest in future studies to take into account sex differences in response to the acute and chronic behavioral effects of nicotine [41,42,64]. Age of exposure to nicotine is also an important variable to be considered when examining its effects in the EPM, as adolescent rodents seem to be more sensitive to the anxiolytic effects of this drug [108].

4.2.2. Exploratory behavior and motor activity

The hole board test offers a simple method with which to evaluate exploratory behavior in a non-familiar environment [72,76], anxiety, or stress response in mice and to measure the "novelty seeking" trait [61,107]. Motor activity in response to new environments is reported to be reduced in enriched mice [76,96]. In our study, the EE group exhibited lower rates of spontaneous locomotor activity in the actimeter and less visits to the holes in the hole board test than the SE group. As shown in Figures 5 and 6, significant differences were not observed between EE and SE animals after chronic oral nicotine exposure in either the hole board or with respect to motor activity. The reduced activity displayed by enriched mice has been related to the fact that actimeter cages are usually smaller and less attractive to animals than their home cages [5]. Some authors, however, have reported increased locomotor and exploratory activity after EE [23]. Previous studies have also confirmed a reduced locomotion in EE mice in the open-field test, associated with lower levels of escape motivation, faster rates of habituation [21] and quicker processing of contextual information [109]. Recently, Viola et al. [110] used the object recognition task to confirm that CF1 mice housed in EE display a better knowledge of the environment and explore it more readily and observed that EE mice spend less time exploring familiar and unfamiliar objects in an object recognition task. EE provokes a significant decrease in motor activity, an initial increase in rearing behavior and a faster habituation to novel environments [13,21,76]. These changes could be related to the fact that the apparatuses in which animals were evaluated in our study (such as the hole board or

actimeter cages) are less attractive than the bigger enriched cages [20]. The exploratory behavior of enriched animals faced with new environments may also be more pronounced if the complexity of the stimulation is increased [2,21] or new objects are introduced [62].

In the current study, chronic administration of nicotine did not induce significant changes in exploratory or motor activity in either SE or EE mice. These results could be influenced by the nicotine dose administered, the duration of the treatment, the age at which the animals began treatment and were tested, the stress associated with the behavioral test, or the characteristics of the subjects [37,95,111]. Earlier studies have reported an increase of motor activity after chronic administration of nicotine [64,83,85], although others have found conflicting results [112]. Chistyakov et al [111] found no significant effects of early exposure to an oral nicotine solution (200 μg/ml) during pregnancy and lactation in mice and attributed the lack of effects to the low level of stress associated with the procedure employed to measure locomotor activity. Furthermore, in the present study the duration of the treatment was shorter than in prior studies [87,109] and mice were tested in late adolescence [85] rather than in adulthood, as was the case in previous studies in which significant changes in motor activity after nicotine exposure were reported.

The effects of nicotine on motor activity are complex. Acute administration induces hypoactivity in rodents [46], whereas repeated exposure to nicotine can induce sensitization to the locomotor stimulant effects of the drug [39]. Previous studies in which nicotine was administered orally have demonstrated an increase of locomotor activity in NMRI mice after a more prolonged exposure to nicotine (50 days) and with higher doses than those used in the current study [113]. Although changes in motor activity were not significant in our study, we can see in Figure 6 that nicotine-treated mice displayed higher locomotor activity than control mice only when they were housed in SE. In contrast, data obtained in the EPM confirm a reduction of motor activity, as nicotine-treated mice performed a lower number of close-arm entries, a measure that usually reflects changes in locomotor activity [71]. This confirms the complex interaction between the effects of housing and nicotine-induced stimulation of locomotor activity [113].

4.2.3. Inhibitory avoidance learning

The inhibitory avoidance task is based on inducing inhibition in rodents through their innate photophobia [114] and has been widely applied in order to evaluate the effect of drugs on long-term memory in rodents [88]. In the current study, although all groups learned the task (they displayed longer crossing latencies during the test than on the day of training), a greater improvement was observed in mice reared in enriched conditions. One of the most significant effects of EE on rodents is the marked improvement observed in different learning tasks [3,115,116], with these effects being more significant in hippocampal-dependent tasks [14,25,117].

The greater stimulation afforded by enriched conditions, the increase in social interactions and in physical activity, and the larger dimensions of the cages could have an influence on the improvement observed in learning tasks [111,118], although some differences have been reported depending on the type of enrichment applied [119,120].

It has also been reported that an EE decreases age-related deficits in the performance of spatial learning tasks due to a heightened attention [26]. The great diversity of paradigms applied in different laboratories must be taken into account when evaluating the effects of EE on learning and memory tasks [117]. The results obtained in the current study are of interest, since they confirm that a short period of enrichment induces a significant improvement in the inhibitory avoidance task in young mice. Future studies should explore the effects of this enrichment model on mice of different ages. Recently, Kazlauckas et al. [121] have confirmed that exposure to an enriched environment for two months improves the performance of CF1 mice in the inhibitory avoidance task, but this improvement is significant only in mice with a low basal level of exploratory behavior.

The present study also indicates that the administration of oral nicotine over the 24 days prior to the behavioral battery does not significantly influence memory in the inhibitory avoidance paradigm. In earlier studies, subcutaneous nicotine (0.125-0.75 mg/kg) did not have significant effects on step-down inhibitory avoidance [122]. There are very few previous studies regarding the cognitive effects of oral nicotine

administration in the inhibitory avoidance learning paradigm. Although we hypothesized that nicotine would enhance the effects of EE on inhibitory avoidance, our data suggest that nicotine, at least at the dose used in the current study, has no significant effects on crossing latencies on the day of the test. These results are in accordance with those obtained by Abreu-Villaca et al. [56] and could be related to the type of administration applied in both studies (via drinking water). These authors did not find that a dose of 50 μg/ml had significant effects on inhibitory avoidance. It is also possible that the effects of EE on inhibitory avoidance learning were extremely potent and could have masked some of the effects of nicotine in this test. Other studies have reported small effects of oral nicotine administration on some cognitive tasks in rodents [123]. Future studies should explore a wider range of nicotine doses and acute drug administration in order to evaluate a possible interaction between housing conditions and the cognitive effects of nicotine, already demonstrated in other behavioral tasks [38].

4.3. General discussion

There are few previous studies about the effects of EE on NMRI mice [124]. The complexity and diversity of physiological and behavioral effects of the EE paradigm have been related to different variables, including enrichment type, duration of exposure to the EE, age at which exposure begins, species (mice or rats) and strain used [1,23,110,115,117,125]. In the current study we selected the NMRI stock since its behavioral profile is well characterized [124,126-129] and is suitable for the oral administration of nicotine [87,95,113,126] and for studies of EE [130].

Several studies suggest that EE improves the performance of mice in different behavioral tasks that evaluate emotional functions and/or learning and in memory tasks [3]. The current study confirms that, following 3-4 weeks of exposure to an enriched environment, mice display lower levels of anxiety and an improved learning. These results reaffirm the usefulness of this experimental paradigm for future studies in which a large number of experimental variables could be manipulated. An enriched environment may offer optimal conditions for promoting exploration, cognitive

activity, social interaction, play behavior and physical activity, thereby "*enriching the environment to empower the brain"*, as indicated by Sale et al. [131].

Previous studies have observed few behavioral changes after chronic nicotine administration in drinking water (in the EPM, locomotor activity and tail-supension tests), although the doses administered were lower than those used in the current study [132]. Chronic nicotine effects vary with the route of administration. Oral nicotine administration is increasingly used in studies [86,126,133], but there are as yet few data regarding its behavioral effects in different housing conditions. This model of nicotine administration is not stressful for animals but has its limitations, including the lack of a precise control of nicotine intake, since it depends on the consumption pattern and level of tolerance to nicotine of each animal [132].

One factor that must be taken into account when interpreting behavioral changes induced by nicotine in EE- and SE-reared mice in the current study is that in all groups nicotine was substituted for tap water 1 hr before behavioral tests were performed. Therefore, the lack of effects observed could be related with some short-term withdrawal effects, although longer periods are usually required in order for withdrawal effects to be evident [113]. Differences in total nicotine intake and cotinine levels in each group may also explain the results obtained in our study. As previously explained, a lower fluid intake and cotinine levels were observed in SE mice than in EE animals. This could imply a lower total nicotine intake in SE mice, which may have masked some of the effects of nicotine in this group and thus may explain the lack of nicotine effects observed. This difference could be related to the fact that the number of cagemates was higher in EE (n=8) than in SE (n=4) cages. On the other hand, this could be a key factor to understand the negligible effects of nicotine obtained in mice housed in standard conditions.

The main novelty of the current study lies in the fact that previous research on the behavioral effects of EE has evaluated exploratory behavior or learning tasks while changes in emotional reactivity or in the response to pharmacological treatments have been less extensively investigated [1,15]. The neurobiological mechanisms that mediate the effects of housing on the behavioral effects of nicotine are not completely

understood [38]. We hope that the current study broadens the knowledge of this field by exploring the combined effects of exposure to chronic oral nicotine and an enriched environment on mice. It also provides data that may allow us to reach a better understanding of the environmental factors that influence the progression of addiction to nicotine [34]. Our study may also help to determine how environmental variables are related to nicotine addiction and other drugs of abuse [29,33] and to develop new therapeutic and treatment approaches [34,134].

4.4. Conclusion

In conclusion, our results suggest that EE induces both physiological (lower increase in body weight and higher fluid intake) and behavioral (decrease in exploratory and motor activity, lower anxiety during plus-maze exposure and improvement of inhibitory avoidance learning) effects. Chronic exposure to nicotine is accompanied by a slight increase in body weight, especially during the first week of treatment, and a decrease in fluid intake. At the behavioral level, the effects of chronic nicotine administration are observed in the EPM and may reflect a lower level of motor activity in mice treated with this drug. We must take in to account the effects of nicotine may be influenced by the order of the different behavioral tests and period of time between them. An interaction between environmental factors and nicotine exposure is observed particularly with respect to emotional behavior. Future studies should explore this interaction in more depth by employing acute administration of nicotine after exposure to enriched environments, a wider range of doses and/or other cholinergic agonists.

REFERENCES

[1] Nithianantharajah J, Hannan AJ. The neurobiology of brain and cognitive reserve: mental and physical activity as modulators of brain disorders. Prog Neurobiol 2009;89:369-82.

[2] Rosenzweig MR, Bennett EL. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 1996;78:57-65.

[3] van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci 2000;1:191-8.

[4] Harburger LL, Nzerem CK, Frick KM. Single enrichment variables differentially reduce age-related memory decline in female mice. Behav Neurosci 2007;121:679-88.

[5] Yuan ZY, Gu P, Liu L, Wang YY, Liu J, Cui DS, et al. Neuroprotective effects of enriched environment in MPTP-treated SAMP8 mice. Neurosci Lett 2009;454:6-10.

[6] Artola A, von Frijtag JC, Fermont PC, Gispen WH, Schrama LH, Kamal A, et al. Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. Eur J Neurosci 2006;23:261-72.

[7] Nithianantharajah J, Hannan AJ. Mechanisms mediating brain and cognitive reserve: Experience-dependent neuroprotection and functional compensation in animal models of neurodegenerative diseases. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35:331-9.

[8] Kempermann G, Gast D, Gage FH. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann Neurol 2002;52:135-43.

[9] Rosenzweig MR. Effects of differential experience on the brain and behavior. Dev Neuropsychol 2003;24:523-40.

[10] Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature 1997;386:493-5.

[11] Viola GG, Rodrigues L, Américo JC, Hansel G, Vargas RS, Biasibetti R, et al. Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice. Brain Res 2009;1274:47-54.

[12] Diniz DG, Foro CA, Rego CM, Gloria DA, de Oliveira FR, Paes JM, et al. Environmental impoverishment and aging alter object recognition, spatial learning, and dentate gyrus astrocytes. Eur J Neurosci 2010;32:509-19.

[13] Laviola G, Hannan AJ, Macri S, Solinas M, Jaber M. Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. Neurobiol Dis 2008;31:159-68.

[14] Mirochnic S, Wolf S, Staufenbiel M, Kempermann G. Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of Alzheimer disease. Hippocampus 2009;19:1008-18.

[15] Pamplona FA, Pandolfo P, Savoldi R, Prediger RD, Takahashi RN. Environmental enrichment improves cognitive deficits in Spontaneously Hypertensive Rats (SHR): relevance for Attention Deficit/Hyperactivity Disorder (ADHD). Prog Neuropsychopharmacol Biol Psychiatry 2009;33:1153-60.

[16] De Bartolo P, Leggio MG, Mandolesi L, Foti F, Gelfo F, Ferlazzo F, et al. Environmental enrichment mitigates the effects of basal forebrain lesions on cognitive flexibility. Neuroscience 2008;154:444-53.

[17] Mandolesi L, De Bartolo P, Foti F, Gelfo F, Federico F, Leggio MG, et al. Environmental enrichment provides a cognitive reserve to be spent in the case of brain lesion. J Alzheimers Dis 2008;15:11-28.

[18] Saucier DM, Yager JY, Armstrong EA. Housing environment and sex affect behavioral recovery from ischemic brain damage. Behav Brain Res 2010;214:48-54.

[19] Fox C, Merali Z, Harrison C. Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. Behav Brain Res 2006;175:1-8.

[20] Brenes JC, Padilla M, Fornaguera J. A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. Behav Brain Res 2009;197:125-37.

[21] Zimmermann A, Stauffacher M, Langhans W, Wurbel H. Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. Behav Brain Res 2001;121:11-20.

[22] Morley-Fletcher S, Rea M, Maccari S, Laviola G. Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. Eur J Neurosci 2003;18:3367-74.

[23] Abramov U, Puussaar T, Raud S, Kurrikoff K, Vasar E. Behavioural differences between C57BL/6 and 129S6/SvEv strains are reinforced by environmental enrichment. Neurosci Lett 2008;443:223-7.

[24] Schloesser RJ, Lehmann M, Martinowich K, Manji HK, Herkenham M. Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. Mol Psychiatry 2010;15:1152-63.

[25] Gresack JE, Kerr KM, Frick KM. Life-long environmental enrichment differentially affects the mnemonic response to estrogen in young, middle-aged, and aged female mice. Neurobiol Learn Mem 2007;88:393-408.

[26] Harati H, Majchrzak M, Cosquer B, Galani R, Kelche C, Cassel JC, et al. Attention and memory in aged rats: Impact of lifelong environmental enrichment. Neurobiol Aging 2011; 32:718-736.

[27] Branchi I, D'Andrea I, Fiore M, Di Fausto V, Aloe L, Alleva E. Early social enrichment shapes social behavior and nerve growth factor and brain-derived neurotrophic factor levels in the adult mouse brain. Biol Psychiatry 2006;60:690-6.

[28] Paban V, Chambon C, Manrique C, Touzet C, Alescio-Lautier B. Neurotrophic signaling molecules associated with cholinergic damage in young and aged rats Environmental enrichment as potential therapeutic agent. Neurobiol Aging 2011; 32: 467-484.

[29] Solinas M, Thiriet N, Chauvet C, Jaber M. Prevention and treatment of drug addiction by environmental enrichment. Prog Neurobiol 2010; 92:572-592.

[30] Redolat R, Perez-Martinez A, Carrasco MC, Mesa P. Individual differences in novelty-seeking and behavioral responses to nicotine: a review of animal studies. Curr Drug Abuse Rev 2009;2:230-42.

[31] Solinas M, Thiriet N, Rawas RE, Lardeux V, Jaber M. Environmental enrichment during early stages of life reduces the behavioral, neurochemical, and molecular effects of cocaine. Neuropsychopharmacology 2008;34:1102-11.

[32] Thiel KJ, Sanabria F, Pentkowski NS, Neisewander JL. Anti-craving effects of environmental enrichment. Int J Neuropsychopharmacol 2009;12:1151-6.

[33] Carroll ME, Anker JJ, Perry JL. Modeling risk factors for nicotine and other drug abuse in the preclinical laboratory. Drug Alcohol Depend 2009;104:S70-8.

[34] Mesa-Gresa P, Pérez-Martínez A, Redolat R. Nicotine and animal models: what does the environmental enrichment paradigm tell us?. Adicciones 2012; 24:87-94.

[35] Chauvet C, Lardeux V, Goldberg SR, Jaber M, Solinas M. Environmental enrichment reduces cocaine seeking and reinstatement induced by cues and stress but not by cocaine. Neuropsychopharmacology 2009, 34:2767-78.

[36] Benowitz NL. Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. Clin Pharmacol Ther 2008;83:531-41.

[37] Levin ED, McClernon FJ, Rezvani AH. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. Psychopharmacology 2006;184:523-39.

[38] Coolon RA, Cain ME. Effects of mecamylamine on nicotine-induced conditioned hyperactivity and sensitization in differentially reared rats. Pharmacol Biochem Behav 2009;93:59-66.

[39] Ericson M, Norrsjo G, Svensson AI. Behavioral sensitization to nicotine in female and male rats. J Neural Transm 2010;117:1033-9.

[40] Gomez MC, Carrasco MC, Redolat R. Differential sensitivity to the effects of nicotine and bupropion in adolescent and adult male OF1 mice during social interaction tests. Aggress Behav 2008;34:369-79.

[41] Isiegas C, Mague SD, Blendy JA. Sex differences in response to nicotine in C57Bl/6:129SvEv mice. Nicotine Tob Res 2009;11:851-8.

[42] Klein LC, Stine MM, Vandenbergh DJ, Whetzel CA, Kamens HM. Sex differences in voluntary oral nicotine consumption by adolescent mice: a dose-response experiment. Pharmacol Biochem Behav 2004;78:13-25.

[43] Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR, et al. Guidelines on nicotine dose selection for in vivo research. Psychopharmacology 2007;190:269-319.

[44] Skwara AJ, Karwoski TE, Czambel RK, Rubin RT, Rhodes ME. Influence of environmental enrichment on hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine by osmotic mini-pumps, and nicotine withdrawal by mecamylamine in male and female rats. Behav Brain Res. 2012;234:1-10.

[45] Green TA, Cain ME, Thompson M, Bardo MT. Environmental enrichment decreases nicotine-induced hyperactivity in rats. Psychopharmacology 2003;170:235-41.

[46] Hamilton DA, Kolb B. Differential effects of nicotine and complex housing on subsequent experience-dependent structural plasticity in the nucleus accumbens. Behav Neurosci 2005;119:355-65.

[47] Zhu J, Bardo MT, Green TA, Wedlund PJ, Dwoskin LP. Nicotine increases dopamine clearance in medial prefrontal cortex in rats raised in an enriched environment. J Neurochem 2007; 103:2575-88.

[48] Caprioli D, Paolone G, Celentano M, Testa A, Nencini P, Badiani A. Environmental modulation of cocaine self-administration in the rat. Psychopharmacology (Berl). 2007;192(3):397-406.

[49] Mesa-Gresa P, Moya-Albiol L. Neurobiology of child abuse: the 'cycle of violence'. Rev Neurol. 2011;52(8):489-503.

[50] Nader J, Claudia C, Rawas RE, Favot L, Jaber M, Thiriet N, Solinas M. Loss of environmental enrichment increases vulnerability to cocaine addiction. Neuropsychopharmacology. 2012;37(7):1579-87.

[51] Renoir T, Pang TY, Hannan AJ. Effects of environmental manipulations in genetically targeted animal models of affective disorders. Neurobiol Dis. 2012; In press.

[52] Verma P, Hellemans KG, Choi FY, Yu W, Weinberg J. Circadian phase and sex effects on depressive/anxiety-like behaviors and HPA axis responses to acute stress. Physiol Behav 2010;99:276-85.

[53] Cirulli F, Berry A, Bonsignore LT, Capone F, D'Andrea I, Aloe L, et al. Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels. Neurosci Biobehav Rev 2010;34:808-20.

[54] Gracceva G, Venerosi A, Santucci D, Calamandrei G, Ricceri L. Early social enrichment affects responsiveness to different social cues in female mice. Behav Brain Res 2009;196:304-9.

[55] Hellemans KG, Benge LC, Olmstead MC. Adolescent enrichment partially reverses the social isolation syndrome. Brain Res Dev Brain Res 2004;150:103-15.

[56] Abreu-Villaca Y, Medeiros AH, Lima CS, Faria FP, Filgueiras CC, Manhaes AC. Combined exposure to nicotine and ethanol in adolescent mice differentially affects memory and learning during exposure and withdrawal. Behav Brain Res 2007;181:136-46.

[57] Deacon RM. Housing, husbandry and handling of rodents for behavioral experiments. Nat Protoc 2006;1:936-46.

[58] Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 1996;54:21-30.

[59] Paylor R, Spencer CM, Yuva-Paylor LA, Pieke-Dahl S. The use of behavioral test batteries, II: effect of test interval. Physiol Behav. 2006;87(1):95-102.

[60] Elliott BM, Grunberg NE. Effects of social and physical enrichment on open field activity differ in male and female Sprague-Dawley rats. Behav Brain Res 2005;165:187-96.

[61] Van de Weerd HA, Van Loo PL, Van Zutphen LF, Koolhaas JM, Baumans V. Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. Physiol Behav 1997;62:1019-28.

[62] Van de Weerd HA, Aarsen EL, Mulder A, Kruitwagen CL, Hendriksen CF, Baumans V. Effects of environmental enrichment for mice: variation in experimental results. J Appl Anim Welf Sci 2002;5:87-109.

[63] Abreu-Villaca Y, Queiroz-Gomes Fdo E, Dal Monte AP, Filgueiras CC, Manhaes AC. Individual differences in novelty-seeking behavior but not in anxiety response to a new environment can predict nicotine consumption in adolescent C57BL/6 mice. Behav Brain Res 2006;167:175-82.

[64] Caldarone BJ, King SL, Picciotto MR. Sex differences in anxiety-like behavior and locomotor activity following chronic nicotine exposure in mice. Neurosci Lett 2008;439:187-91.

[65] Salas R, De Biasi M. Opposing actions of chronic stress and chronic nicotine on striatal function in mice. Neurosci Lett 2008;440:32-4.

[66] Sparks JA, Pauly JR. Effects of continuous oral nicotine administration on brain nicotinic receptors and responsiveness to nicotine in C57Bl/6 mice. Psychopharmacology 1999;141:145-53.

[67] Doremus-Fitzwater TL, Varlinskaya EI, Spear LP. Effects of pretest manipulation on elevated plus-maze behavior in adolescent and adult male and female Sprague-Dawley rats. Pharmacol Biochem Behav 2009;92:413-23.

[68] dos Reis LM, Canto-de-Souza A. Intra-periaqueductal gray matter injections of midazolam fail to alter anxiety in plus-maze experienced mice. Brain Res 2008;1231:93-102.

[69] Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: an ethological perspective. Braz J Med Biol Res 1997;30:289-304.

[70] Benaroya-Milshtein N, Hollander N, Apter A, Kukulansky T, Raz N, Wilf A, et al. Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. Eur J Neurosci 2004;20:1341-7.

[71] Roy V, Chapillon P, Jeljeli M, Caston J, Belzung C. Free versus forced exposure to an elevated plus-maze: evidence for new behavioral interpretations during test and retest. Psychopharmacology 2009;203:131-41.

[72] Boissier JR, Simon P, Lwoff JM. Use of a Particular Mouse Reaction (Hole Board Method) for the Study of Psychotropic Drugs. Therapie 1964;19:571-83.

[73] Boissier JR, Simon P. 'Dissociation de deux composantes dans le comportement d'investigation de la souris'. Arch Int Pharmacodyn 1964;147:3-4.

[74]Rodriguez Echandia EL, Broitman ST, Foscolo MR. Effect of the chronic ingestion of chlorimipramine and desipramine on the hole board response to acute stresses in male rats. Pharmacol Biochem Behav 1987;26:207-10.

[75] Wei XY, Yang JY, Dong YX, Wu CF. Anxiolytic-like effects of oleamide in group-housed and socially isolated mice. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:1189-95.

[76] Zhu SW, Codita A, Bogdanovic N, Hjerling-Leffler J, Ernfors P, Winblad B, et al. Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice. Behav Brain Res 2009;197:339-46.

[77] File SE, Andrews N, Wu PY, Zharkovsky A, Zangrossi H,Jr. Modification of chlordiazepoxide's behavioural and neurochemical effects by handling and plus-maze experience. Eur J Pharmacol 1992;218:9-14.

[78] File SE. Factors controlling measures of anxiety and responses to novelty in the mouse. Behav Brain Res 2001;125:151-7.

[79] Redolat R, Gomez MC, Vicens P, Carrasco MC. Bupropion effects on aggressiveness and anxiety in OF1 male mice. Psychopharmacology 2005;177:418-27.

[80] Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol 1998;350:21-9.

[81] Pogun S, Collins AC, Nesil T, Kanit L. Oral Nicotine Slef-Administration in Rodents. Adicct Res Ther 2012, S2: In press.

[82] King SL, Caldarone BJ, Picciotto MR. Beta2-subunit-containing nicotinic acetylcholine receptors are critical for dopamine-dependent locomotor activation following repeated nicotine administration. Neuropharmacology 2004;47:132-9.

[83] Pauly JR, Sparks JA, Hauser KF, Pauly TH. In utero nicotine exposure causes persistent, gender-dependant changes in locomotor activity and sensitivity to nicotine in C57Bl/6 mice. Int J Dev Neurosci 2004;22:329-37.

[84] Heath CJ, Picciotto MR. Nicotine-induced plasticity during development: modulation of the cholinergic system and long-term consequences for circuits involved in attention and sensory processing. Neuropharmacology 2009;56:254-62.

[85] Adriani W, Macri S, Pacifici R, Laviola G. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. Neuropsychopharmacology 2002;27:212-24.

[86] Andreasen JT, Nielsen EO, Redrobe JP. Chronic oral nicotine increases brain (3H)epibatidine binding and responsiveness to antidepressant drugs, but not nicotine, in the mouse forced swim test. Psychopharmacology 2009;205:517-28.

[87] Gaddnas H, Pietila K, Piepponen TP, Ahtee L. Enhanced motor activity and brain dopamine turnover in mice during long-term nicotine administration in the drinking water. Pharmacol Biochem Behav 2001;70:497-503.

[88] Monleon S, Vinader-Caerols C, Arenas MC, Parra A. Antidepressant drugs and memory: insights from animal studies. Eur Neuropsychopharmacol 2008;18:235-48.

[89] Snedecor GW, Cochran WG. Statistical methods. Ames: The Iowa State University Press; 1980.

[90] Carvalho AF, Araujo AJ, Farias DF, Rocha-Bezerra LC, Cavalheiro MG. Development and reproductive performance of Swiss mice in an enriched environment. Braz J Biol 2009;69:153-60.

[91] Chapillon P, Manneche C, Belzung C, Caston J. Rearing environmental enrichment in two inbred strains of mice: Effects on emotional reactivity. Behav Genet 1999;29:41-6.

[92] Hughes RN, Collins MA. Enhanced habituation and decreased anxiety by environmental enrichment and possible attenuation of these effects by chronic alpha-tocopherol (vitamin E) in aging male and female rats. Pharmacol Biochem Behav 2010;94:534-42.

[93] Grabus SD, Martin BR, Batman AM, Tyndale RF, Sellers E, Damaj MI. Nicotine physical dependence and tolerance in the mouse following chronic oral administration. Psychopharmacology 2005;178:183-92.

[94] Tsai PP, Stelzer HD, Hedrich HJ, Hackbarth H. Are the effects of different enrichment designs on the physiology and behaviour of DBA/2 mice consistent? Lab Anim 2003;37:314-27.

[95] Andreasen JT, Redrobe JP. Antidepressant-like effects of nicotine and mecamylamine in the mouse forced swim and tail suspension tests: role of strain, test and sex. Behav Pharmacol 2009;20:286-295.

[96] Zambrana C, Marco EM, Arranz L, de Castro NM, Viveros MP, de la Fuente M. Influence of aging and enriched environment on motor activity and emotional responses in mice. Ann N Y Acad Sci 2007;1100:543-52.

[97] Rockman GE, Borowski TB, Glavin GB. The effects of environmental enrichment on voluntary ethanol consumption and stress ulcer formation in rats. Alcohol 1986;3(5):299-302.

[98] Rockman GE, Hall AM, Markert LE, Glavin GB. Influence of rearing conditions on voluntary ethanol intake and response to stress in rats. Behav Neural Biol 1988;49(2):184-91.

[99] Rockman GE, Gibson JE. Effects of duration and timing of environmental enrichment on voluntary ethanol intake in rats. Pharmacol Biochem Behav 1992;41(4):689-93.

[100] Wall PM, Messier C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behaviour. Neurosci Biobehav Rev 2001;25:275-86.

[101] Branchi I, Ricceri L. Refining learning and memory assessment in laboratory rodents. An ethological perspective. Ann Ist Super Sanita 2004;40:231-6.

[102] Pereira-da-Silva MS, Cabral-Filho JE, de-Oliveira LM. Effect of early malnutrition and environmental stimulation in the performance of rats in the elevated plus maze. Behav Brain Res 2009;205:286-9.

[103] Sztainberg Y, Chen A. An environmental enrichment model for mice. Nat Protoc 2010;5:1535-9.

[104] Zhu SW, Yee BK, Nyffeler M, Winblad B, Feldon J, Mohammed AH. Influence of differential housing on emotional behaviour and neurotrophin levels in mice. Behav Brain Res 2006;169:10-20.

[105]Brenes JC, Rodriguez O, Fornaguera J. Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. Pharmacol Biochem Behav 2008;89:85-93.

[106] Lin EJ, Choi E, Liu X, Martin A, During MJ. Environmental enrichment exerts sex-specific effects on emotionality in C57BL/6J mice. Behav Brain Res 2011; 216:349-57.

[107] Casarrubea M, Sorbera F, Crescimanno G. Structure of rat behavior in hole-board: I) multivariate analysis of response to anxiety. Physiol Behav 2009;96:174-9.

[108] Elliott BM, Faraday MM, Phillips JM, Grunberg NE. Adolescent and adult female rats differ in sensitivity to nicotine's activity effects. Pharmacol Biochem Behav 2005;80:567-75.

[109] Barbelivien A, Herbeaux K, Oberling P, Kelche C, Galani R, Majchrzak M. Environmental enrichment increases responding to contextual cues but decreases overall conditioned fear in the rat. Behav Brain Res 2006;169:231-8.

[110] Viola GG, Botton PH, Moreira JD, Ardais AP, Oses JP, Souza DO. Influence of environmental enrichment on an object recognition task in CF1 mice. Physiol Behav 2010;99:17-21.

[111] Chistyakov V, Patkina N, Tammimaki A, Talka R, Salminen O, Belozertseva I, et al. Nicotine exposure throughout early development promotes nicotine self-administration in adolescent mice and induces long-lasting behavioural changes. Eur J Pharmacol 2010;25;640:87-93.

[112] Bura SA, Burokas A, Martin-Garcia E, Maldonado R. Effects of chronic nicotine on food intake and anxiety-like behaviour in CB(1) knockout mice. Eur Neuropsychopharmacol 2010;20:369-378.

[113] Gaddnas H, Pietila K, Ahtee L. Effects of chronic oral nicotine treatment and its withdrawal on locomotor activity and brain monoamines in mice. Behav Brain Res 2000;113:65-72.

[114] Detrait ER, Hanon E, Dardenne B, Lamberty Y. The inhibitory avoidance test optimized for discovery of cognitive enhancers. Behav Res Methods 2009;41:805-11.

[115] Redolat R, Mesa-Gresa P. Potential Benefits and Limitations of Enriched Environments and Cognitive Activity on Age-Related Behavioural Decline. Curr Top Behav Neurosci 2012; 10:293-316.

[116] Nithianantharajah J, Hannan AJ. Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci 2006;7:697-709.

[117] Bennett JC, McRae PA, Levy LJ, Frick KM. Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. Neurobiol Learn Mem 2006;85:139-52.

[118] Harburger LL, Lambert TJ, Frick KM. Age-dependent effects of environmental enrichment on spatial reference memory in male mice. Behav Brain Res 2007;185:43-8.

[119] Lambert TJ, Fernandez SM, Frick KM. Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. Neurobiol Learn Mem 2005;83:206-16.

[120] Garcia-Capdevila S, Portell-Cortes I, Torras-Garcia M, Coll-Andreu M, Costa-Miserachs D. Effects of long-term voluntary exercise on learning and memory processes: dependency of the task and level of exercise. Behav Brain Res 2009;202:162-70.

[121] Kazlauckas V, Pagnussat N, Mioranzza S, Kalinine E, Nunes F, Pettenuzzo L, et al. Enriched environment effects on behavior, memory and BDNF in low and high exploratory mice. Physiol Behav 2011;102:475-80.

[122] Rezayof A, Shirazi-Zand Z, Zarrindast MR, Nayer-Nouri T. Nicotine improves ethanol-induced memory impairment: the role of dorsal hippocampal NMDA receptors. Life Sci 2010;86:260-6.

[123] Welzl H, Alessandri B, Oettinger R, Bäting K. The effects of long-term nicotine treatment on locomotion, exploration and memory in young and old rats. Psychopharmacology 1988;96:317-23.

[124] Bouet V, Freret T, Dutar P, Billard JM, Boulouard M. Continuous enriched environment improves learning and memory in adult NMRI mice through theta burst-related-LTP independent mechanisms but is not efficient in advanced aged animals. Mech Ageing Dev 2011;132:240-8.

[125] Pizzorusso T, Berardi N, Maffei L. A richness that cures. Neuron 2007;54:508-10.

[126] Vihavainen T, Piltonen M, Tuominen RK, Korpi ER, Ahtee L. Morphine-nicotine interaction in conditioned place preference in mice after chronic nicotine exposure. Eur J Pharmacol 2008;587:169-74.

[127] Gomez C, Carrasco C, Redolat R. Effects of bupropion, alone or coadministered with nicotine, on social behavior in mice. Addic Biol 2008;13:301-309.

[128] Moragrega I, Carrasco MC, Redolat R. Effects of housing and nicotine on shuttle-box avoidance in male NMRI mice. Behav Brain Res 2005;164:178-87.

[129] Redolat R, Vidal J, Gomez MC, Carrasco MC. Effects of acute bupropion administration on locomotor activity in adolescent and adult mice. Behav Pharmacol 2005;16:59-62.

[130] Freret T, Billard JM, Schumann-Bard P, Dutar P, Dauphin F, Boulouard M, Bouet V. Rescue of cognitive aging by long-lasting environmental enrichment exposure initiated before median lifespan. Neurobiol Aging. 2012 May;33(5):1005.e1-10.

[131] Sale A, Berardi N, Maffei L. Enrich the environment to empower the brain. Trends Neurosci 2009;32:233-9.

[132] Vieyra-Reyes P, Picciotto MR, Mineur YS. Voluntary oral nicotine intake in mice down-regulates GluR2 but does not modulate depression-like behaviors. Neurosci Lett 2008;434:18-22.

[133] Wilking JA, Hesterberg KG, Nguyen VH, Cyboron AP, Hua AY, Stitzel JA. Comparison of nicotine oral consumption and baseline anxiety measures in adolescent and adult C57BL/6J and C3H/lbg mice. Behav Brain Res. 2012;233(2):280-287.

[134] Pang TYC, Hannan AJ. Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. Neuropharmacology 2012;64:515-28.

CAPÍTULO 4

ESTUDIO 3

Efectos conductuales de la aplicación de distintos modelos de enriquecimiento ambiental en ratones tratados con el agonista colinérgico PNU-282987

Publicado en (véase ANEXO 9.6):

Mesa-Gresa P, Ramos-Campos M, Redolat R (2014). Behavioral effects of different enriched environments in mice treated with the cholinergic agonist PNU-282987. Behavioural Processes, 103, 117-124.

BEHAVIORAL EFFECTS OF DIFFERENT ENRICHED ENVIRONMENTS IN MICE TREATED WITH THE CHOLINERGIC AGONIST PNU-282987.

Abstract

Environmental enrichment is an experimental model in which rodents are housed in complex environments that favor lower levels of anxiety-like behavior. PNU-282987 (PNU) is a α7 nicotinic acetylcholine receptor agonist with beneficial effects on learning though its effects on anxiety are unclear. Our main aim was to carry out a study of its effects in NMRI (n=96) mice reared in different environments: Environmental Enrichment (EE), Marlau™ cages (MC) and standard environment (SE). After a 4-month period, mice received acute treatment of PNU (2.5, 5 and 10 mg/kg) and were evaluated in the elevated plus-maze (EPM) and hole-board (HB). In the EPM, both EE and MC reared mice showed an increase in percentage of entries into open arms while those from EE group differed from SE in time spent on open arms. Mice treated with 2.5 and 10 mg/kg of PNU devoted less time to rearing into open arms. In the HB task, MC mice displayed higher exploratory activity reflected in more head-dips (HD) during the first minute than EE and SE, whereas EE displayed low exploration levels reflected in total HD (5 min). Further research is needed in order to clarify the behavioral effects of this nicotinic agonist in interaction with different environmental conditions.

1. INTRODUCTION

Environmental enrichment is an experimental model that consists of housing animals in larger cages containing a variety of objects such as running wheels, toys, tunnels, houses and nesting material (Nithianantharajah and Hannan, 2009; Redolat and Mesa-Gresa, 2012). Animals housed in enriched environments receive higher levels of cognitive, physical, social and somatosensorial stimulation than those reared in standard conditions (Nithianantharajah and Hannan, 2011; Van Praag et al., 2000). It has been suggested that novelty and complexity are the defining characteristics of this type of environment (Pang and Hannan, 2013). Today, environmental enrichment is one of the most commonly employed paradigms for evaluating the effects of different housing conditions and their interaction with other experimental variables.

Exposure to enriched environments has been confirmed to induce different neurobiological and behavioral consequences, particularly an increase of brain size and cortical thickness (Eckert and Abraham, 2012; van Praag et al., 2000), enhanced neurogenesis in the dentate gyrus of the hippocampus (Kempermann, 2008; van Praag et al., 2000), and stimulation of synaptic plasticity (Leger et al., 2012; Nithianantharajah and Hannan, 2011). Behavioral effects reported after environmental enrichment include: improvements in learning and memory in hippocampal-dependent tasks (Leger et al., 2012; Redolat and Mesa-Gresa; 2012; Simpson and Kelly, 2011), lower levels of anxiety-like behavior in the elevated plus-maze (Sztainberg et al., 2010), faster habituation to new environments and reduced exploratory behavior (Brenes et al., 2009; van Praag et al., 2000; Viola et al., 2010). An increase in social behavior and agonistic responses has also been reported in some strains of mice as a consequence of environmental enrichment (Mesa-Gresa et al., 2013a; van Praag et al., 2000), although the results of published studies are contradictory in some cases (Abou-Ismail et al., 2010; Abramov et al., 2008). Variability in the characteristics and application of the environmental enrichment paradigm should be taken into account when evaluating the data obtained and discrepancies between the results of different studies (Redolat and Mesa-Gresa, 2012; Simpson and Kelly, 2012). One way of avoiding discrepancies between the approaches of different laboratories could be the standardization of enriched environments. Different

attempts have been made to regulate the main variables of these housing conditions (Sztainberg and Chen, 2010). Recently, Marlau^{TM} cages have been proposed as a more complex enriched environment including tunnels, ladders, running wheels and inter-changeable labyrinths. This complexity is intended to allow animals (mice or rats) to develop the behavior which is specific to their species (Fares et al., 2012; 2013).

Previous studies suggest that the behavioral effects of drugs can differ depending on the housing conditions in which animals are maintained (Bardo et al., 2013; El Rawas et al., 2009; Moragrega et al., 2005). In fact, there is currently great interest in how environmental enrichment modulates the effects of some drugs of abuse, such as cocaine (Solinas et al., 2010), amphetamines (Thiriet et al., 2011) or heroin (El Rawas et al., 2011), and how preclinical research with regard to this may lead to new therapeutic approaches to drug addiction (Solinas et al., 2008). Few studies, however, have evaluated the influence of exposure to enriched environments on the response to administration of nicotine or other nicotinic agonists (Adams et al., 2013; Mesa-Gresa et al., 2012; Solinas et al., 2010; Zhu et al., 2013). Previous research carried out to explore the interaction of environmental enrichment and nicotine treatment in rodents has reported that this housing condition increases the release of dopamine induced by nicotine in diverse brain areas (Zhu et al., 2012). Exposure of rats to enriched environments also diminishes sensitization to the effects of nicotine-induced motor hyperactivity (Coolon and Cain, 2009; Green et al., 2003) and results in lower levels of hypothalamic-pituitary-adrenal (HPA) axis response to nicotine administration and withdrawal (Skwara et al., 2012). Additionally, it has been shown that exposure to environmental enrichment can alter the molecular mechanisms related to the effects of nicotine (Gomez et al., 2012), but do not block the changes in structural plasticity induced by this drug in rats (Hamilton and Kolb, 2005).

Although reports have been published regarding the effects of nicotine on rodents reared in different housing conditions, few investigations have evaluated how the interaction of these two aspects modifies the behavior of experimental animals (Mesa-Gresa et al., 2012; Mesa-Gresa et al., 2013b, c). A recent study carried out in our laboratory explored the physiological and behavioral effects of chronic oral nicotine treatment (100 µg/ml of nicotine during one month) in NMRI mice maintained in different

housing conditions (Mesa-Gresa et al., 2013b). Nicotine reduced motor activity in the elevated plus-maze (EPM), but there was no significant interaction between housing conditions and nicotine administration. Mice allocated to environmental enrichment conditions displayed less motor and exploratory activity as well as anxiety-like behavior in the EPM. Furthermore, their performance of an inhibitory avoidance task was better than that of standard-housed mice. This prior evidence concerning the interaction between housing conditions and nicotinic acetylcholine receptor (nAChRs) agonists administration, together with a lack of data with respect to their behavioral profile, calls for new research which evaluates how subtle differences in the rearing environment can influence the behavioral response to these drugs. Exploring different doses of nicotine and routes of administration, duration of treatments or more selective nAChRs agonists in interaction with different housing conditions could yield new results about this question. In fact, several studies have analyzed the neurobiological, molecular, structural and functional effects of exposure to nicotine or nicotinic agonists. Clinical and pre-clinical studies have confirmed the important role played by the cholinergic system in attention, learning and memory (Levin et al., 2012). However, results obtained in studies evaluating the effects of more specific nicotinic agonists on stress and anxiety responses are not conclusive (Pandya and Yakel, 2013; Vicens et al., 2013a, b). More specifically, the alpha7 (α7) nAChRs subtype receptor seems to display an important role in the neuroprotective effects of nicotine that seems to be related with the cognitive, motor and sensorial effects of this drug (Hunter et al., 2012; Welch et al., 2013). However, recent studies about the molecular and behavioral actions of different α7 nAChRs are also inconclusive (Thomsen et al., 2010; Vicens et al., 2013b). PNU-282987 (PNU) is a high-affinity full agonist of α7 nAChRs which has been shown to have beneficial effects on cognitive and learning tasks in rodents (Redrobe et al., 2009). Vicens et al. (2011) have reported that PNU (5 mg/kg) decreases motor and exploratory activity in the open field test in C57BL/6J mice when administered acutely or sub-chronically. Regarding cognitive effects, research shows that sub-chronic treatment with low and medium doses (1 and 3 mg/kg) improves acquisition, but not retention, of spatial learning in the Morris water maze. In a recently published study using an animal model of Alzheimer's disease, Vicens et al. (2013a) evaluated motor activity and anxiety-like behavior in open-field and zero-maze tasks. They confirmed that the effects induced by chronic stress were inverted by an acute

treatment of PNU, though no effects were observed in the control group. In contrast, Pandya and Yakel (2013) indicated that a higher dose of PNU (10 mg/kg) induced anxiogenic effects in male rats when their behavior was assessed in an open field. In this study, no significant effects of different doses of PNU (1, 3, 10 mg/kg) on memory or motor coordination tasks were observed.

Although previous research indicates that rearing conditions modulate physiological and behavioral responses to nicotine or nicotinic agonist, no study has evaluated changes in the behavior in rodents reared in different environments after administration of the cholinergic agonist PNU. As a cholinergic agonist with possible neuroprotective properties, the effects of this drug on memory and learning tasks have been evaluated in prior studies, but its effects on other behavioral tasks should also be assessed in order to obtain a more complete behavioral profile of this α7 nAChRs agonist. Until now, few reports have been published and there is a lack of data concerning effects of this drug on exploratory behavior and emotional responses.

In light of the current state of knowledge, the main aim of the present study was to evaluate the behavioral effects of PNU on anxiety-like behavior and exploratory behavior in NMRI male mice and their possible modulation by different housing conditions. Such data could be of use in outlining more clearly the behavioral effects of α7 nAChRs agonists and differences between the diverse models of environmental enrichment that exist. Moreover, the use of different types of enriched environments could shed light on the similarities or differences between these models and their potential applications.

2. MATERIALS AND METHODS

2.1. Animals

Ninety six male NMRI mice (Charles-River, Barcelona, Spain) were used in the current study. The animals arrived at our laboratory on post natal day (PND) 21 (mean weight: 10-12 gr.) and were housed in groups of 4 in a controlled environment (temperature 20-24º and humidity 55±10%) with a 12h light/dark cycle (lights on: 8

hr). On PND 28, mice were randomly allocated to one of three different housing conditions, at which point the experimental procedure began. All animals were allowed free access to food and water. Experimental procedures were approved by the ethical committee of the Universitat de of València and complied with the "Guidelines for the use of animals in research" (Animal Behaviour, 1991; 41, 183-186) and with national (Real Decreto 1201/2005, de 10 de Octubre) and international guidelines (European Directive 2010/63/EU) for the care and handling of animals.

2.2. Drugs

PNU-282987 was obtained from Sigma-Aldrich (Madrid, Spain). It was dissolved in saline (9%) and administered intraperitoneally at doses of 2.5, 5 and 10 mg/kg, 30 min before the behavioral tests. Doses were selected based on pilot studies in our laboratory and previous research about the behavioral effects of this drug (Vicens et al., 2011). Control groups received physiological saline as a vehicle (VEH).

2.3. Housing conditions

During the experimental procedure, animals were randomly allocated to one of three different housing conditions: 1) Standard Environment (SE), in which they were housed in groups of four in standard cages (42x26x14 cm) with only sawdust (n=32); 2) Environmental enrichment cages (EE), in which mice were housed 8 per cage in larger cages (55x36x19 cm) with fixed objects as a house, a running wheel, a tunnel and five different toys that were changed twice a week (n=32) (for more detailed information see Mesa-Gresa et al., 2013b); and 3) Marlau^{TM} Cages (MC), which has recently been proposed as a new model of enriched environment for rodents (Fares et al., 2012; 2013), in which mice are housed 16 per cage (n=32) in a large two-storey cage (59.5x39x32 cm) with running wheels, labyrinths, tunnels and a climbing ladder. Objects included in this cage are fixed, except labyrinths that were changed three times a week. These complex cages are composed of two floors (first floor contains running wheels, tunnels and food and water access; the second floor contains the

labyrinth). The two floors are connected by a climbing ladder and a tunnel (more specific information can be obtained in Fares et al., 2012). Sawdust was changed once a week for all the different housing conditions.

In each housing condition (SE, EE and MC), sub-groups of mice received different pharmacological treatments (PNU2.5, PNU5, PNU10 or VEH). In this way, we established a total of twelve different experimental groups named according to their housing condition and pharmacological treatment: SE-VEH, SE-PNU2.5, SE-PNU5, SE-PNU10, EE-VEH, EE-PNU2.5, EE-PNU5, EE-PNU10, MC-VEH, MC-PNU2.5, MC-PNU5 and MC-PNU10.

2.4. Procedure

Mice underwent a battery of behavioral tests on PND 120. Thirty minutes after acute administration of PNU, mice were exposed to EPM and, immediately after, to the HB test. This schedule was established based on the sensitivity of the EPM and HB test to factors such as the prior manipulation of animals (Deacon et al., 2006; Mesa-Gresa et al., 2013b; Paylor et al., 2006). Thirty min before each behavioral test, mice were transported to the testing room in order to become habituated to it and received the designated drug treatment. Test apparatuses were cleaned before the first test began and between animals with a solution containing diluted ethanol (2%). During behavioral testing, a researcher remained in the room outside the scope of the animals' vision in order to avoid interferences in their behavior.

2.4.1. Elevated plus-maze

The EPM was constructed of Plexiglas and was placed at a height of 45 cm from the floor. It has two closed or protected arms opposite to two open or unprotected arms which are 30 cm long and 5 cm wide, with a central platform (5x5 cm) in the intersection of open and closed arms. The two closed arms are considered protected zones. At the beginning of each session, each animal was positioned on the central platform facing an open arm and was permitted to explore the maze freely for 5 min.

Its behavior was videotaped and analysed by a blind observer to the experimental conditions using a computerized program in order to evaluate both classic and ethological measures. Main classic parameters considered were the number of entries into protected and unprotected arms and into the central platform, total entries (open and closed arms), time spent in open and closed arms, percentage of open entries and percentage of time spent in open and closed arms and in central platform; whereas ethological measures were rearing, head-dipping (exploratory movement of the head and neck in the direction of the bottom of the maze), stretched attend posture (SAP) (exploratory behavior, in which the animal stretches its body forward and then returns to its original position without any forward locomotion), peeping-out (exploratory behavior in which the animal stretches its head into the central platform while standing on a closed arm), and end-arm maze (number of times the mice reaches the end of the open arms). Entries into the open and closed arms were considered when mice placed all four paws on the arm (Roy et al., 2009). Ethological analysis was carried out according to that described by other authors for evaluating risk assessment and more specific behaviors (Carrasco et al., 2006; dos Reis and Canto-de-Souza, 2008; Mesa-Gresa et al., 2013b; Rodgers et al., 1997).

2.4.2. Hole board

The apparatus employed in the current study (Cibertec, Barcelona, Spain) was an acrylic black board (31.5x31.5x20.5 cm) with 16 holes (hole diameter: 2 cm; distance between holes: 5 cm). The hole sensors were situated at a depth of 1 cm and automatically recorded the number of head-dips (HD) performed by animals. At the beginning of each session (5 min), mice were placed in the central area of the HB. The following parameters were analysed: 1) latency to the first head-dip (measured by the experimenter); 2) total number of head-dips during the first minute (HD min 1); and 3) total number of head-dips by the end of the test (5 minutes) (HD min 5).

2.5. Statistical analysis

All statistical analyses were performed using IBM® SPSS® Statistics 19.0 (IBM corp, NY, USA) for Windows. Behavioral data obtained in the EPM and in the HB tests were

analyzed by ANOVA using “Housing conditions” and “PNU Treatment” as between-subject factors. Post-hoc comparisons were conducted when appropriate using the Tukey HSD test. Significance levels were set at $p<0.05$. Pearson’s correlation coefficient between the variables of “HD min 5” obtained in the HB and “percentage of open entries” measured in the EPM was obtained, in order to determine a possible relation in the activity displayed by animals in these behavioral tests. Data are presented as mean ± standard error of the mean (SEM).

3. RESULTS

3.4. Elevated plus-maze

In relation to classic measures, ANOVA showed a statistically significant effect of Housing conditions on several behavioral categories. This effect reached significance in open-arm entries [$F(2,62)=6.09$, $p<0.01$]. A post-hoc Tukey test revealed that animals allocated to EE ($p<0.05$) and MC ($p<0.01$) cages performed a higher number of open-arms entries than SE mice. This variable was also significant for central platform entries [$F(2,92)=3.20$, $p<0.05$] and total entries [$F(2,92)=3.19$, $p<0.05$]. A post-hoc test indicated that MC mice performed more central platform entries ($p=0.05$) and total entries than mice reared in SE ($p<0.05$). No significant differences in these categories were observed between EE and MC groups, or between EE and SE groups (See Figure 1). Differences were also observed in the percentage of open-arm entries [$F(2,92)=6.30$, $p<0.01$]. A post-hoc test showed that EE- and MC-housed mice performed a higher percentage of open-arm entries than SE mice ($p<0.01$ and $p<0.05$, respectively) (See Figure 2). Housing conditions also produced differences in the time spent in the open arms [$F(2,92)=6.32$, $p<0.01$] and percentage of time in the open arms [$F(2,92)=6.32$, $p<0.01$]. A post-hoc Tukey test indicated that animals allocated to EE conditions spent significantly more time and a higher percentage of time in the open arms than mice housed in SE ($p<0.01$) (See Figure 3). In the same way, statistically significant differences were detected in time [$F(2,92)=5.33$, $p<0.01$] and percentage of time in the closed arms [$F(2,92)=5.33$, $p<0.01$]. Post-hoc tests indicated that mice in the

EE group spent less time ($p<0.05$) and displayed a lower percentage of time in the closed arms ($p<0.05$) than SE and MC housed mice. PNU treatment and the interaction between Housing conditions and PNU treatment were not significant for any of the classic behavioral categories measured in the EPM (See Figure 1). These data are summarized in Table 1.

Regarding ethological measures, results indicated that the main factor Housing conditions was statistically significant for different measures. The total number of head-dips reached statistical significance [$F(2,92)=18.69$, $p<0.0001$]; a post-hoc test showed that mice allocated to EE and MC conditions performed a higher total number of head-dips ($p<0.0001$) than SE mice. With respect to the percentage of protected SAP, results indicated that the factor Housing conditions [$F(2,92)=6.33$, $p<0.01$] produced significant differences in this variable. Post-hoc tests indicated that EE and MC mice exhibited lower percentages of this behavior that SE mice ($p<0.01$). The behavioral variable of total number of rearings was also statistically significant [$F(2,92)=5.05$, $p<0.01$]. A post-hoc test revealed a higher total number of rearings by MC mice than by EE ($p<0.01$) or SE mice ($p<0.05$). The variable of time spent on peeping-out behavior also reached statistical significance [$F(2,92)=10.79$, $p<0.0001$]; a post-hoc Tukey test showed that EE and MC mice spent less time in this behavior than SE mice ($p<0.01$). In terms of PNU treatment, the time each group engaged in rearing behavior in the open arms reached statistical significance [$F(3,91)=3.603$, $p<0.05$]. A post-hoc test indicated that mice treated with PNU doses of 2.5 and 10 mg/kg allocated less time to rearing behavior than VEH-treated mice ($p<0.05$). If we notice Table 1, some interesting observations appear in some classical measures of anxiety (values of % open time and % open entries). There seems to be a U inverted shape regarding the effect of treatment in both variables since those animals treated with the intermediate dose displayed an increase in these measures, although the differences did not reach statistical significance. In the behavioral variable of rearing into open arms, MC reared mice treated with VEH displayed more time to this behavior than groups treated with PNU ($p<0.01$). When the frequency of protected head-dips were considered, it was observed that in the group of EE mice, those treated with VEH displayed more head-dips than those treated with PNU at doses of 5 and 10 mg/kg ($p<0.05$). There were also an interaction between Housing conditions and PNU treatment

when grooming frequency in closed arms was observed: in SE-reared mice, sub-groups treated with PNU 5 and PNU 2.5 displayed more grooming than VEH-treated ($p<0.01$).

3.5. Hole Board

Neither Housing conditions nor PNU treatment reached significance in the variable "Latency to the first head-dip". When the variable "Total number of head-dips at min 1" was considered, an ANOVA revealed that the effect of Housing conditions was significant [$F(2,92)=22.05$, $p<0.0001$)]. Post-hoc Tukey tests revealed that mice allocated to MC cages performed a higher total number of head-dips during the first minute of exposure to the HB than mice housed in EE ($p<0.0001$) or SE conditions ($p<0.0001$). Regarding the variable "Total number of head-dips at min 5", data revealed statistical differences for Housing conditions [$F(2,92)=10.44$, $p<0.0001$]. Mice in the group EE performed less head-dips than SE animals ($p<0.0001$) and MC-housed mice ($p<0.0001$). Neither the factor PNU treatment nor the interaction Housing conditions and PNU treatment was statistically significant for any of the variables evaluated in the HB test (See Figure 4).

Pearson's correlation coefficient between the variables of "HD min 5" obtained in the HB task and "percentage of open entries" measured in the EPM was analysed for the experimental groups of MC, EE and SE. None of these correlations reached statistical significance.

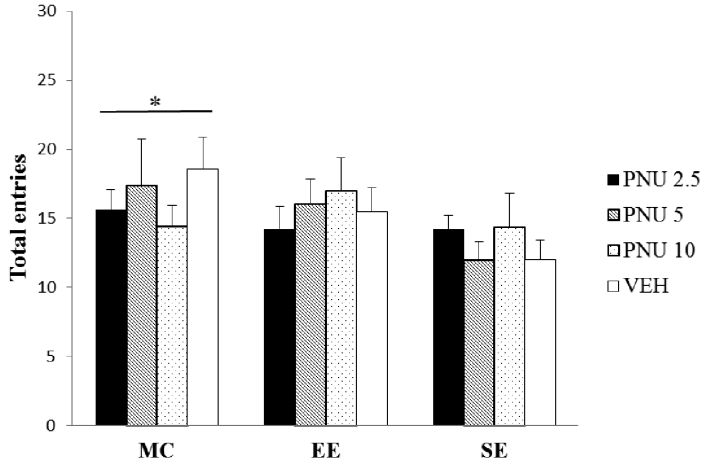

Figure 1. Total entries (open+closed arms) in the elevated plus maze test in each housing condition (MC, EE and SE) and sub-groups of mice that received different pharmacological treatments (PNU2.5, PNU5, PNU10 or VEH). Data are presented as mean ± SEM.
(*) p <0.05 MC vs SE

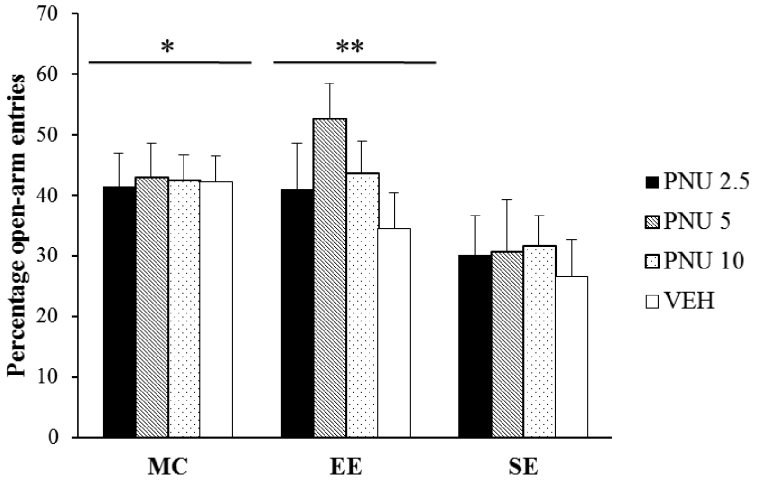

Figure 2. Percentage of entries into open arms in the elevated plus maze test in each housing condition (MC, EE and SE) and sub-groups of mice that received different pharmacological treatments (PNU2.5, PNU5, PNU10 or VEH). Data are presented as mean ± SEM.
(*) p <0.05 MC vs SE
(**) p <0.01 EE vs SE

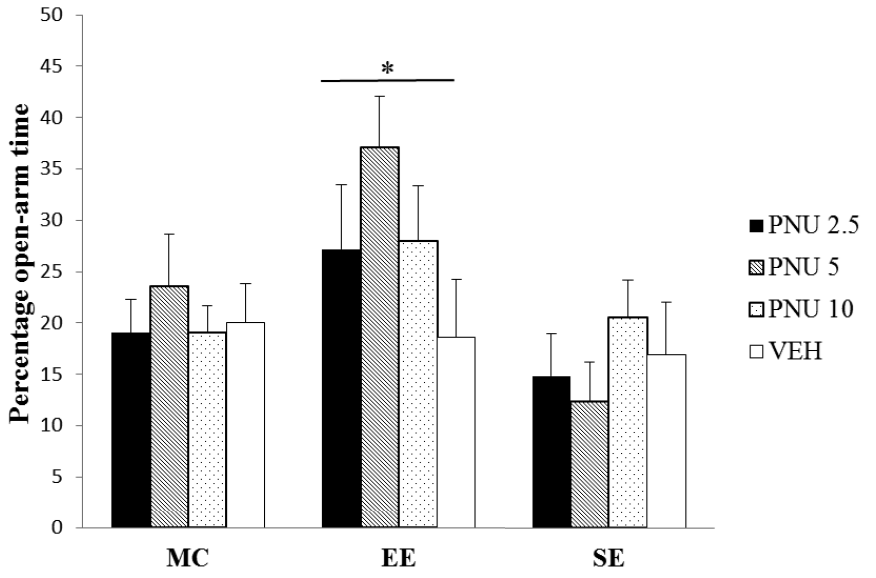

Figure 3. Percentage of time spent in the open arms in the elevated plus maze test in each housing condition (MC, EE and SE) and sub-groups of mice that received different pharmacological treatments (PNU2.5, PNU5, PNU10 or VEH). Data are presented as mean ± SEM.
(*) p <0.01 EE vs SE

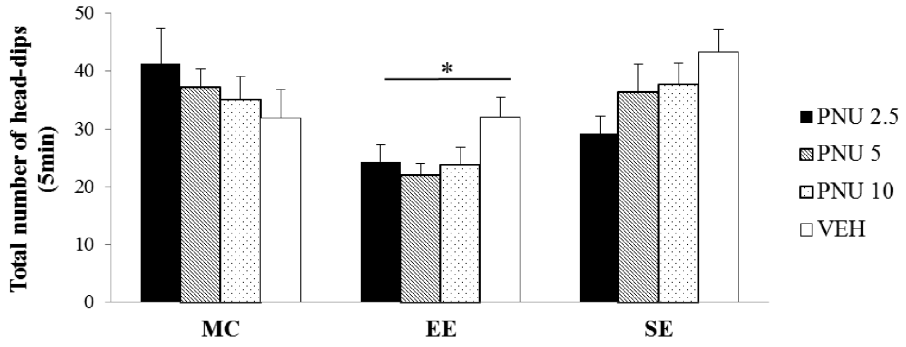

Figure 4. Total number of head-dips by the end of the test (5 minutes) in the hole board test in each housing condition (MC, EE and SE) and sub-groups of mice that received different pharmacological treatments (PNU2.5, PNU5, PNU10 or VEH). Data are presented as mean ± SEM.
(*) p <0.0001 EE vs SE; EE vs MC

Table 1. Effects of housing in environmental enrichment (EE), Marlau™ cages (MC) or standard environment (SE) on the behavior displayed in the elevated plus-maze by male NMRI mice under acute treatment with PNU-282987 at different doses (PNU 2.5 mg/kg, PNU 5 mg/kg, PNU 10 mg/kg) or saline (VEH).

	EE				MC				SE			
BEHAVIORAL CATEGORIES	**PNU2.5**	**PNU5**	**PNU10**	**VEH**	**PNU2.5**	**PNU5**	**PNU10**	**VEH**	**PNU2.5**	**PNU5**	**PNU10**	**VEH**
TOTAL ENTRIES	14.25±1.59	16±1.84	17±2.4	15.5±1.73	15.62±1.5	17.37±3.36	14.62±1.31	19±2.66	14.25±0.99	12±1.28	14.33±2.51	12±1.41
OPEN ENTRIES	6±1.3	8.5±1.41	7.75±1.54	5.87±1.33	6.75±1.13	8.62±3.1	6.37±0.98	8.5±1.87	4.37±1.11	3.87±1.14	5±1.21	3.25±0.98
CLOSED ENTRIES	8.25±1.32	7.5±1.09	9.25±1.25	9.62±0.92	8.87±0.81	8.75±0.94	8.25±0.82	10.5±0.96	8.75±1.11	8.12±0.97	9.33±1.49	8.75±1.11
% OPEN ENTRIES	40.93±7.75	52.62±5.83	43.67±5.34	34.47±5.87	41.47±5.347	42.92±5.75	42.53±4.2	42.24±4.19	30.09±6.56	30.7±8.59	31.62±5.06	26.61±6.1
% OPEN TIME	27.22±6.23	37.12±4.94	27.97±6.23	18.57±5.65	19.04±3.3	23.53±5.1	17.73±2.37	21.25±4.27	14.81±4.11	12.26±3.87	20.53±3.61	16.86±5.12
% CENTER TIME	39.53±5.11	30.33±3.81	39.58±4.2	45.18±3.5	34.88±2.57	38.57±4.06	41.76±3.86	34.59±1.69	46.54±2.44	44.12±3.35	38.44±3.43	43.77±4.33
TOTAL HD	27.5±3.48	37.37±3.9	27±4.36	30±4.35	26.25±3.29	29.37±5.17	27.37±2.43	26.83±3.1	17.87±3.7	15.62±2.24	18.5±2.15	12.55±2.69
% p HD	48.6±9.09	30.6±5.86	44.46±8.05	64.47±7.88	57.67±5.88	57.96±7.44	54.48±5.36	56.39±6.25	68.31±8.52	57.26±13.35	61.1±10.24	45.78±10.57
TOTAL SAP	16.5±10.09	15.75±9.62	12.37±8.43	11.75±9.05	12.25±9.21	13.87±7.97	11.12±40.06	13±10.37	14.37±8.29	15.5±6.19	13.75±11.84	11.22±6.22
% p SAP	61.42±10.09	41.05±9.62	46.98±8.43	70.27±9.05	50.66±9.21	50.69±7.97	59.46±4.06	57.64±10.37	79.17±8.29	80.42±6.19	52.63±11.84	79.8±6.21
TOTAL REARS	12±2.56	13.5±1.88	12.62±3.39	12.5±1.99	18.37±3.27	15.37±2.63	16.62±2.64	21.67±3.66	13.87±1.44	15±2.3	11.75±2.05	14.22±2.66
PEEPING-OUT	13.62±2.09	9.75±1.32	10.87±1.43	13.12±0.93	13.37±1.1	15.37±1.88	13.12±0.81	15.33±1.63	16±1.46	13.75±1.16	12.25±1.21	13.22±1.67
ACTIVITY END MAZE	4.12±1.23	7.62±1.99	4±1.03	3±1.02	6.12±1.25	5.37±1.27	3.37±0.53	2.67±1.36	2.37±1.08	1.62±0.65	3.87±1.22	2.89±1.25

Data are presented as mean values ± SEM.

Abbreviations: HD: head-dip; SAP: stretched attend posture; % p: percentage protected.

4. DISCUSSION

In the present study, we have evaluated the behavioral effects of the α7 nAChRs agonist PNU in mice reared in various environments. Responses to the acute administration of this drug were compared in different groups of NMRI male mice maintained in three different housing conditions (standard housing; a model of enriched environment developed in our laboratory and the more complex Marlau™ cage). To our knowledge, this is the first study that combines environmental manipulation of housing conditions and administration of a specific α7 nAChRs agonist in the same group of mice. The stand-out data of the current study suggest that housing mice under EE conditions decreases anxiety-like behavior and diminishes exploratory behavior, while allocating animals to Marlau™ cages induces an increase in exploratory behavior in the HB and greater motor activity in the EPM.

The EPM is a task mainly used to explore anxiety-like behavior in preclinical studies, as it is sensitive to both behavioral and pharmacological manipulations (Elliott and Grunberg, 2005; Roy et al., 2009; Wall and Messier, 2001). The results obtained in the current study indicate that rearing animals in an enriched environment diminishes anxiogenic-like behavior. EE-reared mice also showed a higher percentage of entries and time spent in the open arms than standard-housed mice, which is suggestive of a decrease in anxiety-like behavior (Roy et al., 2009). Ethological analysis of the behavioral response displayed by mice during the 5 min of the test supports such an EE-induced anxiolytic-like profile (Roy et at., 2009). The main ethological categories (SAP, head-dips and peeping-out behaviors) have been evaluated previously as measures of risk assessment behavior (dos Reis and Canto-de-Souza, 2008; Mesa-Gresa et al., 2013b; Roy et al., 2009). In the current study, a decrease in peeping-out behavior and in the percentage of protected SAP was observed in EE and MC-reared mice in comparison with SE mice. These changes were accompanied by an increase in the total number of head-dips displayed in the EPM. This profile with respect to both classic and ethological measures supports the well-established anxiolytic effect induced by enriched environments (Fox et al., 2006; Mesa-Gresa et al., 2013b; Ragu Varman et al., 2013; Sztainberg et al., 2010; Sztainberg and Chen, 2010). However, the

reasons for this observed decrease in anxiety-like behavior are not totally clear. One possible interpretation is that this type of enriched environment offers greater possibilities for physical exercise than a standard environment, which could reduce the level of anxiety-like behavior (Ragu Varman et al., 2013). On the other hand, it can be suggested that weekly changes of the toys in the EE cages (twice a week) may act as a mild repeated stressor for the animals that influence adaptation to subsequent situations of stress (such as exposure to the EPM). For example, prior studies have observed that exposure to enriched environments may counteract the main effects produced by maternal separation (higher levels to corticosterone and behavioral response to stress) in rodents (Francis et al., 2002).

At a behavioral level, some of the contradictory changes observed when animals are reared in enriched environments could be related to the different types of paradigms employed (Mesa-Gresa et al., 2013c; Simpson and Kelly, 2012; Solinas et al., 2008; van Praag et al., 2000). Although a recent study reported a clear anxiolytic effect after exposure to Marlau^{TM} cages (Fares et al. 2013), the effects of this type of housing were not so conclusive regarding anxiety-like behavior in the current study. In the EPM, we observed that animals allocated to MC displayed a significant increase in the percentage of entries into open arms and total number of head-dips as well as a decrease in the percentage of protected SAP when compared to SE mice. This behavioral profile could be interpreted as anxiolytic-like behavior. It is important to consider that MC-reared mice did not obtain significant differences with standard mice for the categories related to the time spent in each part of the maze. However, variations in classical variables (increased entries in central platform and total entries) and ethological measures (increased head-dips, rearing or peeping-out behaviors) in MC mice could be related to an increase in motor activity (Roy et al., 2009). These variations may also be related to the high levels of activity displayed by these animals in their own cages (Fares et al., 2012). While results obtained in mice allocated to EE clearly point to a decrease on anxiety-like behavior, data for MC mice also show possible effects on motor activity. Such hyperactivity could explain why MC mice performed a significantly higher percentage of entries into the open arms and that this measure did not correlate with the percentage of time spent in this area of the maze.

However, if we directly compare the behavioral profile between animals allocated to the two types of enriched environments (EE and MC) no clear differences appear in risk assessment behaviors (peeping-out behavior and percentage of protected SAP). These results suggest that although the two housing conditions may have anxiolytic effects, there are subtle differences between them. The only significant difference between the two housing conditions (MC and EE) was obtained in total rearing, a measure which reflects higher motor activity in the maze for MC group.

The HB test is a classic task based on exposing animals to a non-familiar environment. Depending on the paradigm employed, exploratory behavior, response to stress, anxiety-like behavior, novelty-seeking and memory have been evaluated (Boissier et al., 1964; Casarrubea et al., 2009; Redolat et al., 2009; Simpson and Kelly, 2011; Zhu et al., 2009). In the current study, we have observed that EE clearly reduces exploratory behavior, in contrast with results obtained for animals allocated to MC or SE conditions. These results are in accordance with those reported in prior studies evaluating habituation to new environments and changes in exploratory response in mice reared in EE (Brenes et al., 2009). Recent research carried out in our laboratory indicated that exposure of NMRI mice to EE conditions for 4 weeks was enough to induce a decrease in exploratory behavior and motor activity (Mesa-Gresa et al., 2013b). Previously published data has also shown a decrease in locomotor activity in animals exposed to EE conditions (Zambrana et al., 2007; Zhu et al., 2009). This decrease in motor and exploratory behavior in enriched-reared animals has been attributed to faster habituation to the new environment and better knowledge of it (Viola et al., 2010; Zimmermann et al., 2001) accompanied by higher contextual processing (Barbelivien et al., 2006). In contrast with their EE counterparts, MC-reared mice displayed higher levels of exploration and motor activity during the first minute of the HB test than EE- and SE- housed mice, and the level of this activity did not wane significantly during the rest of the task (5 min). This slower habituation rate could be related, as previously reported, to the higher levels of activity which MC-reared mice usually display in their own cages. Furthermore, the higher number of animals allocated to each cage (n=16) is likely to hinder a high level of activity in terms of reduced access to the resources and activity accessories available in the cage. Fares et

al. (2013) observed that rats allocated in Marlau^{TM} cages displayed a higher level of activity immediately after the maze change (which occurs three times a week). It is important to consider that the main characteristic of this type of cage is that food pellets and water bottles are located in separated compartments, and the only way animals can access them is through the maze allocated in the top of the cage. For that reason, the design of these cages seems to prompt to these animals displaying high levels of exploratory behavior (Fares et al., 2013). The hyperactivity observed during the first minute of the HB task in MC group would also support the possible interpretation about the increase in motor activity established previously for data obtained in the EPM, although correlation between the variables of "HD min 5" obtained in the HB and "percentage of open entries" measured in the EPM did not reach statistical significance. Despite of these results contrast with those that show a reduction of motor activity in rodents housed under enriched conditions (Mesa-Gresa et al., 2013b; Zhu et al., 2009), the discrepancy between studies could be attributed to different reasons. In fact, in our prior study motor activity was measured directly with an actimeter, whereas in the present one only indirect measures were taken. In addition to the different paradigms employed in each study, the species or strain of rodents used, sex of the animals, level of activity displayed by rodents, complexity of the environment or behavioral tests analyzed could modify the results obtained (Abramov et al., 2008; Lin et al., 2011; Simpson and Kelly, 2011, 2012).

One of the main aims of the current study was to evaluate behavioral effects of pharmacological treatment with PNU in interaction with the type of housing environment. In terms of the effects of PNU on anxiety-like behavior, the only difference observed for the main factor Drug Treatment was in the number of rearings displayed by mice in the open arms of the EPM. Alterations in this behavior are difficult to interpret and could be related to the locomotor effects of the drug administered. Prior research carried out by Vicens et al. (2011) found that a high dose of PNU (5 mg/kg) decreased motor activity in mice in the open-field test whereas these researchers did not obtain conclusive results for anxiety-like behavior. However, in another study carried out recently by Pandya and Yakel (2013), rats treated with 10 mg/kg were displayed an anxiogenic-like response in the open field test. Different

reports have suggested that the effects of nicotine on anxiety are dose-dependent (Pandya and Yakel, 2013); in this way, an anxiogenic response is induced with high doses and the opposite response with lower doses.

In the HB task, neither the main factor PNU treatment nor the interaction with Housing conditions reached statistical significance for any of the variables considered. Prior studies that have evaluated the effects of this cholinergic agonist in relation to exploratory behavior have obtained discrepant results. Redrobe et al. (2009) observed that a treatment with 10 mg/kg of PNU attenuated the scopolamine-induced effects on exploratory behavior in rats and mice exposed to an unfamiliar maze. Doses and time of administration should be taken into account when evaluating results obtained in mice treated with α7 nAChRs agonists. More recently, Vicens et al. (2013a) failed to observe changes in the behavioral consequences after acute treatment with a low dose of PNU (1 mg/kg) in transgenic mice exposed to stress, but not in control animals. These results, and those obtained in the current study, underlie the difficulty of determining the optimal dose for treatments with this nicotinic agonist. Taking into account the lack of a clear knowledge about the behavioral profile of this α7 nAChRs agonist, more studies are needed in order to clarify its effects at different doses and according to acute, sub-chronic and/or chronic treatment and applying different behavioral models (zero-maze test for anxiety assessment, social interaction test, direct measures of motor activity or cognitive tasks).

In the current study, behavioral effects of the exposure to enriched environments were similar to those reported in prior studies. We consider that the two housing conditions compared in the current research may diminish anxiety-like behavior, although new studies with longer periods of housing and taking into account ethological measures and direct measures of motor activity may aid to confirm the subtle differences between EE and MC. On the other hand, effects of acute administration of PNU were few and showed some interaction with the type of housing conditions. For that reason, future studies are needed in order to evaluate the behavioral effects of a broader range of doses and the response to other nicotinic agonists administered during longer periods of time in order to observe if behavioral

differences are maintained over time. These effects need to be compared in groups of mice maintained in different housing conditions.

Further research should seek to establish the possible applications and extrapolations of results obtained in different paradigms of environmental enrichment. The possible interaction between environmental and pharmacological manipulations with nicotinic agonists could lead to new therapeutic possibilities in different fields as aging, addiction, traumatic brain injury or neurodegenerative disorders, etc (Gomez et al., 2012; Nithianantharajah and Hannan, 2009; Redolat and Mesa-Gresa, 2012; Solinas et al., 2010).

REFERENCES

Abou-Ismail, U. A., Burman, O. H., Nicol, C. J., Mendl, M., 2010. The effects of enhancing cage complexity on the behaviour and welfare of laboratory rats. Behav Processes. 85:172-80.

Abramov, U., Puussaar, T., Raud, S., Kurrikoff, K., Vasar, E., 2008. Behavioural differences between C57BL/6 and 129S6/SvEv strains are reinforced by environmental enrichment. Neurosci Lett. 443:223-7.

Adams, E., Klug, J., Quast, M., Stairs, D. J., 2013. Effects of environmental enrichment on nicotine-induced sensitization and cross-sensitization to d-amphetamine in rats. Drug Alcohol Depend. 129:247-53.

Barbelivien, A., Herbeaux, K., Oberling, P., Kelche, C., Galani, R., Majchrzak, M., 2006. Environmental enrichment increases responding to contextual cues but decreases overall conditioned fear in the rat. Behav Brain Res. 169:231-8.

Bardo, M. T., Neisewander, J. L., Kelly, T. H., 2013. Individual differences and social influences on the neurobehavioral pharmacology of abused drugs. Pharmacol Rev. 65:255-90.

Boissier, J. R., Simon, P., Lwoff, J. M., 1964. Use of a particular mouse reaction (hole board method) for the study of psychotropic drugs. Therapie. 19:571-83.

Brenes, J. C., Padilla, M., Fornaguera, J., 2009. A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. Behav Brain Res. 197:125-37.

Carrasco, M. C., Vicens, P., Vidal, J., Redolat, R., 2006. Effects of co-administration of bupropion and nicotinic agonists on the elevated plus-maze test in mice. Prog Neuropsychopharmacol Biol Psychiatry. 30:455-62. doi:10.1016/j.pnpbp.2005.11.018

Casarrubea, M., Sorbera, F., Crescimanno, G., 2009. Structure of rat behavior in hole-board: I) multivariate analysis of response to anxiety. Physiol Behav. 96:174-9.

Coolon, R. A., Cain, M. E., 2009. Effects of mecamylamine on nicotine-induced conditioned hyperactivity and sensitization in differentially reared rats. Pharmacol Biochem Behav. 93:59-66.

Deacon, R. M., 2006. Housing, husbandry and handling of rodents for behavioral experiments. Nat Protoc. 1:936-46.

dos Reis, L. M., Canto-de-Souza, A., 2008. Intra-periaqueductal gray matter injections of midazolam fail to alter anxiety in plus-maze experienced mice. Brain Res. 1231:93-102.

Eckert, M. J., Abraham, W. C., 2012. Effects of environmental enrichment exposure on synaptic transmission and plasticity in the hippocampus. Curr Top Behav Neurosci. In press.

El Rawas, R., Thiriet, N., Lardeux, V., Jaber, M., Solinas, M., 2009. Environmental enrichment decreases the rewarding but not the activating effects of heroin. Psychopharmacology (Berl). 203:561-70.

El Rawas, R., Thiriet, N., Nader, J., Lardeux, V., Jaber, M., Solinas, M., 2011. Early exposure to environmental enrichment alters the expression of genes of the endocannabinoid system. Brain Res. 1390:80-9.

Elliott, B. M., Grunberg, N. E., 2005. Effects of social and physical enrichment on open field activity differ in male and female sprague-dawley rats. Behav Brain Res. 165:187-96.

Fares, R. F., Kouchi, H., Bezin, L., 2012. Standardized environmental enrichment for rodents in marlau cage. Protocol Exchange. In press.

Fares, R. P., Belmeguenai, A., Sanchez, P. E., Kouchi, H. Y., Bodennec, J., Morales, A., Georges, B., Bonnet, C., Bouvard, S., Sloviter, R. S., Bezin, L., 2013. Standardized environmental enrichment supports enhanced brain plasticity in healthy rats and prevents cognitive impairment in epileptic rats. PLoS One. 8:e53888.

Fox, C., Merali, Z., Harrison, C., 2006. Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. Behav Brain Res. 175:1-8.

Francis, D. D., Diorio, J., Plotsky, P. M., Meaney, M. J., 2002. Environmental enrichment reverses the effects of maternal separation on stress reactivity. J Neurosci. 22:7840-3.

Gomez, A. M., Midde, N. M., Mactutus, C. F., Booze, R. M., Zhu, J., 2012. Environmental enrichment alters nicotine-mediated locomotor sensitization and phosphorylation of DARPP-32 and CREB in rat prefrontal cortex. PLoS One. 7:e44149.

Green, T. A., Cain, M. E., Thompson, M., Bardo, M. T., 2003. Environmental enrichment decreases nicotine-induced hyperactivity in rats. Psychopharmacology (Berl). 170:235-41.

Hamilton, D. A., Kolb, B., 2005. Differential effects of nicotine and complex housing on subsequent experience-dependent structural plasticity in the nucleus accumbens. Behav Neurosci. 119:355-65.

Hunter, R. G., 2012. Stress and the alpha7 nicotinic acetylcholine receptor. Curr Drug Targets. 13:607-12.

Kempermann, G., 2008. The neurogenic reserve hypothesis: What is adult hippocampal neurogenesis good for?. Trends Neurosci. 31:163-9.

Leger, M., Bouet, V., Freret, T., Darmaillacq, A. S., Dacher, M., Dauphin, F., Boulouard, M., Schumann-Bard, P., 2012. Environmental enrichment improves recent but not remote memory in association with a modified brain metabolic activation profile in adult mice. Behav Brain Res. 228:22-9.

Levin, E. D., 2012. alpha7-nicotinic receptors and cognition. Curr Drug Targets. 13:602-6.

Lin, E. J., Choi, E., Liu, X., Martin, A., During, M. J., 2011. Environmental enrichment exerts sex-specific effects on emotionality in C57BL/6J mice. Behav Brain Res. 216:349-57.

Mesa-Gresa, P., Perez-Martinez, A., Redolat-Iborra, R., 2012. Nicotine and animal models: What does the environmental enrichment paradigm tell us? Adicciones. 24:87-94.

Mesa-Gresa, P., Perez-Martinez, A., Redolat, R., 2013a. Environmental enrichment improves novel object recognition and enhances agonistic behavior in male mice. Aggress Behav. 39:269-79.

Mesa-Gresa, P., Perez-Martinez, A., Redolat, R., 2013b. Behavioral effects of combined environmental enrichment and chronic nicotine administration in male NMRI mice. Physiol Behav. 114-115:65-76.

Mesa-Gresa, P., Ramos-Campos, M., Redolat-Iborra, R., 2013c. Enriched environments for rodents and their interaction with nicotine administration. Curr Drug Abuse Rev. 6:191-200.

Moragrega, I., Carmen Carrasco, M., Redolat, R., 2005. Effects of housing and nicotine on shuttle-box avoidance in male NMRI mice. Behav Brain Res. 164:178-87.

Nithianantharajah, J., Hannan, A. J., 2009. The neurobiology of brain and cognitive reserve: Mental and physical activity as modulators of brain disorders. Prog Neurobiol. 89:369-82.

Nithianantharajah, J., Hannan, A. J., 2011. Mechanisms mediating brain and cognitive reserve: Experience-dependent neuroprotection and functional compensation in animal models of neurodegenerative diseases. Prog Neuropsychopharmacol Biol Psychiatry. 35:331-9.

Pandya, A. A., Yakel, J. L., 2013. Activation of the alpha7 nicotinic ACh receptor induces anxiogenic effects in rats which is blocked by a 5-HT(1a) receptor antagonist. Neuropharmacology. 70C:35-42.

Pang, T. Y., Hannan, A. J., 2013. Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. Neuropharmacology. 64:515-28.

Paylor, R., Spencer, C. M., Yuva-Paylor, L. A., Pieke-Dahl, S., 2006. The use of behavioral test batteries, II: Effect of test interval. Physiol Behav. 87:95-102.

Ragu Varman, D., Marimuthu, G., Emmanuvel Rajan, K., 2013. Environmental enrichment upregulates micro-RNA-183 and alters acetylcholinesterase splice variants to reduce anxiety-like behavior in the little indian field mouse (mus booduga). J Neurosci Res. 91:426-35.

Redolat, R., Perez-Martinez, A., Carrasco, M. C., Mesa, P., 2009. Individual differences in novelty-seeking and behavioral responses to nicotine: A review of animal studies. Curr Drug Abuse Rev. 2:230-42.

Redolat, R., Mesa-Gresa, P., 2012. Potential benefits and limitations of enriched environments and cognitive activity on age-related behavioural decline. Curr Top Behav Neurosci. 10:293-316.

Redrobe, J. P., Nielsen, E. O., Christensen, J. K., Peters, D., Timmermann, D. B., Olsen, G. M., 2009. Alpha7 nicotinic acetylcholine receptor activation ameliorates scopolamine-induced behavioural changes in a modified continuous Y-maze task in mice. Eur J Pharmacol. 602:58-65.

Rodgers, R. J., Cao, B. J., Dalvi, A., Holmes, A., 1997. Animal models of anxiety: An ethological perspective. Braz J Med Biol Res. 30:289-304.

Roy, V., Chapillon, P., Jeljeli, M., Caston, J., Belzung, C., 2009. Free versus forced exposure to an elevated plus-maze: Evidence for new behavioral interpretations during test and retest. Psychopharmacology. 203:131-41.

Simpson, J., Kelly, J. P., 2011. The impact of environmental enrichment in laboratory rats--behavioural and neurochemical aspects. Behav Brain Res. 222:246-64.

Simpson, J., Kelly, J. P., 2012. The effects of isolated and enriched housing conditions on baseline and drug-induced behavioural responses in the male rat. Behav Brain Res. 234:175-83.

Skwara, A. J., Karwoski, T. E., Czambel, R. K., Rubin, R. T., Rhodes, M. E., 2012. Influence of environmental enrichment on hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine by osmotic mini-pumps, and nicotine withdrawal by mecamylamine in male and female rats. Behav Brain Res. 234:1-10.

Solinas, M., Chauvet, C., Thiriet, N., El Rawas, R., Jaber, M., 2008. Reversal of cocaine addiction by environmental enrichment. Proc Natl Acad Sci U S A. 105:17145-50.

Solinas, M., Thiriet, N., Chauvet, C., Jaber, M., 2010. Prevention and treatment of drug addiction by environmental enrichment. Prog Neurobiol. 92:572-92.

Sztainberg, Y., Chen, A., 2010. An environmental enrichment model for mice. Nat Protoc. 5:1535-9.

Sztainberg, Y., Kuperman, Y., Tsoory, M., Lebow, M., Chen, A., 2010. The anxiolytic effect of environmental enrichment is mediated via amygdalar CRF receptor type 1. Mol Psychiatry. 15:905-17.

Thiriet, N., Gennequin, B., Lardeux, V., Chauvet, C., Decressac, M., Janet, T., Jaber, M., Solinas, M., 2011. Environmental enrichment does not reduce the rewarding and neurotoxic effects of methamphetamine. Neurotox Res. 19:172-82.

Thomsen, M. S., Hansen, H. H., Timmerman, D. B., Mikkelsen, J. D., 2010. Cognitive improvement by activation of alpha7 nicotinic acetylcholine receptors: From animal models to human pathophysiology. Curr Pharm Des. 16:323-43.

van Praag, H., Kempermann, G., Gage, F. H., 2000. Neural consequences of environmental enrichment. Nat Rev Neurosci. 1:191-8.

Vicens, P., Ribes, D., Torrente, M., Domingo, J. L., 2011. Behavioral effects of PNU-282987, an alpha7 nicotinic receptor agonist, in mice. Behav Brain Res. 216:341-8.

Vicens, P., Ribes, D., Heredia, L., Torrente, M., Domingo, J. L., 2013a. Motor and anxiety effects of PNU-282987, an alpha7 nicotinic receptor agonist, and stress in an animal model of alzheimer's disease. Curr Alzheimer Res. 10:516-23.

Vicens, P., Ribes, D., Heredia, L., Torrente, M., Domingo, J. L., 2013b. Effects of an alpha7 nicotinic receptor agonist and stress on spatial memory in an animal model of alzheimer's disease. Biomed Res Int. 2013:952719.

Viola, G. G., Botton, P. H., Moreira, J. D., Ardais, A. P., Oses, J. P., Souza, D. O., 2010. Influence of environmental enrichment on an object recognition task in CF1 mice. Physiol Behav. 99:17-21.

Wall, P. M., Messier, C., 2001. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neurosci Biobehav Rev. 25:275-86.

Welch, K. D., Pfister, J. A., Lima, F. G., Green, B. T., Gardner, D. R., 2013. Effect of alpha(7) nicotinic acetylcholine receptor agonists and antagonists on motor function in mice. Toxicol Appl Pharmacol. 266:366-74.

Zambrana, C., Marco, E. M., Arranz, L., de Castro, N. M., Viveros, M. P., de la Fuente, M., 2007. Influence of aging and enriched environment on motor activity and emotional responses in mice. Ann N Y Acad Sci. 1100:543-52.

Zhu, S. W., Codita, A., Bogdanovic, N., Hjerling-Leffler, J., Ernfors, P., Winblad, B., Dickins, D. W., Mohammed, A. H., 2009. Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice. Behav Brain Res. 197:339-46.

Zhu, J., Bardo, M. T., Dwoskin, L. P., 2012. Distinct effects of enriched environment on dopamine clearance in nucleus accumbens shell and core following systemic nicotine administration. Synapse. 67:57-67.

Zimmermann, A., Stauffacher, M., Langhans, W., Wurbel, H., 2001. Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. Behav Brain Res. 121:11-20.

CAPÍTULO 5

ESTUDIO 4

Impacto de la edad y del periodo de exposición al modelo de enriquecimiento ambiental

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Artículo en revisión (Revista: Developmental Psychobiology):

Mesa-Gresa P, Ramos-Campos M, Redolat R. Impact of age and duration of exposure to enriched environments on social interactions and anxiety-like behavior in NMRI male mice.

IMPACT OF AGE AND DURATION OF EXPOSURE TO ENRICHED ENVIRONMENTS ON SOCIAL INTERACTIONS AND ANXIETY-LIKE BEHAVIOR IN NMRI MALE MICE

Abstract

Prior studies have suggested that short periods of exposure to environmental enrichment (EE) improve cognitive tasks but increase agonistic interactions between male mice. Our aim was to evaluate if these changes in aggressive behavior are maintained after longer periods exposed to EE and if they can be related to changes in motor activity, anxiety or exploratory behavior. NMRI male mice (n=64) arrived at our laboratory on post-natal day (PND) 21 and were exposed to EE or to a standard environment (SE). Groups compared were: 1) SE-6: exposure to SE on PND 28 and lasting 6 months; 2) EE-6: exposure to EE on PND 28 and lasting 6 months; 3) EE-4: exposure to EE on PND 90 and lasting 4 months; 4) EE-2: exposure to EE on PND 155 and lasting 2 months. Behavior displayed in hole board (HB), elevated plus-maze (EPM), social interaction (SI) and a novel object recognition task (NOR) was evaluated. Results indicated that in HB the decrease in exploratory behavior reached significance only when exposure to EE was initiated at adolescence. In the EPM, anxiolytic effects tend to diminish after a long period exposed to EE. The group EE-4 devoted more time to explore from a distance during SI, but no significant effects on NOR were obtained. In contrast with prior studies, no significant increase of aggressive behavior was observed at any of the ages tested indicating that this effect may disappear when housing in enriched conditions is maintained during longer periods.

1. INTRODUCTION

Environmental Enrichment (EE) is an experimental paradigm in which rodents are housed in new and complex environments consisting of large cages containing colorful toys, houses, ropes, balls, ladders, nesting materials, tunnels and running wheels (Hannan, 2014; Nithianantharajah & Hannan, 2009; Redolat & Mesa-Gresa, 2012). It has been reported that this type of housing condition enhances physical activity, cognitive functions, somato-sensorial stimulation and social interactions (Madroñal et al., 2010; Mesa-Gresa, Perez-Martinez & Redolat-Iborra, 2012, Mesa-Gresa, Ramos-Campos & Redolat, 2013a). EE paradigm has been evaluated as a mean of counteracting age-related cognitive decline (Lazarov, Mattson, Peterson, Pimplikar & van Praag, 2010; Mora, 2013; Patel, 2012), depressive symptoms (Richter, Zeuch, Riva, Gass & Vollmayr, 2013) and some consequences of chronic stress situations (Garrido et al., 2013; McQuaid, Audet, Jacobson-Pick & Anisman, 2013). The exposure to this type of allocation has also positive effects on cognition in animal models of Alzheimer's disease (Barak et al., 2013; Beauquis et al., 2013, Freret et al., 2012), Parkinson's disease (Yuan et al., 2009) and other neurodegenerative disorders (Hannan, 2014; Nithianantharajah & Hannan, 2006; Wood et al., 2010), and has even been proposed as a neuroprotective strategy against traumatic brain injury (Cheng et al., 2012; Johnson, Traver, Hoffman, Harrison & Herman, 2013; Frasca, Tomaszczyk, McFadyen & Green, 2013) or addiction (Mesa-Gresa, Perez-Martinez & Redolat 2013b; Solinas, Thiriet, Chauvet & Jaber, 2010; Pang & Hannan, 2013). This complex type of housing conditions also induces positive effects on neonatal hypoxia-ischemia in animal models (Rojas et al., 2013) or recovery after amblyopia (Baroncelli et al., 2012; Sale, Hannan, Maffei & Guzzetta, 2013). The need for addressing the consequences of exposure to enriched environments in preclinical studies with different drugs has recently been underscored (Burrows & Hannan, 2013; Mesa-Gresa, Ramos-Campos & Redolat, 2014; Simpson & Kelly, 2012).

There is experimental evidence suggesting that this type of allocation induces neurobiological changes including neurogenesis in the hippocampus, increased cortical thickness, dendritic branching and higher levels of brain-derived neurotrophic factor (BDNF) (Hendriksen et al., 2012; Kempermann, Gast & Cage, 2002; Petrosini et al., 2009;

Ramírez-Rodriguez al., 2014; Simpson & Kelly, 2011; van Praag, Kempermann & Cage 2000). The results of various studies evaluating behavioral consequences of EE have differed depending on variables assessed and the behavioral task applied (Pang & Hannan, 2013), age of the animals (Freret et al., 2012; Patel, 2012), strain (Abramov, Puusaar, Raud, Kurrikoff & Vasar, 2008; Branchi et al., 2006), duration of exposure (Bouet, Freret, Dutar, Billard & Boulouard, 2011) and type of complex housing conditions (Fares, Kouchi & Bezin, 2012; Mesa-Gresa et al., 2013a; Xie et al., 2013). Behavioral changes reported after exposure to EE include less pronounced stereotypic behaviors (Gross, Richter, Engel & Wurbel, 2012), decreased basal locomotor activity (Bowling, Rowlett & Bardo, 1993; Mesa-Gresa et al., 2013b; Varty, Paulus, Braff & Geyer, 2000), enhanced performance in learning and memory tests such as the inhibitory avoidance task (Mesa-Gresa et al., 2013b), Morris water maze (Leggio et al., 2005) or novel object recognition tasks (Bechara & Kelly, 2013; Leger et al., 2012; Mesa-Gresa, Perez-Martínez & Redolat, 2013c), diminished exploratory behavior (Brenes, Rodriguez, & Fornaguera, 2008) and more rapid habituation to novel environments (Renner & Rosenzweig, 1986; Vedovelli et al., 2011; Zimmerman, Stauffacher, Laughans & Wurbel, 2001). Although most studies have reported changes in learning and memory tasks after exposure to different periods of EE (Bechara & Kelly, 2013; Birch, McGarry & Kelly, 2013), some of them have also documented changes in emotional responses. For example, levels of anxiety-like behavior have been reported to be reduced in the elevated plus-maze (Benaroya-Milshtein et al., 2004; Fernandez-Teruel, Escorihuela, Castellano, Gonzalez & Tobena, 1997; Hughes & Otto, 2013; Mesa-Gresa et al., 2014), though the results have been contradictory at times (Brenes, Padilla & Fornaguera, 2009; Zhu et al., 2006). Recently, the need to evaluate how agonistic interactions and dominance relationships between male rodents may change as a consequence of being housed in enriched environments has been highlighted (Hutchinson et al., 2012; Mesa-Gresa et al., 2013a, 2013c). Housing animals in these complex environmental conditions implies larger groups (usually 8-10, though some studies include up to 20) that share a cage for several weeks or months (Redolat & Mesa-Gresa, 2012; Nithianantharajah & Hannan, 2009). Although evidence about the effects of long-term exposure to enriched housing on social and agonistic interactions is inconclusive, some studies have suggested that the presence of novel objects in the cage and the complexity of the environment enhance aggressive behaviors, due to the animals

competition for the resources or the physical proximity between them (Abou-Ismail, 2011; Haemisch & Gartner, 1997; Haemisch, Voss & Gartner, 1994; McQuaid et al., 2013). Moreover, recent research performed in our laboratory has indicated that NMRI male mice maintained in EE housing conditions for 4 weeks display enhanced sociability and increased agonistic behavior (Mesa-Gresa et al., 2013b). Some reports, however, have concluded that EE housing reduce aggressive behavior in comparison with standard housing conditions (Abou-Ismail, Burman, Nicol & Mendi, 2010; Abramov et al., 2008; Chamove, 1989; Pietropaolo et al., 2004).

There is debate about how each component of the EE paradigm is most responsible for its effects (Bechara & Kelly, 2013; Lee et al., 2013; Mesa-Gresa et al., 2013a; Pang & Hannan, 2013; Rojas et al., 2013). Some recent studies suggest that physical activity is the main neurogenic component present in enriched environments (Bechara & Kelly, 2013; Kobilo et al., 2011; Mustroph et al., 2012) and induces significant changes in plasticity (Nichol, Deeny, Seif, Camaclang & Cotman, 2009; Sale et al., 2013), although cognitive activity (Birch et al., 2013) and social interaction (Kulesskaya, Rauvala & Voikar, 2011) or the interaction between all of them may have also a relevant role (Mesa-Gresa et al., 2013a, 2014). However, we do not know all the neurobiological mechanisms by which EE improves memory or decreases anxiety (Patel, 2012). Xie et al. (2013) have recently suggested that there are two mechanisms by which enriched environments may induce behavioral changes, one is dependent on exercise in the cages whereas the other is exercise-independent. However, the most adequate duration and age of initiation of EE is not clear. Several studies have attempted to address this question by comparing the performance of animals maintained in enriched conditions during different time periods in several behavioral tests or by measuring their physiological variables (Freret et al., 2012; Leal-Galicia, Castaneda-Bueno, Quiroz-Baez & Arias, 2008, Leger et al., 2012; Sampedro-Piquero, Begega, Zancada-Menendez, Cuesta & Arias, 2013). If enriched environments potentiate physical activity, somato-sensorial stimulation, cognitive function and social interactions (Benaroya-Milshtein et al., 2004; Mesa-Gresa et al., 2013a; Mohammed et al., 2002), we can expect that duration of the allocation to complex housing conditions may influence the behavior displayed by each group of mice.

Different protocols of enrichment have been applied in past research, obtaining positive effects when exposure is initiated at different stages of the lifespan (Freret et al., 2012; Rosenzweig & Bennett, 1996; Workman, Fonken, Gusfa, Kassouf & Nelson, 2011). The age at which animals are first exposed to EE seems to be of great relevance in determining its effects, with the consequences being more evident when this type of complex housing is initiated at adolescence (Pietropaolo et al., 2008) or adulthood (Ramirez-Rodriguez et al., 2013). Some results also suggest that plasticity is maintained during cognitive aging (Mora, 2013; Paban, Chambon, Manrique, Touzet & Alescio-Lautier, 2009; Speisman et al., 2013). Furthermore, the influence of the total period during which animals are submitted to EE has also been addressed in some studies. Short periods (3-4 weeks) may have benefits in comparison with impoverished environments (isolation or standard housing) (Mesa-Gresa et al., 2013b, 2013c). The minimum period necessary to obtain effects may be related with the type of tasks evaluated (Brenes & Fornaguera, 2008; Leggio et al., 2005; Simpson & Kelly, 2011) or the species/strain of the animals (Abramov et al., 2008). In a prior study, Bouet et al. (2011) have evaluated the consequences of exposure of NMRI mice to EE for 3 months at different ages. Results indicated that said exposure induces positive effects on memory and learning capabilities and diminishes anxiety-like response in adult but not in aged mice.

Recently, Simpson and Kelly (2012) have written a comprehensive review about the influence of different variables, such as size cage, number of animals in each cage, mice or rat strain, gender, age and total time of exposure, on the results obtained in experiments performed with rodents in enriched environments. Their conclusions indicate that, in the majority of studies, the EE protocol has been initiated before the age of one month, with only 2% of the studies initiating it from 1 year onwards. Considering previous research, it has been suggested that both neurobiological and behavioral effects are more evident if exposure to complex environments is initiated at an early age or maintained during long periods of time (Baldini et al., 2013; Kempermann et al., 2002). Taking this question into account, the main goal of the current study was to characterize the behavioral changes in exploratory and anxiety-like response, novel object recognition test, social interaction and aggressive behavior induced by exposure to enriched environments initiated at different ages and of varying duration in NMRI male mice.

2. MATERIALS AND METHODS

2.1. Subjects

Sixty-four NMRI male mice (Charles River, Barcelona, Spain), which arrived at our laboratory at 21 days of age (mean weight: 10-12 gr.), were employed for the longitudinal study. Prior studies suggest that this strain is sensitive to environmental changes (Bouet et al., 2011; Freret et al., 2012; Mesa-Gresa et al., 2013b,c;2014).

On their arrival on post-natal day (PND) 21, mice were housed in groups of 4 in standard cages (42 x 26 x 14 cm) in order to allow them to adapt to the new conditions and, one week later, they were randomly assigned to different housing conditions. During the whole experimental procedure, mice were maintained in a controlled environment (lights off at 08:00 hr), at a constant temperature (20-24ºC) and humidity (55±10%), with food and water access ad libitum.

All procedures were approved by the local ethics committee and complied with local, national (Real Decreto 1201/2005; Decreto 13/2007) and international guidelines (European Community's Council Directive of November 24, 1986 -86/609/EEC; 2007/526/CE; 2010/63/EU) for the care and treatment of animals.

2.2. Housing condition and procedure

At 28 days of age (PND 28), mice were randomly allocated to different housing conditions: 1) Environmental Enrichment (EE), consisting of a large cage (55 x 36 x 19 cm) (n=8) containing different toys, a house, tunnels and a running wheel; or 2) Standard Environment (SE) (42 x 26 x 14 cm), consisting of a smaller cage containing only sawdust (n=4) (for a more detailed description see Mesa-Gresa et al., (2014)). In order to favor novelty and complexity, toys in the EE cages were changed twice a week. Four experimental groups were evaluated in different behavioral tests at the end of the varying housing periods (PND 216): 1) Group SE, exposure to SE began on PND 28 and lasted a total period of 6 months; 2) Group EE-6, exposure to EE began on PND 28 and lasted 6 months; 3) Group EE-4, exposure to EE began on PND 91 and

lasted 4 months; 4) Group EE-2, exposure to EE began on PND 154 and lasted 2 months.

The elevated plus-maze and hole-board tests were performed on PND 217, the novel object recognition test took place on PND 218-219, and the social interaction test took place on PND 220-221. During experimental procedures, mice were taken to the testing room 30 min before tests in order to allow them to adapt to the surroundings. All the apparatus were cleaned between animals with a solution containing ethanol (2%).

2.2.1. Hole board (HB)

The test used in our experiments consists of a Plexiglas board (31.5 x 31.5 x 20.5 cm) with 16 holes (2 cm diameter) and transparent walls. This task allows novelty seeking behavior, locomotion and exploration to be measured (Boissier, Simon & Lwoff, 1964). Each animal was placed in the center of the board and permitted to explore it freely for 5 min., during which the latency to the first head-dip (HD) and total number of HD were measured at min 1 (HD 1 min) and 5 (HD 5 min). It has been suggested that a lower number of HD implies lower levels of anxiety (Simpson & Kelly, 2011), novelty seeking and exploratory behavior (Mesa-Gresa et al., 2013b,2014; Zhu et al., 2009).

2.2.2. Elevated plus-maze (EPM)

The EPM is an experimental procedure employed to measure changes in anxiety-like behavior after pharmacological and behavioral interventions (Elliott & Grunberg, 2005; Roy, Chapillon, Jeljeli, Caston & Belzung, 2009). The apparatus used in the current study consisted of 4 arms (two open and two closed) and a central platform positioned 45 cm above the floor. The maze was made of black Plexiglas and the walls of the closed arms were transparent. At the beginning of the test, each animal was placed in the central platform facing an open arm. The classical and ethological parameters measured in this test have been described with more detail in Mesa-Gresa et al. (2013b).

2.2.3. Novel object recognition test

The novel object recognition test (NOR) is widely used to assess learning in both mice and rats. The task was performed in three different phases following the procedure reported by Greco et al. (2010) and Mesa-Gresa et al. (2013c). During the habituation phase, which took place one day prior to testing, mice were allowed to explore the neutral cage (60x33x30 cm) for 5 min. Twenty-four hours later, the training phase took place and two identical novel objects were placed in the open field and mice were freely allowed to explore them (10 min). Finally, 1 hour after training, the test phase began. Mice were allowed to explore two different objects: the one they had previously explored during the training phase and a new one. The test phase was video-recorded and the total time spent exploring the new object with respect to total exploration time and time allocated to rearing or grooming was calculated by a blind observer. An object was considered to have been explored when the head of the animal was ½ cm from the object or touching it (Ennaceur, 2010; Greco et al., 2010; Mesa-Gresa et al., 2013c). Object location was counterbalanced across experimental conditions in order to avoid object and location preference. A discrimination index (total time spent exploring new object/total time of object exploration) was calculated for each experimental group. The advantages of this behavioral paradigm are the absence of positive or negative reinforcers and the possibility of retention interval manipulation (Leger et al., 2012) and the fact that it seems to be sensitive to changes induced by exposure to EE (Mesa-Gresa et al., 2013c).

2.2.4. Social interaction test

The social interaction test consisted of encounters in which EE or SE mice were confronted with a standard opponent (group-housed mice) of a similar age and weight (32-35 gr) marked with fur dye in order to identify them in the video recordings. Tests took place in a neutral cage made of clear Plexiglas (60x33x30 cm), to which both experimental and opponent animals were allowed 1 min to adapt, during which time they were separated by a white plastic barrier. Following the procedure reported by Chistyakov et al. (2010) and Mesa-Gresa et al. (2013c), the total duration of agonistic encounters was limited to four minutes in order to avoid serious injuries.

The behavior displayed by mice in the plus maze and NOR, and during their social encounters was analysed by a blind researcher using the "Raton-time" software, a program which allows ethological analysis of behavior. In social encounters, two different analysis were performed: one following the behavioral categories reported by Brain, McAllister and Walmsley (1989) and another one according to the categories set out by Chistyakov et al. (2010). Both methods have been extensively reported in a prior study (Mesa-Gresa et al., 2013c).

2.3. Statistical analysis

All statistical analyses were performed using IBM SPSS®Statistics (Version 19.0) for Windows. In the hole board task, data for HD (1 min), HD (5 min) and latency for the first HD were analysed with ANOVA taking the factor "Housing condition" into consideration. For the EPM, data for each behavioral category was also analysed with ANOVA. During social encounters, the time allocated to each behavioral category and the number of times that mice displayed each behavior were submitted to an analysis of variance (ANOVA), using "Housing Group" as a factor. Recognition memory in the test phase of the object recognition test was measured using a discrimination index (total time spent exploring the new object/total exploration time) and comparison within groups were performed with ANOVA. All data are presented as mean ± standard error of the mean (SEM). In all cases, significance levels were set at $p<0.05$.

3. RESULTS

3.2. Hole Board

There were no significant differences in the latency to the first HD or total number of HD at the first minute. However, when the total number of head dips at the end of the test (HD 5 min) was considered, ANOVA indicated significant differences between groups [$F(3,63)=2.979$, $p<0.05$]. Post hoc tests showed that the EE-6 group performed a lower number of total HD (10±1.09) than the SE-6 group (16.37±2.31) (see Figure 1).

3.3. Elevated plus maze

ANOVA revealed significant differences in Total number of entries into the closed arms [$F(3,63)= 6.592$, $p<0.001$], indicating that groups EE-4 and EE-2 performed a lower number of closed entries than group SE-6 ($p<0.01$). There were also significant differences in the percentage of entries into open arms [$F(3,63)=5.668$, $p<0.01$]. The post-hoc test indicated that the EE-4 group performed a higher percentage of entries into the open area of the maze (64.41±1.40) than EE-6 (52.20±3.74) and SE (49.43±3.00) mice (see Table 1 and Figure 2).

The results for ethological measures obtained in the EPM indicated significant differences for the variables of total number of SAP [$F(3,60)=2.83$, $p<0.05$] and time [$F(3,60)=6.08$, $p<0.001$] and frequency of peeping-out behavior [$F(3,60)=3.30$, $p<0.05$]. Post-hoc Tukey tests showed that all the groups of animals allocated to EE conditions (EE-6, EE-4 and EE-2) engaged less frequently in this behavior and devoted less time to it ($p<0.05$) than mice allocated to standard conditions. Regarding the total number of SAP, post-hoc tests did not show statistical significance, although a tendency towards a significant difference ($p=0.056$) was detected between EE-2 (11.31±1.18) and SE-housed (16.94±1.64) mice.

3.4. Novel object recognition test

ANOVA for the results of this test indicated significant differences in the time exploring novel object novel object [$F(3,57)=3.044$, $p<0.05$]. Post-hoc tests showed statistically significant differences between groups EE-4 and EE-2 and a trend towards significant differences with respect to the SE-6 group ($p<0.05$). Animals housed in EE conditions for 4 months (group EE-4) spent more time exploring the new object during the test phase than kept in the same conditions for two months (EE-2 group). No significant effects were found in the discrimination index between groups. In this comparison, a discrimination index of >50% indicates a preference for exploring novel objects (Siopi et al., 2012) (see Figure 3).

3.5. Social Interation test

If we consider the different behaviors according to the ethogram reported by Brain et al. (1989), the analysis of our results revealed statistical differences in the time dedicated to the behavioral categories of digging [$F(3,57)=2.99$, $p<0.05$], non-social exploration [$F(3,57)=2.979$, $p<0.05$] and exploration from a distance [$F(3,57)=2.88$, $p<0.05$] and for the frequency of digging behavior [$F(3,57)=3.348$, $p<0.05$]. Post-hoc Tukey tests indicated that EE-6 mice engaged more frequently and during longer periods in digging behavior than EE-2 mice ($p<0.05$). Statistical differences were detected for EE-4 mice, which engaged more frequently in exploration from a distance and devoted more time to it than SE-6 mice ($p<0.05$). However, the post-hoc test did not reveal statistical differences for the time spent engaged in non-social exploration (see Table 2).

When the frequencies and time devoted to each behavioral category set out by Chistyakov et al. (2010) were analysed, results did not reach statistical significance for any of the variables assessed: “individual behavior” “agonistic behaviour”, “sociability” or “social behaviour”.

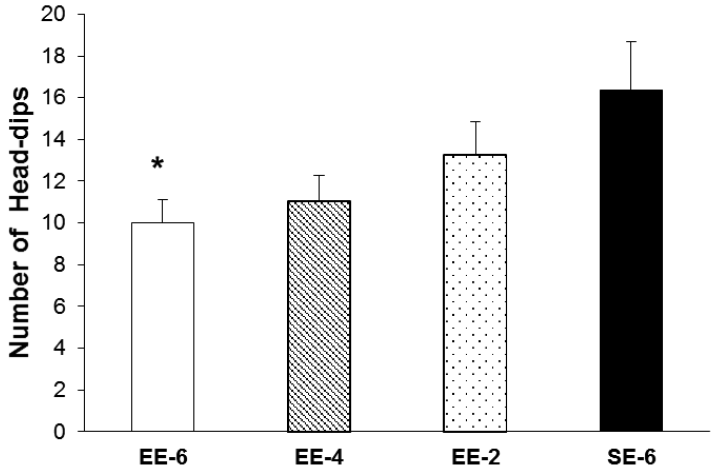

Figure 1. Number of Head-dips in hole-board test by the four experimental groups: Environmental Enrichment (EE-6; EE-4; EE-2) and Standard Environment (SE-6). Data are presented as mean values ± SEM.
(*) $p<0.05$ EE-6 vs SE-6.

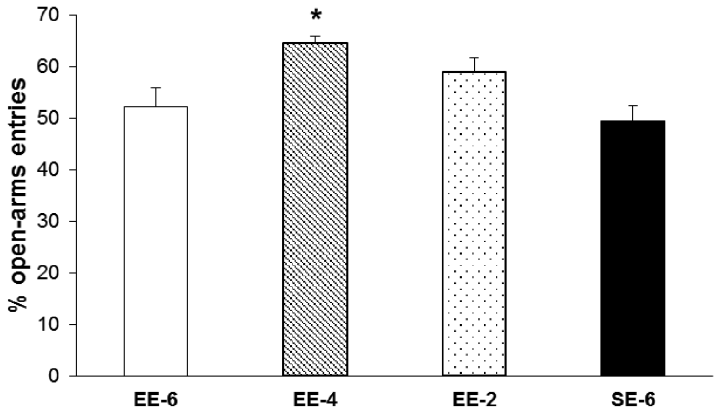

Figure 2. Percentage of entries into the open arms in the elevated plus maze test in the four experimental groups: Environmental Enrichment (EE-6; EE-4; EE-2) and Standard Environment (SE-6). Data are presented as mean ± SEM.

(*) $p<0.01$ EE-4 vs EE-6 and SE-6.

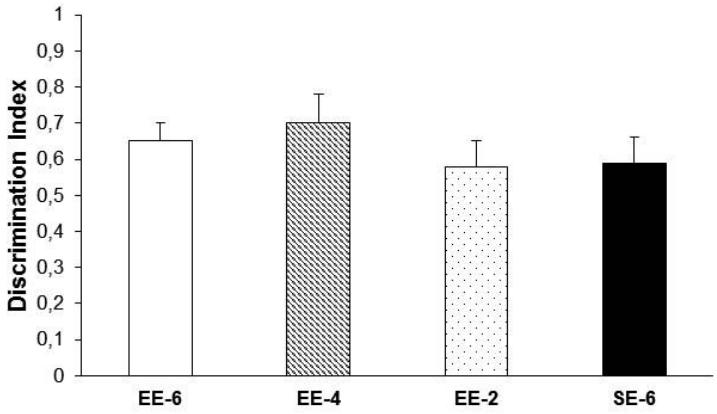

Figure 3. Discrimination Index obtained in the novel object recognition task (total time spent with new object / total time of object exploration) by the four experimental groups: Environmental Enrichment (EE-6; EE-4; EE-2) and Standard Environment (SE-6). Data are presented as mean values ± SEM.

Table 1. Effects of exposure to an enriched (EE-6, EE-2 y EE-2) or standard environment (SE-6) displayed by male NMRI male mice in the elevated plus-maze test. Median time (in seconds) with ranges allocated to broad behavioral categories is shown. Data are presented as mean values ± SEM. Abbreviations: HD: head-dip; SAP: stretched attend posture; % p: percentage protected.

BEHAVIORAL CATEGORIES	EE-6	EE-4	EE-2	SE-6
TOTAL ENTRIES	15.18±1.24	15.75±1.39	14.44±1.52	18.44±1.04
OPEN ENTRIES	8.19±0.94	10.12±0.91	8.44±1.01	9.25±0.79
CLOSED ENTRIES	7±0.6	5.62±0.57	6±0.68	9.19±0.63
% OPEN ENTRIES	52.19±3.74	64.42±1.4	58.9±2.69	49.43±3
% OPEN TIME	31.04±3.16	53.58±12.66	40.61±3.52	32.03±2.59
% CENTER TIME	39.09±2.46	31.73±1.89	31.92±3.3	38.36±2.16
TOTAL HD	41.56±3.29	45.75±3.35	41.13±3.08	33.31±2.88
% p HD	46.82±5.18	37.27±3.13	40.84±4.52	52.98±3.77
TOTAL SAP	12.13±1.6	12.06±1.61	11.31±1.25	16.94±1.64
% p SAP	40.91±5.88	41.9±6.28	33.5±5.66	51.85±5.68
TOTAL REARS	15.5±2.78	10.63±2.21	7.31±1.65	10.25±1.24
PEEPING-OUT	12.19±0.97	11.31±0.7	11.19±1.18	15.19±1.18
ACTIVITY END MAZE	5.94±0.97	8.06±0.81	7.25±0.74	7.63±1.06

Table 2. Effects of exposure to an enriched (EE-6, EE-2 y EE-2) or standard environment (SE-6) displayed by male NMRI male mice during a social encounter with a conspecific. Median time (in seconds) with ranges allocated to broad behavioral categories is shown. Data are presented as mean values ± SEM.

BEHAVIORAL CATEGORIES	EE-6	EE-4	EE-2	SE-6
SELF-GROOMING	1.23±0.50	9.01±5.05	4.91±3.20	1.35±0.48
DIGGING#	7.85±1.46	4.52±1.03	2.81±0.86	4.84±1.35
NON-SOCIAL EXPLORATION	148.31±5.67	130.69±9.79	117.37±9.46	149.86±10.49
EXPLORE FROM A DISTANCE##	1.91±1.07	6.13±2.63	1.83±0.43	0.87±0.19
SOCIAL INVESTIGATION	48.72±4.53	53.68±5.24	68.49±7.65	57.79±10.17
THREAT	7.16±4.85	8.83±4.54	5.37±1.63	2.65±1.49
ATTACK	0.72±0.40	0.61±0.29	1.24±0.85	0.37±0.25
AVOIDANCE/FLEE	0.02±0.02	0.00±0.00	0.00±0.00	0.00±0.00
DEFENSIVE/SUBMISSIVE	0.60±0.47	0.08±0.08	0.02±0.02	1.09±0.77

(#) $p<0.05$ EE-6 vs. EE-2;
(##) $p<0.05$ EE-4 vs. SE-6.

4. DISCUSSION

The current study assesses the impact of different periods of housing in enriched environments on social interaction, exploratory behavior, anxiety response and learning in male NMRI mice. We have observed that the effects of differential rearing depend on both the age at which allocation to complex housing conditions is initiated and the total duration of exposure. Few studies have taken into account both parameters simultaneously, and none has evaluated the impact of varying periods of enriched environments on agonistic encounters between male mice. Our data support the findings of prior studies showing that early experience of EE can have long-term behavioral consequences (Amaral, Vargas, Hansel, Izquierdo & Souza, 2008; Freret et al., 2012; Lee et al., 2013; Mustroph et al., 2012). As recently reported, this type of housing may also have relevant consequences for behavioral and pharmacological studies (Macri, Ceci, Altabella, Canese & Laviola, 2013).

The physiological and molecular mechanisms which underlie the behavioral changes induced by EE are unclear (Bouet et al., 2011; Patel, 2013; Redolat & Mesa-Gresa, 2012; Petrosini et al., 2009; Sale, Berardi & Maffei, 2014), and studies such as the present one may help to determine if there is a critical period within which neurobiological and behavioral changes are induced by EE (Pietropaolo et al., 2008; Renner & Rosenzwieg, 1987; Sale et al., 2013; van Praag et al., 2000). As discussed above, the paradigm of EE involves a combination of social, cognitive and physical stimulation (Rojas et al., 2013). The effects associated with an enriched environment, however, appear to depend on the form of enrichment, the species/strain and sex of rodents used and, importantly, whether animals are housed in groups or individually (Fox, Merali & Harrison, 2006; Simpson & Kelly, 2011). In our study, there was no specific handling protocol for the EE group, but it is possible that repeated handling (twice a week, when the toys were substituted) influenced the behavior of the animals, since evidence suggests that handling is a relevant variable in the effects of enriched environments (Pritchard, van Kempen & Zimmerberg, 2013).

Emotional reactivity to the hole-board task has been shown to vary with age (Laviola, Macri, Morley-Fletcher, & Adriani, 2003; Tirelli, Tambour, & Michel, 2003). When exploratory behavior was evaluated in the HB test in the current study, we observed a decrease in exploratory activity that was more evident in mice housed in enriched conditions from an early age and during a longer period of time (6 months). Some previous studies have evaluated the impact of different periods of exposure to EE and age at initiation of exposure on motor, exploratory and emotional behavior. Brenes et al. (2008) assessed the behavioral effects of EE from PND 30 to PND 114 and found that enriched rats showed the lowest locomotion and highest grooming rates on all the PNDs tested. Bouet et al. (2011) observed that 3 months of continuous EE decreased anxiety-like behavior in adult mice but not in aged mice. In general, previous reports have shown that exposure to enriched housing conditions diminishes anxiety response both in the EPM (Abramov et al, 2008; Mesa-Gresa et al., 2013a; Zhu et al., 2006) and during social interaction tests (Mesa-Gresa et al., 2013c). In the EPM, the behavioral changes displayed by NMRI mice in the current study reflect lower levels of anxiety in mice reared in enriched conditions during 4 months. This response could have been related to a greater ability to cope with different stressful conditions and changes in HPA axis sensitivity (Harati et al., 2011). Our results obtained in the EPM are in accordance with previous observations in animals of different ages indicating that EE diminishes the anxiety response in the EPM (Harati et al., 2013; Leal-Galicia, Saldivar-Gonzalez, Morimoto & Arias, 2007; Peña, Prunell, Dimitsantos, Nadal & Escorihuela, 2006). However, in our experiments, this effect was more significant in the EE-4 group than in the EE-6 group, suggesting that anxiolytic effects diminish when animals are housed in enriched conditions during a long period of time. Further studies evaluating different periods of rearing in enriched conditions and employing aging animals are needed in order to establish if there is a critical window within which clear anxiolytic effects are obtained.

Recently, there has been growing interest in evaluating the effects of EE on the object recognition test (Bechara & Kelly, 2013; Dolaumes, Lee & Shea, 2013; Mesa-Gresa et al., 2013c). This task has previously been employed to assess the effects of different experimental manipulations on learning and memory (Dere, Huston & de

Souza-Silva, 2007; Ennaceur, 2010; Heyser & Chemero, 2012), and we have used it with the aim of evaluating cognitive changes after EE exposure. As explained previously, the main advantage of this experimental task is that it does not rely on positive or negative reinforcers (Leger et al., 2012), as it is sensitive to changes induced by exposure to enriched environments during short periods of time (Mesa-Gresa et al., 2013c). In prior studies, it has been observed that EE can improve learning and memory in both spatial (Leggio et al., 2005; Van Praag et al., 2000,) and non-spatial tasks (Bouet et al., 2011; Mesa-Gresa et al., 2013b; Viola et al., 2009). However, the positive effects of EE on learning and memory may have different causes. Recently, Harati et al. (2013) have demonstrated that enriched housing protects against the significant loss of basal forebrain cholinergic neurons. Moreover, Rojas et al. (2013) observed that EE counteracted the impairment in the object recognition task observed in hypoxic ischemic rats, though the animals in their study were submitted to EE for only 1h/day for 9 weeks. In the current study, we have seen how mice allocated to EE conditions for 4 months explored the novel object more than those which had been housed in the same conditions for only two months, though there were no significant differences in the discrimination index. This lack of significant effects of EE on memory of the task could have been related with the short interval between training and tests phases in our study (only 24 hr). Different mechanisms seem to be implicated in the cognitive effects induced by EE (Patel, 2012), and more studies are needed to confirm if there is a critical period for their beneficial effects on non-spatial tasks such as the novel object recognition test.

The results of our study confirm previously published data indicating that the age at which EE begins has a bearing on subsequent changes in learning tasks. Harati et al. (2011) compared behavioral changes in rats maintained in EE conditions over different periods of time (3, 12 or 24 months, and tested at 4, 13 or 25 months) and found that longer periods of enrichment attenuated age-related cognitive deficits to a greater extent. The age at which enrichment begins also seems to be relevant when explaining the beneficial effects of EE (Bouet et al., 2011; Freret et al., 2012). Sampedro-Piquero et al. (2013) have recently compared the effects of 2-month exposure to an enriched environment on 3-month-old and 18-month-old Wistar rats, reporting an improved

performance in a spatial memory task in both groups, though benefits were greater among the younger animals. Analyisis of Cox histochemistry suggests that this improvement is mediated by differential brain mechanisms in young versus old rats. Vazquez-Sanroman et al. (2013) have also observed that the effects of EE on BDNF expression in the cerebellum of Balb/c mice differ depending on the length of exposure to the enriched environment and cell-type. The duration of exposure to EE seemed to influence BDNF expression in Purkinje cells, as longer periods (4-8 weeks) were needed to produce a significant increase. Some studies have suggested an interaction between length of exposure to EE and the age at which it begins. For example, in adult rats, the effects of short-term (3 months) or long-term (24 months) EE on spatial memory in a water-maze task have been shown to be similar, while in aged rats the beneficial effects of EE were found to be greater when exposure was of a shorter duration (only 3 months) (Harati et al., 2011). No studies, however, have addressed the question of how these two variables (age at initiation and duration of exposure) or their possible interaction may have an impact on social interaction and agonistic behavior.

In addition to the influence of EE on anxiety and learning in experimental animals, it is important to consider the variations in social interaction that it may produce, as the majority of EE procedures involve a large group of animals housed together in the same cage for several weeks or months (Redolat & Mesa-Gresa, 2012; Swetter, Karpiak & Cannon, 2011). A previous study carried out by our group suggested that 4 weeks of EE initiated at an early age is sufficient for increasing agonistic interactions between NMRI male mice (Mesa-Gresa et al., 2013b). On the other hand, Lee et al. (2013) observed that maintaining mice in an enriched environment for 2 months induced an enhancement of motor and cognitive functions. However, few research groups have evaluated the effects of long-term exposure to an enriched environment on social and aggressive interactions between male mice, and the evidence which does exist is somewhat inconsistent, with some studies reporting a reduction of aggressive interactions (Abou-Ismail, 2011; Kaliste, Mering & Huuskonen, 2006) and others reporting an increase (Haemisch & Gartner, 1997; Mesa-Gresa et al., 2013c, Richter et al., 2013). The results of the current study indicate that there were no significant

increases of agonistic behavior after exposure to EE for 2, 4 or 6 months, though changes in other behavioral categories were confirmed. This would support the conclusions of previous research carried out in our laboratory showing that the increase in aggressive behavior observed after exposure to enriched environments during short periods of time was mainly territorial and did not reflect any atypical or pathological aggression (Mesa-Gresa et al., 2013c). We consider that effects exerted on agonistic behaviors or social interaction may depend on the docility of the strain evaluated (Mesa-Gresa et al., 2013c), the total duration of exposure to EE, the age at which this exposure begins, or the behavior in question. For example, Workman et al. (2011) recently reported that enriched housing from PND 21 to 60 did not alter overt measures of aggressive behavior (biting or boxing) in young C57BL mice. In fact, some authors have implemented different strategies in order to diminish aggressive interactions between mice exposed to EE conditions. Tanti et al. (2013) have recently described how they substituted two identical objects for two existing objects twice a week in order to enhance curiosity and exploration, thereby diminishing the possibility of aggressive interactions between animals. Age also influences the results obtained in agonistic interactions, although Richter et al. (2013) recently found that exposure to enriched environments for 5 weeks had no significant effects on agonistic interactions or other spontaneous behavior displayed by 14-week- or 28-week-old Sprague-Dawley rats ones in their own cage.

5. CONCLUSIONS

Our data confirms previous research (Bouet et al., 2011, Leger et al., 2012: Leal-Galicia et al., 2008; Sale et al., 2013) showing that both age of initiation and total duration of exposure to EE determine the effects of this paradigm on exploratory, social, agonistic and anxiety behaviors and on cognition, and that there is a complex interaction between these two variables.

Different studies have attempted to clarify the main neurobiological and behavioral effects of components of EE and the possible interaction between them

(Mesa-Gresa et al., 2013a). It is difficult to define the relative contribution of each of the different elements of the EE paradigm (Mustroph et al., 2012). For that reason, future studies should employ animals of different ages at initiation of exposure and varying periods of exposure with the aim of manipulating the different components of the EE paradigm (i.e. objects introduced into the cage). Physical and cognitive activity need to be distinguished in order to determine the contribution of each one (Mesa-Gresa et al., 2013a; Pang & Hannan, 2013). Taking this into account, it would be advisable to standardize procedures of exposure to EE in order to obtain more consistent and reliable results (Bouet et al., 2011; Simpson & Kelly, 2012). It is also necessary to perform more studies in order to evaluate the benefits of the EE paradigm against age-related memory decline and other neurodegenerative disorders, whether alone or in interaction with enviromimetics (Hannan, 2014; Patel, 2013; Redolat & Mesa-Gresa, 2012).

REFERENCES

Abou-Ismail, U. A. (2011). The effects of cage enrichment on agonistic behaviour and dominance in male laboratory rats (rattus norvegicus). *Research in Veterinary Science*, 90, 346-51.

Abou-Ismail, U. A., Burman, O. H., Nicol, C. J., & Mendl, M. (2010). The effects of enhancing cage complexity on the behaviour and welfare of laboratory rats. *Behavioural Processes,* 85, 172-80.

Abramov, U., Puussaar, T., Raud, S., Kurrikoff, K., & Vasar, E. (2008). Behavioural differences between C57BL/6 and 129S6/SvEv strains are reinforced by environmental enrichment. *Neuroscience Letters,* 443, 223-27.

Amaral, O. B., Vargas, R. S., Hansel, G., Izquierdo, I., & Souza, D. O. (2008). Duration of environmental enrichment influences the magnitude and persistence of its behavioral effects on mice. *Physiology & Behavior,*93, 388-94.

Baldini, S., Restani, L., Baroncelli, L., Coltelli, M., Franco, R., Cenni, M. C., … Berardi, N. (2013). Enriched early life experiences reduce adult anxiety-like behavior in rats: A role for insulin-like growth factor 1. *The Official Journal of the Society for Neuroscience,* 33, 11715-23.

Barak, B., Shvarts-Serebro, I., Modai, S., Gilam, A., Okun, E., Michaelson, D., … Ashery, U. (2013). Opposing actions of environmental enrichment and Alzheimer's disease on the expression of hippocampal microRNAs in mouse models. *Translational Psychiatry,* 3, e304.

Baroncelli, L., Bonaccorsi, J., Milanese, M., Bonifacino, T., Giribaldi, F., Manno, I., … Sale, A. (2012). Enriched experience and recovery from amblyopia in adult rats: Impact of motor, social and sensory components. *Neuropharmacology,* 62, 2388-97.

Beauquis, J., Pavia, P., Pomilio, C., Vinuesa, A., Podlutskaya, N., Galvan, V., & Saravia, F. (2013). Environmental enrichment prevents astroglial pathological changes in the hippocampus of APP transgenic mice, model of alzheimer's disease. *Experimental Neurology,* 239, 28-37.

Bechara, R. G., & Kelly, A. M. (2013). Exercise improves object recognition memory and induces BDNF expression and cell proliferation in cognitively enriched rats. Behavioural *Brain Research,* 245, 96-100.

Benaroya-Milshtein, N., Hollander, N., Apter, A., Kukulansky, T., Raz, N., Wilf, A., … Pick, C. G. (2004). Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. *The European Journal of Neuroscience,* 20,1341-47.

Birch, A. M., McGarry, N. B., & Kelly, A. M. (2013). Short-term environmental enrichment, in the absence of exercise, improves memory, and increases NGF concentration, early neuronal survival, and synaptogenesis in the dentate gyrus in a time-dependent manner. *Hippocampus,* 23, 437-50.

Boissier, J. R., Simon, P., & Lwoff, J. M. (1964). Use of a particular mouse reaction (Hole Board Method) for the study of psychotropic drugs. *Therapie,* 19, 571-83.

Bouet, V., Freret, T., Dutar, P., Billard, J. M., & Boulouard, M. (2011). Continuous enriched environment improves learning and memory in adult NMRI mice through theta burst-related-LTP independent mechanisms but is not efficient in advanced aged animals. *Mechanisms of Ageing and Development,* 132, 240-248.

Bowling, S. L., Rowlett, J. K., & Bardo, M. T. (1993). The effect of environmental enrichment on amphetamine-stimulated locomotor activity, dopamine synthesis and dopamine release. *Neuropharmacology,* 32, 885-93.

Brain, P. F., McAllister, K. H., & Walmsley, S. (1989). Drug effects on social behavior. In A. A. Boulton, G. B. Baker, & A. J. Greenshaw (Eds.), Methods in ethopharmacology, neuromethods (Vol. 13, pp. 687–739 Psychopharmacology). Clifton, NJ: Humana Press.

Branchi, I., D'Andrea, I., Fiore, M., Di Fausto, V., Aloe, L., & Alleva, E. (2006). Early social enrichment shapes social behavior and nerve growth factor and brain-derived neurotrophic factor levels in the adult mouse brain. *Biological Psychiatry,* 60, 690-96.

Brenes, J. C., & Fornaguera, J. (2008). Effects of environmental enrichment and social isolation on sucrose consumption and preference: Associations with depressive-like behavior and ventral striatum dopamine. *Neuroscience Letters,* 436, 278-82.

Brenes, J. C., Rodriguez, O., & Fornaguera, J. (2008). Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. *Pharmacology, Biochemistry, and Behavior,* 89, 85-93.

Brenes, J. C., Padilla, M., & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behavioural Brain Research,* 197, 125-37.

Burrows, E. L., & Hannan, A. J. (2013). Towards environmental construct validity in animal models of CNS disorders: Optimizing translation of preclinical studies. *CNS & Neurological Disorders Drug Targets,* 13; 7,157.

Chamove, A.S. (1989).Cage design reduces emotionality in mice.*Laboratory Animals,*23,215-19.

Cheng, J. P., Shaw, K. E., Monaco, C. M., Hoffman, A. N., Sozda, C. N., Olsen, A. S., & Kline, A. E. (2012). A relatively brief exposure to environmental enrichment after experimental traumatic brain injury confers long-term cognitive benefits. *Journal of Neurotrauma,* 29, 2684-88.

Chistyakov, V., Patkina, N., Tammimaki, A., Talka, R., Salminen, O., Belozertseva, I., … Zvartau, E. (2010). Nicotine exposure throughout early development promotes nicotine self-administration in adolescent mice and induces long-lasting behavioural changes. *European Journal of Pharmacology,* 640, 87-93.

de Witt, B. W., Ehrenberg, K. M., McAloon, R. L., Panos, A. H., Shaw, K. E., Raghavan, P. V… Kline, A. E. (2011). Abbreviated environmental enrichment enhances neurobehavioral recovery comparably to continuous exposure after traumatic brain injury. *Neurorehabilitation and Neural Repair,* 25, 343-50.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience and Biobehavioral Reviews,* 31, 673-704.

Doulames, V., Lee, S., & Shea, T. B. (2013). Environmental enrichment and social interaction improve cognitive function and decrease reactive oxidative species in normal adult mice. *International Journal of Neuroscience,* 124, 369-76.

Elliott, B. M., & Grunberg, N. E. (2005). Effects of social and physical enrichment on open field activity differ in male and female sprague-dawley rats. *Behavioural Brain Res,* 165, 187-96.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research,* 215, 244-54.

Fares, R. F., Kouchi, H., & Bezin, L. (2012). Standardized environmental enrichment for rodents in marlau cage. *Protocol Exchange.* In press.

Fernandez-Teruel, A., Escorihuela, R. M., Castellano, B., Gonzalez, B., & Tobena, A. (1997). Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: Focus on the roman rat lines. *Behavior Genetics,* 27, 513-26.

Fox, C., Merali, Z., & Harrison, C. (2006). Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. *Behavioural Brain Res,* 175, 1-8.

Frasca, D., Tomaszczyk, J., McFadyen, B. J., & Green, R. E. (2013). Traumatic brain injury and post-acute decline: What role does environmental enrichment play? A scoping review. *Frontiers in Human Neuroscience,* 7, 31.

Freret, T., Billard, J. M., Schumann-Bard, P., Dutar, P., Dauphin, F., Boulouard, M., & Bouet, V. (2012). Rescue of cognitive aging by long-lasting environmental enrichment exposure initiated before median lifespan.*Neurobiology of Aging,* 33, 1005.e1-1005.10.

Garrido, P., De Blas, M., Ronzoni, G., Cordero, I., Anton, M., Gine, E., … Mora, F. (2013). Differential effects of environmental enrichment and isolation housing on the hormonal and neurochemical responses to stress in the prefrontal cortex of the adult rat: Relationship to working and emotional memories. *Journal of Neural Transmission*, 120, 829-43.

Greco, S. J., Bryan, K. J., Sarkar, S., Zhu, X., Smith, M. A., Ashford, J. W., … Casadesus, G. (2010). Leptin reduces pathology and improves memory in a transgenic mouse model of alzheimer's disease. *Journal of Alzheimer's Disease,* 19 1155-67.

Gross, A. N., Richter, S. H., Engel, A. K., & Wurbel, H. (2012). Cage-induced stereotypies, perseveration and the effects of environmental enrichment in laboratory mice. *Behavioural Brain Research,* 234, 61-68.

Haemisch, A., & Gartner, K. (1997). Effects of cage enrichment on territorial aggression and stress physiology in male laboratory mice. *Acta Physiologica Scandinavica*, 640, 73-76.

Haemisch, A., Voss, T., & Gartner, K. (1994). Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J mice. *Physiology & Behavior*, 56, 1041-48.

Hannan, A. J. (2014). Environmental enrichment and brain repair: Harnessing the therapeutic effects of cognitive stimulation and physical activity to enhance experience-dependent plasticity. *Neuropathology and Applied Neurobiology,* 40, 13-25.

Harati, H., Majchrzak, M., Cosquer, B., Galani, R., Kelche, C., Cassel, J. C., & Barbelivien, A. (2011). Attention and memory in aged rats: Impact of lifelong environmental enrichment. *Neurobiology of Aging,* 32, 718-36.

Harati, H., Barbelivien, A., Herbeaux, K., Muller, M. A., Engeln, M., Kelche, C., … Majchrzak, M. (2013). Lifelong environmental enrichment in rats: Impact on emotional behavior, spatial memory vividness, and cholinergic neurons over the lifespan. *Age,* 35, 1027-43.

Hendriksen, H., Bink, D. I., Daniels, E. G., Pandit, R., Piriou, C., Slieker, R., … Oosting, R. S. (2012). Re-exposure and environmental enrichment reveal NPY-Y1 as a possible target for post-traumatic stress disorder. *Neuropharmacology,* 63, 733-42.

Heyser, C. J., & Chemero, A. (2012). Novel object exploration in mice: Not all objects are created equal. *Behavioural Processes,* 89, 232-38.

Hughes, R. N., & Otto, M. T. (2013). Anxiolytic effects of environmental enrichment attenuate sex-related anxiogenic effects of scopolamine in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 40, 252-259.

Hutchinson, K. M., McLaughlin, K. J., Wright, R. L., Bryce Ortiz, J., Anouti, D. P., Mika, A., … Conrad, C. D. (2012). Environmental enrichment protects against the effects of chronic stress on cognitive and morphological measures of hippocampal integrity. *Neurobiology of Learning and Memory,* 97, 250-60.

Johnson, E. M., Traver, K. L., Hoffman, S. W., Harrison, C. R., & Herman, J. P. (2013). Environmental enrichment protects against functional deficits caused by traumatic brain injury. *Frontiers in Behavioral Neuroscience,* 7, 44.

Kaliste, E. K., Mering, S. M., & Huuskonen, H. K. (2006). Environmental modification and agonistic behavior in NIH/S male mice: Nesting material enhances fighting but shelters prevent it. *Comparative Medicine,*56, 202-8.

Kempermann, G., Gast, D., & Gage, F. H. (2002). Neuroplasticity in old age: Sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Annals of Neurology,* 52, 135-43.

Kobilo, T., Liu, Q. R., Gandhi, K., Mughal, M., Shaham, Y., & van Praag, H. (2011). Running is the neurogenic and neurotrophic stimulus in environmental enrichment. *Learning & Memory*, 18, 605-9.

Kulesskaya, N., Rauvala, H., & Voikar, V. (2011). Evaluation of social and physical enrichment in modulation of behavioural phenotype in C57BL/6J female mice*. PloS One,* 6, e24755.

Laviola, G., Macri, S., Morley-Fletcher, S., & Adriani, W. (2003). Risk-taking behavior in adolescent mice: Psychobiological determinants and early epigenetic influence. *Neuroscience and Biobehavioral Reviews,* 27, 19-31.

Lazarov, O., Mattson, M. P., Peterson, D. A., Pimplikar, S. W., & van Praag, H. (2010). When neurogenesis encounters aging and disease. *Trends in Neurosciences,* 33, 569-79.

Leal-Galicia, P., Saldivar-Gonzalez, A., Morimoto, S., & Arias, C. (2007). Exposure to environmental enrichment elicits differential hippocampal cell proliferation: Role of individual responsiveness to anxiety. *Developmental Neurobiology,* 67, 395-405.

Leal-Galicia, P., Castaneda-Bueno, M., Quiroz-Baez, R., & Arias, C. (2008). Long-term exposure to environmental enrichment since youth prevents recognition memory decline and increases synaptic plasticity markers in aging. *Neurobiology of Learning and Memory,* 90, 511-18.

Lee, M., Yu, J. H., Kim, J. Y., Seo, J. H., Park, E. S., Kim, C. H., … Cho, S. (2013). Alteration of synaptic Activity–Regulating genes underlying functional improvement by long-term exposure to an enriched environment in the adult brain. *Neurorehabilitation and Neural Repair,* 27, 561-74.

Leger, M., Bouet, V., Freret, T., Darmaillacq, A. S., Dacher, M., Dauphin, F., … Schumann-Bard, P. (2012). Environmental enrichment improves recent but not remote memory in association with a modified brain metabolic activation profile in adult mice. *Behavioural Brain Research,* 228, 22-29.

Leggio, M. G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research,* 163, 78-90.

Macri, S., Ceci, C., Altabella, L., Canese, R., & Laviola, G. (2013). The directive 2010/63/EU on animal experimentation may skew the conclusions of pharmacological and behavioural studies. *Scientific Reports,* 3, 2380.

Madronal, N., Lopez-Aracil, C., Rangel, A., del Rio, J. A., Delgado-Garcia, J. M., & Gruart, A. (2010). Effects of enriched physical and social environments on motor performance, associative learning, and hippocampal neurogenesis in mice. *PloS One,* 5, e11130.

McQuaid, R. J., Audet, M., Jacobson-Pick, S., & Anisman, H. (2013). Environmental enrichment influences brain cytokine variations elicited by social defeat in mice. *Psychoneuroendocrinology,* 38, 987-96.

Mesa-Gresa, P., Perez-Martinez, A., & Redolat-Iborra, R. (2012). Nicotina y modelos animales: ¿qué nos aporta el paradigma de enriquecimiento ambiental? *Adicciones,* 24, 87-94.

Mesa-Gresa, P., Ramos-Campos, M., & Redolat, R. (2013a). Enriched Environments for Rodents and their Interaction with Nicotine Administration. *Current Drug Abuse Rew,* 6, 191-200.

Mesa-Gresa, P., Perez-Martinez, A., & Redolat, R. (2013b). Behavioral effects of combined environmental enrichment and chronic nicotine administration in male NMRI mice. *Physiology & Behavior,* 114-115C, 65-76.

Mesa-Gresa, P., Perez-Martinez, A., & Redolat, R. (2013c). Environmental enrichment improves novel object recognition and enhances agonistic behavior in male mice. *Aggressive Behavior,* 39, 269-79.

Mesa-Gresa, P., Ramos-Campos, M., & Redolat, R. (2014). Behavioral effects of different enriched environments in mice treated with the cholinergic agonist PNU-282987. *Behavioural Processes,* 103, 117-24.

Mohammed, A. H., Zhu, S. W., Darmopil, S., Hjerling-Leffler, J., Ernfors, P., Winblad, B., … Bogdanovic, N. (2002). Environmental enrichment and the brain. *Progress in Brain Research,* 138, 109-33.

Mora, F. (2013). Successful brain aging: Plasticity, environmental enrichment, and lifestyle. *Dialogues in Clinical Neuroscience,* 15, 45-52.

Mustroph, M. L., Chen, S., Desai, S. C., Cay, E. B., DeYoung, E. K., & Rhodes, J. S. (2012). Aerobic exercise is the critical variable in an enriched environment that increases hippocampal neurogenesis and water maze learning in male C57BL/6J mice. *Neuroscience,* 219, 62-71.

Nichol, K., Deeny, S. P., Seif, J., Camaclang, K., & Cotman, C. W. (2009). Exercise improves cognition and hippocampal plasticity in APOE epsilon4 mice. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association,* 5, 287-94.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews. Neuroscience,* 7, 697-709.

Nithianantharajah, J., & Hannan, A. J. (2009). The neurobiology of brain and cognitive reserve: Mental and physical activity as modulators of brain disorders.*Progress in Neurobiology,* 89, 369-82.

Paban, V., Chambon, C., Manrique, C., Touzet, C., & Alescio-Lautier, B. (2009). Neurotrophic signaling molecules associated with cholinergic damage in young and aged rats environmental enrichment as potential therapeutic agent.*Neurobiology of Aging,*32,470-85.

Pang, T. Y., & Hannan, A. J. (2013). Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. *Neuropharmacology,* 64, 515-28.

Patel, T. R. (2013). Environmental enrichment: Aging and memory. *The Yale Journal of Biology and Medicine,* 85, 491-500.

Pena, Y., Prunell, M., Dimitsantos, V., Nadal, R., & Escorihuela, R. M. (2006). Environmental enrichment effects in social investigation in rats are gender dependent. *Behavioural Brain Research,* 174, 181-87.

Petrosini, L., De Bartolo, P., Foti, F., Gelfo, F., Cutuli, D., Leggio, M. G., & Mandolesi, L. (2009). On whether the environmental enrichment may provide cognitive and brain reserves. *Brain Research Reviews,* 61, 221-39.

Pietropaolo, S., Branchi, I., Cirulli, F., Chiarotti, F., Aloe, L., & Alleva, E. (2004). Long-term effects of the periadolescent environment on exploratory activity and aggressive behaviour in mice: Social versus physical enrichment. *Physiology & Behavior,* 81, 443-53.

Pietropaolo, S., Sun, Y., Li, R., Brana, C., Feldon, J., & Yee, B. K. (2008). The impact of voluntary exercise on mental health in rodents: A neuroplasticity perspective. *Behavioural Brain Research,* 192, 42-60.

Pritchard, L., Van Kempen, T., & Zimmerberg, B. (2013). Behavioral effects of repeated handling differ in rats reared in social isolation and environmental enrichment. *Neuroscience Letters,* 536, 47-51.

Ramírez-Rodríguez, G., Ocaña-Fernández, M., Vega-Rivera, N., Torres-Pérez, O., Gómez-Sánchez, A., Estrada-Camarena, E., & Ortiz-López, L. (2014). Environmental enrichment induces neuroplastic changes in middle age female BalbC mice and increases the hippocampal levels of BDNF, p-akt and p-MAPK1/2. *Neuroscience,* 260, 158-70.

Redolat, R., & Mesa-Gresa, P. (2012). Potential benefits and limitations of enriched environments and cognitive activity on age-related behavioural decline. *Current Topics in Behavioral Neurosciences,* 10, 293-316.

Renner, M. J., & Rosenzweig, M. R. (1987). Enriched and impoverished environments effects on brain and behavior recent research in psychology. New York: Springer-Verlag.

Renner, M. J., & Rosenzweig, M. R. (1986). Social interactions among rats housed in grouped and enriched conditions. *Developmental Psychobiology,* 19, 303-13.

Richter, S. H., Zeuch, B., Riva, M. A., Gass, P., & Vollmayr, B. (2013). Environmental enrichment ameliorates depressive-like symptoms in young rats bred for learned helplessness. *Behavioural Brain Research,* 252, 287-92.

Rojas, J. J., Deniz, B. F., Miguel, P. M., Diaz, R., Hermel Edo, E., … Pereira, L. O. (2013). Effects of daily environmental enrichment on behavior and dendritic spine density in hippocampus following neonatal hypoxia-ischemia in the rat. *Experimental Neurology,* 241, 25-33.

Rosenzweig, M. R., & Bennett, E. L. (1996). Psychobiology of plasticity: Effects of training and experience on brain and behavior. *Behavioural Brain Research,* 78, 57-65.

Roy, V., Chapillon, P., Jeljeli, M., Caston, J., & Belzung, C. (2009). Free versus forced exposure to an elevated plus-maze: Evidence for new behavioral interpretations during test and retest. *Psychopharmacology,* 203, 131-41.

Sale, A., Berardi, N., & Maffei, L. (2009). Enrich the environment to empower the brain. *Trends in Neurosciences,* 32, 233-39.

Sale, A., Hannan, A. J., Maffei, L., & Guzzetta, A. (2013). Noninvasive strategies to optimise brain plasticity: From basic research to clinical perspectives. *Neural Plasticity,*2013: 863970.

Sale, A., Berardi, N., & Maffei, L. (2014). Environment and brain plasticity: Towards an endogenous pharmacotherapy. *Physiological Reviews,* 94, 189-234.

Sampedro-Piquero, P., Begega, A., Zancada-Menendez, C., Cuesta, M., & Arias, J. L. (2013). Age-dependent effects of environmental enrichment on brain networks and spatial memory in wistar rats. *Neuroscience,* 248C, 43-53.

Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats--behavioural and neurochemical aspects. *Behavioural Brain Research,* 222, 246-64.

Simpson, J., & Kelly, J. P. (2012). The effects of isolated and enriched housing conditions on baseline and drug-induced behavioural responses in the male rat. *Behavioural Brain Research,* 234, 175-83.

Siopi, E., Llufriu-Daben, G., Fanucchi, F., Plotkine, M., Marchand-Leroux, C., & Jafarian-Tehrani, M. (2012). Evaluation of late cognitive impairment and anxiety states following traumatic brain injury in mice: The effect of minocycline. *Neuroscience Letters,* 511, 110-15.

Solinas, M., Thiriet, N., Chauvet, C., & Jaber, M. (2010). Prevention and treatment of drug addiction by environmental enrichment. *Progress in Neurobiology,* 92, 572-92.

Speisman, R. B., Kumar, A., Rani, A., Pastoriza, J. M., Severance, J. E., Foster, T. C., & Ormerod, B. K. (2013). Environmental enrichment restores neurogenesis and rapid acquisition in aged rats. *Neurobiology of Aging,* 34, 263-74.

Swetter, B. J., Karpiak, C. P., & Cannon, J. T. (2011). Separating the effects of shelter from additional cage enhancements for group-housed BALB/cJ mice. *Neuroscience Letters,* 495, 205-9.

Tanti, A., Westphal, W. P., Girault, V., Brizard, B., Devers, S., Leguisquet, A. M., … Belzung, C. (2013). Region-dependent and stage-specific effects of stress, environmental enrichment and antidepressant treatment on hippocampal neurogenesis. *Hippocampus,* 252, 234-52.

Tirelli, E., Tambour, S., & Michel, A. (2003). Sensitised locomotion does not predict conditioned locomotion in cocaine-treated mice: Further evidence against the excitatory conditioning model of context-dependent sensitisation. *European Neuropsychopharmacology,* 13, 289-296.

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews.Neuroscience,* 1, 191-98.

Varty, G. B., Paulus, M. P., Braff, D. L., & Geyer, M. A. (2000). Environmental enrichment and isolation rearing in the rat: Effects on locomotor behavior and startle response plasticity. *Biological Psychiatry,* 47, 864-73.

Vazquez-Sanroman, D., Sanchis-Segura, C., Toledo, R., Hernandez, M., Manzo, J., & Miquel, M. (2013). The effects of enriched environment on BDNF expression in the mouse cerebellum depending on the length of exposure. *Behavioural Brain Research,* 243, 118-28.

Vedovelli, K., Silveira, E., Velho, E., Stertz, L., Kapczinski, F., Schroder, N., & Bromberg, E. (2011). Effects of increased opportunity for physical exercise and learning experiences on recognition memory and brain-derived neurotrophic factor levels in brain and serum of rats. *Neuroscience,* 199, 284-91.

Viola, G. G., Rodrigues, L., Americo, J. C., Hansel, G., Vargas, R. S., Biasibetti, R., … Amaral, O. B. (2009). Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice. *Brain Research*, 1274, 47-54.

Wood, N. I., Carta, V., Milde, S., Skillings, E. A., McAllister, C. J., Ang, Y. L., … Morton, A. J. (2010). Responses to environmental *enrichment differ with sex and genotype in a transgenic mouse model of huntington's disease. PloS One,* 5, e9077.

Workman, J. L., Fonken, L. K., Gusfa, J., Kassouf, K. M., & Nelson, R. J. (2011). Post-weaning environmental enrichment alters affective responses and interacts with behavioral testing to alter nNOS immunoreactivity. *Pharmacology, Biochemistry, and Behavior,* 100, 25-32.

Xie, H., Wu, Y., Jia, J., Liu, G., Zhang, Q., Yu, K., … Hu, R. (2013). Enrichment-induced exercise to quantify the effect of different housing conditions: A tool to standardize enriched environment protocols. *Behavioural Brain Research,* 249, 81-89.

Yuan, Z. Y., Gu, P., Liu, L., Wang, Y. Y., Liu, J., Cui, D. S., … Wang, M. W. (2009). Neuroprotective effects of enriched environment in MPTP-treated SAMP8 mice. *Neuroscience Letters,* 454, 6-10.

Zhu, S. W., Yee, B. K., Nyffeler, M., Winblad, B., Feldon, J., & Mohammed, A. H. (2006). Influence of differential housing on emotional behaviour and neurotrophin levels in mice. *Behavioural Brain Research,* 169, 10-20.

Zhu, S. W., Codita, A., Bogdanovic, N., Hjerling-Leffler, J., Ernfors, P., Winblad, B., … Mohammed, A. H. (2009). Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice.*Behavioural Brain Research,* 197, 339-46.

Zimmermann, A., Stauffacher, M., Langhans, W., & Wurbel, H. (2001). Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. *Behavioural Brain Research,* 121, 11-20.

CAPÍTULO 6

ESTUDIO 5

Aplicación del paradigma de enriquecimiento ambiental a un modelo de estrés social crónico: efectos fisiológicos y conductuales en ratones

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En preparación:

Mesa-Gresa P, Ramos-Campos M, Redolat R. Physiologycal and behavioral efects of the exposure to an enviromental enrichment and chronic social stress in NMRI male mice.

PHYSIOLOGICAL AND BEHAVIORAL EFECTS OF SIMULTANEOUS EXPOSURE TO ENRICHED ENVIRONMENTS AND CHRONIC SOCIAL STRESS IN NMRI MALE MICE

Abstract

Environmental enrichment (EE) is an experimental paradigm which is believed to counteract some of the effects induced by stressors, although few studies have exposed rodents simultaneously to EE and stress. Our aim was to compare the short- and long- term effects of different housing conditions in mice submitted to chronic social stress. 128 NMRI male mice arrived at our laboratory on postnatal day 21 (PND 21). During Phase I of the study (PND 28), half of the animals were submitted to a social stress protocol following the procedure reported by Schmidt et al. (2008) and based on a complete disruption of the established hierarchy in each cage. Animals were assigned to four experimental groups: 1) EE+STRESS: mice housed in EE and submitted to stress (n=32); 2) EE+NO STRESS: no stress was applied while mice were kept in EE conditions (n=32); 3) SE+STRESS: mice maintained in standard housing conditions (SE) and submitted to stress (n=32); and 4) SE+NO STRESS (n=32). At the end of Phase I (PND 77), one cohort of 32 animals was used for behavioral assessment (elevated plus-maze test, hole-board, running wheel and novel object recognition task) whereas another cohort of 32 was sacrificed in order to obtain blood samples for corticosterone analysis. Results indicated that the factor "Housing" was significant indicating that EE animals showed less body weight, higher water and food intake, diminished anxiety response and decreased motor and exploratory behavior. The "Stress" factor was also significant indicating that animals exposed to stress gained less body weight, showed higher food and fluid intake and displayed decreased exploratory behavior. Furthermore, EE+STRESS group displayed significantly higher corticosterone levels than EE+NO STRESS group whereas EE+NO STRESS group showed lower levels than SE+NO STRESS. On PND 83 (when the stress procedure had finished), Phase II of the study began. Animals (n=96) were assigned to two different housing conditions: EE (n=48) and SE (n=48). On PND 112, a cohort of mice was sacrificed in order to obtain corticosterone analysis (n=32) whereas other group of mice (n=64) were used for behavioral study. Results indicated that EE animals showed lower body weight and higher food and fluid intake, as well as some effects on emotional response assessed in the elevated plus maze test. Further studies are needed to evaluate the long-term effects of exposure to EE during social stress situations.

1. INTRODUCTION

Experimental evidence indicates that exposure to chronic stress situations, including prenatal exposure, induces alterations in the nervous system (e.g. changes in the prefrontal cortex, hippocampus and amygdala) and modifications in neurochemical, endocrine and immune function, as well as complex behavioral responses (De Miguel et al., 2011; Emack & Mathews, 2011; Franklin et al., 2012; Malter Cohen et al., 2013; McEwen et al., 2012; McQuaid et al., 2013), though data concerning long-term consequences are still limited (Garrido et al., 2013; Scharf et al., 2013). Chronic stress may influence emotional systems and induce a dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis that controls emotional response, leaving the individual more vulnerabe to affective and anxiety disorders (Malter Cohen et al., 2013; Sterlemann et al., 2008; Wang et al., 2012). Different studies have also reported a less effective performance of memory and learning tasks as a consequence of exposure to stress (Buwalda et al., 2011; Solinas et al., 2008), as well as changes in synaptic plasticity, cell proliferation and BDNF levels in the hippocampus (Scharf et al., 2013; Schmidt et al., 2013). A stressful experience early in life, in turn, increases the individual's vulnerability to environmental challenges and may interact with genetic risk factors related to susceptibility to anxiety during adulthood (Scharf et al., 2013; Wang et al., 2012). Moreover, early exposure to stress has been shown to accelerate the rate of cognitive decline and hippocampal damage during aging (Epel, 2009; Fox et al., 2010; Pardon, 2009).

The model of social stress established by Müller and colleagues seems to be an interesting paradigm for the study of the behavioral effects of exposure to chronic social stress. This model has numerous advantages, including high construct and predictive validity and the possibility of analyzing individual vulnerability in response to stressful events (Buwalda et al., 2010; Kalueff & Schmidt, 2011; Schmidt, 2010a, 2010b; Sterlemann et al., 2010). Moreover, this paradigm initiates exposure to stressful situations in adolescence and into early adulthood, allowing the consequences of adverse experiences at an early age (an important period of brain development) to be studied (Schloesser et al., 2008). This model of chronic social

stress is based on a complete disruption of the social hierarchy of animals, which creates an unstable social environment for an extended period of time. It has been observed that a week after exposure to these stressful conditions, the HPA axis is still hyperactive, which induces a downregulation of glucocorticoid receptors in the hippocampus. In addition, mice show an anxiety-like response in tasks such as the open field or elevated plus maze (Schmidt et al., 2007). The long-term effects of exposure to this chronic stress situation include physiological and behavioral consequences, changes in the distribution of body fat in stressed animals related to a greater possibility of suffering metabolic illness, even one whole year after the exposure to stress (Schmidt et al., 2009), an increase in the basal corticosterone levels (Scharf et al., 2013), and an impairment of spatial memory and exploratory behavior (Scharf et al., 2013; Sterlemann et al., 2010).

Diverse studies have identified some protective and modulatory variables which may regulate stress responses, such as pharmacological treatments (Pérez-Tejada et al., 2013; Scharf et al., 2013; Sterlemann et al., 2008) and environmental conditions (Garrido et al., 2013, Malter Cohen et al., 2013; McQuaid et al., 2013; Sale et al., 2014; Schloesser et al., 2010). The Environmental Enrichment (EE) paradigm has been recognized as a suitable model to investigate the "protective" effects of maintaining an active lifestyle (Laviola et al., 2008; Mora, 2013), and has also been related to regulation of the stress response (Emack and Matthews, 2011; McQuaid et al., 2013; Schloesser et al., 2010). The EE model is based on the exposure of animals to higher levels of physical, cognitive, social and somatosensorial stimulation than they receive in standard housing conditions (Mesa-Gresa et al., 2013a; Sale et al., 2014). Research has shown that allocating animals to these complex conditions induces beneficial effects at neurobiological, physical and behavioral levels (Hannan, 2014; Sale et al., 2014), including a reduction of negative effects of exposure to psychological and/or chronic stress (Burrows et al., 2010; Schloesser et al., 2010).

Some previous studies have shown opposite effects between exposure to stress and enriched environments (Barak et al., 2013; McQuaid et al., 2013; Mitra & Sapolsky, 2008; Schloesser et al., 2010), observing that EE ameliorates some of the effects of stress on brain structure and chemistry (Garrido et al., 2013; Segovia et al., 2009) and

has benefits for emotional response and copying behaviors (Schloesser et al., 2010), as well as for learning and memory performance (Mitra & Sapolsky, 2008; Garrido et al., 2013). Housing animals in enriched environments has positive effects on synaptic plasticity and brain reward systems (Artola et al., 2006; Mitra & Sapolsky, 2009; Schloesser et al., 2010) and appears to promote resilience to the negative effects of stress (Fox et al., 2006). Moreover, EE conditions affect stress hormone release, reducing the HPA axis response and acetylcholine release, and improving emotional reactivity and memory deficits related to exposure to different stressors such as isolation, immobilization or prenatal stress (Chen et al., 2010; Cymerblit-Sabba et al., 2013; Hendriksen et al., 2010).

Recent research suggests that subjects do not respond in the same way to changes in social environment, as some are more resilient or vulnerable with respect to specific experiences (Franklin et al., 2012; Illin & Richter-Levin, 2009). Allocating animals to enriched environment at different ages can produce important changes in stress vulnerability and resilience response (Garrido et al., 2013; Hutchinson et al., 2012; Ravenelle et al., 2013). Thus, the EE paradigm can be considered a useful model that increases psychological resilience to chronic social stress (Lehmann & Henkermann, 2011). In a recent study, Hutchinson et al. (2012) evaluated the physiological and behavioral impact of exposure to EE and chronic stress in rats during adulthood, observing that EE diminished some of the impairing effects induced by chronic stress on hippocampal function and learning capabilities, independently of the moment of exposure to the enriched environment (before or after the stress exposure).

Despite this evidence, few studies have evaluated if exposure to enriched environments involving models with high predictive validity can counteract the effects of social stress or how changes in emotionality, which is known to modulate cognitive function, may contribute to resilience or vulnerability to the effects of stress in animals housed in different environments. More specifically, few studies have assessed the physiological and behavioral consequences of exposure to chronic social stress in interaction with environmental manipulations, or the mechanisms underlying these effects. Taking this question into account, the main aim of the current research was to evaluate the physiological and behavioral consequences of exposure of NMRI male

mice to a chronic social stress situation at an early age, after which the animals were housed in different environmental conditions. Physical parameters involved corticosterone levels, weight gain, and fluid and food intake, while behavioral assessment included tasks related to anxiety-like response, exploratory behavior, motor activity and learning and memory. The experiment referred to as "Phase I" was performed to evaluate the effects of exposure to chronic social stress in mice housed in different environmental conditions. The experiment referred to as "Phase II" evaluated the long-term consequences of maintaining animals in enriched or standard housing conditions after the initial stressing conditions (accompanied or not by enriched housing) had terminated.

2. MATERIALS AND METHODS

The experimental procedure of the current research was performed in two phases. In order to clarify their nature, they will be referred to as Phase I (exposure to a chronic social stress condition, accompanied or not by exposure to EE conditions) and Phase II (evaluation of long-term consequences of prior exposure to stress in animals maintained in enriched or standard housing conditions).

2.1. Phase I: Physiological and behavioral effects of exposure to chronic social stress in mice housed in different environmental conditions.

2.1.1. Animals

NMRI male mice (Charles River, Barcelona, Spain) were used in the present study. Animals arrived at our laboratory at 21 days of age (PND 21) weighing between 10-12 gr. After a week of adaptation to laboratory conditions (temperature 20-24°C, humidity 55±10%, 12-h light-dark cycle, lights on 8:00h, and water and food access ad libitum), mice were randomly allocated to one of four different experimental conditions: 1) Environmental Enrichment + Social Stress (EE+STRESS): n=32; 2) Environmental Enrichment + No Social Stress (EE+NO STRESS): n=32; 3) Standard Environment + Social Stress (SE+STRESS): n=32; 4) Standard Environment + No Social

Stress (SE+NO STRESS). All animals were marked on both ears and on the tail in order to identify them during the entire process of the experiment. Experimental procedures were approved by the local ethical commitee (University of Valencia) and complied with national (Real Decreto 1201/2005, de 10 de Octubre) and international guidelines (European Community's Council Directive of November 24, 1986 — 86/609/EEC) for the care and handling of animals. At the end of Phase I of the study, one cohort of 32 animals was used for behavioral assessment whereas another cohort of 32 was sacrificed in order to obtain blood samples for hormonal analysis.

2.1.2. Housing conditions

At 28 days of age, animals were randomly allocated to one of four different experimental conditions, as indicated previously. The main characteristics of the two different housing conditions were: 1) Environmental Enrichment (EE), animals housed in groups of 4 in larger cages (55x36x19 cm) with assorted toys including a house, tunnels and a running wheel (n=64) (For more detailed description please see Mesa-Gresa et al., 2013b). Although mice were allocated to groups of 8 per cage in similar EE procedures previously performed in our laboratory, in the current study animals were housed in fours in order to apply the social stress protocol described below; and 2) Standard Environment (SE), mice were allocated in groups of 4 to standard cages (42x26x19 cm) containing only sawdust (n=64).

2.1.3. Social stress procedure

The procedure of chronic stress employed in Phase I of the current study was based on the social stress model of Sterlemann et al. (2008) and Schmidt et al. (2007), and was adapted to the main purpose of our study, which was to evaluate interaction between stress and housing conditions. This model exposes animals to an “unstable social environment” owing to a complete disruption of the social hierarchy of the mice. On PND 28, animals were randomly assigned to one of two different experimental “stress” conditions: 1) Social Stress (STRESS): Mice were divided into groups of four and underwent the social stress procedure. The composition and hierarchy of each cage were changed twice a week for 7 weeks. In each of these changes, four mice belonging to four different cages were housed together in a new, clean cage with

sawdust and/or toys (depending on the housing group of the animals). Rotation of mice during the social stress procedure was randomized in order to minimize the probability of encounters between animals that had been housed together at some point during the previous 7 weeks (n=64); and 2) No Social Stress (NO STRESS): Mice were divided into groups of four which remained together in the same cage during the whole of Phase I of the experimental procedure (n=64).

2.1.4. Experimental procedure

In Phase I of the study, animals were allocated to the abovementioned experimental conditions from PND 28 to PND 77. On PND 77, a cohort of 32 mice were sacrificed in order to take corticosterone measures from blood samples, and 32 were exposed to a battery of behavioral tests from PND 77 to PND 84. The order of the behavioral tests employed in the present study was as follows: elevated plus maze (PND 77), hole board (PND 77), running wheel task (PND 78) and object recognition test (PND 79 and 80). In addition, further physiological measures were obtained twice a week during the whole experimental procedure (PND 28-77), including body weight of the animals and fluid and food intake.

Animals were taken to the experimental room one hour before behavioral testing began. All tests were carried out under light conditions and cages were cleaned at the beginning of the tests and between subjects with a solution containing water and ethanol (2%).

2.1.5. Body weight and fluid and food intake

Physiological parameters obtained during the experimental procedure of Phase I were animal body weight and fluid and food intake. Measurements were taken twice a week during the course of the seven weeks of social stress procedure at the time of manipulation of the cages. Average fluid (ml/mouse) and food intake (gr/mouse) was calculated as the mean of fluid and food consumption measurements, each of which was calculated as the total consumption divided by the number of mice allocated to each cage (Van de Weerd et al., 1997; 2002).

2.1.6. Analysis of corticosterone levels

Serum corticosterone levels were obtained on PND 78. Mice were anesthetized with halothane and their blood collected via inferior vena cava in order to determine concentrations of corticosterone. The blood samples were centrifuged in heparin-containing tubes (MP Biomedicals: LLC) and the plasma was frozen at -20°C until the assay was carried out. The technique used for the analysis was radioimmunoassay (RIA). All samples were tested in duplicate and the average value of the two tests was considered for the statistical analyses. The inferior limit of sensitivity was 25 ng/ml and the inter-intra assay coefficients of variation were 3.24% and 5.93%, respectively.

2.1.7. Behavioral tests

Elevated plus-maze (EPM), hole-board (HB), running wheel task (RW) and object recognition test (NOR).

2.1.8. Statistical analysis

All statistical analysis was carried out using IBM SPSS Statistics 19.0 for Windows. Body weight and fluid and food intake were analyzed using a repeated measures analysis of variance (ANOVA) and post-hoc Tukey tests. Corticosterone measures and behavioral data obtained in EPM, HB, RW and NOR were analyzed with ANOVA using "Housing Phase I" and "Social Stress" as between-subjects factors. Significance level was set at $p<0.05$ and assumptions for ANOVA were taken into account. All data are presented as mean ± standard error of the mean (SEM).

2.2. Phase II: Physiological and behavioral effects of exposure to different environmental conditions on mice previously submitted to chronic social stress

2.2.1. Animals

96 NMRI male mice (Charles River, Barcelona, Spain), which had arrived at our laboratory at 21 days of age (PND 21) and had undergone Phase I under one of the previously reported "Initial Conditions" (see section 2.1), were evaluated in a second phase of the study. On PND 83 (when the stress procedure had finished) these animals

were assigned to two different housing conditions in which they were maintained until the end of the experiment. Half of the animals which had been housed in enriched cages in Phase I remained in these conditions, whereas the other half was exposed to standard housing conditions. In a similar way, half of the animals which had been housed in standard housing conditions in Phase I were maintained in the same conditions while the other half was exposed to enriched housing. No stress procedure was applied during Phase II of the study, since our aim was to observe the long-term consequences of the initial stress. In this phase, 64 animals were used in behavioral studies and 32 were used for hormonal analysis.

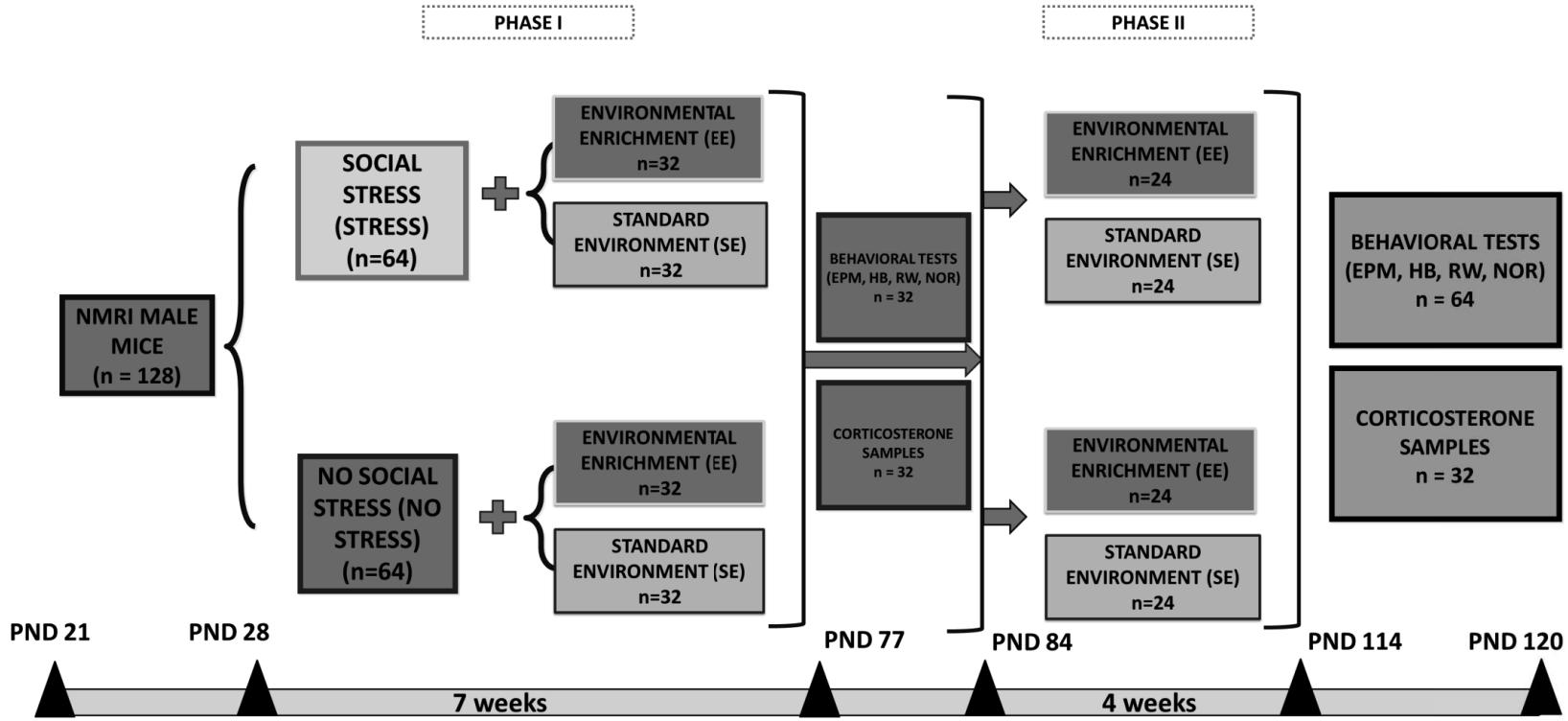
2.2.2. Experimental procedure

During Phase II (from PND 83 to PND 118), physiological measures were obtained twice a week. On PND 112, 64 mice were assessed in a battery of behavioral tests similar to that employed at the end of Phase I of the study: EPM (PND 113-114), HB (PND 113-114), RW (PND 115), and NOR (PND 116-118). On the same day (PND 112), 32 mice were sacrificed in order to obtain corticosterone samples. The behavioral tests and physiological variables obtained were the same as those in Phase I.

2.2.3. Statistical analysis

Statistical analysis was performed with SPSS in a similar manner to that in Phase I of the current study. The following factors were taken into account for analysis: “Initial Condition”, which refers to the conditions animals were exposed to during Phase I of the study (EE+STRESS, EE+NO STRESS, SE+STRESS, SE+NO STRESS), and “Housing Phase II” (for which EE and SE conditions during the second phase of the study were compared). Post-hoc Tukey or Dunet T3 were used when required.

Figure 1. Experimental procedure.



3. RESULTS

3.1. Phase I

3.1.1. Body weight, fluid intake and food consumption

ANOVA for repeated measures indicated that the factor "Housing Phase I" reached statistical significance for body weight [$F(1,130)=14.88$, $p<0.0001$]. Mice housed in EE conditions gained less body weight than mice allocated to SE. The "Stress" factor also showed statistical significance [$F(1,130)=6.41$, $p<0.05$], as mice submitted to social stress weighed less than their non-stressed counterpart. The interaction "Housing Phase I x Stress" was also significant [$F(3,128)=12.67$, $p<0.001$]. Post-hoc Tukey tests indicated that mice in the EE+STRESS group were lighter than those in EE+NO STRESS, SE+STRESS and SE+NO STRESS groups ($p<0.0001$).

ANOVA for repeated measures of fluid intake indicated that the factors "Housing Phase I" [$F(1,130)=283.11$, $p<0.0001$] and "Stress" [$F(1,130)=430.44$, $p<0.0001$] reached statistical significance. Mice allocated to EE conditions displayed higher fluid intake than SE-housed mice. Moreover, animals subjected to stress also showed higher fluid consumption than non-stressed mice. The interaction "Housing Phase I x Stress" reached statistical significance [$F(3,128)=104.42$, $p<0.0001$]. A Post-hoc Tukey test indicated that the EE+STRESS group displayed higher fluid intake than EE+NO STRESS, SE+STRESS and SE+NO STRESS mice ($p<0.0001$). Furthermore, EE+NO STRESS groups showed lower fluid intake than SE+STRESS animals ($p<0.05$) and higher fluid consumption than SE+NO STRESS animals ($p<0.0001$), whereas the SE+STRESS group displayed higher water intake than SE+NO STRESS mice ($p<0.0001$).

Regarding food consumption data, ANOVA reached statistical significance for "Housing Phase I" [$F(1,130)=39.32$, $p<0.0001$] and "Stress" factors [$F(1,130)=138.14$, $p<0.0001$]. EE-housed mice displayed higher food intake than SE-housed mice, whereas stressed mice displayed higher food consumption than non-stressed ones. The interaction "Housing Phase I x Stress" was significant [$F(3,128)=10.99$, $p<0.001$]. A Post-hoc test indicated that the EE+STRESS group exhibited a higher food intake than EE+NO STRESS and SE+NO STRESS groups ($p<0.0001$), whereas the EE+NO STRESS group showed lower food consumption than the SE+STRESS group but higher food

intake than the SE+NO STRESS group ($p<0.001$). SE+STRESS mice also showed higher food consumption than their SE+NO STRESS counterparts ($p<0.0001$).

3.1.2. Corticosterone levels

Neither "Housing Phase I" nor "Stress" factors reached statistical significance with respect to corticosterone measures [$F(1,30)= 0.483$, n.s. and $F(1,30)=2.786$, n.s., respectively]. When the interaction "Housing Phase I x Stress" was considered, ANOVA revealed significant results [$F(3,28)=11.346$ ($p<0.01$)]. A Post-hoc Tukey test indicated that the EE+STRESS group displayed significantly higher corticosterone levels than the EE+NO STRESS group ($p<0.01$). In contrast, animals allocated to the EE+NO STRESS group presented lower levels than those reared in SE+NO STRESS conditions ($p<0.05$). Corticosterone levels for the four experimental groups are shown in Figure 2.

3.1.3. Elevated Plus Maze

ANOVA showed that the factor "Housing Phase I" was significant in relation to closed-arm entries [$F(1,30)=7.21$, $p<0.05$] and percentage of open-arm entries [$F(1,30)=4.273$, $p<0.05$]. Animals housed under EE conditions performed fewer entries into the closed arms and a higher percentage of entries into the open area of the maze than animals allocated to standard housing (See Table 1). Regarding ethological parameters, the variable of total number of head-dips proved significant with respect to the factor "Housing Phase I" [$F(1,30)=4.93$, $p<0.05$], as EE-housed mice performed a higher total number of head-dips than standard-housed mice. Neither the factor "Stress" nor the interaction between factors obtained statistical significance for any of the variables evaluated.

3.1.4. Hole Board

Neither "Housing Phase I" nor "Stress" showed significant results in relation to "Latency to the first HD". The variable "number of HD at 1 minute of the test" reached statistical significance for the factor "Housing Phase I" [$F(1,30)=7.81$, $p<0.01$]. When the variable "total number of HD" were considered (5 min), ANOVA revealed statistical significance for the factors "Housing Phase I" [$F(1,30)=7.82$, $p<0.01$] and "Stress" [$F(1, 30)=4.89$, $p<0.05$], but not for the interaction "Housing Phase I" x "Stress". Animals

allocated to EE conditions performed a lower total number of HD at 1 and 5 minutes than those housed in SE. On the other hand, animals exposed to Stress conditions also performed a lower number of HD at 5 minutes of the test than control animals (See Figure 3).

3.1.5. Running Wheel

Analysis of the turnings of the running wheel during 5-min periods in each group indicated significant results for the factor "Housing Phase I" for measures obtained at 10 [$F(1,30)=4.55$, $p<0.05$] and 15 minutes of the test [$F(1,30)=6.98$, $p<0.05$]. Motor activity in enriched mice was lower than in standard-housed mice. No statistically significant differences were detected for the factor "Stress" or interaction between factors in this task.

3.1.6. Novel object recognition test

When animals were evaluated during the Test phase of the task, no statistical significance was obtained for the variable "discrimination index" for any of the factors evaluated. However, frequency of grooming behavior reached statistical significance for "Housing Phase I" [$F(1,30)=4.13$, $p<0.05$], "Stress" [$F(1,30)=4.93$, $p<0.05$] and for the interaction "Housing Phase I x Stress" [$F(3,28)=8.62$, $p<0.01$]. Animals housed under EE conditions and those exposed to social stress showed a higher frequency of grooming in the test phase than those allocated to standard cages or not exposed to stress. A Post-hoc Tukey test indicated that mice in the EE+STRESS group engaged in more grooming than animals in the EE+NO STRESS and SE+STRESS groups ($p<0.01$).

3.2. Phase II

3.2.1. Body weight, fluid intake and food consumption

ANOVA for repeated measures indicated that the main factor "Housing Phase II" [$F(1,95)=4.746$, $p<0.05$] reached statistical significance for body weight. Animals allocated to EE conditions gained less weight than their standard-house counterparts. Neither the factor "Initial condition" nor the interaction between factors reached significance.

Regarding fluid consumption, the factor "Housing Phase II" [$F(1,96)=16.12$, $p<0.001$] was significant, indicating that fluid intake was higher in EE animals than in those allocated to SE. The factor "Initial condition" was also significant [$F(3,94)=40.752$, $p<0.0001$]. A Post-hoc Tukey test indicated that intake was higher in the EE+STRESS group than the EE+NO STRESS, SE+STRESS and EE+NO STRESS groups ($p<0.001$), whereas it was higher in the SE+STRESS group than the EE+NO STRESS and SE+NO STRESS groups ($p<0.0001$). However, when the interaction between factors was considered, results did not prove to be significant.

ANOVA for repeated measures of food consumption also revealed that "Housing Phase II" was a significant factor [$F(1,96)=19.30$, $p<0.0001$], indicating that consumption was higher among EE animals than standard-ones. The factor "Initial condition" was also significant [$F(3,94)=59.06$, $p<0.0001$]. Animals assigned to EE+STRESS conditions during Phase I of the study displayed higher intake than those belonging to the EE+NO STRESS and SE+NO STRESS groups ($p<0.0001$), but lower intake than SE+STRESS mice ($p<0.05$). Moreover, consumption was higher among animals initially exposed to SE+STRESS conditions than those exposed to EE+NO STRESS and SE+NO STRESS conditions ($p<0.0001$), while it was higher in the group SE+NO STRESS group than in the EE+NO STRESS group ($p<0.05$)

3.2.2. Corticosterone levels

Neither "Housing Phase II" nor "Initial condition" factors nor the interaction between them reached significant effects with respect to the corticosterone measures obtained in Phase II of the study (See Figure 4).

3.2.3. Elevated Plus Maze

ANOVA showed significant results for "Housing Phase II" in relation to the percentage of time spent in the closed arms [$F(1,62)=6.15$, $p<0.05$]. Animals reared in EE conditions spent more time in the closed arms than SE-mice. Regarding the factor "Initial condition", the number of entries into the closed arms was also statistically significant [$F(3,60)=3.25$, $p<0.05$], as mice exposed to EE+STRESS in Phase I of the study

performed a lower number of entries into the closed arms than those exposed to the EE+NO STRESS condition.

When ethological measures were considered, the factor "Housing Phase II" reached statistical significance for the variables of time [$F(1,62)=5.10$, $p<0.05$] and frequency of peeping-out behavior [$F(1,62)=4.71$, $p<0.05$] and total SAP displayed by animals [$F(1,62)=9.16$, $p<0.001$]. Mice housed in EE conditions during Phase II spent less time engaged in peeping-out behavior ($p<0.05$) and less frequently, and also performed a lower total number of SAP ($p<0.001$) than animals in the SE group. The "Initial condition" factor also proved to be statistically significant for the variables of number of peeping-out [$F(3,60)=4.63$, $p<0.001$] and percentage of protected head-dips [$F(3,60)=3.44$, $p<0.05$]. The main results indicated that mice whose initial condition in Phase I of the study was EE+STRESS or SE+NO STRESS performed a lower number of peeping-out than those who were initially exposed to EE+NO STRESS ($p<0.05$). Moreover, animals in the EE+NO STRESS group performed a higher percentage of protected HD than SE+NO STRESS animals during Phase I ($p<0.05$) (See Table 2).

3.2.4. Hole Board

Neither "Housing Phase II" nor interaction between factors produced significant results for this second phase of the study. However, when the factor "Initial condition" was considered, the variables "Latency to the first HD" [$F(3,60)=5.23$, $p<0.01$] and "total number of HD at 1 min" [$F(3,60)=4.43$, $p<0.001$] "and at 5 min "[$F(3,60)=8.56$, $p<0.0001$] reached statistical significance. Animals assigned to the EE+STRESS condition in Phase I of the study displayed a higher latency to the first HD than EE+NO STRESS, SE+STRESS and SE+NO STRESS ($p<0.05$) groups when evaluated at the end of the second phase of the study (See Figure 5). Furthermore, animals whose initial condition during Phase I was EE+STRESS performed a lower number of HD at the first minute of the test than those initially in the EE+NO STRESS ($p<0.01$) and a lower number of HD at 5 min than those who had been in EE+NO STRESS and SE+NO STRESS groups in Phase I ($p<0.001$). Furthermore, those initially exposed to SE+STRESS performed a lower total number of HD than those initially in the SE+NO STRESS group ($p<0.01$).

3.2.5. Running Wheel

ANOVA for “turnings of the running wheel” detected significant differences for the “Initial Condition” factor with respect to data obtained at 5 min of the test [$F(3,60)=4.78$, $p<0.01$]. Motor activity of mice whose initial group was EE+STRESS was lower than those in the SE+STRESS and SE+NO STRESS groups ($p<0.05$). Neither “Housing Phase II” nor interaction reached statistical significance for the measures assessed.

3.2.6. Novel object recognition test

Regarding the test phase, ANOVA reached statistical significance for “Housing Phase II” with respect to time devoted to the new object [$F(1,62)=5.33$, $p<0.05$]. Mice housed in EE conditions spent less time engaged in this behaviour than standard-housed mice. Neither “Initial Condition” nor interaction between factors showed statistically significant differences.

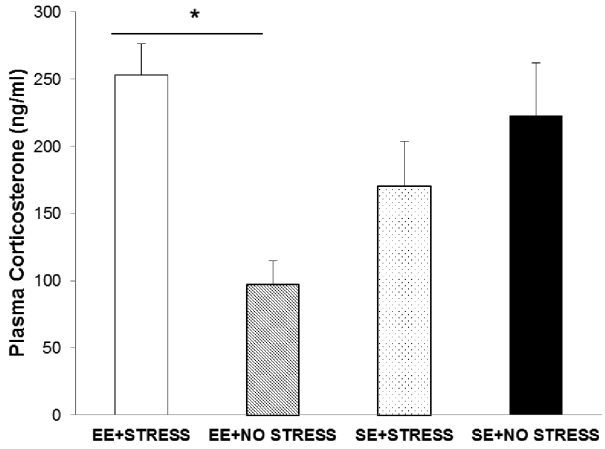


Figure 2. Plasma corticosterone levels (ng/ml) in the four experimental groups during Phase I of the study: Environmental Enrichment + Stress (EE+STRESS), Environmental Enrichment + No Stress (EE+NO STRESS), Standard Environment + Stress (SE+STRESS) and Standard Environment + No Stress (SE+NO STRESS). Data are presented as mean ± SEM.

(*) p<0.01; EE+STRESS vs. EE+NO STRESS

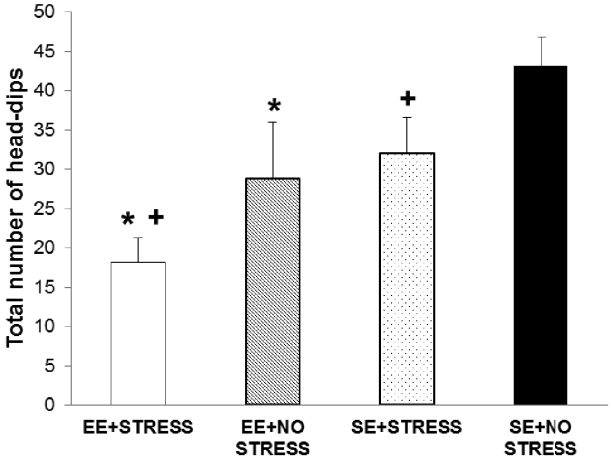


Figure 3. Total number of head-dips displayed in the hole-board test by the four experimental groups during Phase I of the study: Environmental Enrichment + Stress (EE+STRESS), Environmental Enrichment + No Stress (EE+NO STRESS), Standard Environment + Stress (SE+STRESS) and Standard Environment + No Stress (SE+NO STRESS). Data are presented as mean ± SEM.

(*) p<0.01; EE vs. SE. (+) p<0.05; STRESS vs. NO STRESS

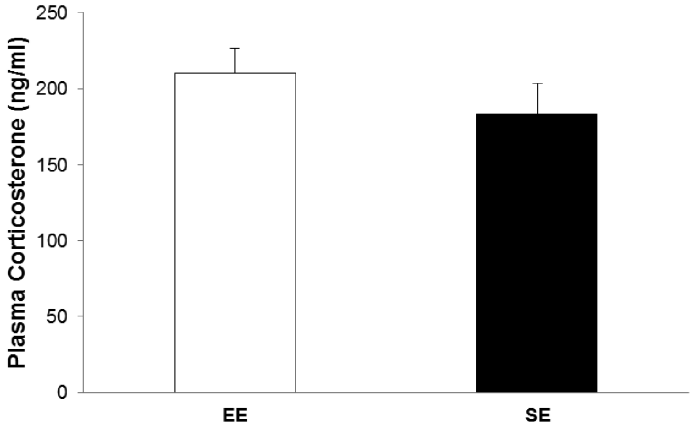


Figure 4. Plasma corticosterone levels (ng/ml) in the two experimental groups during Phase II of the study: Environmental Enrichment (EE) and Standard Environment (SE). Data are presented as mean ± SEM.

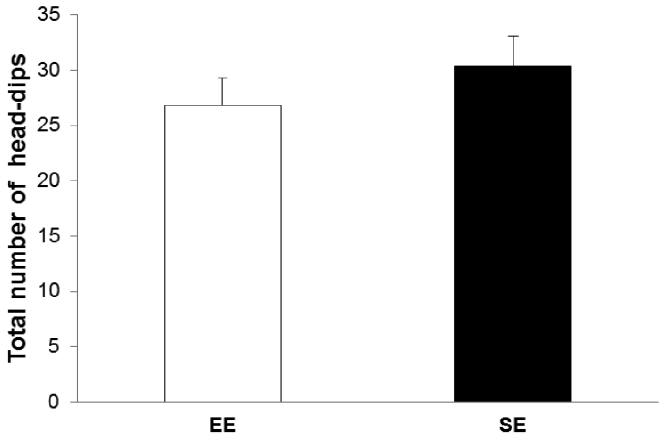


Figure 5. Total number of head-dips displayed in the hole-board test by the two experimental groups during Phase II of the study: Environmental Enrichment (EE) and Standard Environment (SE). Data are presented as mean ± SEM.

Table 1. Effects of housing in enriched environment (EE) or standard environment (SE) on the behavior displayed in the elevated plus-maze test by male NMRI mice exposed to chronic social stress (STRESS) or no exposed to stress conditions (No STRESS) during the Phase I of the study.

	EE		SE	
BEHAVIORAL CATEGORIES	**STRESS**	**NO STRESS**	**STRESS**	**NO STRESS**
TOTAL ENTRIES	14.75±1.52	15.13±2.50	14.75±1.46	15.25±1.01
OPEN ENTRIES	7.75±1.11	7.50±2.55	5.63±1.00	5.88±0.74
CLOSED ENTRIES	7.00±0.53	7.63±0.38	9.13±0.91	9.38±0.91
% OPEN ENTRIES	51.47±2.79	46.68±5.59	37.34±5.15	38.50±13.17
% OPEN TIME	35.59±3.95	23.94±4.87	29.26±5.74	26.81±2.97
% CENTER TIME	37.86±2.93	43.53±4.98	36.51±2.85	36.64±1.95
TOTAL HD	37.00±4.87	28.88±3.73	20.88±5.81	23.13±5.07
% p HD	44.35±6.31	49.19±8.01	35.44±8.71	50.12±6.59
TOTAL SAP	12.37±2.64	11.88±1.26	12.00±0.98	14.50±1.02
% p SAP	45.82±8.31	55.19±10.94	56.42±9.48	45.87±8.03
TOTAL REARS	17.63±2.41	17.38±1.35	15.88±1.89	20.38±2.24
PEEPING-OUT	11.00±1.02	14.00±2.18	11.38±1.27	11.13±0.79
ACTIVITY END MAZE	6.75±1.83	3.88±1.43	4.88±0.99	6.00±1.48

Data are presented as mean values ± SEM.

Abbreviations: HD: head-dip; SAP: stretched attend posture; % p: percentage protected.

Table 2. Effects of housing in enriched environment (EE) or standard environment (SE) on the behavior displayed in the elevated plus-maze test by male NMRI mice during the Phase II of the study. The factor "Initial condition", which refers to the conditions animals were exposed to during Phase I: Environmental Enrichment + Stress (EE+STRESS), Environmental Enrichment + No Stress (EE+NO STRESS), Standard Environment + Stress (SE+STRESS) and Standard Environment + No Stress (SE+NO STRESS). Data are presented as mean values ± SEM.

	EE				SE			
BEHAVIORAL CATEGORIES	**EE+STRESS**	**EE+NO STRESS**	**SE+STRESS**	**SE+NO STRESS**	**EE+ STRESS**	**EE+NO STRESS**	**SE+STRESS**	**SE+NO STRESS**
TOTAL ENTRIES	11.38±0.84	16.13±1.61	13.50±1.20	12.50±1.79	11.38±1.61	14.00±2.15	13.50±1.05	13.63±2.20
OPEN ENTRIES	3.88±0.48	5.50±1.21	4.50±0.82	5.50±1.27	4.25±1.01	4.00±0.87	4.13±0.88	5.13±1.09
CLOSED ENTRIES	7.50±0.89	10.63±1.25	9.00±1.07	7.00±0.89	7.13±0.95	10.00±1.31	9.38±0.73	8.50±1.35
% OPEN ENTRIES	35.34±4.59	32.96±5.05	33.35±5.35	41.97±5.09	35.07±5.32	26.59±2.65	29.19±5.16	37.63±3.95
% OPEN TIME	21.94±4.57	17.98±5.69	17.99±5.48	24.19±3.15	21.85±3.28	15.02±3.19	21.58±5.44	32.94±9.04
% CENTER TIME	35.83±1.62	41.90±3.75	39.40±3.38	39.90±4.29	48.52±2.96	48.47±3.97	43.75±3.59	32.53±5.86
TOTAL HD	20.75±1.81	27.25±6.91	31.00±3.67	15.50±2.48	28.63±6.43	22.50±2.69	24.38±3.93	38.13±5.10
% p HD	52.30±6.53	56.56±9.38	54.25±4.90	56.22±8.84	65.17±8.23	43.78±5.03	71.87±6.35	44.71±9.91
TOTAL SAP	4.13±1.09	1.88±0.52	4.88±0.72	5.88±1.57	4.63±1.60	3.13±0.69	7.50±1.54	6.88±2.07
% p SAP	45.94±13.65	50.00±16.67	69.82±9.10	54.79±14.15	46.91±14.46	41.67±11.36	75.75±7.37	40.79±13.42
TOTAL REARS	11.13±1.92	12.00±2.20	11.75±2.34	11.13±2.36	15.88±3.31	8.13±2.30	18.63±3.66	14.88±1.47
PEEPING-OUT	11.38±1.78	11.63±1.24	11.38±1.16	14.00±1.63	14.63±1.68	9.38±0.73	18.50±1.75	13.13±2.47
ACTIVITY END MAZE	2.63±0.71	2.00±0.33	2.25±0.68	3.50±1.38	4.13±1.47	4.00±0.85	2.50±0.89	4.25±1.39

4. DISCUSSION

In the present study, we have assessed the short- and long-term effects of exposure to chronic social stress in mice allocated to different housing conditions, taking into account physiological and behavioral consequences. Below we discuss the main results obtained during each phase of the study.

4.1. Phase I: Short- term physiological and behavioral effects of exposure to chronic social stress in mice housed in different environmental conditions.

Physiological variables evaluated during Phase I indicated variations both in body weight and in food and water intake in mice exposed to different housing conditions. These results support those of previous studies carried out in our laboratory in NMRI male mice in which we have observed that those housed in EE conditions gain less body weight and intake more food and fluid than standard-housed mice (Mesa-Gresa et al., 2013b). Similar data have been obtained by another research groups in rodents (Hutchinson et al., 2012b; Moncek et al., 2004), although discrepant results have also been reported (Hutchinson et al., 2012a; Carvalho et al., 2009). The bigger size and complexity of the cages in enriched environments, accompanied by an increase of physical activity in this type of housing has been proposed by way of explaining these changes (Harati et al., 2011; Mesa-Gresa et al., 2013b). Our results also indicate that mice submitted to a chronic stress situation gained less body weight and displayed increased water and food consumption. These results are in accordance with prior evidence of a decrease in body weight gain in chronically stressed rodents (Hutchinson et al., 2012b), although other past studies do not support this conclusion (Scharf et al., 2013) or have observed a reduction of food intake as a consequence of chronic restraint stress. In situations of social stress, Kumar et al. (2013) have suggested that increased food intake is related to a decrease in satiation mediated by ghrelin levels.

When the interaction between type of housing and stress was considered, the most significant changes regarding body weight and intake behavior were observed in the EE+STRESS group. These results are in accordance with studies that have reported decreased body weight gain in male mice under EE conditions and exposed to stress induced by social defeat (McQuaid et al., 2012). These authors of the study in question did not observe a similar decrease in stressed mice that were housed individually or in standard conditions. In fact, it has been reported that exposure of rodents to a social stress paradigm induces changes in body fat distribution (increase in visceral and subcutaneous fat ratio) that can be observed as long as one year after the stress condition has terminated, which has been related to a higher risk of developing some diseases (Schmidt et al., 2010a; Sterlemann et al., 2008).

Regarding changes observed in corticosterone levels, the main factors of stress or housing conditions were not found to be significant. However, when the interaction between exposure to stress and type of rearing was considered, some interesting data emerged. In mice exposed to stress conditions, those housed in enriched environments displayed higher levels of corticosterone than standard-housed animals. Nevertheless, contrasting results were obtained in non-stressed mice, with lower levels of corticosterone being reported in animals allocated to EE conditions. These results may be interpreted as a consequence of the cumulative effects of stress and exposure to enriched environments or, in contrast, to a protective effect of complex environments on animals in non-stressed conditions. Previously published data obtained in enriched environments have yielded controversial results, with both increases (Emack and Matthews, 2001; Hutchinson et al., 2012; McQuaid et al., 2013) and decreases (Garrido et al., 2013; Hutchinson et al., 2012b) of corticosterone levels being reported. McQuaid et al. (2013) reported that male mice reared under EE displayed higher levels of corticosterone than standard-housed mice, suggesting that exposure to complex environments represents a stressful situation due to the increase in territorial behavior induced by access to the different resources of the cage and the constant pressure to establish a social hierarchy. In fact, it has been hypothesized that the increase in corticosterone levels and the decrease in weight gain observed in male rodents is a result of increased agonistic behavior induced by EE exposure (Emack and

Matthews, 2011; McQuaid et al., 2013). Nevertheless, other studies have attributed some of the behavioral changes related to EE –e.g. better coping with stress– to a lower release of corticosterone hormones (Garrido et al., 2013). By contrast, Ravenelle et al. (2013) have reported that rats exposed to EE display higher levels of corticosterone but a faster return to baseline level after exposure to stress, indicating that animals housed in EE conditions are better suited to these complex situations than standard-housed ones.

Regarding the behavioral measures obtained in the current study, some interesting results emerged. The behavioral categories evaluated included anxiety-like behavior, exploratory and motor activity, and learning. Behavior displayed in the elevated plus-maze test, measured taking into account both ethological and classical measures, suggested that housing animals under enriched conditions has diminished their anxiety-like response. Animals allocated to EE conditions performed more open-arm entries and fewer closed-arm entries, and a higher total number of head-dips than their standard-housed counterparts. Such changes have been interpreted as an index of anxiolytic-like response (dos Reis et al., 2008; Redolat et al., 2005; Roy et al., 2009). As we have previously reported, exposure of male mice to enriched conditions similar to those employed in the current study may produce modifications in their emotional response (Mesa-Gresa et al., 2013b; 2014). Similar results have been obtained with different tasks for measuring the anxiety-like response in rodents exposed to EE; e.g. the elevated-zero maze (Sampedro-Piquero et al., 2014).

When the effects of the main factor “Stress” were evaluated in the elevated plus-maze, results did not prove to be significant. In fact, previous studies have demonstrated that exposure to chronic stress situations are related to a dysregulation of the HPA axis and increased vulnerability to different diseases, especially anxiety disorders (Sterlemann et al., 2008; Pérez-Tejada et al., 2013). In a study carried out by Sterlemann et al. (2008), increased anxiety responses were observed in the elevated plus-maze. Similar results were obtained in rats, indicating enhanced anxiety-like behavior after exposure to EE, especially in those classified with a “High anxiety” phenotype (Ravenelle et al., 2013). Elizalde et al. (2008) obtained similar results when

they evaluated the anxiety response in the elevated plus-maze, but not in the light-dark exploration test.

In the current study, both exploratory and motor activity were reduced in EE groups, in line with previous results obtained in our laboratory. (Mesa-Gresa et al., 2013b; 2014). Such changes have been related to faster habituation to new environments, diminished stimulation received through the test apparatus in comparison with that received through habitual cages, or more rapid processing of contextual information (Barbelivien et al., 2006; Brenes et al., 2009; Zhu et al., 2009; Zimmermann et al., 2001; Hannan, 2014), although they could also reflect a greater capability of enriched animals to cope with stressful situations (Fox et al., 2006; Scholoesser et al., 2010). It is well established that the main characteristics of EE, and the basis of its potential benefits, is that it provides animals with an environment full of novelty and complexity (Nithianantharajah and Hannan, 2011; Redolat and Mesa-Gresa, 2012). Moreover, in our research stressed animals displayed less exploratory behavior in the hole-board test, supporting those of prior studies (Elizalde et al., 2008). Although no effects in motor activity were obtained in stressed mice evaluated in the current study.

In addition, we did not obtain a significant effect of housing conditions or stress exposure on learning in the novel object recognition task when evaluated through the discrimination index. This test, which mainly measures working and visual recognition memory, is very sensitive to different procedural variables (including the presence of objects, light and assessment procedure), to the exploratory behavior of the animal and to cognitive and sensorial processing information (Antunes & Biala, 2011; Elizalde et al., 2008; van Goethem et al., 2012). These variables may explain some of the discrepancies between the findings of studies of the effects of enriched environments on this task (Bechara & Kelly, 2013; Viola et al., 2010). Differences between the procedures applied (i.e. light conditions during the test) could underlie the absence of significant effects of the complex environment observed in the present study versus the improving effects of enriched environment on this task previously reported by our (Mesa-Gresa et al., 2013c; Viola et al., 2010) and other research groups (Bechara &

Kelly, 2013; Kazlauckas et al., 2011; Kulesskaya et al., 2011; Mesa-Gresa et al., 2013b; Pang & Hannan, 2013; Vedovelli et al., 2011).

4.2. Phase II: Long-term physiological and behavioral effects of exposure to different environmental conditions on mice previously submitted to a chronic social stress

The physiological variables assessed in our study indicate that mice exposed to EE conditions during Phase II had lower body weight and higher fluid and food consumption than standard-reared mice. However, differences in corticosterone plasma levels were not observed between EE and SE groups. Data previously reported in male mice suggest that, in the absence of defeat, mice allocated to EE and SE conditions do not exhibit differences in corticosterone levels (McQuaid et al., 2013).

With respect to behavioral measures, EE mice spent a higher percentage of time in the closed arms of the EPM than SE mice. Although this difference could be interpreted as evidence of an anxiogenic response, certain authors who have obtained similar results have related this behavior to the more intense exploration of the environment displayed by EE-housed mice (Brenes et al., 2009; Zhu et al., 2006; 2009). Regarding ethological measures, values for total SAP and time and frequency of peeping-out behavior were lower in EE mice, a result that has previously been related to decreased anxiety (Dos Reis & Canto de Souza, 2008; Mesa-Gresa et al., 2013b). Thus, it could be hypothesized that ethological measures are more sensitive to alterations in anxiety levels induced by long-term exposure to EE, although other interpretations are also possible. The results obtained for classical and ethological measures could also be related to the lower motor activity displayed by EE animals. Although the great majority of previous studies have confirmed a reduction in anxiety levels as a consequence of exposure to enriched environments, some discrepant results have been published. For example, McQuaid et al. (2013) observed an increase in anxiety-like behavior and plasma corticosterone levels; though these changes could have been related to the EE paradigm applied by the authors, which increases

territorial and competitive behaviors of male mice and could, thus, have been a source of stress for them.

In this second phase of our study, enriched environments also had no clear effects on motor and exploratory behavior or the novel recognition task. These results contrast with the decrease in motor and exploratory activity usually reported after exposure to EE (Solinas et al., 2008, Mesa-Gresa et al., 2013a), and which we confirmed in the first phase of our study. Although this absence of clear behavioral effects is difficult to explain, particularly since few prior studies have employed enrichment and stress procedures simultaneously, we can hypothesize that they are related with the long-term effects of the stress procedure applied during the first phase. Furthermore, it should be taken into account that emotional responses and other behavioral effects induced by exposure to EE may be attenuated if animals are maintained in the same condition during long periods of time (Mesa-Gresa et al., 2014; unplublished data).

In the current study we have observed short- and long-term effects of stress and exposure to enriched environments, applied both individually and simultaneously to the same group of animals at an early age. Since no previously published studies have employed a similar design, we set out to perform an exploratory study although some interesting questions arise. Although a direct comparison between the two phases of the study is not possible, we do have access to data concerning the effects of an enriched environment on animals submitted to social stress early in life; namely, the results for the “Initial condition” factor (group to which animals were assigned during Phase I: EE+STRESS, EE+NO STRESS, SE+STRESS and SE+NO STRESS). The most interesting effect, both at physiological and behavioral levels, was obtained in the EE+STRESS group, in which food and water intake was higher than in the other initial groups. Moreover, this group (EE+STRESS) displayed less exploratory behavior and motor activity than the other groups, especially the EE+NO STRESS group. No clear effects, however, were observed on emotional responses or learning task when “Initial condition” factor was taken into account. The absence of emotional response could have been related to the amelioration of the long-term effects of EE when housing conditions were changed (Schmidt et al., 2007; Sterlemann et al., 2008). Nevertheless,

other research groups have observed that exposure to EE conditions can enhance the response of the HPA axis to chronic stress, making it more adaptive and promoting emotional stability and stress resiliency (Schloesser et al., 2010).

Taking into account the housing condition in which animals were maintained during the second phase of the study, the small number of animals per group in each condition should be considered with respect to the factor “Initial condition”. Previous studies have suggested that deviations in variables are usually larger in animals reared in enriched conditions, which undermines statistical power (Hutchinson et al., 2012a). Therefore, future studies will need to increase the sample number (of subjects) in order to further explore the complex interactions between stress and early and adulthood housing conditions observed in the current research. Although the question of short- and long-term effects of chronic social stress has previously been explored by different research groups, few studies have evaluated it in interaction with enriched housing. Sterlemann et al. (2008) observed short-term physiological and behavioral effects induced by exposure to social chronic stress (higher levels of corticosterone and anxiety-like behavior). Structural brain changes were reported one year after cessation of the stressor (when the mice were 15 months old), while results in the elevated plus-maze were unclear. The main findings of that study indicated that the effects of early exposure to stressful situations can be observed during late adulthood, which is of relevance in light of human studies regarding early trauma and long-term effects (Sterlemann et al., 2008).

Different studies suggest that rodents display more pronounced responses to chronic stress experienced during adolescence due to the difficulty of adjusting behavior to repeated stressors (Buwalda et al., 2011; Franklin et al., 2012; Schmidt, 2010b). In human subjects, early stressors may be a risk factor for many psychiatric disorders and addiction (Nithianantharajah & Hannan, 2010; Mesa-Gresa et al., 2012; Solinas et al., 2010). However, the possible existence of subjects who are resilient to stress early in life has not been taken into consideration in preclinical research (Elliott et al., 2010; Schmidt, 2010b).

Some prior data have suggested that exposure to EE can reverse changes induced by stress. However, few studies have used models with high predictive validity to evaluate whether exposure to enriched environments counteracts the effects of social stress. In addition, the mechanisms by which EE decreases the adverse effects of exposure to stressors are still not well known. Different studies in animal models suggest that not all individuals are equally responsive to changes in their social environment, as some are more resilient or vulnerable to specific experiences (Coppens et al., 2010; Curley et al., 2010; De Miguel et al., 2011; Franklin et al., 2012). It is important to identify the phenotype of subjects with increased susceptibility to chronic stress, as this phenotype may be related with anxiety, behavioral reactivity or greater social inhibition (Touma et al., 2008). Moreover, differences between strains of animals in response to stress have been reported (Dadomo et al., 2010). Therefore, it would seem necessary to incorporate individual variation into the design of research projects (Curley et al., 201; Elliott et al., 2010). For example, Koolhaas et al (2010) proposed the concept of "*coping styles*" to designate the different patterns of response that exist among animals in response to a stressor. So far, there is relatively little information on molecular mechanisms that may contribute to resilience (the ability of some individuals to escape the deleterious effects of stress).

In our study, the data obtained regarding the short-term effects of exposure to EE and social stress point to physiological and behavioral effects that, in most cases, are in accordance with the findings of previous studies. However, long-term changes are more difficult to interpret, since effects of prior housing conditions and exposure to stress seem to interact with housing conditions during the second phase of the study, when the mice were adults. For that reason, future studies are needed in order to determine if EE prevents or counteracts some of the effects of social stress. In particular, it would be of interest to apply stress procedures at different developmental stages with the aim of evaluating vulnerability to the consequences of chronic stress and the possible protective effect of an active lifestyle.

REFERENCES

Antunes, M., & Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cognitive Processing,* 13, 93-110.

Artola, A., von Frijtag, J. C., Fermont, P. C., Gispen, W. H., Schrama, L. H., Kamal, A., & Spruijt, B. M. (2006). Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. *The European Journal of Neuroscience,* 23, 261-72.

Barak, B., Shvarts-Serebro, I., Modai, S., Gilam, A., Okun, E., Michaelson, D., … Ashery, U. (2013). Opposing actions of environmental enrichment and Alzheimer's disease on the expression of hippocampal microRNAs in mouse models. *Translational Psychiatry,* 3, e304.

Barbelivien, A., Herbeaux, K., Oberling, P., Kelche, C., Galani, R., & Majchrzak, M. (2006). Environmental enrichment increases responding to contextual cues but decreases overall conditioned fear in the rat.*Behavioural Brain Research,* 169, 231-38.

Bechara, R. G., & Kelly, A. M. (2013). Exercise improves object recognition memory and induces BDNF expression and cell proliferation in cognitively enriched rats. *Behavioural Brain Research,* 245, 96-100.

Brenes, J. C., Padilla, M., & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behavioural Brain Research,* 197, 125-37.

Burrows, E. L., McOmish, C. E., & Hannan, A. J. (2010). Gene-environment interactions and construct validity in preclinical models of psychiatric disorders. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 35, 1376-82.

Buwalda, B., van der Borght, K., Koolhaas, J. M., & McEwen, B. S. (2010). Testosterone decrease does not play a major role in the suppression of hippocampal cell proliferation following social defeat stress in rats. Physiology & Behavior, 101(5), 719-725.

Buwalda, B., Geerdink, M., Vidal, J., & Koolhaas, J. M. (2011). Social behavior and social stress in adolescence: a focus on animal models. Neuroscience and Biobehavioral Reviews, 35, 1713-21.

Carvalho, A. F., Araujo, A. J., Farias, D.F., Rocha-Bezerra, L.C., & Cavalheiro, M. G. (2009). Development and reproductive performance of Swiss mice in an enriched environment. *Brazilian Journal of Biology*, 69, 153–60.

Chen, Y., Mao, Y., Zhou, D., Hu, X., Wang, J., & Ma, Y. (2010). Environmental enrichment and chronic restraint stress in ICR mice: Effects on prepulse inhibition of startle and Y-maze spatial recognition memory.*Behavioural Brain Research,* 212, 49-55.

Coppens, C. M., de Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: Towards underlying mechanisms. *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences,* 365, 4021-28.

Curley, J., Rock, V., Moynihan, A., Bateson, P., Keverne, E., & Champagne, F. (2010). Developmental shifts in the behavioral phenotypes of inbred mice: The role of postnatal and juvenile social experiences.*Behavior Genetics,* 40, 220-32.

Cymerblit-Sabba, A., Lasri, T., Gruper, M., Aga-Mizrachi, S., Zubedat, S., & Avital, A. (2013). Prenatal enriched environment improves emotional and attentional reactivity to adulthood stress. *Behavioural Brain Research,* 241, 185-90.

De Miguel, Z., Vegas, O., Garmendia, L., Arregi, A., Beitia, G., & Azpiroz, A. (2011). Behavioral coping strategies in response to social stress are associated with distinct neuroendocrine, monoaminergic nas immune response profiles in mice. *Behavioural Brain Research,* 225, 554-61.

dos Reis, L. M., & Canto-de-Souza, A. (2008). Intra-periaqueductal gray matter injections of midazolam fail to alter anxiety in plus-maze experienced mice. *Brain Research,* 1231, 93-102.

Elizalde, N., Gil-Bea, F. J., Ramirez, M. J., Aisa, B., Lasheras, B., Del Rio, J., & Tordera, R. M. (2008). Long-lasting behavioral effects and recognition memory deficit induced by chronic mild stress in mice: Effect of antidepressant treatment. *Psychopharmacology,* 199, 1-14.

Elliott, E., Ezra-Nevo, G., Regev, L., Neufeld-Cohen, A., & Chen, A. (2010). Resilience to social stress coincides with functional DNA methylation of the crf gene in adult mice. *Nature Neuroscience,* 13, 1351-53.

Emack, J., & Matthews, S. G. (2011). Effects of chronic maternal stress on hypothalamo–pituitary–adrenal (HPA) function and behavior: No reversal by environmental enrichment. *Hormones and Behavior,* 60, 589-598.

Epel, E. S. (2009). Psychological and metabolic stress: A recipe for accelerated cellular aging? *Hormones,* 8, 7-22.

Fox, C., Merali, Z., & Harrison, C. (2006). Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress.*Behavioural Brain Research*,175,1-8.

Fox, S. E., Levitt, P., & Nelson, C. A. (2010). How the timing and quality of early experiences influence the development of brain architecture. *Child Development,* 81, 28-40.

Franklin, T. B., Saab, B. J., & Mansuy, I. M. (2012). Neural mechanisms of stress resilience and vulnerability. *Neuron,* 75, 747-61.

Garrido, P., De Blas, M., Ronzoni, G., Cordero, I., Anton, M., Gine, E., … Mora, F. (2013). Differential effects of environmental enrichment and isolation housing on the hormonal and neurochemical responses to stress in the prefrontal cortex of the adult rat: Relationship to working and emotional memories. *Journal of Neural Transmission,* 120, 829-43.

Hannan, A. J. (2014). Environmental enrichment and brain repair: harnessing the therapeutic effects of cognitive stimulation and physical activity to enhance experience-dependent plasticity. *Neuropathology and Applied Neurobiology,* 40, 13-25.

Harati, H., Majchrzak, M., Cosquer, B., Galani, R., Kelche, C., Cassel, J.C., et al. (2011). Attention and memory in aged rats: impact of lifelong environmental enrichment. *Neurobiology of Aging,* 32, 718–36.

Hendriksen, H., Prins, J., Olivier, B., & Oosting, R. S. (2010). Environmental enrichment induces behavioral recovery and enhanced hippocampal cell proliferation in an antidepressant-resistant animal model for PTSD. *PloS One,* 5, e11943.

Hutchinson, E.K., Avery, A.C., Vasewoude, S. (2012a). Environmental Enrichment during rearing alters corticosterone levels, thymocyte numbers, and aggression in female BALB/c mice. *Journal of American Association for Laboratory Animal Science,* 51, 18-24.

Hutchinson, K. M., McLaughlin, K. J., Wright, R. L., Bryce Ortiz, J., Anouti, D. P., Mika, A., … Conrad, C. D. (2012b). Environmental enrichment protects against the effects of chronic stress on cognitive and morphological measures of hippocampal integrity. *Neurobiology of Learning and Memory,* 97, 250-60.

Ilin, Y., & Richter-Levin, G. (2009). Enriched environment experience overcomes learning deficits and depressive-like behavior induced by juvenile stress. *PloS One,* 4, e4329.

Kalueff, A. V., & Schmidt, M. V. (2011). Novel experimental models and paradigms for neuropsychiatric disorders: Editorial. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 35, 1355-56.

Kazlauckas, V., Pagnussat, N., Mioranzza, S., Kalinine, E., Nunes, F., Pettenuzzo, L., … Lara, D. R. (2011). Enriched environment effects on behavior, memory and BDNF in low and high exploratory mice.*Physiology & Behavior,* 102, 475-80.

Kulesskaya, N., Rauvala, H., & Voikar, V. (2011). Evaluation of social and physical enrichment in modulation of behavioural phenotype in C57BL/6J female mice. *PloS One,* 6, e24755.

Kumar, J., Chuang, J.C., Na, E.S., Kuperman, A., Gillman, A.G., … Lutter, M. (2013). Differential effects of chronic social stress and fluoxetine on meal patterns in mice. *Appetite,* 64, 81-8.

Koolhaas, J., De Boer, S., Coppens, C., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology,* 31, 307-21.

Laviola, G., Hannan, A. J., Macri, S., Solinas, M., & Jaber, M. (2008). Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. *Neurobiology of Disease,* 31, 159-68.

Lehmann, M. L., & Herkenham, M. (2011). Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway. *The Official Journal of the Society for Neuroscience,* 31, 6159-73.

Malter Cohen, M., Tottenham, N., & Casey, B. (2013). Translational developmental studies of stress on brain and behavior: Implications for adolescent mental health and illness? *Neuroscience,* 249, 53-62.

McEwen, B. S., Eiland, L., Hunter, R. G., & Miller, M. M. (2012). Stress and anxiety: Structural plasticity and epigenetic regulation as a consequence of stress. *Neuropharmacology,* 62, 3-12.

McQuaid, R. J., Audet, M., Jacobson-Pick, S., & Anisman, H. (2013). The differential impact of social defeat on mice living in isolation or groups in an enriched environment: Plasma corticosterone and monoamine variations. *The International Journal of Neuropsychopharmacology,* 16, 351-63.

Mesa-Gresa, P., Pérez-Martínez, A., & Redolat-Iborra, R. (2012). Nicotina y modelos animales: ¿qué nos aporta el paradigma de enriquecimiento ambiental? *Adicciones*, 24, 87-94.

Mesa-Gresa, P., Ramos-Campos, M., & Redolat, R. (2013a). Enriched Environments for Rodents and their Interaction with Nicotine Administration. *Current Drug Abuses,* 6, 191-200.

Mesa-Gresa, P., Perez-Martinez, A., & Redolat, R. (2013b). Behavioral effects of combined environmental enrichment and chronic nicotine administration in male NMRI mice. *Physiology & Behavior,* 114-115C, 65-76.

Mesa-Gresa, P., Pérez-Martinez, A., & Redolat, R. (2013c). Environmental enrichment improves novel object recognition and enhances agonistic behavior in male mice. *Aggressive Behavior,* 39, 269-79.

Mesa-Gresa, P., Ramos-Campos, M., & Redolat, R. (2014). Behavioral effects of different enriched environments in mice treated with the cholinergic agonist PNU-282987. *Behavioural Processes,* 103, 117-24.

Mitra, R., & Sapolsky, R. M. (2008). Effects of enrichment predominate over those of chronic stress on fear-related behavior in male rats. *Stress*, 1.

Mitra, R., & Sapolsky, R. M. (2009). Effects of enrichment predominate over those of chronic stress on fear-related behavior in male rats. *Stress: The International Journal on the Biology of Stress,* 12, 305-12.

Moncek, F., Duncko, R., Johansson, B., & Jezova, D. (2004). Effect of environmental enrichment on stress related systems in rats*. Journal of Neuroendocrinology,* 16, 423-31.

Mora, F. (2013). Successful brain aging: Plasticity, environmental enrichment, and lifestyle. *Dialogues in Clinical Neuroscience,* 15, 45-52.

Nithianantharajah, J., & Hannan, A. J. (2011). Mechanisms mediating brain and cognitive reserve: Experience-dependent neuroprotection and functional compensation in animal models of neurodegenerative diseases. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 35, 331-39.

Pang, T. Y., & Hannan, A. J. (2013). Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. *Neuropharmacology,* 64, 515-28.

Pardon, M.C. (2009). Hormesis is applicable as a pro-healthy aging intervention in mammals and human beings. *Dose Response,* 8, 22-27.

Pérez-Tejada, J., Arregi, A., Gómez-Lázaro, E., Vegas, O., Azpiroz, A., & Garmendia, L. (2013). Coping with chronic social stress in mice: Hipothalamic-pituitary-adrenal/ Sympathetic-adrenal-medullary axis activity, behavioral changes and effects of antalarmin treatment: Implication for the study of stress-related psychopathologies. *Neuroendocrinology,* 98, 73-88.

Ravenelle, R., Byrnes, E. M., Byrnes, J. J., McInnis, C., Park, J. H., & Donaldson, S. T. (2013). Environmental enrichment effects on the neurobehavioral profile of selective outbred trait anxiety rats. *Behavioural Brain Research,* 252, 49-57.

Redolat, R., Gomez, M. C., Vicens, P., & Carrasco, M. C. (2005). Bupropion effects on aggressiveness and anxiety in OF1 male mice. *Psychopharmacology,* 177, 418-27.

Roy, V., Chapillon, P., Jeljeli, M., Caston, J., & Belzung, C. (2009). Free versus forced exposure to an elevated plus-maze: Evidence for new behavioral interpretations during test and retest. *Psychopharmacology,*203, 131-41.

Sale, A., Berardi, N., & Maffei, L. (2014). Environment and brain plasticity: Towards an endogenous pharmacotherapy. *Physiological Reviews,* 94, 189-234.

Sampedro-Piquero, P., Begega, A., & Arias, J. L. (2014). Increase of glucocorticoid receptor expression after environmental enrichment: Relations to spatial memory, exploration and anxiety-related behaviors.*Physiology & Behavior,* 129C, 118-29.

Scharf, S. H., Sterlemann, V., Liebl, C., Müller, M. B., & Schmidt, M. V. (2013). Chronic social stress during adolescence: Interplay of paroxetine treatment and ageing. *Neuropharmacology,* 72, 38-46.

Schloesser, R., Lehmann, M., Martinowich, K., Manji, H., & Herkenham, M. (2010). Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. *Molecular Psychiatry,*15, 1152-63.

Schmidt, M., Sterlemann, V., Ganea, K., Liebl, C., Alam, S., Harbich, D., … Müller, M. (2007). Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. *Psychoneuroendocrinology,* 32, 417-29.

Schmidt, M. V., Sterlemann, V., & Muller, M. B. (2008). Chronic stress and individual vulnerability. *Annals of the New York Academy of Sciences,* 1148, 174-83.

Schmidt, M. V., Czisch, M., Sterlemann, V., Reinel, C., Samann, P., & Muller, M. B. (2009). Chronic social stress during adolescence in mice alters fat distribution in late life: Prevention by antidepressant treatment.*Stress,* 12, 89-94.

Schmidt, M. V. (2010a). Animal models for depression and the mismatch hypothesis of desease. *Psychoneuroendocrinology,* 36, 330-38.

Schmidt, M. V. (2010b). Molecular mechanisms of early life stress: lessons from mouse models*. Neuroscience and Biobehavioral Reviews,* 34, 845-52.

Schmidt, M. V., Scharf, S. H., Liebl, C., Harbich, D., Mayer, B., Holsboer, F., & Muller, M. B. (2010a). A novel chronic social stress paradigm in female mice. *Hormones and Behavior,* 57, 415-20.

Schmidt, M. V., Scharf, S. H., Sterlemann, V., Ganea, K., Liebl, C., Holsboer, F., & Muller, M. B. (2010b). High susceptibility to chronic social stress is associated with a depression-like phenotype.*Psychoneuroendocrinology,* 35, 635-43.

Schmidt, M., Abraham, W., Maroun, M., Stork, O., & Richter-Levin, G. (2013). Stress-induced metaplasticity: From synapses to behavior. *Neuroscience*, 250, 112-20.

Segovia, G., Del Arco, A., de Blas, M., Garrido, P., & Mora, F. (2008). Effects of an enriched environment on the release of dopamine in the prefrontal cortex produced by stress and on working memory during aging in the awake rat. *Behavioural Brain Research,* 187, 304-11.

Segovia, G., Del Arco, A., Garrido, P., de Blas, M., & Mora, F. (2008). Environmental enrichment reduces the response to stress of the cholinergic system in the prefrontal cortex during aging. *Neurochemistry International,* 52, 1198-203.

Segovia, G., Del Arco, A., & Mora, F. (2009). Environmental enrichment, prefrontal cortex, stress, and aging of the brain. *Journal of Neural Transmission,* 116, 1007-16.

Solinas, M., Thiriet, N., Rawas, R. E., Lardeux, V., & Jaber, M. (2008). Environmental enrichment during early stages of life reduces the behavioral, neurochemical, and molecular effects of cocaine.Neuropsychopharmacology.

Solinas, M., Thiriet, N., Chauvet, C., & Jaber, M. (2010). Prevention and treatment of drug addiction by environmental enrichment. *Progress in Neurobiology,* 92, 572-92.

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., … Schmidt, M. V. (2008). Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: Implications for stress-related disorders. *Hormones and Behavior,* 53, 386-94.

Sterlemann, V., Rammes, G., Wolf, M., Liebl, C., Ganea, K., Muller, M. B., & Schmidt, M. V. (2010). Chronic social stress during adolescence induces cognitive impairment in aged mice. *Hippocampus,* 20, 540-49.

Touma, C., Bunck, M., Glasl, L., Nussbaumer, M., Palme, R., Stein, H., … Holsboer, F. (2008). Mice selected for high versus low stress reactivity: A new animal model for affective disorders.*Psychoneuroendocrinology,* 33, 839-62.

Van de Weerd, H. A., Aarsen, E. L., Mulder, A., Kruitwagen, C. L., Hendriksen, C. F., & Baumans, V. (2002). Effects of environmental enrichment for mice: Variation in experimental results. *Journal of Applied Animal Welfare Science,* 5, 87-109.

Van de Weerd, H. A., Van Loo, P. L., Van Zutphen, L. F., Koolhaas, J. M., & Baumans, V. (1997). Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice.*Physiology & Behavior,* 62, 1019-28.

van Goethem, N. P., Rutten, K., van der Staay, F. J., Jans, L. A., Akkerman, S., Steinbusch, H. W., … Prickaerts, J. (2012). Object recognition testing: Rodent species, strains, housing conditions, and estrous cycle.*Behavioural Brain Research,* 232, 323-34.

Vedovelli, K., Silveira, E., Velho, E., Stertz, L., Kapczinski, F., Schroder, N., & Bromberg, E. (2011). Effects of increased opportunity for physical exercise and learning experiences on recognition memory and brain-derived neurotrophic factor levels in brain and serum of rats. *Neuroscience,* 199, 284-91.

Viola, G. G., Botton, P. H., Moreira, J. D., Ardais, A. P., Oses, J. P., & Souza, D. O. (2010). Influence of environmental enrichment on an object recognition task in CF1 mice. *Physiology & Behavior,* 99, 17-21.

Wang, X., Labermaier, C., Holsboer, F., Wurst, W., Deussing, J. M., Müller, M. B., & Schmidt, M. V. (2012). Early-life stress-induced anxiety-related behavior in adult mice partially requires forebrain corticotropin-releasing hormone receptor 1. *European Journal of Neuroscience,* 36, 2360-67.

Zhu, S. W., Yee, B. K., Nyffeler, M., Winblad, B., Feldon, J., & Mohammed, A. H. (2006). Influence of differential housing on emotional behaviour and neurotrophin levels in mice. *Behavioural Brain Research,* 169, 10-20.

Zhu, S. W., Codita, A., Bogdanovic, N., Hjerling-Leffler, J., Ernfors, P., Winblad, B., … Mohammed, A. H. (2009). Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice.*Behavioural Brain Research*, 197, 339-46.

Zimmermann, A., Stauffacher, M., Langhans, W., & Wurbel, H. (2001). Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. *Behavioural Brain Research,* 121, 11-20.

CAPÍTULO 7

DISCUSIÓN

1. RESUMEN DE LOS PRINCIPALES RESULTADOS OBTENIDOS

Los estudios preclínicos contenidos en la presente Tesis Doctoral incluyen diferentes investigaciones entre las que se encuentra el desarrollo de un modelo válido de EE así como diferentes experimentos que tratan de evaluar la posible interacción de los ambientes enriquecidos con la administración de agonistas colinérgicos como la nicotina o el PNU-282987. Además, se ha abordado la cuestión de si existe variabilidad en los cambios conductuales y fisiológicos observados en función de la edad en la que los animales son expuestos al ambiente enriquecido y/o el periodo total de exposición al mismo. Otro de nuestros objetivos, llevado a cabo en el último estudio presentado, ha sido valorar los efectos fisiológicos y conductuales de la interacción entre dos factores aplicados de modo simultáneo, como son, el alojamiento en ambientes enriquecidos y la exposición a un procedimiento de estrés social crónico inducido mediante inestabilidad social. Estos efectos se han evaluado tanto a corto plazo (inmediatamente después de finalizar las 7 semanas del procedimiento de estrés social) como a largo plazo (cuando los animales son mantenidos en diferentes tipos de ambiente -enriquecido o estándar- durante 4 semanas una vez finalizado el procedimiento de estrés).

A continuación se presenta una breve descripción de los estudios realizados, de los principales resultados obtenidos así como sus principales conclusiones.

1.1. Estudio 1

Diversos estudios han evaluado los posibles efectos conductuales y fisiológicos del alojamiento de roedores en ambientes enriquecidos, observándose generalmente mejoras en sus funciones cognitivas reflejadas en la obtención de resultados positivos en pruebas conductuales de aprendizaje y memoria. Sin embargo, los resultados relacionados con los posibles efectos de estos ambientes complejos sobre alteraciones en cambios emocionales, conducta agresiva e interacción social son más limitados y contradictorios. El principal objetivo del presente estudio fue desarrollar un modelo de

EE en ratones macho de la cepa NMRI, validando sus principales efectos en una tarea de aprendizaje y explorando posibles alteraciones inducidas en la conducta agonística. En base a los datos previos, nuestra hipótesis era que la exposición a EE mejoraría el rendimiento cognitivo y podría incrementar ligeramente la conducta agonística. El procedimiento seguido en este estudio fue el siguiente: los ratones (n=64) llegaron al laboratorio el día post-natal (PND) 21, y tras una semana adaptación, fueron divididos en dos grupos experimentales, alojamiento en EE (n=16) y en condiciones estándar (SE) (n=16). El resto de los animales (n=32) fueron mantenidos en las mismas condiciones estándar de laboratorio con el fin de ser utilizados como oponentes en el test de interacción social. Tras cuatro semanas de alojamiento en estas condiciones (PND 56-58), se realizaron los tests conductuales, utilizándose la tarea de reconocimiento de objetos (RO) con el fin de evaluar memoria visual y de trabajo mediante un índice de discriminación y el test de interacción social (IS) para el estudio de la conducta agonística y social de los ratones. Los principales resultados obtenidos en este estudio indicaron que el alojamiento en condiciones de EE tuvo efectos positivos sobre la memoria de reconocimiento evaluada, puesto que los animales alojados en el ambiente enriquecido pasaron más tiempo explorando el objeto nuevo que los mantenidos en SE. Además, se incrementaron las conductas de sociabilidad y agonística en esta cepa de ratones. Estos resultados corroboran los obtenidos previamente sobre los efectos positivos del EE sobre la cognición, y añaden evidencia sobre sus posibles efectos en la conducta agresiva en ratones macho.

1.2. Estudio 2

Las consecuencias de la exposición combinada del alojamiento en ambientes enriquecidos y la administración de nicotina ha sido un tema abordado en pocos estudios, especialmente durante periodos críticos para el desarrollo como la adolescencia. Por tanto, se llevó a cabo una investigación más amplia con el fin de evaluar de forma sistemática los principales cambios a nivel fisiológico (peso, consumo de fluidos y niveles plasmáticos de cotinina) y conductual (actividad motora, conducta exploratoria, ansiedad y aprendizaje) de la exposición a nuestro modelo de EE en ratones NMRI. Otro objetivo de nuestra investigación fue evaluar los efectos

combinados de la exposición al ambiente enriquecido y la administración crónica de nicotina. Teniendo en cuenta estudios previos, se hipotetizó que el tratamiento crónico con nicotina podría modular los efectos del alojamiento en ambientes complejos. En el PND 28, la mitad de los ratones (n=32) fueron alojados en condiciones de EE y la otra mitad en condiciones de SE (n=32). Dentro de cada grupo, la mitad de los animales fueron tratados con nicotina oral (100 µg/ml) y la otra mitad recibió una solución oral con sacarina. Tras tres semanas de alojamiento en estas condiciones, los ratones fueron evaluados en una batería conductual que incluyó el test de laberinto elevado (EPM), *hole-board* (HB), actímetro (ACT) y tarea de evitación pasiva (EP). Los niveles de cotinina en plasma fueron medidos en un grupo complementario de 32 animales con el fin de confirmar el consumo de nicotina en los animales tratados. Los principales resultados de este estudio indicaron que los ratones alojados en EE ganaron menos peso corporal y mostraron un mayor consumo de fluidos que los alojados en condiciones estándar. Además, las pruebas conductuales confirmaron que el EE reduce la respuesta de ansiedad y la conducta exploratoria y motora, mejorando la adquisición en una tarea de aprendizaje de evitación inhibitoria. Por lo que respecta a los efectos del tratamiento crónico con nicotina oral, los datos obtenidos en medidas fisiológicas sugieren que los animales que recibieron de forma crónica nicotina oral en dosis de 100 µg/ml diluida en el agua de bebida mostraron menor peso corporal y menor consumo de fluidos, así como mayores niveles de cotinina en sangre que los ratones del grupo control. En las pruebas conductuales únicamente se obtuvieron efectos significativos en el EPM, observándose principalmente un efecto motor en los animales tratados con nicotina. En este estudio se muestran más efectos fisiológicos y conductuales de la exposición a EE en ratones NMRI, confirmando en general los datos obtenidos en estudios previos de otros grupos de investigación en diferentes cepas de ratones. Sin embargo, en el presente estudio no se pudieron demostrar efectos significativos del tratamiento crónico con nicotina en la dosis utilizada, a excepción de la disminución de la conducta motora observada en el test de laberinto elevado. Tan solo se observó una interacción entre el alojamiento en EE y el tratamiento con nicotina en la respuesta emocional. Estos resultados muestran la necesidad de realizar más estudios ampliando el rango de dosis de nicotina u otros agonistas colinérgicos en interacción con EE.

1.3. Estudio 3

Como ya se ha observado previamente, son escasos los estudios que han evaluado la posible interacción entre las condiciones de EE y la administración de agonistas nicotínicos. El PNU-282987 (PNU) es un agonista colinérgico de los receptores α7 que ha mostrado efectos positivos en tareas de aprendizaje y memoria, no obteniéndose datos concluyentes respecto a otras conductas como la respuesta emocional. El principal objetivo del presente estudio fue evaluar y comparar los efectos conductuales del alojamiento en distintos tipos de ambientes complejos y su posible interacción con el tratamiento agudo de PNU. 96 ratones macho NMRI llegaron a nuestro laboratorio y, tras una semana de adaptación (PND 28), fueron alojados en distintos tipos de ambiente: EE, cajas Marlau^{TM} y SE. Tras cuatro meses de exposición a estas condiciones ambientales, los animales recibieron tratamiento agudo con PNU (2.5, 5 y 10 mg/kg) y fueron evaluados en el EPM y en el HB. Los resultados obtenidos mostraron que el alojamiento de los animales en el modelo de EE desarrollado en nuestro laboratorio indujo resultados acordes a los obtenidos previamente, observándose una disminución en la respuesta de ansiedad y en la conducta exploratoria de los ratones. Sin embargo, el alojamiento en las cajas Marlau^{TM} produjo un aumento en la conducta exploratoria evaluada en el HB y en la actividad motora registrada en el EPM. La administración farmacológica de PNU, en las dosis evaluadas, mostró efectos poco concluyentes en el EPM que pueden estar relacionados con cambios en la actividad motora. Futuros estudios son necesarios para evaluar en mayor profundidad los efectos de este agonista colinérgico en interacción con diferentes condiciones ambientales.

1.4. Estudio 4

Tal y como se ha observado en los estudios 1 y 2 de la presente Tesis Doctoral, la exposición a EE durante periodos cortos de tiempo (3-4 semanas) a edades tempranas puede inducir efectos fisiológicos y conductuales en ratones macho NMRI. Además, estos efectos pueden sufrir variaciones en base a la edad y/o periodo de exposición a

ambientes complejos. Teniendo en cuenta esta cuestión y los resultados obtenidos previamente en las conductas evaluadas con el modelo de EE desarrollado en nuestro laboratorio, el principal objetivo del cuarto estudio fue el de evaluar si los cambios observados previamente en la conducta exploratoria, motora, respuesta de ansiedad, interacción social y memoria, se mantenían tras exposiciones más largas o con inicio a diferentes edades. 64 ratones macho NMRI llegaron a nuestro laboratorio en el PND 28 y fueron expuestos a EE o a SE. Los grupos experimentales comparados en el presente estudio fueron: 1) SE-6: la exposición a SE fue iniciada en el PND 28 y duró 6 meses; 2) EE-6: la exposición a EE se inició en el PND 28 y duró 6 meses; 3) EE-4: la exposición a EE se inició en el PND 90 y duró 4 meses; y 4) EE-2: la exposición a EE se inició en el PND 155 y duró 2 meses. Los tests conductuales empleados en este estudio fueron el HB, EPM, SI y RO. Los principales resultados obtenidos indicaron una disminución de la conducta exploratoria evaluada en el HB únicamente observable cuando la exposición al ambiente enriquecido se inició durante la adolescencia. En el EPM se observó que el efecto ansiolítico inducido por EE tiende a disminuir tras largos periodos de exposición. Por lo que respecta a la tarea de IS, los animales del grupo EE-4 estuvieron más tiempo haciendo exploración social a distancia, no observándose efectos significativos en la tarea cognitiva de RO. A diferencia de lo observado en el estudio 1, no se obtuvo incremento en la conducta agonística en ninguno de los grupos experimentales, concluyéndose que éste y otros efectos observados previamente podrían verse atenuados por el alojamiento en EE durante largos periodos de tiempo o la exposición en edad adulta.

1.5. Estudio 5

El modelo de EE ha sido planteado como un paradigma experimental capaz de revertir algunos de los efectos perjudiciales de la exposición a situaciones de estrés, aunque son pocos los estudios que han evaluado la administración simultánea de ambos factores. Teniendo en cuenta esta cuestión, nuestro principal objetivo fue comparar los efectos a corto y largo plazo inducidos por la exposición a un procedimiento de estrés social en ratones alojados en distintos tipos de ambientes. 128 ratones macho NMRI llegaron a nuestro laboratorio a los 21 días de edad. Durante

la Fase I del estudio (PND 28), la mitad de los animales fueron sometidos a protocolo de estrés social crónico introducido por Schmidt y cols. (2008) y basado en la completa disrupción de la jerarquía social establecida en cada una de las cajas. Los ratones fueron alojados en distintas condiciones ambientales y asignados a 4 grupos experimentales: 1) EE+STRESS: animales alojados en condiciones de EE y sometidos a estrés (n=32); 2) EE+NO STRESS: animales alojados en EE pero sin recibir estrés (n=32); 3) SE+STRESS: animales mantenidos en SE y sometidos a estrés (n=32); y 4) SE+NO STRESS (n=32). Al finalizar la Fase I, en el PND 77, un subgrupo de 32 animales fue sometido a las pruebas conductuales (HB, EPM, RO y rueda de actividad) y otro subgrupo de 32 ratones fue sacrificado con el fin de obtener muestras de sangre para la medición de los niveles de corticosterona. Los resultados obtenidos en esta Fase del estudio indicaron que los animales alojados en condiciones de EE mostraron menor peso corporal, mayor ingesta de agua y comida, así como menor respuesta de ansiedad y en la actividad motora y exploratoria. Por lo que respecta a los resultados de la exposición a estrés, se observó que los animales estresados pesaban menos y consumían más agua y comida, mostrándose una disminución en la conducta exploratoria. Los niveles de corticosterona indicaron resultados significativos para la interacción entre alojamiento y estrés: el grupo EE+STRESS tenía niveles superiores de corticosterona que los animales del grupo EE+NO STRESS, mientras que este grupo (EE+NO STRESS) a su vez, presentaba menores niveles que el grupo SE+NO STRESS. Una vez finalizado el protocolo de estrés y realizadas las pruebas de evaluación pertinentes (PND 83), se inició la Fase II, en la que los animales (n=96) fueron asignados a condiciones de EE (n=48) y SE (n=48). Un mes después, al igual que se había hecho en la Fase I, un subgrupo de animales (n=32) se sacrificaron para obtener muestras de sangre y el otro subgrupo restante (n=64) se sometió a evaluación conductual. Los resultados de esta Fase indicaron que los animales alojados en EE mostraron menor peso corporal y mayor ingesta, así como efectos en la respuesta emocional evaluada en el EPM. El resto de variables evaluadas no mostró resultados concluyentes, haciéndose patente la necesidad de realizar más estudios sobre los efectos a largo plazo de la exposición a situaciones de estrés y EE.

2. CONCLUSIONES

Los principales resultados obtenidos en la presente Tesis Doctoral han sido publicados en revistas científicas de impacto, contribuyendo mediante nuevas evidencias, a la literatura existente sobre el paradigma de EE y su posible aplicación en diferentes ámbitos.

Tras una extensa revisión de la literatura existente, se diseñó un modelo validado de EE basado en la utilización de jaulas grandes en las que se alojaban 8 animales, y que incluyen objetos fijos (rueda de correr, casa y túnel) y objetos variables (5 en total) que se cambian dos veces por semana. Las principales conclusiones alcanzadas aplicando este paradigma de EE se resumen a continuación:

- Los ratones expuestos a este modelo de ambiente enriquecido muestran cambios a nivel fisiológico, neurobiológico y conductual, acordes con la literatura existente. Los principales efectos fisiológicos observados son la disminución del peso corporal y un aumento en la ingesta de comida y bebida. A nivel conductual, se confirma que el alojamiento en este ambiente complejo induce una disminución en la actividad motora, exploratoria así como en la respuesta emocional de ansiedad. También se observó una mejora en tareas de aprendizaje y memoria evaluadas mediante distintas tareas y un aumento en la conducta social y agonística.
- Los resultados acerca de la posible interacción entre los efectos del EE y la administración farmacológica de agonistas colinérgicos, no confirman una clara modulación y/o potenciación de los efectos del ambiente enriquecido. Los resultados obtenidos acerca de la posible interacción entre la administración crónica de nicotina oral con el ambiente en que se mantiene el animal resultan complejos. A nivel fisiológico se observan cambios en las medidas de peso corporal de los animales, principalmente al inicio del tratamiento. A nivel conductual, el tratamiento con nicotina afecta especialmente a los resultados obtenidos en el laberinto elevado, reflejando menor actividad motora de los animales tratados con este fármaco, aunque este efecto no parece estar influido directamente por el ambiente enriquecido. Por lo que respecta a la

administración aguda de PNU-282987, los resultados no mostraron efectos concluyentes en la conducta exploratoria y respuesta de ansiedad para las dosis evaluadas, ni tampoco se observó interacción con el alojamiento en distintos tipos de ambiente.

- La comparación entre los efectos inducidos en ratones NMRI por nuestro propio modelo de EE, desarrollado en los estudios anteriores, y el modelo estandarizado de las cajas Marlau™ muestra efectos diferenciales de ambos tipos de alojamiento: los ratones expuestos a EE mostraron menor conducta exploratoria y respuesta de ansiedad mientras que los animales alojados en cajas Marlau™ presentaron un incremento en la conducta exploratoria y mayor actividad motora, no observándose resultados concluyentes sobre la disminución de la respuesta de ansiedad. Estas diferencias muestran los posibles efectos de la variabilidad de modelos de EE y la necesidad de tener en cuenta este aspecto a la hora de valorar e interpretar los resultados obtenidos.
- Los efectos del alojamiento en EE dependen de la edad de inicio y del periodo de exposición. La disminución en la conducta exploratoria es más evidente en aquellos animales en los que el EE se inicia a edades tempranas y durante periodos más largos de tiempo, mientras que el efecto ansiolítico del EE parece ser atenuado por el tiempo de exposición y edad de los animales. Estos resultados indican que los efectos del enriquecimiento sobre conducta exploratoria y emocional parecen ser más pronunciados cuando la exposición al ambiente enriquecido se inicia a edades tempranas mientras que otros efectos conductuales tienden a disminuir después de 6 meses de alojamiento.
- La exposición a estrés social crónico induce efectos significativos en ratones macho NMRI. A corto plazo, se observa que los animales estresados muestran menor peso corporal y mayor ingesta de fluidos y comida que aquellos no estresados, mientras que a nivel conductual el estrés reduce la actividad exploratoria. A largo plazo, los efectos fisiológicos y conductuales del estrés quedan atenuados, no habiéndose obtenido en el estudio realizado datos concluyentes.
- La interacción entre EE y la exposición a estrés social muestra también efectos interesantes. A corto plazo se observa que los animales alojados en condiciones

de EE y sometidos a un ambiente social inestable muestran menor peso corporal y mayor ingesta, observándose además una clara interacción en los niveles de corticosterona en sangre. En cuanto a los efectos a largo plazo, se observó que aquellos animales que habían permanecido en ambas condiciones (EE y estrés) mostraron mayor ingesta así como menor actividad motora y exploratoria principalmente en comparación con aquellos que permanecieron en EE sin estrés, no observándose efectos claros respecto a la respuesta emocional o de aprendizaje.

En conjunto, los resultados aquí expuestos indican que la exposición a ambientes enriquecidos y complejos, iniciada en edades tempranas, puede tener efectos positivos a nivel fisiológico, neurobiológico y conductual. Diversos factores como la edad de inicio, el periodo de exposición, la administración de fármacos o la interacción con situaciones de estrés pueden modificar los efectos de la exposición. Cabe destacar que en los estudios preclínicos realizados, cuestiones como la especie, el sexo y la cepa de los animales utilizados, el tipo de manipulación y tareas experimentales, las dosis y fármacos utilizados o incluso las condiciones ambientales de evaluación pueden influir sobre los resultados obtenidos, por lo que éstos deben ser interpretados con cautela principalmente en lo referente a la posible extrapolación a los efectos de un estilo de vida activo en seres humanos. Las principales limitaciones para la extrapolación de los resultados incluyen el hecho de que la intensidad, la duración y la edad de inicio de la exposición en humanos es difícil de controlar y cuantificar. Además, la sinergia entre los distintos componentes del EE parece ser imprescindible para obtener todos sus efectos beneficiosos tanto en animales como en sujetos humanos. Futuros estudios son necesarios con el fin de profundizar y perfilar estas cuestiones, de modo que se analicen las posibles interacciones entre la exposición a ambientes enriquecidos y otro tipo de manipulaciones, añadiendo evidencia científica sobre sus efectos positivos y/o neuroprotectores.

3. PERSPECTIVAS DE FUTURO Y POSIBLES APLICACIONES DEL MODELO DE ENRIQUECIMIENTO AMBIENTAL

Los resultados obtenidos en la presente Tesis Doctoral han abierto nuevas cuestiones y perspectivas de futuro en la investigación del modelo de EE. La aplicación de este paradigma ha mostrado claros efectos neurobiológicos y conductuales, pero ha planteado nuevas hipótesis y preguntas respecto a su posible interacción con factores como la administración de otros agonistas colinérgicos o en distintas dosis, sus efectos neuroprotectores frente el envejecimiento y el declive cognitivo asociado a la edad, así como sus posibles efectos a largo plazo frente a la exposición a estrés social crónico, marcando la necesidad de plantear nuevos estudios longitudinales. Además, se ha hecho patente la necesidad de diferenciar entre los distintos componentes del modelo de EE, principalmente la estimulación cognitiva y actividad física, y su contribución y/o interacción frente a cuestiones como el envejecimiento y otras patologías asociadas. Una meta en futuros estudios podría ser incrementar la validez de los modelos animales con el fin de explicar mejor los datos obtenidos en sujetos humanos, de modo que puedan implementarse estrategias óptimas de intervención.

A pesar de las limitaciones analizadas, el modelo de EE y la extrapolación de los principales resultados obtenidos a los estudios en humanos ha mostrado su aplicación y efectos positivos, como tratamiento no invasivo en distintas áreas como envejecimiento, estrés, adicción, enfermedades neurodegenerativas, trastornos psicológicos, daño cerebral e incluso tratamiento contra el cáncer. Recientemente, Sale y colaboradores (2014) han definido el EE como un tipo de estrategia no farmacológica de tratamiento concluyendo que *"la aplicación de paradigmas de EE puede abrir el camino para una nueva era de farmacoterapia endógena, por lo que la administración de sustancias externas no sea necesaria para la estimulación de las vías moleculares de la neuroplasticidad, sino la utilización del potencial de las estrategias no invasivas de estimulación ambiental para para mejorar la capacidad de reparación espontánea del cerebro".* Consideramos que esta perspectiva concuerda con los principales resultados obtenidos en la presente Tesis Doctoral y podría ser una aproximación de interés en futuros estudios realizados en este ámbito de investigación.

FONDOS DE FINANCIACIÓN

La presente Tesis Doctoral ha sido realizada en el marco de un proyecto de investigación denominado “El enriquecimiento ambiental como estrategia protectora frente al estrés social y el envejecimiento y su posible potenciación mediante agonistas colinérgicos”, llevado a cabo gracias a la financiación obtenida del Ministerio de Economía y Competitividad y Plan E (PSI2009-10410) y de la “Conselleria d’Educació i Ciència” de la Generalitat Valenciana (GVACOMP2010-273, GERÓNIMO FORTEZA FPA/2012/101). Otra fuente de financiación que ha contribuido a la realización de los estudios de esta Tesis, obtenida de la Generalitat Valenciana, se enmarca en las ayudas para grupos de excelencia (PROMETEO/2011/048).

ANEXO

ANEXO 9.1

Redolat R., & Mesa-Gresa P. (2012)

POTENTIAL BENEFITS AND LIMITATIONS OF ENRICHED ENVIRONMENTS AND COGNITIVE ACTIVITY ON AGE-RELATED BEHAVIOURAL DECLINE

Current Topics in Behavioral Neurosciences, 10: 293-316

ANEXO 9.2

Mesa-Gresa P., Ramos-Campos M. & Redolat R. (2013)

ENRICHED ENVIRONMENTS FOR RODENTS AND THEIR INTERACTION WITH NICOTINE ADMINISTRATION

Current Drug Abuse Reviews, 6: 191-200

ANEXO 9.3

Mesa-Gresa P., Pérez-Martinez A. & Redolat R. (2012)

NICOTINA Y MODELOS ANIMALES: ¿QUÉ NOS APORTA EL PARADIGMA DE ENRIQUECIMIENTO AMBIENTAL?

Adicciones, 24: 87-94

ANEXO 9.4

Mesa-Gresa P., Pérez-Martinez A. & Redolat R. (2013)

ENVIRONMENTAL ENRICHMENT IMPROVES NOVEL OBJECT RECOGNITION AND ENHANCES AGONISTIC BEHAVIOR IN MALE MICE

Aggressive Behavior, 39: 269-279

ANEXO 9.5

Mesa-Gresa P., Pérez-Martinez A. & Redolat R. (2013)

BEHAVIORAL EFFECTS OF COMBINED ENVIRONMENTAL ENRICHMENT AND CHRONIC NICOTINE ADMINISTRATION IN MALE NMRI MICE

Physiology & Behavior, 114-115: 65-76

ANEXO 9.6

Mesa-Gresa P., Ramos-Campos M. & Redolat, R. (2014)

BEHAVIORAL EFFECTS OF DIFFERENT ENRICHED ENVIRONMENTS IN MICE TREATED WITH THE CHOLINERGIC AGONIST PNU-282987

Behavioural Processes, 103: 117-124