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ESCUELA INTERNACIONAL DE DOCTORADO

Rendimiento Cognitivo en Envejecimiento: Efecto del Sueño, del Ejercicio y de la TMS en *Octodon degus*

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Lda. Dña. Cristina Estrada Esteban

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Esta tesis doctoral ha sido dirigida por:

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La que suscribe, Doña María Trinidad Herrero Ezquerro, Catedrática de Universidad del Área de Anatomía y Embriología Humana en el Departamento de Anatomía Humana y Psicobiología de la Facultad de Medicina de la Universidad de Murcia,

AUTORIZA la presentación de la Tesis Doctoral titulada "*Rendimiento cognitivo en envejecimiento: efecto del sueño, del ejercicio y de la TMS en Octodon degus*", realizada por **Dña. Cristina Estrada Esteban** y que constituye el trabajo de investigación realizado bajo mi dirección y supervisión en el grupo de Neurociencia Clínica y Experimental (NiCE-CIBERNED) de la Facultad de Medicina.

CONFIRMA que: esta Tesis Doctoral reúne la calidad y el rigor científico necesarios para ser defendida para la obtención del grado de Doctor por la Universidad de Murcia dentro del Programa de Doctorado "Integración y Modulación de Señales en Biomedicina" conforme al RD 99/2011.

Y para que así conste dónde fuere menester firmo la presente en Murcia, a siete de julio de dos mil diecisiete,

PÉCSI TUDOMÁNYEGYETEM
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Anatómiai Intézet

INFORME VALORACIÓN TESIS DOCTORAL

Doctoranda: Cristina Estrada Esteban

Título de la Tesis: "*Rendimiento cognitivo en envejecimiento: efecto del sueño, del ejercicio y de la TMS en Octodon degus*"

Doña Dora Reglodi, como miembro de la Universidad Pècs de Hungría y como evaluadora de la tesis doctoral "**Rendimiento cognitivo en envejecimiento: efecto del sueño, del ejercicio y de la TMS en *Octodon degus***" con mención de doctorado internacional, presentada por Doña Cristina Estrada Esteban en la Universidad de Murcia (España),

Informa que:

La línea de investigación en la que se encuadra la tesis de Cristina Estrada Esteban pertenece al campo de la neurociencia y en ella se evalúa el papel terapéutico de la Estimulación Magnética Transcraneal en la función cognitiva de *Octodon degus* (jóvenes y mayores).

Los objetivos de este trabajo son de gran interés en futuras aplicaciones humanas ya que el deterioro cognitivo asociado a la edad es uno de los principales problemas de la sociedad actual. Por ello, estos estudios que evalúan la mejora del deterioro cognitivo con Estimulación Magnética Transcraneal, y/o con la práctica de ejercicio físico voluntario serían útiles y ventajosos en el futuro en la práctica diaria.

Asimismo, el uso de *Octodon degus* jóvenes y añosos como modelo experimental en el estudio de envejecimiento y de enfermedades asociadas al mismo aporta gran originalidad al trabajo.

La contribución de la estudiante en este trabajo de investigación es relevante y se refleja en el número de publicaciones ya realizadas (y evaluadas por pares) que forman parte del manuscrito presentado.

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En consideración con todo lo descrito, esta tesis cumple el grado de satisfacción en cuanto a calidad, contenido y presentación de resultados para su defensa y obtención del grado de Doctor "Mención International".

En Pècs, a 25 de agosto de 2017

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INFORME VALORACIÓN TESIS DOCTORAL

Doctoranda: Cristina Estrada Esteban

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Doña __Rita Raisman-Vozari_como miembro de la Universidad Pierre et Marie Curie de Paris y como evaluadora de la tesis doctoral "**Rendimiento cognitivo en envejecimiento: efecto del sueño, del ejercicio y de la TMS en *Octodon degus***" con mención de doctorado internacional, presentada por Doña Cristina Estrada Esteban en la Universidad de Murcia (España),

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En_Paris_, a 20 de agosto de 2017

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A todos los animales

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I. Introducción

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1. Envejecimiento

1.1 Definición y situación actual

El envejecimiento es un fenómeno complejo, diversificado y multidisciplinar, que lo podemos definir como un proceso multidimensional e irreversible que recoge los cambios acumulados en un organismo con el paso del tiempo, desde el momento de su nacimiento. Hoy en día, estamos ante una sociedad que está padeciendo un envejecimiento progresivo, es más, no ha existido una época anterior con un número tan grande de personas mayores(Seyyedraasooli y cols., 2013). El crecimiento de la esperanza de vida, sobre todo en los países tecnológicamente avanzados (DuGoff y cols., 2014)y el declive en la tasa de fertilidad se han establecido como los principales factores que determinan la llamada “transición demográfica” (Kalache, 1997). El progreso del desarrollo humano, la medicina moderna, la educación y la mejoría de las condiciones socioeconómicas de la población en general, son algunos de los factores que han hecho posible mejorar el estilo de vida y de calidad en el envejecimiento.

Desde mediados del siglo pasado, con el aumento de la natalidad y la disminución de la mortalidad, asociado al avance médico en prevención y cura de enfermedades, se ha producido un gran aumento de la cifra de individuos que viven por encima de los límites de edad establecidos por la especie humana (DuGoff y cols., 2014). Se estima que la población senil mundial tiende a crecer un 2,4% por año (Busciglio y cols., 1998). No todo es positivo en el aumento de la esperanza de vida, el envejecimiento de la población conlleva una nueva problemática y nuevos desafíos tanto en lo referente a la investigación, como en el aspecto socioeconómico (Lorenzo, 2004). Prueba de esto, es que este proceso ha conducido al aumento en la prevalencia de enfermedades asociadas a la vejez(Seyyedraasooli y cols., 2013). Si bien, cabe señalar que ha habido un gran avance en la farmacoterapia de algunas de ellas, como es el caso del cáncer, cardiopatías y enfermedades vasculares, inclusive las cerebrales (Busciglio y cols., 1998). Sin embargo, para las enfermedades neurodegenerativas, el tratamiento y prevención aún carecen de

avances significativos, existen algunas terapias paliativas, pero no curativas, aunque sí se conocen bastantes aspectos de las bases fisiopatológicas, metabólicas y moleculares de la mayoría de ellas (Ahmad y cols., 2017).

1.2. Envejecimiento cerebral fisiológico y patológico

El ciclo vital nacimiento-desarrollo-envejecimiento-muerte se cumple en todos los seres vivos. Todas las células, órganos y sistemas de los seres del reino animal también cumplen este ciclo desde la formación de su primordio hasta el fallecimiento del ser que los alberga, aunque las características de este proceso de desarrollo e involución sean muy diferentes ya que son específicas para cada especie y tejido celular de cualquier ser vivo (Mitteldorf, 2016, Mason y cols., 2007).

En concreto, el Sistema Nervioso Central (SNC) presenta un desarrollo de diseño muy especial (Martin-Padilla 2014). En el período embrionario de cada especie, se crea una ingente cantidad de neuronas, pero sólo sobreviven las que emigran hacia lugares predeterminados, los “núcleos grises” del SNC, y son capaces de establecer conexiones correctas con otras neuronas del propio núcleo al que pertenecen, o de otros núcleos más o menos alejados, que también están predeterminados para cada especie. Se crea así la citoarquitectonia básica del SNC, fundamento de los circuitos neuronales, que se presenta al nacimiento del ser (Basak y Taylor, 2009). En la época postnatal, el desarrollo se centra en el establecimiento y refuerzo de nuevas conexiones neuronales que funcionarán de manera dinámica para cumplir todas las funciones encomendadas al SNC, desde las sencillas respuesta monosinápticas que son la base de algunos movimientos reflejos, hasta las muy complejas respuestas multisinápticas donde intervienen millones de neuronas para cumplir las funciones cognoscitivas superiores (Toro y Deakin, 2007). Además, se ha demostrado que las sinapsis son “plásticas” (Sala y Segal., 2014., Vitureira y cols., 2013, Bhalla, 2014, Toledano, 1999), es decir, cambian morfológicamente en número, forma y tamaño, así como funcionalmente, aumentando o disminuyendo los componentes macromoleculares específicos para su función y regulando la producción de

neurotransmisores, neurorreguladores y segundos mensajeros intracelulares (Ming y Song, 2011).

Esta plasticidad sináptica anteriormente descrita, es una manifestación de la capacidad de adaptación de las neuronas para cumplir los objetivos de cada parte del SNC (Toledano, 1999, Raven y cols., 2017). Esta adaptación ocurre a lo largo de toda la vida incluso en la fase senil: el envejecimiento cerebral debe entenderse como un proceso involutivo-adaptativo (Frolkis y cols., 1979, Van Praag y cols., 2005) donde no existe reemplazamiento de neuronas, por ser células postmitóticas incapaces de dividirse, pero sí de funciones para paliar la pérdida funcional de las dañadas (French y cols., 2017).Neuronas sobrevivientes pueden suplantar la función de desaparecidas sin alterarse la función cognoscitiva(Radad y cols., 2017) Se ha comprobado de manera experimental como aumentan las sinapsis de muchas regiones del SNC de diversos animales seniles con entrenamiento o bien con su permanencia en “ambientes enriquecidos” (Frolkis y cols., 1979, Connor y cols., 1980).

En un cerebro senil “normal” encontramos un menor número de neuronas en diversas regiones del SNC, una importante pérdida de sinapsis sobre muchas neuronas, y una disminución de moléculas para la conexión sináptica y la neurotransmisión (Daulatzai, 2010). En el proceso de involución senil, se pueden apreciar neuronas atróficas y disatróficas, pero también hipertróficas e hiperactivas debidas a la adaptación neuronal. Aquellos individuos cuyo cerebro tiene estas características, no presentan alteraciones muy importantes de las funciones cognoscitivas y comportamentales, es decir, no padecen ninguna demencia cuya principal característica según la OMS, es la pérdida de múltiples funciones cognoscitivas como son la memoria, el pensamiento abstracto, o el juicio, entre otras (Golub, 2017).

Por el contrario, existen individuos seniles que manifiestan un grado variable de demencia (de leve a grave), con alteraciones importantes de todas las funciones cognoscitivas, aunque no todos los deterioros cognoscitivos se hayan iniciado simultáneamente ni su progresión curse en paralelo. El estudio del cerebro de estos individuos muestra que además de las alteraciones antes mencionadas del cerebro senil “normal”, aparecen lesiones o cambios aberrantes en las neuronas y en las células gliales (Sala-Llonch y cols., 2017).

Nos encontramos en presencia de nuevos elementos que consideramos neuropatológicos dentro y fuera de las células como los [ovillos neurofibrilaresintracelulares y placas amiloides extracelulares en el caso dela Enfermedad de Alzheimer (EA)] (Sergeant y cols., 2008). La manifestación clínica de la demencia se corresponde con una neurodegeneración o involución neurodegenerativa. Bajo estas circunstancias nos referiremos a un "envejecimiento senil patológico" por contraposición al anteriormente reseñado, el "envejecimiento senil normal o fisiológico".

1.2.1 Sistema visual: cambios asociados al envejecimiento

El sistema visual, al igual que es resto del organismo no es indiferente al paso del tiempo, y prueba de ello son las patologías visuales asociadas a la edad.Estas alteraciones requieren una atención temprana para poder prevenir las consecuencias negativas que pueden desencadenar posteriormente. Entre los problemas más habituales destacamos la presbicia, miodesopsias, cataratas, glaucoma o degeneración macular (Jackson y Owsley, 2003). Mención especial requiere en nuestro caso la retina, ya que es un tejido especialmente sensible al paso del tiempo (Cunha y cols., 2016, Boya y cols., 2016). La retina es una membrana situada en el interior del ojo que recibe las impresiones luminosas que son transmitidas al cerebro mediante una serie de fenómenos químicos y eléctricos. Cubre la coroides hasta el iris y está formada esencialmente por expansiones del nervio óptico, una estructura compleja formada por varias capas de neuronas interconectadas mediante sinapsis. Las únicas células sensibles directamente a la luz son los conos y los bastones,Los bastones funcionan principalmente en condiciones de baja luminosidad y proporcionan la visión en blanco y negro, por el contrario, los conos están adaptados a las situaciones de mucha luminosidad y proporcionan la visión en color (Moore y cols., 2013).

A nivel macroscópico, la retina se divide en dos áreas:

-Área central de la retina: parte de la retina que rodea la fóvea y donde se produce la fotorrecepción. La fóvea y el área que la rodea contiene un pigmento amarillo denominado mácula lútea.

-Área periférica de la retina: parte con menor capacidad de fotorrecepción, por poseer menor número de conos y bastones.

A nivel microscópico, la retina contiene una serie de capas paralelas (figura 1):

- Epitelio pigmetario: capa más externa. Está formada por células cúbicas que no son neuronas y tienen unos gránulos de melanina con la que adquieren una pigmentación característica.
- Capa de células fotorreceptoras: está formada por los segmentos más externos de los conos y bastones.
- Capa limitante externa: No es una membrana, sino un conjunto de uniones intercelulares entre las células fotorreceptoras y las células de Müller.
- Capa nuclear externa: está formada por los núcleos celulares de las células fotorreceptoras.
- Capa plexiforme externa: es la zona de conexión sináptica entre las células bipolares y las células fotorreceptoras.
- Capa nuclear interna: está formada por los núcleos celulares de las células bipolares, las células horizontales y las células amacrinas.
- Capa plexiforme interna: es la región de conexión sináptica entre las células bipolares amacrinas y ganglionares.
- Capa de células ganglionares: está formada por los núcleos de las células ganglionares.
- Capa de fibras del nervio óptico: está formada por los axones de células ganglionares que forman el nervio óptico.
- Capa limitante interna: separa la retina del humor vítreo.

Además, está formada por tres tipos de células: i) pigmentadas, son las encargadas del metabolismo de los fotorreceptores, ii) de sostén, cuya función es dar soporte, sintetizar glucógeno y ceder glucosa a otras células nerviosas, y iii) neuronas (Figura 1).

Las neuronas se dividen en:

- Células fotorreceptoras (conos y bastones): transforman los impulsos luminosos en señales eléctricas.
- Células bipolares de la retina: conectan las células fotorreceptoras con las células ganglionares

- Células amacrinas: son interneuronas moduladoras
- Células horizontales: tienen una función similar a las anteriores.
- Células ganglionares de la retina: de las mismas, parte el nervio óptico que conecta la retina con el cerebro

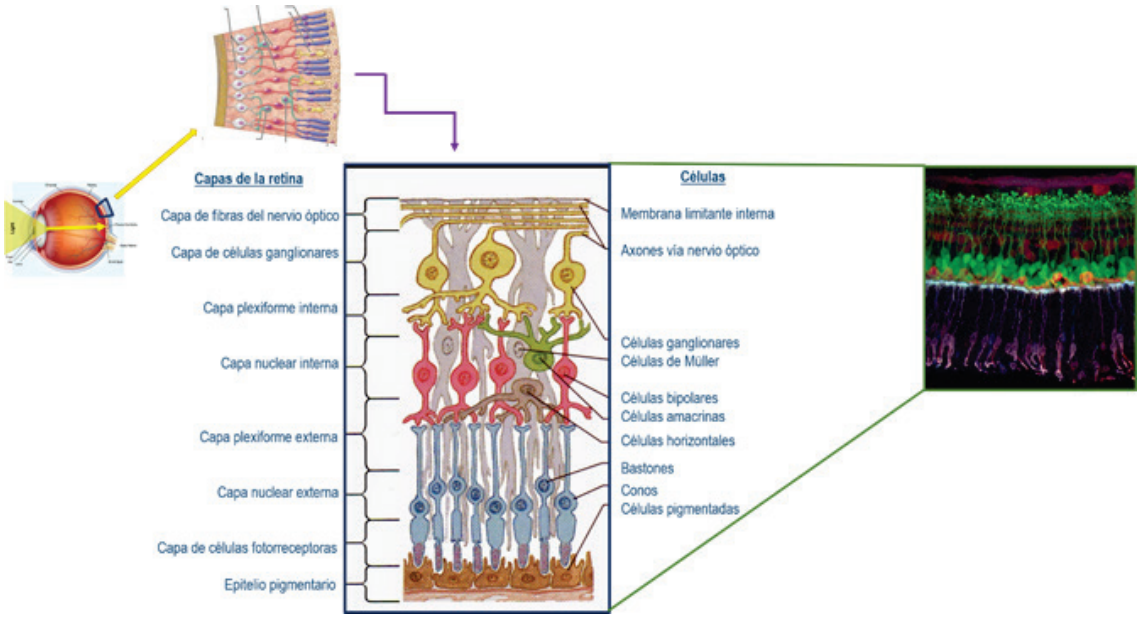

Figura 1. La luz entra por la izquierda y debe atravesar todas las capas celulares hasta llegar a los conos y bastones como se aprecia en el esquema.

Las complicaciones relacionadas con la edad en la retina se han vistas en diferentes mamíferos, como primates, gatos, ovejas, rata y ratón, e inclusive en *Octodon degus.*

1.3 Deterioro cognitivo asociado a la enfermedad de Alzheimer

La primera causa de demencia a nivel mundial es la EA (alz.org). Esta patología es especialmente paradigmática por las características de la discapacidad que acarrea, al tratarse de una patología que actualmente no tiene prevención ni tratamiento eficaz, y en la que el enfermo requiere la continua presencia de un familiar o cuidador, con lo que esto implica desde un punto de vista sociosanitario (alz.org).

En al año 1907, el psiquiatra y neurólogo Alois Alzheimer, fue el primero en describir una serie de alteraciones neuropatológicas en el cerebro de una mujer que había padecido demencia.Aunque inicialmente lo consideró como signos de senilidad precoz, luego lo describió como signos de una enfermedad nueva o senilidad patológica frente la senilidad “normal” de la mayoría de los

individuos (Alzheimer, 1907). Posteriormente, se confirmó por estudios llevados a cabo por Kraepelin (Kraepelin, 1910, Schorer, 1985) y por la investigación científica básica y clínica. A lo largo de los años han ido evolucionando muchos de los conceptos sobre la EA, pero esta idea de que se trata de un envejecimiento cerebral patológico permanece inalterable (Dubois y cols., 2016).

En España, si consideramos cierta la dualidad del curso involutivo, sin demencia o con demencia, habría que incluir en los cálculos asistenciales solo el porcentaje de población susceptible de padecer la EA (alrededor de 1,6 millones de personas en 2050), pero si se considera que existe un *continuum*, se deberían establecer medidas preventivas y sistemas asistenciales para más de 13,5 millones de españoles que se verán afectados hacia mitad del presente siglo (De Pedro-Cuesta y cols., 2009).

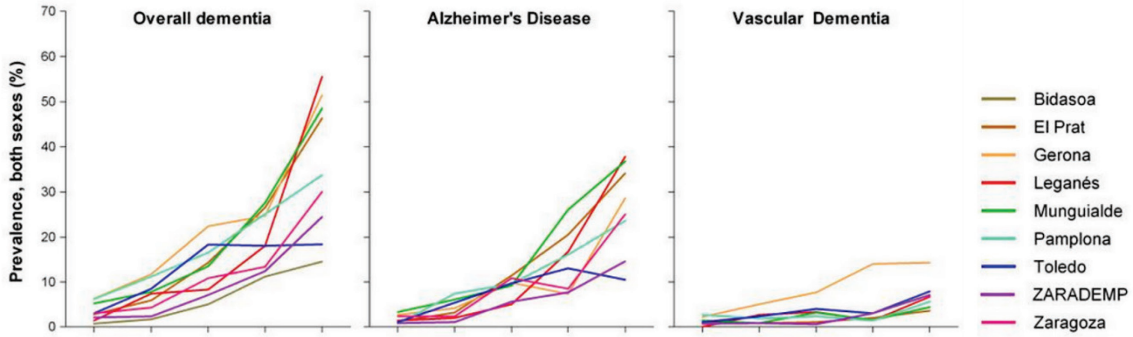

Figura 2. Representación de la prevalencia de demencia, Enfermedad de Alzheimer (EA) y demencia vascular específica de la edad en distintas zonas de la población española *(Adaptado de Pedro-Cuesta y cols., 2009).*

Por otro lado, si los nuevos postulados sobre la diversidad de procesos patogénicos incluidos en el universo de la EA (Dubois y cols., 2007, Dubois y cols., 2010, De Pedro-Cuesta y cols., 2009) se confirman, como por ejemplo, la pérdida de la actividad colinérgica en el SNC o la elevada producción de β-amiloide (Figura 3 c), neurotoxicidad, estrés oxidativo o la inflamación, el número de afectados debería revisarse al alza, aunque habría que considerar como elemento corrector la posible eficacia de nuevas medidas preventivas específicas, que actúen sobre las nuevas dianas terapéuticas que va ofreciendo el desarrollo de la investigación (Shah y cols., 2016). Además,

habría que intensificar las medidas preventivas en individuos asintomáticos en edad adulta (40-50 años) para combatir las alteraciones patológicas más precoces que pudieran aparecer en el inicio del envejecimiento (Lazarczyk y cols., 2012). La figura 3 nos ilustra cómo se afectan las neuronas y las terminaciones sinápticas con la edad (Figura 3 a y b). Actualmente, el diseño de métodos y herramientas para definir la posible población en riesgo para ser especialmente tratada contra la neurodegeneración es un gran reto, de aquí la intensa búsqueda de marcadores tempranos de la enfermedad (Porteri y cols., 2017, Boccardi y cols., 2017, Dourlen y cols., 2017, Huang y cols., 2017).

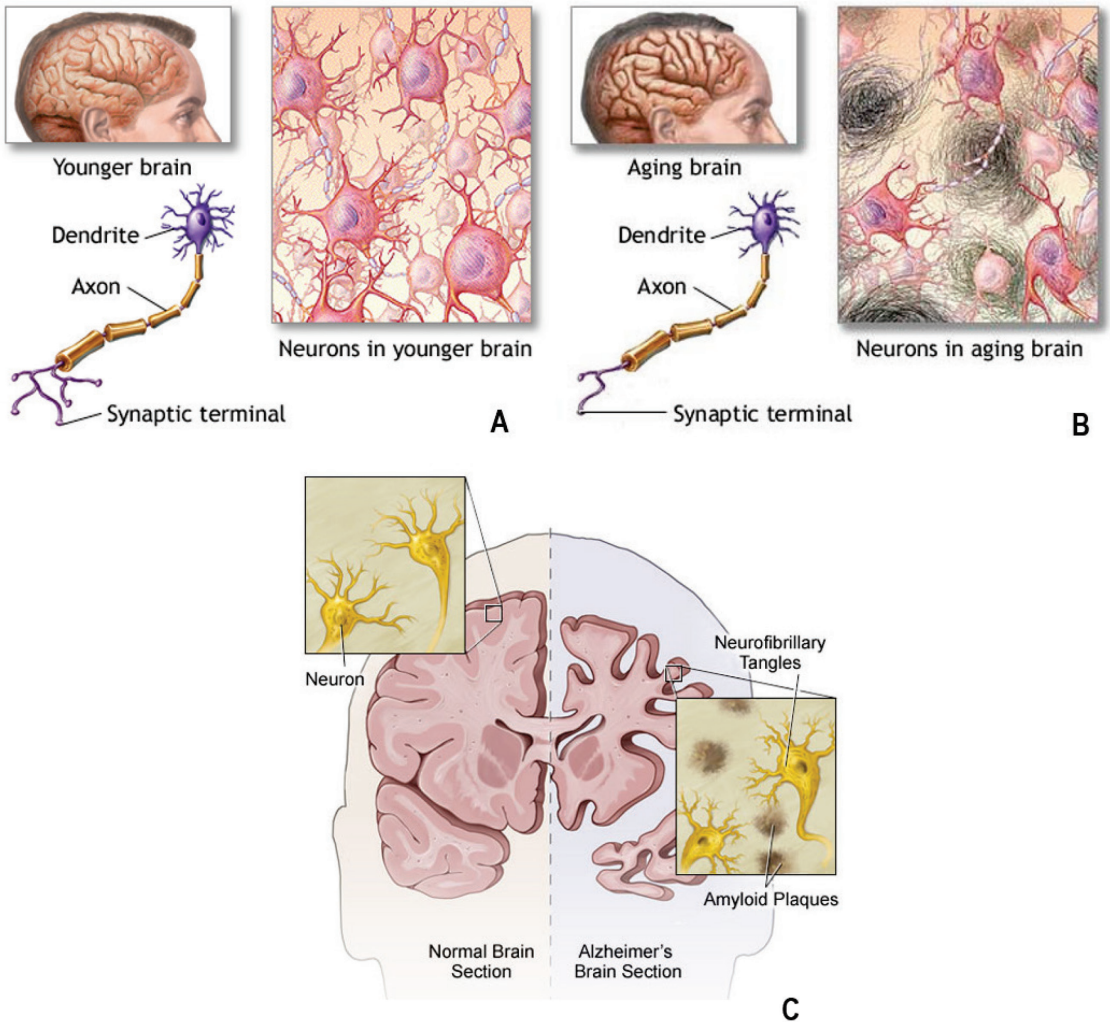

Figura 3. Cambios en el SNC asociados a la edad. Se puede observar la evolución de las neuronas y de las terminaciones sinápticas con la edad al comparar el cerebro de una persona joven (A) con el de una persona añosa (B). En la imagen C, se compara una sección cerebral normal con una sección cerebral con EA, donde se aprecian las placas β-amiloides y los anillos neurofibrilares. *(Adaptada de ADAM)*

Los estadíos iniciales de la EA, se asocian con una disfunción en la memoria causada por la degeneración del sistema límbico, y una vez que ésta alcanza la neocorteza, la progresión del deterioro cognitivo va en aumento (Rathore y cols., 2017). Por el momento, hay algunos tratamientos para la mejora de la enfermedad, pero los resultados no son del todo efectivos.

A nivel histopatológico la EA se caracteriza por una atrofia cortical, una marcada pérdida de neuronas y sinápsis, apoptosis, gliosis y placas extra e intracelulares de β-amiloide (Ballard y cols., 2011, Hardy y Higgins, 1992) e hiper- y anormal fosforilación de la proteína tau (Braak y Braak, 1995, Delacourte y cols., 1999). Hay muchos estudios que han relacionado la alteración de diferentes sistemas de neurotransmisión con el deterioro cognitivo de las enfermedades neurodegenerativas, y su relación con demencia y envejecimiento. Además, se conoce que neurotransmisores como glutamato, acetilcolina o dopamina, están implicados en estos procesos(Xu y cols., 2012, Wu y cols., 2017).

El glutamato, también conocido como el neurotransmisor excitatorio por excelencia, tiene una función importante en la patogénesis de la muerte neuronal en las enfermedades neurodegenerativas (Proctor y cols., 2011).En concreto en las que desarrollan demencias donde la modulación de los receptores glutamatérgicos N-Metil-D-Aspartato (NMDA) parece presentar un papel fundamental. En la EA, la acumulación y depósito de proteína β-amiloide y tau respectivamente, hiperactivan los receptores glutamatérgicos de tipo NMDA, provocando la entrada masiva del ion Ca^{2+} hacia el interior de las neuronas, y la consecuente activación de enzimas diana (Armstrong., 2011). Igualmente, también se ha descrito que la proteína tau causa por sí misma la activación de los receptores NMDA, dando lugar a diversos procesos de neurotoxicidad (Xu y cols., 2012).

La acetilcolina (ACh) es un neurotransmisor implicado en los problemas cognitivos de las distintas afectaciones cerebrales asociadas a la edad, debido a su participación en varias funciones cerebrales, como el control voluntario del movimiento, memoria o atención (Lotfipour y cols., 2017). Existen dos tipos de receptores colinérgicos: nicotínicos (nACh) y muscarínicos (mACh). La distribución de estos últimos abarca distintas áreas del SNC, como corteza

motora primaria, prefrontal, periforme y somatosensorial, y varias regiones límbicas, como el núcleo de accumbens y el hipocampo (Xu y cols., 2012). El hecho de que estos receptores se encuentren en estas zonas, nos da una idea de la importancia que tienen para el buen funcionamiento de muchas de las funciones superiores (Sato y cols., 2017). La relevancia de este neurotransmisor en nuestro estudio, queda de manifiesto en el apartado del análisis de la actividad acetil- y butiril colinesterasa en nuestro modelo experimental (Punto III.I.VI-Resultados).

En el caso de la dopamina, es un neurotransmisor de la familia de las catecolaminas, implicado en la regulación de la plasticidad sináptica y en la modulación de la disfunción cognitiva a través de distintas vías de señalización (Chang y Berg, 2001). Además, existen interacciones entre los sistemas dopaminérgico-colinérgico y dopaminérgico-glutamatérgico, lo que podría explicar los cambios de diversos procesos psicológicos, como la ansiedad, el aprendizaje o la memoria (El-Ghundi y cols., 2007).

El papel de los distintos neurotransmisores tanto en la aparición como en el desarrollo de la EA, están en continuo estudio con el fin de confirmar su relación entre los distintos sistemas de neurotransmisión y los problemas cognitivos que aparecen en las demencias asociadas a la edad (Jankowska y cols., 2017). En la actualidad, la farmacoterapia de la EA se centra principalmente, en los sistemas glutamatérgico y colinérgico, que son los que han mostrado resultados positivos hasta la fecha. Existen dos tipos de fármacos aprobados por la *Food and Drug Administration* (FDA) utilizados para el tratamiento de la EA:

- Inhibidores de la acetilcolinesterasa (AChE): la AChEes una enzima responsable de la degradación de la ACh en el espacio sináptico. Dada la importancia de este neurotransmisor en el envejecimiento cerebral, los fármacos que actúen inhibiendo la actividad de esta enzima tiene como objetivo maximizar la función del neurotransmisor cuando está presente. El tratamiento con inhibidores de la acetilcolinesterasa es de elección puesto que logra reducir significativamente manifestaciones conductuales de la EA (Birks y cols., 2006). Además, se ha demostrado su validez en paciente con EA de sintomatología moderada (Ballard y cols., 2011).En España

podemos encontrar comercializados los medicamentos donepezilo (Aricept®), rivastigmina (Exelon®, Prometax®) y galantamina (Reminyl®).

- Antagonistas de los receptores NMDA: son otro de los fármacos utilizados en el tratamiento de la sintomatología de la EA, en concreto la memantina (Rodríguez y cols., 2012). El importante papel que tienen los receptores de NMDA en el aprendizaje y la memoria, hace que la utilización de estos fármacos resulte en un deterioro de estas funciones. Sin embargo, a pesar de que el antagonismo de los receptores NMDA es capaz de provocar deterioro en el aprendizaje y memoria, también son utilizados como tratamiento en la EA, logrando un retraso en la aparición y el desarrollo de los problemas cognitivos asociados a esta enfermedad (Danysz y Parsons, 2003). En España registrada como Ebixa®.

La FDA ha aprobado recientemente un nuevo medicamento: Namzaric®. Sin embargo, se trata más bien de un ejemplo de innovación en su formulación, ya que se trata de una cápsula que combina memantina y donezepilo, fármacos que se prescriben conjuntamente en el 70% de los pacientes de EA.

	Donepezilo	**Rivastigmina**	**Galantamina**	**Memantina**
Indicación	EA leve-moderada EA grave (EE.UU)	EA leve-moderada Demencia asociada a enfermedad de Parkinson	EA leve-moderada	EA leve-moderada
Galénica	Comp. 5 y 10 mg Bucodispersable5 y 10 mg Retard 23 mg (EE.UU)	Caps. 1,5-3-4,5-6 mg Sol. Oral 2mg/ml parches 4,6-9,5-13,3 mg/24 h	Caps. Lib. Prolongada 8-16-24 mg Sol. Oral 4 mg/ml	Comp. 10-20 mg Sol.oral
Vida media	70 horas	2-4 horas	6-8 horas	10-20 horas
Metabolismo	70% hepático (vía CYP450) 30% renal	100% hepático (no citocrómico)	60% hepático 40% renal	10% hepático 90% renal
Mecanismo de acción	Inhibidor reversible de acetilcolinesterasa (> 80%)	Inhibidor pseudoirreversible de acetil y butirilcolinesterasa	Inhibidor reversible de acetilcolinesterasa (60%) Modulador de receptores nicotínicos	Bloqueante parcial de receptores NMDA del glutamato
Biodisponibilidad	100%	Oral: 60-70% Cutánea: 100%	80%	100%
Ensayos clínicos	Caídas en	Demencia con	Enfermedad de	

positivos en otras indicaciones no aprobadas*	parkinsonianos con demencia Emergencia de síntomas conductuales	cuerpos de Lewy	Alzheimer con componente cerebrovascular	
Ensayos clínicos negativos**	Estimulación cognitiva en controles Demencia asociada a síndrome de Down Deterioro cognitivo postraumático	Deterioro postraumático	Demencia vascular pura Deterioro cognitivo asociado a esquizofrenia	Demencia asociada a enfermedad de Parkinson Demencia frontotemporal

Tabla 1. Fármacos comercializados en el tratamiento de la EA en fase de demencia. En esta tabla se resumen las características del donepezilo, rivastigmina, galantamina y memantina como principales fármacos utilizados en el tratamiento de la EA.*(Tabla adaptada de https://knowalzheimer.com/profesionales/tratamiento-farmacologico-actual-de-la-enfermedad-de-alzheimer/)*

** Estos ensayos clínicos no tienen suficiente nivel de evidencia para que las autoridades reguladoras consideren suficientemente comprobada la eficacia y/o seguridad en estos pacientes*.

** Se citan algunos de los ejemplos más relevantes. Algunos ensayos clínicos no han sido publicados.

2. Memoria

2.1 Procesos básicos de la memoria

El ser humano presenta tres procesos cognitivos fundamentales: la percepción, el aprendizaje y la memoria. La percepción es la forma en la que interpretamos la información que percibimos a través de nuestros sentidos, el aprendizaje consiste en adquirir conocimientos sobre el mundo a través de la experiencia, y la memoria es la retención y evocación de esos conocimientos(Deiana y cols., 2011). La memoria a su vez posee tres funciones básicas: recogida de nueva información, su organización para que tenga un significado y su recuperación cuando se necesita recordar algo (Squire, 1986).

2.2 Estructura y funcionamiento de la memoria

La memoria consta de tres etapas: codificación, almacenamiento y recuperación. Ésta como función cerebral superior, registra los sucesos como recuerdos. Sin embargo, ¿cómo asociamos unos recuerdos con otros?Hasta la fecha se han descrito tres sistemas de memoria (Figura 3) que se comunican e interactúan entre sí(Deiana y cols., 2011):

a) Memoria sensorial: registra las sensaciones y permite reconocer las características físicas de los estímulos
b) Memoria a corto plazo: guarda la información que necesitamos en el momento presente
c) Memoria a largo plazo: conserva nuestros conocimientos del mundo para utilizarlos posteriormente.

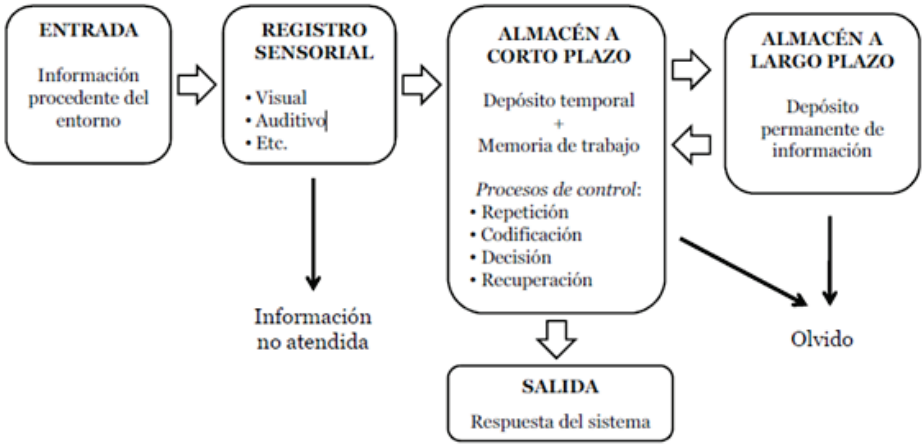

Figura 4. Estructura y funcionamiento de la memoria. *(Adaptada del grupo Rúbrica)*

La memoria sensorial registra la información que proviene del ambiente externo (imágenes, sonidos, olores, sabores) durante un tiempo muy breve (segundos), pero el suficiente para que esa información sea transmitida a la memoria de corto plazo. Al mismo tiempo esta memoria, explora las características físicas de los estímulos y registra sensaciones.Los rasgos físicos de los estímulos, su forma, color o intensidad son determinantes en el registro de la información (Diaz-Orueta y cols., 2016). La capacidad de la memoria sensorial es grande y subsistema para cada sentido, de este modo la memoria icónica registra la información en forma de iconos (imágenes) y la memoria ecoica registra sonidos y palabras. Además, la duración de esta información depende del sentido, así enla memoria ecoica, la información permanece durante dos segundos, mientras que la memoria icónica guarda la información un segundo. Si la información que llega a la memoria sensorial no es transferida a la memoria de corto plazo, decae rápidamente (Cherkin, 1969).La memoria a corto plazo (MCP) se puede definir como el mecanismo de memoria que nos permite retener una cantidad limitada de información durante un período corto de tiempo (Squire, 1982). La

información almacenada en la memoria sensorial se transfiere en parte a la memoria a corto plazo, antes de pasar a la memoria de largo plazo. La función de esta memoria es organizar y analizar la información e interpretar experiencias. En la memoria a corto plazo, la información se codifica de forma visual y acústica, y en menor medida por signos semánticos. Se trata de una memoria de trabajo que integra todos los conocimientos y recuerdos que importan en la situación presente, pero sucapacidad de almacenamiento es limitada, no puede retener más de siete ítems a la vez y los recuerdos de ésta se pueden alterar por nuevas experiencias. La duración temporal de esta información es breve, oscilando entre 18 y 20 segundos. Si la información se interpreta y organiza de forma lógica, se puede recordar por más tiempo (Squire, 1982).

La memoria a largo plazo (MLP) es la capacidad de mantener la información desde unos días hasta incluso décadas(Deiana y cols., 2011). Contiene nuestros conocimientos de la realidad social y cultural, recuerdos autobiográficos, así como el lenguaje y los significados de los conceptos. En este tipo de memoria, la información está bien organizada, facilitando su acceso cuando es oportuno. La información es semántica cuando el material es verbal y visual cuando se trata de figuras o gráficos. El código semántico permite establecer relaciones significativas entre la diversidad de conocimientos almacenados. Sin embargo, la MLP tiene una capacidad ilimitada(Deiana y cols., 2011).El cerebro no almacena los recuerdos en una única estructura. Por el contrario, los diferentes tipos de memoria se almacenan en regiones cerebrales diferenciadas. La MLP se divide en dos grandes subtipos: la memoria explícita y la memoria implícita.

La memoria explícita o memoria declarativa tiene que ver con todos los recuerdos que se encuentran conscientemente disponibles. Las estructuras cerebrales que participan en su codificación son el hipocampo, corteza entorrinal y la corteza perirrinal, pero el almacenamiento de la información tiene lugar en la corteza temporal. A su vez la memoria explícita se divide en:*i)Memoria episódica: se refiere a los recuerdos relacionados con los*

sucesos vividos y ii) Memoria semántica: se refiere a los conocimientos generales sobre el mundo.

La memoria implícita o memoria procedimental se refiere a la habilidad para realizar movimientos o utilizar objetos (Arroyo-Anlló y cols., 2013).

A lo largo de este manuscrito trataremos tanto la memoria episódica como la espacial, perteneciendo ambas a la rama semántica de la memoria declarativa(Deiana y cols., 2011).Recientemente,se ha demostrado que la memoria declarativa es dependiente del hipocampo (Crystal, 2010). En este sentido se considera que la memoria espacial es una de las formas de procesamiento cognitivo más avanzado en los mamíferos, e incluye la capacidad para almacenar información acerca del entorno de uno mismo y su orientación en el espacio (Begega y cols., 2012). De esta manera, la orientación espacial puede servirse de claves ambientales para la formación y reconstrucción posterior de mapas cognitivos, esto es, una representación interna del entorno (O´Keefe y Nadel, 1978, Rubio y cols., 2012). Para la formación de estas representaciones, se hanpostulado principalmente dos tipos de estrategia: una egocéntrica, que es aquella que define al individuo en relación a los objetos, el objetivo y las claves disponibles dentro del propio paradigma, y otra alocéntrica, la cual define al individuo en función de la localización de los objetos dentro del entorno global que los rodea (Ball y cols., 2017).

En numerosos estudios con animales se ha demostrado que ambos tipos de estrategia son evaluables mediante diferentes paradigmas conductuales, entre los que se encuentra tanto el laberinto radial (Begega y cols., 2012), como el laberinto de Barnes (Popovic y cols., 2010). Es por ello, que ambos paradigmas han sido utilizados a lo largo de este trabajo con el fin de evaluar la memoria de los animales con los que hemos realizado los estudios. En otros trabajos se ha demostrado que tanto el hipocampo como el sistema colinérgico son fundamentales para la adquisición de información cuando se sigue una estrategia alocéntrica (Deiana y col., 2011).

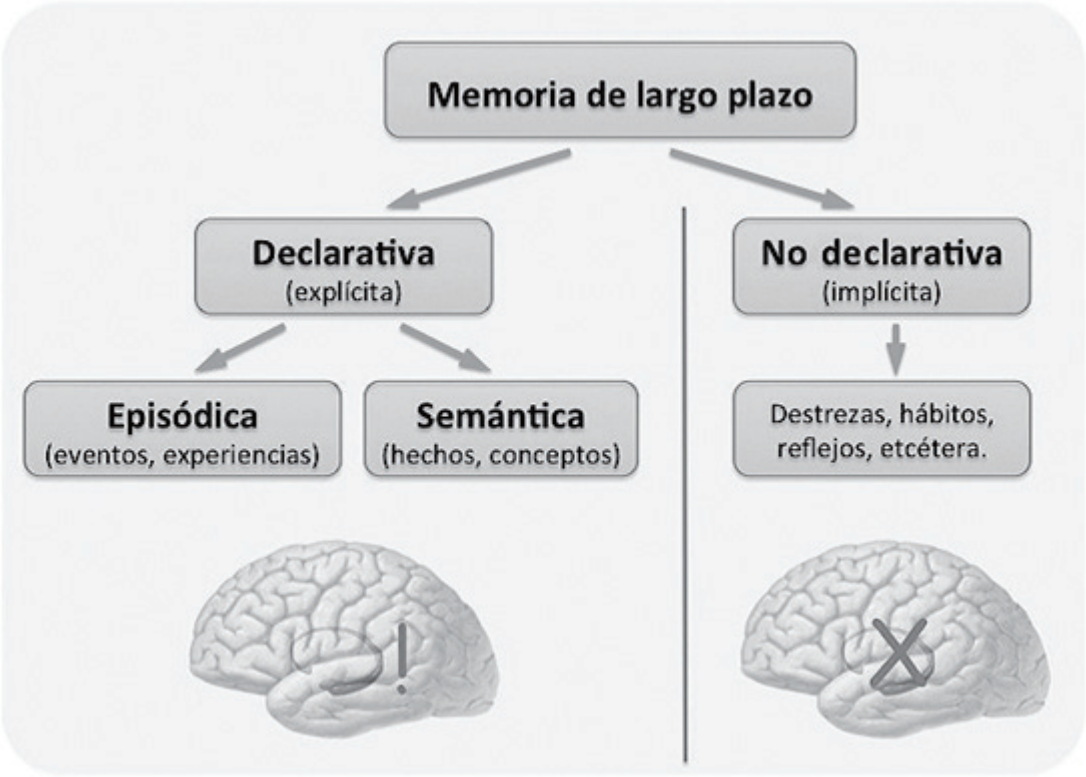

Figura 5. Taxonomía de la MLP. La memoria declarativa incluye los recuerdos accesibles de forma consciente, basados en hechos (saber "qué"). La memoria episódica (experiencias propias pasadas) y la memoria semántica (recuerdos de conocimiento general), forman parte de la misma. La memoria no declarativa incluye la memoria implícita y lo relacionado con el conocimiento procedural (saber "cómo"). (Squire y Wixted, 2011).*(Imagen de Rodrigo Quian)*

2.3 La función cognitiva y atención

La función cognitiva engloba un grupo de funciones complejas llevadas a cabo por los hemisferios cerebrales, denominadas funciones superiores o ejecutivas. Se definen como un conjunto de procesos cognitivos que deben coordinarse para conseguir con éxito la ejecución de tareas complejas, e implica esencialmente planificación, generación de estrategias, monitorización de la ejecución repaso y valoración de las estrategias elegidas, además de la capacidad de cambio o cese de una determinada conducta (Squire y Wixted, 2011).

La cantidad de información a la que constantemente nos vemos sometidos desde el exterior, excede por lo general a la capacidad de nuestro SNC para poder procesarla por completo. Es necesario que exista un mecanismo regulador neuronal que seleccione u organice las percepciones para una efectiva recepción. Este mecanismo regulador es la atención, que además de

organizar la entrada de información está implicada en el procesamiento de la misma (Moscovitch y cols., 2006). Es un mecanismo neuronal que regula y focaliza el organismo, seleccionando y organizando la percepción partiendo que el estímulo pueda dar lugar a un “impacto”, es decir que se pueda desarrollar un proceso electroquímico. Es el resultado de una red de conexiones corticales y subcorticales de predominio de hemisferio derecho (Moscovitch y cols., 2006). Los aspectos que caracterizan una correcta capacidad atencional son percepción selectiva y dirigida (orientación y exploración), esfuerzo de concentración por una tarea con un interés por una fuente particular (vigilancia). Cabe destacar que la atención es un proceso neurocognitivo que precede a la percepción y a la acción, y sin atención, no se podría dar lugar a la memoria y el aprendizaje (Cabeza y Nyberg, 2000). La atención se encarga de focalizar selectivamente nuestra conciencia, regulando la entrada de información, ejerciendo funciones de filtrado y desecho de información, ayuda a resolver la competencia entre estímulos para resolverlos en paralelo, activando áreas cerebrales y reclutando o activando otras para dar respuestas adecuadas, de esta manera facilita la percepción, la memoria y el aprendizaje (Unsworth y Robison, 2017).

2.4 Afección de la memoria con la edad: envejecimiento cognitivo

Es evidente que la edad afecta al rendimiento de la mayoría de los procesos cognitivos, como demuestran los datos epidemiológicos de la incidencia de demencias (Sosa-Ortiz y cols., 2012, Crane y cols., 2017, Rafnsson y cols., 2017). En la actualidad, los estudios relacionados con los cambios en la memoria asociados al envejecimiento no la contemplan como un proceso unitario. Tanto esasí, que el envejecimiento produce efectos diferenciales de deterioro en los distintos tipos de memoria (Park y cols., 2002).En un estudio llevado a cabo con 345 adultos con edades entre los 20 y 80 años, se evaluó la memoria operativa (amplitud de cálculo), memoria episódica (recuerdo de una lista de palabras) y memoria semántica (definir palabras en una prueba de vocabulario). En él, se observaron cambios a lo largo de toda la vida adulta y no sólo limitados al grupo más envejecido. También, se muestra como aspecto interesante que el envejecimiento tiene un

efecto selectivo y así, la memoria operativa y la memoria episódica decaen significativamente a lo largo de la vida, mientras que, la memoria semántica llega a aumentar significativamente. En la Figura 5 se muestra una disminución de todas las medidas de la función cognitiva excepto el conocimiento, que podría mostrar incluso alguna mejoría (Park y cols., 2002).

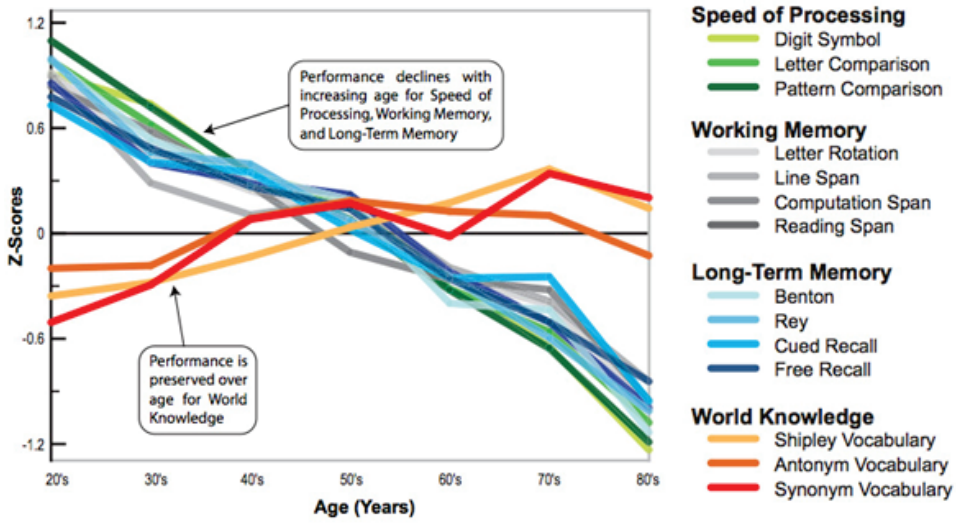

Figura 6. Representación de los datos de comportamiento en medidas de velocidad de procesamiento, memoria de trabajo, memoria a largo plazo y conocimientos, en sujetos de entre 20 y 80 años. *(Adaptado de Park y cols., 2002)*

Un estudio publicado en 2014 revela que la multitarea y la inteligencia fluida son habilidades cognitivas distintas que muestran diferencias divergentes relacionadas con la edad(Kievit y cols., 2014). Además, están mediadas por distintos subsistemas neuronales, mostrando patrones diferenciales de comportamiento cerebral en individuos mayores frente a individuos más jóvenes(Kievit y cols., 2014). Estos resultados muestran que la relación entre las diferencias relacionadas con la edad en la función ejecutiva y los cambios en el curso de la vida de la estructura neural es multidimensional. Como se representa en la figura 7, el fórceps menor (FM) muestra mayores diferencias asociadas a la edad, seguido de la radiación talámica anterior (ATR). Las dos áreas de materia gris (MD, BA10) muestran diferencias similares (Kievit y cols., 2014).

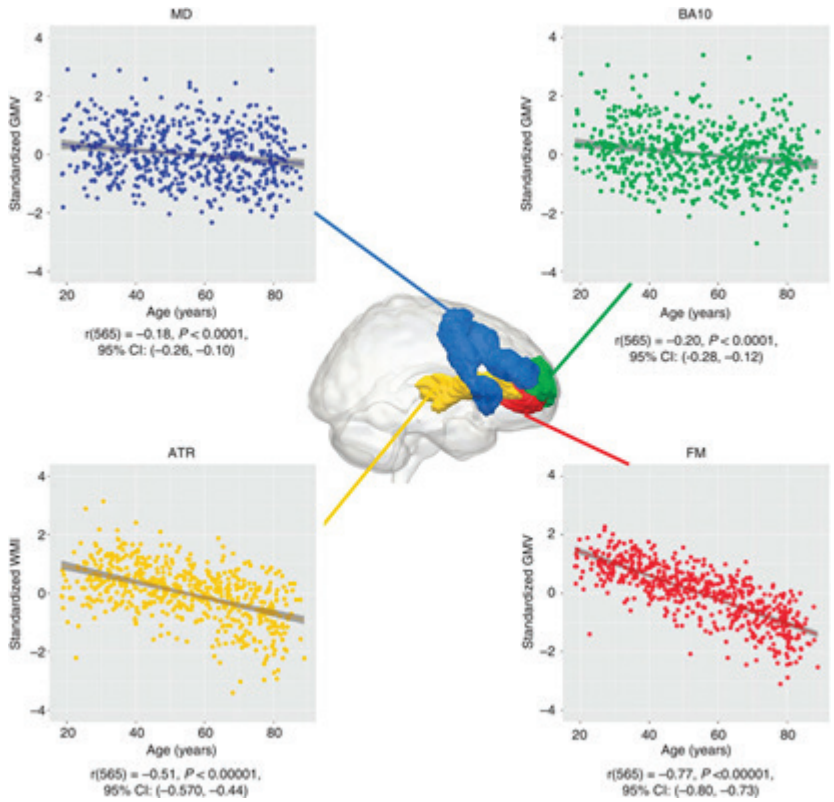

Figura 7. Diferenciación del área prefrontal asociado a la edad. Los diagramas muestran diferencias según el patrón de envejecimiento de las cuatro estructuras de interés; materia gris (área de brodmann, sistema de demanda múltiple) y materia blanca (fórceps menor, radiación talámica anterior). *(MD = Multiple Demand System; BA 10 = Brodmann Area 10; ATR = Anterior Thalamic Radiations; FM = Forceps Minor) (Adaptado de Kievit y cols., 2014)*

2.5 Anatomía de la memoria

El aprendizaje y la memoria son dos procesos cognitivos transcendentales para la adaptación y la supervivencia de los organismos. Ambas conductas son procesadas en el SNC y su regulación requiere la participación de diversas estructuras cerebrales, siendo una de estas estructuras es el hipocampo.

El hipocampo deriva de la región medial del telencéfalo, forma parte del sistema límbico y tiene un papel importante en la adquisición del aprendizaje espacial y la consolidación de la memoria a largo y corto plazo. Anatómicamente, está organizado en el cuerpo de Amón (hipocampo propio) y el giro dentado; el complejo subicular formado por *el presubiculum*, el *subiculum* y el *parasubiculum*; y la corteza entorrinal (Amaral y Witter, 1989, Lavenex y cols., 2007, Kivisaari y cols., 2013). El cuerpo de Amón está dividido en tres áreas: CA1, CA2 y CA3. La mayor entrada de fibras en el hipocampo

proviene de la corteza parahipocampal que es la principal vía de entrada de aferencias neocorticales de procesamiento provenientes de distintas áreas dorsales, como la corteza parietal posterior, la corteza retrosparietal, la corteza prefrontal dorsolateral o de la parte dorsal del surco temporal superior estructuras estrechamente asociadas a la codificación de la localización espacial de los estímulos (Witter y cols., 2000, Lavenex y cols., 2004). Estas aferencias son distribuidas hacia la corteza entorrinal. Las células de las capas II y III de esta corteza envían sus axones hasta el giro dentado y el hipocampo a través de la vía perforante, atravesando la capa de células piramidales del *subiculum* (Khalaf-Nazzal y cols., 2013, Laurberg y cols., 1981). Por otra parte, las neuronas piramidales de la región CA3 proyectan sus axones hacia las dentritas de las neuronas piramidales de las CA1 mediante los colaterales de Schaffer. Así mismo, los axones provenientes de la región CA3 proyectan hacía todo el hipocampo mediante proyecciones comisurales entre hemisferios y/o asociativas, en el mismo hemisferio (Laurberg y cols., 1981, Ishizuka y cols., 1990, Frotscher y cols., 1991). Mientras que las neuronas granulares del giro dentado, proyectan sus axones o fibras musgosas hacia las dentritas proximales de las neuronas piramidales de la región CA3, atravesando el hilus (Chicurel y Harris, 1999, Suzuki y Amaral, 2003)

3. Sueño

3.1 Bases estructurales del ciclo vigilia-sueño

A pesar de que se encuentra ampliamente aceptado que el sueño es un estado de consciencia, es una conducta en sí misma, de la que se pueden distinguir dos estados: i) vigilia y ii) sueño. Aunque existen diversas teorías, no se conoce la causa principal por la que dormimos, y probablemente exista más de una, pero lo que sí resulta evidente es que diversos y muy importantes procesos fisiológicos, están estrechamente relacionados o incluso determinados por el sueño.Entre ellos el restablecimiento de la energía, eliminación de los radicales libres acumulados durante el día, regulación de la actividad eléctrica cortical, regulación térmica, regulación metabólica y endocrina, homeostasis sináptica, activación inmunológica o consolidación de la memoria, entre muchas otras (Mirescu y cols., 2006, Graves y cols., 2001).

Desde el punto de vista funcional, el sueño se conceptualiza que en la regulación global del mismo participan tres subsistemas anatómico-funcionales: i) un sistema homeostático que regula la duración, la cantidad y la profundidad del sueño (en este sistema se ha involucrado especialmente el área preóptica de hipotálamo), ii) un sistema responsable de la alternancia cíclica entre el sueño sin movimientos oculares rápidos (NREM) y sueño con movimientos oculares rápidos (REM), que ocurre en cada episodio de sueño (en el que se ha implicado primordialmente al tallo cerebral rostral), iii) un sistema circadiano que regula el momento en el que ocurre el sueño y el estado de alerta (con la participación del hipotálamo anterior) (Rosenwasser, 2009). Así mismo, se ha demostrado que paralelamente a la participación de distintas estructuras cerebrales, también diferentes neurotransmisores participan en las fases del sueño y vigilia (Carillo-Mora y cols., 2013).

El ciclo de sueño-vigilia se ha estudiado mediante el uso de electroencefalografía (EEG), debido a que ambos estados presentan tipos de ondas cerebrales diferentes y claramente diferenciables (Korotchikova y cols., 2016).La vigilia no es una fase del sueño propiamente dicha, pero es el estado de partida, durante la cual las ondas cerebrales muestran dos patrones de actividad: alfa y beta (Iber y cols., 2007). La actividad alfa consiste en ondas regulares de frecuencia media (8-12 Hz), y aparecen bajo estados de relajación que no requieren actividad mental exigente. Están asociadas con estados de relajación y se registran cuando la persona está con los ojos cerrados o momentos antes de dormir. En cambio, la actividad beta consiste en ondas irregulares de baja amplitud (13-30 Hz), siendo más frecuente en momentos de exigencia cognitiva y alerta. Ésta, se registra cuando la persona se encuentra despierta y en plena actividad mental, con muchos circuitos trabajando simultáneamente. Consecuentemente, la actividad beta es asincrónica (irregular)(Iber y cols., 2007).

La etapa del sueño se divide en dos fases bien diferenciadas que, de forma normal, ocurren siempre en la misma sucesión: todo episodio de sueño comienza con el llamado sueño NREM, que tiene tres estadios y después se pasa a un sueño REM (Figura 8).

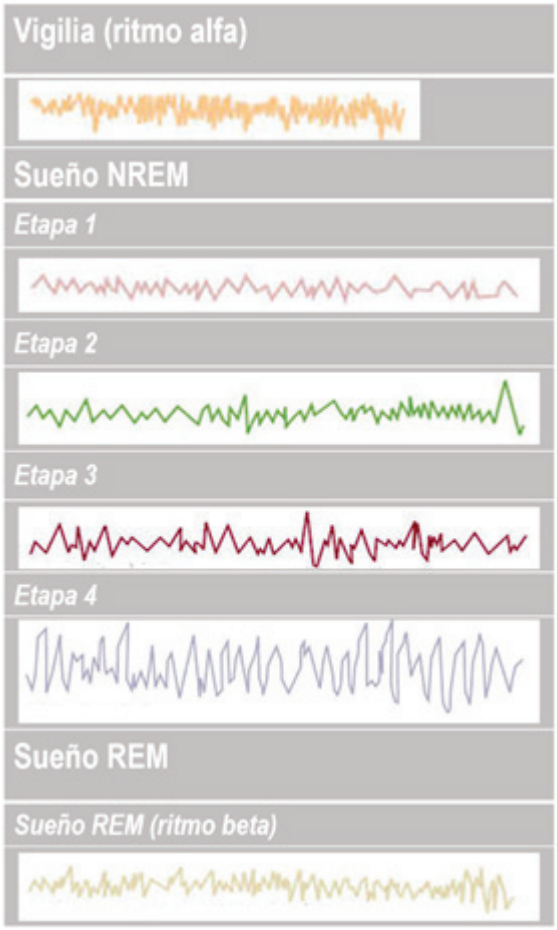

Figura 8. Fases del sueño y vigilia. Se ilustra la señal de EEG durante las diferentes fases del sueño y la vigilia.

La etapa 1 podemos considerarla de adormecimiento (Figura 8). Empiezan a aparecer ondas theta con una frecuencia de 3,5-7,5 Hz, lo que indica que la descarga de neuronas del neocortex se va haciendo más sincronizada. Las ondas theta son de mayor amplitud y menos frecuencia, alcanzándose cuando se entra en un estado de calma profunda.La etapa 2 aparece 10 minutos después del adormecimiento, en la cual el EEG en general es irregular, incluyendo períodos de actividad theta, *spindles* del sueño y complejos k. Los *spindles* son breves ondas de 12-14 Hz que ocurren de 2 a 5 veces por minuto durante las fases 1 a 4 del sueño REM. Los complejos k aparecen en esta fase y se trata de ondas lentas y de gran amplitud que se dan espontáneamente aproximadamente una vez por minuto, aunque también pueden provocarse por un ruido inesperado. Tiene una duración aproximada de 15 minutos (Susmáková y Krakovska, 2008).

Las etapas 3 y 4 se caracterizan por la presencia de ondas delta que constituyen entre 20%-50% de la actividad EEG, en la etapa 3 y más del 50% en la etapa 4. La actividad de estas ondas consiste en oscilaciones muy sincronizadas de gran amplitud y con una frecuencia ligeramente menor a 1 Hz. Cada oscilación consta de una sola onda bifásica. En la fase descendente de la onda, las neuronas corticales están en reposo, hiperpolarizadas y no descargan, en la fase ascendente de la onda, las neuronas corticales descargan brevemente con una frecuencia alta. De forma intercalada a estas ondas, pueden aparecer de forma esporádica complejos k y *spindles* del sueño(Susmáková y Krakovska, 2008).

La etapa 5 (REM) aparece aproximadamente 90 minutos después del comienzo del sueño y unos 45 minutos después de iniciada la etapa 4. En esta etapa, se presenta la mayor frecuencia e intensidad de ensoñaciones. Los ojos se mueven rápidamente y la actividad de las ondas cerebrales se asemeja a la de la etapa 1 del sueño. Sin embargo, aunque la actividad cerebral es intensa, se da una casi total pérdida del tono muscular, con inhibición de neuronas motoras, exceptuando las que controlan la respiración y el movimiento de los ojos, y el sujeto está prácticamente paralizado (Susmáková y Krakovska, 2008).

Un ciclo completo de sueño con las cinco etapas definidas comprende unos 90 minutos de sueño NREM, más unos 20-30 minutos de sueño REM.

3.2 Efectos de la privación de sueño

3.2.1 ¿Por qué dormimos?

El sueño es un fenómeno universal entre los vertebrados. Peces y anfibios entran en períodos de inactividad, los reptiles duermen, y los mamíferos y aves no sólo duermen, sino que también entran en fase REM (Hutchison y cols., 2015). El hecho de que el sueño se mantenga en especies que estarían mejor sin él, nos indica que no es un mecanismo simplemente adaptativo, sino esencial para la supervivencia. Es evidente que diversos y muy importantes procesos fisiológicos, están estrechamente relacionados por el sueño o la periodicidad del mismo. Algunas de las funciones del sueño son el restablecimiento de energía y de la actividad eléctrica cortical, regulación

térmica, metabólica y endocrina y uno de los aspectos más interesantes es su importancia en la memoria (Vassalli y Dijk, 2009, Diekelmanm y cols., 2010). Se ha descrito que la retención de conocimientos era mejor después de una noche de sueño que tras un intervalo de descanso similar, pero manteniéndose despierto.

3.3 Sueño y memoria

Está ampliamente descrito Está más que demostrado que la necesidad e importancia del período de sueño para la buena consolidación y recuperación de determinados tipos de memoria: procedimental (Diekelmann y cols., 2009), declarativa (Pace-Schoot y cols., 2009) o de trabajo (Voderholzer y cols., 2011) y la falta de sueño afecta de manera severa a la ejecución de tareas cognitivas (Durmer y Dinges, 2005). Ciertas tareas son más sensibles a la privación de sueño que por otras: en la memoria asociada al reconocimiento facial, permanece intacta en personas privadas de sueño durante períodos de hasta 35 horas, aunque sí se altera el recuerdo del contexto en que estas caras aparecieron (Sheth y cols., 2009).

El deterioro de la memoria causado por la privación de sueño viene dado por la interrupción del sueño REM, provocando una menor consolidación de una tarea aprendida (Foster y Wulff., 2005). Varios autores sugieren diferentes mecanismos por los cuales este fenómeno puede ocurrir (Patti y cols., 2010), atribuyéndolo a reducciones en el tiempo de reacción (Polzella y cols., 1975), o a alteraciones motoras (Gruat-Masso y cols., 1995).

3.4 Sueño y estrés

Tanto el estrés crónico como el agudo poseen un efecto sobre la arquitectura del sueño y su ritmicidad circadiana, tanto en humanos como en modelos roedores (Cano y cols., 2008). Existen estudios en humanos y animales que desvelan la existencia de fuertes interacciones entre el sistema circadiano y la respuesta al estrés (Gonnissen y cols., 2012, Olbrich y Dittmar, 2012). Estos datos indican que la respuesta de un organismo a un efecto estresor agudo varía en función del momento del día en que el estímulo se presenta por lo que está relacionado con el sistema circadiano (Cano y cols.,

2008). El estrés se ha estudiado ampliamente desde el campo de la investigación básica. Existe una gran variedad de paradigmas para inducirlo, no sólo como un objetivo final, sino como forma de activación de una vía que induce otras condiciones relacionadas con el estrés, como la depresión o la ansiedad (Anisman y Matheson, 2005). Experimentos de estrés agudo llevados a cabo en distintos roedores confirman los datos, y más aún algunos estudios llevados a cabo con hamsters indican que bajo condiciones de estrés agudo, estos animales son capaces de reiniciar la señal del reloj biológico (Van Reeth y cols., 1991), lo cual apoya la idea de una relación entre estrés y sistemas fisiológicos circadianos de carácter adaptativo (Simons y cols., 2015).

4. Ejercicio físico

La actividad física regular es una posibilidad de mejora de la calidad de vida(Hassmén y cols., 2000, Lee y cols., 1995, Espeland y cols., 2017, Zhang y cols., 2016).En humanos se ha demostrado que no sólo puede alargar la esperanza de vida, sino que aumenta el bienestar general y la percepción de salud: i) mantiene el peso corporal y mejora la resistencia física, ii) incrementa la densidad ósea, iii) aumenta tono y fuerza muscular, iv) mejora la flexibilidad y la movilidad de las articulaciones, v) reduce la sensación de fatiga, vi) aumenta la autoestima, vii) rebaja tensión y estrés, y viii) disminuye la aterosclerosis y el riesgo de enfermedades cardiovasculares (Lee y cols., 2014, Rhodes y cols., 2017). Asimismo, el ejercicio previene y mejora de los síntomas de ansiedad y depresión (Larson y cols., 2006, Peluso y cols., 2005), mejora las funciones cognitivas, especialmente en personas mayores (Kramer y cols., 2006, Larson y cols., 2006) y aumenta la neuroplasticidad dando lugar a una mejora cognitiva (Voelcker-Rehage y Niemann, 2013). Especial atención merecen deportes de contacto-contacto, como el fútbol, hockey sobre hielo, rugby americano o artes marciales, cuyos beneficios físicos y psíquicos pueden verse condicionados por la aparición de lesiones cerebrales, desde hace poco llamada la “epidemia silenciosa” (Giza y cols., 2017).

4.1 Ejercicio físico y envejecimiento

Como se ha descrito anteriormente, el envejecimiento no es sólo un fenómeno biológico, sino también antropológico y ecológico. No depende exclusivamente de las modificaciones de la estructura celular, sino que existe una estrecha relación con el medio ambiente y el sistema de vida, lo cual nos indica la importancia de lo psicológico en este proceso y en la calidad de vida durante esta etapa, sobre todo en lo referente al tema que nos abarca, lasdemencias (Guure y cols., 2017). El envejecimiento en el ser humano viene determinado por una serie de modificaciones orgánicas psicológicas y sociales, que configuran como envejece el individuo (Niu y cols., 2017). Se ha demostrado que las funciones cognitivas pueden ser influenciadas por la práctica de ejercicio(Tabei y cols., 2017, Barha y cols., 2017, Sáez de Asteasu y cols., 2017). En los estudios llevados a cabo con roedores, se observa un mejor rendimiento cognitivo como resultado de la práctica de ejercicio físico (Samorajski y cols., 1985, Fordyce y Farrar, 1991, Anderson y cols, 2000). En un modelo de ratón" tipo-Alzheimer" (THY-Tau22), se evaluaron los efectos del ejercicio voluntario a largo plazo practicado por estos animales, un modelo transgénico apropiado y fiable que presentaba un desarrollo progresivo y paralelo de la patología de la Tau y el consecuente deterioro de la memoria, pero en ausencia de déficits motores,demostró los efectos beneficiosos del ejercicioincluso a nivel colinérgico (Belarbi y cols., 2011).

En medicina humana, se ha demostrado desde hace tiempo cómo los individuos que practican ejercicio tienen un mejor rendimiento cognitivo y memoria en comparación con los que no lo practican (Diesfeldt y Diesfeldt-Groenendijk, 1977, Young, 1979 y Emery y cols., 1995). En apoyo a esto se ha estudiado los efectos del ejercicio en la neurogénesis (Van Praag y cols., 1999) y en la regulación de la expresión de factores tróficos (Neeper y cols., 1996 y Ang y cols., 2003).En 2008, un estudio demostró que los niveles de atención y concentración de un grupo de estudiantes incrementaron tras la realización de 10 minutos de ejercicio físico. La mejora, tanto de la precisión como de la velocidad en las tareas cognitivas parece tener según los autores, una relación con el hecho de que exista una conexión entre el cerebelo, principalmente especializado en tareas motoras y la corteza frontal (Budde y cols., 2008). La actividad motora hace variar el patrón de activación cerebral y el grado de

información que se está procesando. Es decir, cuanto mayor sea la actividad motora, más actividad de la corteza prefrontal se requiere. El ejercicio físico activaría de esta manera, los lóbulos frontales, responsables de múltiples tareas cognitivas. En este tipo de estudios, en los que se evalúan diversos procesos perceptivos o cognitivos mientras se realiza actividad física o poco después de haberla finalizado, demuestran que se producen ciertas mejoras cognitivas que revierten poco después de haber finalizado el ejercicio, y éstas suelen tener mayor duración si se realiza ejercicio de manera regular, más que si se realiza de manera aislada (Chan y cols., 2011).

Las repercusiones de la actividad física en los mayores parecen en ocasiones incluso más notables que en los jóvenes. Son muchas las publicaciones que determinan que el deporte en mayores actúa como neuroprotector frenando el declive asociado a la edad, e incluso retrasar la aparición de algunas enfermedades neurodegenerativas (Andel y cols., 2008, Larson y cols, 2006, Rovio y cols, 2005, Kirk-Sanchez y McGough y cols., 2014). La práctica regular de actividad física se ha convertido en uno de los factores más importantes sobre el que se centran múltiples estudios, no solo para mejorar la salud física, reducir el riesgo de enfermedades cardiovasculares y neurodegenerativas, y retrasar la mortalidad (Thompson y cols., 2003), sino además para retrasar el enlentecimiento perceptivo y cognitivo que generalmente se produce con la edad (Hötting y Röder, 2013, Voelcker-Rehage y Niemann, 2013,Cooper y cols., 2017).

Ese factor neuroprotector que a menudo se le atribuye al ejercicio físico, es consistente con el hecho de que algunas de las estructuras cerebrales implicadas en la memoria cognitiva cuando se realiza actividad física, como el hipocampo (Cooper y cols., 2017), a menudo estén relacionadas con la EA. El hipocampo es una estructura que pierde volumen con la edad, produciendo a su vez, pérdidas en la memoria episódica y aumentando el riesgo de demencia (Erickson y Kramer, 2009, Erickson y cols., 2010, Raz y Rodríguez., 2006). En 2010, se realizó un estudio en el que un grupo de personas mayores practicaba durante un año actividad física de tipo aeróbico mientras que otro grupo realizaba ejercicios de estiramiento. Los resultados mostraron que hubo un incremento en el volumen del hipocampo anterior de aproximadamente un 2%

en el grupo que realizó ejercicio aeróbico como se observa en la figura 9. Cabe destacar que antes de empezar el estudio, no había diferencias entre grupos del volumen hipocampal. En el grupo que practico ejercicios de estiramiento obtuvo, tras este período de un año, una pérdida en el volumen hipocampal de aproximadamente un 1,4 %, dato que sugiere que no todos los tipos de actividad física actúan retrasando el declive cognitivo asociado a la edad (Erickson y cols., 2010). También, se observó que la actividad aeróbica incrementó los niveles de factor neurotrófico de origen cerebral (BDNF), proteína que mide la expansión dentrítica (Lee y cols, 2001) y cumple con un papel fundamental en la memoria a largo plazo (Bekinschtein y cols, 2008, Pang y cols., 2004).

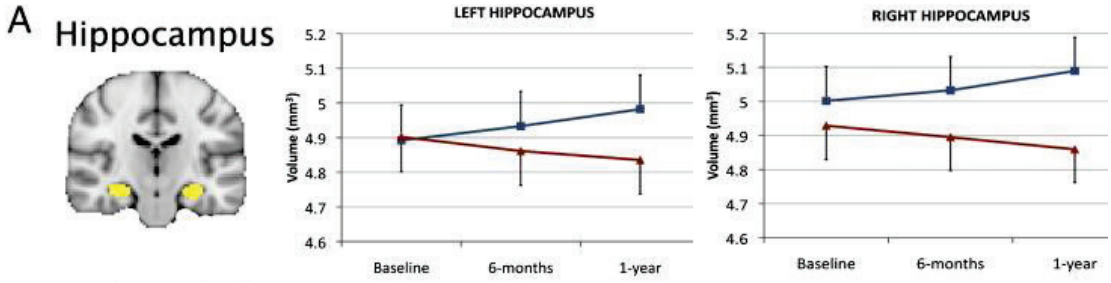

Figura 9. Representación gráfica del incremento del volumen hipocampal en el grupo que practican ejercicio aeróbico y un descenso en el volumen del grupo control. *(Adaptado de Erickson y cols., 2010)*

Se han estudiado diferentes paradigmas en la eficacia, como podría ser el sexo o el tipo de ejercicio. Son dos factores posibles que se han analizado, y donde se ha realizado una revisión sistemática simultánea de adultos mayores cognitivamente saludables(Barha y cols., 2017). En humanos, se examinaron las funciones ejecutivas, la memoria episódica, la función visuoespacial, la fluidez de la palabra, la velocidad de procesamiento y la función cognitiva global para los efectos dependientes del ejercicio y del sexo. Para las funciones ejecutivas, tres tipos de intervenciones de ejercicio [i) entrenamiento aeróbico, ii) entrenamiento de resistencia, iii) entrenamiento multimodal (entrenamiento aeróbico y de resistencia)] se llevaron a cabo, y en comparación con los grupos control, los tres enfoques de entrenamiento de ejercicio mejoraban la función visuoespacial, pero sólo el entrenamiento multimodal mejoró la memoria episódica. En general, el entrenamiento aeróbico

condujo a mayores beneficios que el entrenamiento de resistencia en función cognitiva global y funciones ejecutivas, mientras que el entrenamiento combinado multimodal condujo a mayores beneficios que el entrenamiento aeróbico para la función cognitiva global, memoria episódica y fluidez de palabras(Barha y cols., 2017). Recientemente, también se ha analizado el efecto de la frecuencia de la práctica de ejercicio en gente mayor. Se demostró que las personas que practican ejercicio 2 y 3 veces por semana tuvieron un efecto positivo en la salud mental y el funcionamiento social de los ancianos que residen en centros de cuidados. A pesar de ello, se vio que la realización de ejercicio 3 veces semanales fue más eficaz no sólo mejorando la salud mental, si no que presenta beneficios adicionales en la vitalidad. Esto podría ayudar a que las personas mayores pueden preservar su independencia y llevar a cabo actividades de la vida diaria de manera segura y efectiva (Rugbeer y cols., 2017).

El beneficio cognitivo asociado al ejercicio físico se puede explicar por muchos mecanismos tales como aumento de la perfusión cerebral con estímulo de angiogénesis, aumento de la neurogénesis (Snowden y cols., 2011), y la plasticidad sináptica, con aumento de síntesis del factor vascular de crecimiento endotelial (VEGF) y del BDNF (Carro y cols., 2000). Debido a estos efectos tan beneficiosos, el ejercicio físico se puede enfocar como un tratamiento no farmacológico prometedor para mitigar los efectos deletéreos del envejecimiento en la salud del cerebro (Lazarov y cols., 2010, Lautenschlager y cols., 2008, Dedeyne y cols., 2017).

4.2 Neurogénesis: memoria y aprendizaje

Uno de los hallazgos más importantes de los últimos 50 años ha sido el descubrimiento que determinadas áreas cerebrales fabrican nuevas neuronas a lo largo de la vida, proceso conocido como neurogénesis. El hipocampo es el lugar donde más se da este proceso, donde las células hipocampales en la zona subventricular llegan a madurar, fundamentalmente integrando células neuronales granuladas (Kemperman y cols., 2004, Abrous y cols., 2005). Generalmente, este término hace referencia al proceso de proliferación, migración, supervivencia y diferenciación de nuevas células (Figura 10)

(Fernandes y cols., 2015, Aimone y cols., 2015). La reciente investigación sobre los mecanismos que controlan la progresión de las neuronas a estos estados de madurez, además del desarrollo electrofisiológico de las células en cada estado, indica tanto la importancia de las propiedades intrínsecas de las células como el microambiente de la zona subventricular (Castro-García y cols., 2015).Varias líneas de investigación sugieren que la neurogénesis hipocampal adulta es importante en desórdenes psiquiátricos y neurológicos, como la adicción, depresión, epilepsia y esquizofrenia. El hipocampo participa en la regulación de la memoria y el estado de humor de las personas y puede tener una mayor influencia sobre las vías cerebrales (Totterdell y Smith, 1989, Floresco y cols, 2001). La estructura y la función del hipocampo estás desreguladas en el cerebro de pacientes con esquizofrenia, adicción o epilepsia (Sapolsky, 2000, Geuze y cols., 2005, Lucassen y cols., 2006, Keller y Roberts, 2008). En modelos animales con estos desórdenes donde la neurogénesis adulta se ve alterada, se ha visto que con las terapias apropiadas a menudo se normalizan estos cambios (Chen y cols., 2000, Abrous y cols., 2005, Pittenger y Duman, 2008).

Una de las preguntas más frecuentes en la investigación de la neurogénesis hipocampal es, si la producción de nuevas neuronas en el giro dentado podría ser relevante en el aprendizaje espacial asociado al hipocampo. La posible implicación de la neurogénesis hipocampal en el aprendizaje podría explicarse, considerando que la neurogénesis es estimulada por el aprendizaje (Gould y cols., 2001, Fabel y cols., 2009). Estudios previos han demostrado que el aprendizaje espacial, el ambiente enriquecido y el ejercicio físico voluntario incrementan las tasas de neurogénesis en el giro dentado (Kempermann., 2015, Zhao y cols., 2008, Clellenad y cols., 2009, Speisman y cols., 2013). Esto está asociado al aumento en el rendimiento cognitivo, probablemente a través de la incorporación de las nuevas neuronas a las redes neurales del hipocampo. El aprendizaje espacial dependiente de hipocampo es uno de los principales reguladores de la neurogénesis hipocampal. Específicamente, la neurogénesis en el giro dentado se incrementa por el aprendizaje de tareas dependientes de hipocampo (Merritt y cols., 2015, Deng y Gage, 2015). Por contrario, el aprendizaje no dependiente

del hipocampo no favorece la neurogénesis en el giro dentado. Se ha reportado que el aprendizaje *per se*, y no el entrenamiento, es el factor que induce la activación y la regulación de la neurogénesis hipocampal (Opendak y Gould, 2015).

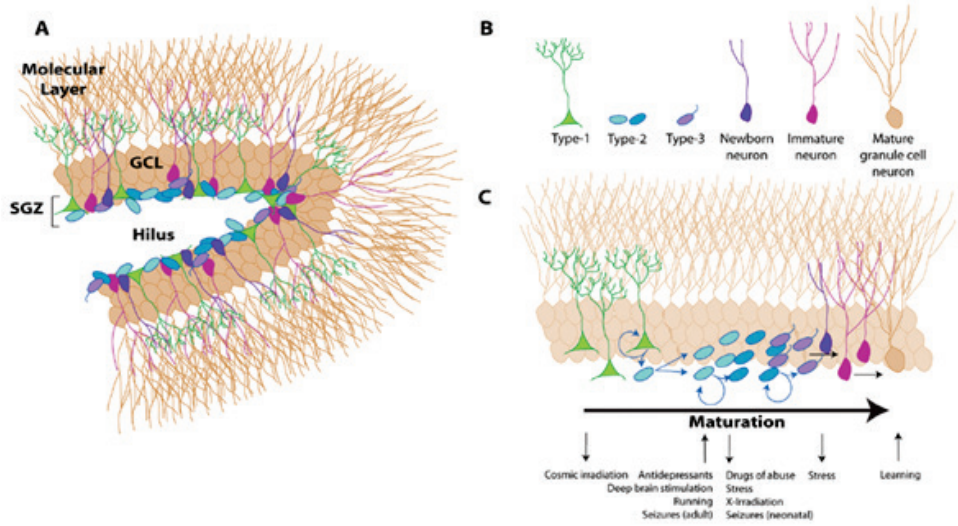

Figura 10. Esquema de los estados de la neurogénesis hipocampal adulta*(Adaptado de Eisch y cols, 2008).*

5. Estimulación Magnética Transcraneal

5.1 Estimulación Magnética Transcraneal: nueva terapia

Actualmente, los tratamientos farmacológicos para el tratamiento de las demencias como la EA, tienen efectividad limitada, son caros y, en ocasiones, pueden inducir efectos secundarios importantes (Reeve y cols., 2017), más si cabe teniendo en cuenta que gran parte de estos pacientes están medicados (Parameswaran y cols., 2016). En este sentido las nuevas estrategias terapéuticas, como la estimulación magnética, están siendo útiles como tratamientos complementarios (Vahabzadeh-Hagh y cols., 2011). Así, el desarrollo de nuevos métodos no invasivos de estimulación cerebral ha incrementado el interés en técnicas neuromoduladoras como herramientas terapéuticas para rehabilitación cognitiva en EA (Nardone y cols., 2012). Uno de estos métodos es la Estimulación Magnética Transcraneal (TMS). La TMS es una técnica que mediante la aplicación de pulsos magnéticos es capaz de inducir cambios en la corriente eléctrica, excitando o inhibiendo la corteza

cerebral, y afectando su actividad (Hallet, 2007), por lo que ha sido utilizada para reproducir varios procesos neurofisiológicos incluyendo excitabilidad, inhibición o plasticidad sináptica/cerebral (Zafiris y cols., 2012). En individuos adultos, la TMS induce una mejoría transitoria en la memoria asociativa, y modifica positivamente el lenguaje de pacientes con EA (Nardone y cols., 2012).

La TMS actúa mediante la generación de potentes y breves pulsos magnéticos que penetran a través de los huesos del cráneo con sólo una leve atenuación por parte de éstos (Barker y cols., 1987). El sistema nervioso reproduce con despolarizaciones neuronales a los impulsos magnéticos inducidos mediante la bobina de la TMS colocada sobre el área del cerebro que se desea estimular. Los cambios inducidos en el sistema nervioso por parte de la TMS pueden verse reflejados tras la estimulación del área motora primaria o sobre la raíz espinal cervical en forma de potenciales motores evocados (Bartres-Faz y cols., 2000).

El circuito básico de un estimulador magnético incluye un condensador y su circuito de carga, y un circuito de descarga que utiliza un interruptor electrónico, capaz de hacer fluir miles de amperios en milisegundos a través de una bobina de estimulación (Pascual-Leone y cols., 2001). Este circuito básico puede modificarse para producir pulsos repetitivos de TMS. La corriente necesaria para generar un campo magnético de intensidad suficiente como para estimular la corteza cerebral es aproximadamente 7-10 kA. Esta corriente se aplica en un pulso muy breve a través de la bobina. Se transfieren aproximadamente 500 J a la bobina en menos de 100 μs. El pulso puede ser monofásico o polifásico, lo que determina ciertas propiedades biológicas del estímulo. La variación en el tiempo es alta, ya que ésta determina la magnitud del campo magnético y de la corriente secundaria inducida. El campo magnético pasa de 0 a 2,5 T en aproximadamente 50 μs. El campo inducido interactúa con el tejido y, por lo tanto, hay que tener en cuenta las corrientes de inducción generadas directamente por la corriente que fluye por la bobina y corrientes de condensación generadas por la acumulación de carga en toda la interfase de tejidos de resistencia y conductividad diferentes (Wagner y cols., 2004, Wagner y cols., 2007). La despolarización de neuronas y la generación de un potencial

de acción dependen de la diferencia de potencial a través de la membrana axonal o dentrítica. La probabilidad de que un campo inducido active una neurona es una función de la derivada espacial del campo a lo largo de la membrana neuronal. La distinta orientación de las neuronas en la corteza cerebral y sus axones impide una traslación sencilla de las observaciones en conductores homogéneos al volumen de tejido nervioso afectado por la TMS en un cerebro(Pascual-Leone y Tormos-Muñoz, 2008). Así, el conocimiento de la anatomía de las áreas corticales estimuladas es crítico para una correcta interpretación de los efectos de la TMS.

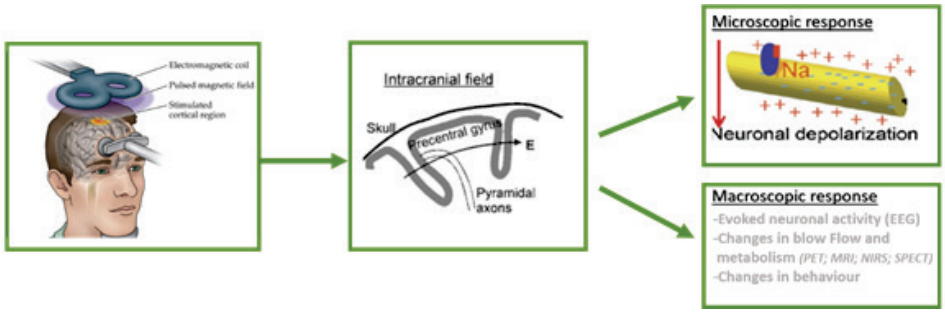

Figura 11. Representación gráfica de la aplicación de TMS en humanos y su respuesta microscópica y macroscópica.

5.2 Aplicaciones en neurología

La accesibilidad al córtex motor primario por parte de las bobinas utilizadas en la TMS, ha propiciado que el primer y más utilizado enfoque de esta herramienta sea en el sistema motor. El estudio de las distintas funciones superiores involucra una mayor viabilidad de regiones relevantes de la corteza cerebral y de los núcleos subcorticales. Los pulsos magnéticos inducidos por la TMS penetran una profundidad de 2-3 cm en el cerebro, afectando a las capas internas de la corteza cerebral o a las zonas más extensas de la sustancia blanca (Bartres-Faz y cols., 2000). La aplicación de TMS para el estudio de la neurociencia cognitiva podría no ser apropiada por la no afectación directa de zonas tan importantes como el hipocampo o los ganglios basales, lejos del alcance de los pulsos magnéticos. Pero las bases neurofisiológicas a través de las cuales la TMS es capaz de modular aspectos cognitivos, están empezando a entenderse por la aplicación simultánea de TMS junto con métodos de neuroimagen funcional. Gracias a, la posibilidad de combinar ambas técnicas se ha visto que la aplicación de TMS sobre el córtex prefrontal dorsolateral induce un cambio en la señal dependiente del nivel de oxígeno, recogida por

resonancia magnética funcional en esta estructura y en el núcleo caudado (Keenan y cols., 1999, Nakano, 2017).

5.3 Estimulación Magnética Transcraneal: memoria y aprendizaje

La aplicación de TMS como tratamiento en los déficits cognitivos es una diana terapéutica prioritaria en las enfermedades mentales (Estrada y cols., 2015). Bridgers y Delaney estudiaron los efectos de la TMS sobre la memoria declarativa en una muestra de 30 sujetos normales. Se les administraron pulsos simples de TMS a distintas intensidades sobre zonas frontocentrales de ambos hemisferios, tras la ejecución de diversas pruebas neuropsicológicas. Después de la sesión de TMS se midieron de nuevo los mismos parámetros conductuales, y los resultados mostraron que la TMS produjo una mejoría significativa en la prueba de pares verbales asociados de la escala de memoria de Wechsler(Bridgers y Delaney, 1989). El diseño de este estudio no permite descartar que las diferencias encontradas se debieran a un efecto re-test puesto que a pesar de utilizar listas distintas aparentemente equivalentes antes y después de la TMS, los autores no las contrabalancearon en distintos ensayos (Bridgers y Delaney, 1989). Otros estudios clínicos demuestran que la TMS podría conducir a la preservación e incluso la mejora de las funciones cognitivas al menos durante el tiempo de tratamiento en personas con EA (Avirame y cols., 2016). Igualmente, se ha probado los efectos en el rendimiento cognitivo del tratamiento con TMS tras realizar una privación de sueño. Se ha demostrado que la aplicación de TMS tanto en humanos (Luber y cols, 2013, Martínez-Cancino y cols., 2016) como en animales (Estrada y cols., 2015, Estrada y cols., 2015) sometidos a privación de sueño previene el impacto que ésta causa en la memoria. En la línea de nuestras observaciones, otros grupos han descrito que la aplicación de TMS en ratones añosos, tras un tratamiento con 4 sesiones diarias de TMS durante 14 días consecutivos,una mejoría en el rendimiento cognitivo sin verse influenciada la habilidad motora y visual (Zhang y cols., 2015)

Cada vez son más las evidencias de que la TMS es capaz de ejercer cierta acción sobre la autoinmunidad y las células inmunes. El tratamiento con TMS contribuye a una activación general de macrófagos, dando como resultado cambios de autoinmunidad y varias reacciones inmunológicas, tales como aumento de la actividad fagocítica o de la producción de quimiocinas (Onodera y cols., 2003). La TMS ejerce un efecto beneficioso sobre la función cerebral y puede deberse a un efecto neuroprotector sobre el daño celular oxidativo, ya que la TMS podría contrarrestar las respuestas pro-inflamatorias presentes en los trastornos neurodegenerativos, reduciendo su aparición (Guerriero y Ricevuti, 2016). Además, su utilización resulta terapéutica en enfermedades neuropsiquiátricas ligadas a alteraciones de la excitabilidad (Rossini y Rossi, 2007).Igualmente, ha sido utilizada para el tratamiento de la depresión, y para evaluar sus efectos entre la EA, Enfermedad de Parkinson (EP) o dolor crónico (Martinez-Cancino y cols., 2016).

6. Octodon degus

6.1 Generalidades

El *Octodon degus (O. degus)* es una especie de roedor nativo de América del Sur, que representa un modelo único para estudiar rasgos fisiológicos y comportamentales, incluyendo las habilidades cognitivas y sensoriales. Viven en colonias con una organización social bien estructurada en grupos de 5-10 jóvenes y 2-5 individuos adultos, compartiendo un sistema de madrigueras. Presentan un patrón de actividad circadiana diurna-crepuscular dependiente de la temperatura (Vivanco y cols., 2010).

En libertad, suelen vivir poco más de 2 años debido mayoritariamente a la depredación. Sin embargo, en cautividad se reproducen y viven entre 5 y 7 años, y muestran rasgos distintivos de enfermedades neurodegenerativas, la diabetes y el cáncer (Ardiles y cols., 2013). Las hembras se reproducen durante 4-5 años, mientras que los machos son fértiles toda su vida. Los individuos de *O. degus* criados en cautividad requieren una serie de cuidados para mantenerse sanos, evitar las enfermedades diarreicas y alcanzan la madurez de al menos el 90% de la prole nacida en cautividad (Palacios y cols., 2013).

El área de expansión de los *O. degus* está fuertemente influenciada por la disponibilidad y la calidad del alimento. Durante la primavera austral (septiembre-octubre), la disponibilidad de alimentos de gran calidad favorece la generación de descendencia. Por el contrario, disminuye durante el verano austral (enero-marzo), cuando la comida es escasa y de baja calidad, favoreciendo el aumento del área de expansión de la población de *O. degus* hacia lugares con mayor disponibilidad de alimento (Quirici y cols., 2010).

6.2 Locomoción

Las secuencias diagonales y laterales en la marcha de los *O. degus* les permiten contrarrestar algunos niveles de inestabilidad locomotora en terrenos inclinados. La marcha al trote con secuencias laterales ocurre a lo largo de todo el rango de velocidades, mientras que los patrones de marcha con secuencias diagonales se producen a velocidades más altas. Además, los cambios en los parámetros de las extremidades anteriores (más que los cambios en las extremidades posteriores) parecen desempeñar un papel importante en la selección del tipo de marcha de estos roedores (Schmidt y cols., 2014).

6.3 Dentición

Los *O. degus* nacen con los incisivos permanentes completamente erupcionados y en oclusión funcional. Los dientes premolares aparecen en los días 2-3, mientras que todos los primeros molares surgen a los 4-5 días de vida. Asimismo, se ha demostrado que los *O. degus* son un buen modelo animal experimental para el estudio de las alteraciones en el desarrollo de la dentición humana. El desarrollo del esmalte y la dentina se ve alterado bajo una dieta alta en fósforo y una ratio inadecuada de calcio-fósforo, causando despigmentación, hipoplasia y picaduras en el esmalte, así como alteraciones en la morfología de la dentina (Jekl y cols., 2011). Por este motivo, se debe prestar especial atención al contenido de fósforo en la dieta cuando aparecen alteraciones en la estructura dental de pacientes jóvenes con una dentición decidual o permanente (Richardson, 2003).

6.4 Sistemas

6.4.1 Sistema digestivo

La anatomía digestiva del *O. degus* puede presentar distintas variantes, en especial la posición del ciego y el colon ascendente (González y cols., 1997). El ciego se ubica en el lado izquierdo, en dirección craneal o caudal, pudiendo extenderse sobre el nivel medio hacia el lado derecho o permanecer con la cabeza y el cuerpo en la derecha, mientras que el vértice aparece en el lado izquierdo frente a la entrada de la pelvis. Por su parte, el colon ascendente se dispone en dos pliegues superpuestos, a menudo en espiral, que pueden diferir en cuanto a forma y extensión dependiendo de la posición del ciego. Se ha descrito además una amplia gama de variaciones interespecíficas en la vascularización arterial de las regiones abdominal y pélvica de distintos roedores. En concreto, se han encontrado diferencias entre los *Histricognatos* (p.ej. *O. degus*, conejillo de Indias) y los *Sciurognatos* en la disposición del tronco cólico-mesentérico, así como de las arterias abdominal craneal, mesentérica caudal y la ilíaca circunfleja profunda (Ventura y cols., 1996).

6.4.2 Sistema visual

La lente del O*. degus* contiene azúcares como el heptitol y el octitol, presentes también en las lentes humanas (Barker y cols., 1983). Además de la D-glucosa, la D-fructosa, el sorbitol y el mioinositol, otros monosacáridos que forman parte de la lente del *O. degus* son glicerol, eritriol, treitol, ribitol, xilitol y manitol (Tomana y cols., 1984). Es por esto, que lo convierte en un modelo adecuado para estudiar enfermedades oculares, como las cataratas o aquellas asociadas a la edad (Szabadfi y cols., 2015). Los roedores son sensibles a la radiación ultravioleta(UV), por lo que las marcas territoriales de orina (al igual que las feromonas) son señales visuales que podrían haber favorecido la evolución de los conos-UV (Chávez y cols., 2003).Como roedor diurno, el *O. degus* presenta una serie de adaptaciones visuales a la luz (Jacobs y cols., 2003). La lente del ojo del *O. degus* absorbe selectivamente la luz de onda corta y experimenta un aumento progresivo en la densidad óptica en función de la edad. La retina de estos roedores tiene unos 9 millones de fotorreceptores, que pueden ser de tres tipos: bastones con un pico de sensibilidad alrededor

de los 500 nm y dos clases de conos, con picos a los 362 nm y los 507 nm respectivamente. Un tercio de los fotorreceptores son bastones, mientras que el resto corresponde a los dos tipos de conos (507 nm/362 nm), en una proporción 13:1. Para efectuar la transición completa de la visión con bastones a la visión con conos se requieren niveles relativamente altos de adaptación a la luz. Además, los *O. degus* son capaces de hacer discriminaciones de color entre la luz ultravioleta y la luz visible.

6.4.3 Sistema endocrino

El *O. degus* tiene un interés especial en el campo de la endocrinología, ya que desarrolla *diabetes mellitus* espontáneamente y presenta amiloidosis de los islotes pancreáticos. Además, parece ser que podría existir alguna forma de presión evolutiva positiva en las hormonas del metabolismo de los carbohidratos en los roedores histricomorfos. Esto queda reflejado en la similitud que hay en la secuencia de la amilina o polipéptido amiloide de los islotes (IAPP) del *O. degus* y la forma no amiloide del IAPP del conejillo de Indias. En cambio, la insulina y la región C-terminal del glucagón del *O. degus* es muy diferente a las de otros mamíferos, pero no del conejillo de Indias (Nishi y cols., 1990). El principal glucocorticoide en estos animales es el cortisol, cuyos niveles más elevados en ambos sexos se producen en primavera, coincidiendo con el periodo de lactancia de las hembras, momento en el que la movilización de energía, la producción y la masa corporal alcanzan su valor máximo. El cortisol disminuye en verano y alcanza el valor medio más bajo durante la temporada de apareamiento. En el caso de los machos, esta disminución del cortisol podría deberse al efecto supresor de la testosterona que, pese a mantenerse en niveles bajos todo el año, alcanza su máximo valor (0.16 ng/ml) en la temporada de apareamiento (Kenagy y cols., 1999).

Los niveles circulantes de hormonas del estrés experimentan variaciones estacionales, adaptándose a las distintas necesidades en cada etapa de la vida. Los niveles de cortisol inducidos por el estrés son más elevados durante los periodos con mayor riesgo de exposición al estresor o en momentos de

balance energético positivo, siendo siempre superiores en las hembras en comparación con los machos (Bauer y cols., 2014).

6.4.4 Sistema nervioso

El *O. degus* es un tipo de roedor histricomorfo cada vez más utilizado en el campo de la neurociencia debido a que presenta comportamientos más complejos que las ratas y ratones, tales como conductas sociales complejas, comunicaciones vocales, y uso de herramientas con gran destreza manual. Cada uno de estos comportamientos complejos correlaciona con regiones cerebrales específicas (Kumazawa y cols., 2013). De acuerdo a los estudios neuroanatómicos realizados en el *O. degus*, aparentemente no existe un dimorfismo sexual en cuanto al tamaño cerebral se refiere (Wright y Kern, 1992). Además, el prosencéfalo y el mesencéfalo del *O. degus* son generalmente más compactos que las regiones correspondientes del cerebro en la rata (suborden *Myomorpha*) y el conejillo de Indias. El complejo amigdalino se extiende más hacia delante en el telencéfalo. Asimismo, los principales núcleos del mesencéfalo y los tractos de fibras ocupan una posición más rostral. Sin embargo, los colículos superior e inferior son mucho más largos en el *O. degus* que en la rata.

6.5 *Octodon degus* como modelo de deterioro cognitivo

El *O.degus* es un modelo valioso para muchas enfermedades diferentes, entre ellas las relacionadas con la neurodegeneración, como ya señalamos en la revisión realizada por nuestro grupo (Tarragon y cols., 2013)puesto que este animal desarrolla de manera espontánea varios síntomas que se pueden vincular a un número de condiciones patológicas similares a estas dolencias. Frecuentemente, también se ha utilizado en estudios circadianos por su ciclo diurno (Vivanco y cols., 2010). Además el *O.degus*es un animal muy sociable, lo que explica su papel en la investigación social y neuroafectiva para trastornos como enfermedades neurodegenerativas, metabólicas, oftalmológicas, entre otras (Figura 12) (Tarragon y cols., 2013, Colonnello y cols., 2010). El *O. degus* vive una media de hasta 7 años en cautiverio (Lee,

2004), por lo que lo hace un modelo interesante para su uso en estudios relacionados en el campo de la neurobiología del envejecimiento.

El deterioro cognitivo asociado a la edad, así como el declive en la memoria es el principal aspecto que cualquier modelo debe ser capaz de reproducir para ser válido su uso en la investigación. En este sentido, Ardiles y colaboradores demostraron en 2012 que con la edad, se produce una acumulación de β-amiloide y proteína Tau que se correlaciona con el deterioro cognitivo en la memoria de reconocimiento espacial y la de objeto, así como en la disfunción de la plasticidad sináptica y neural en el *O. degus* (Ardiles y cols., 2012).

El deterioro de la memoria es la primera manifestación de los síntomas de las enfermedades neurodegenerativas, y uno de los requisitos que cumple el *O. degus* es la capacidad de discriminar en diferentes tests cognitivos y los diferentes tipos de memoria clásicamente afectadas en este tipo de enfermedades (Popovic y cols., 2010, Tarragon y cols., 2014, Estrada y cols., 2015).

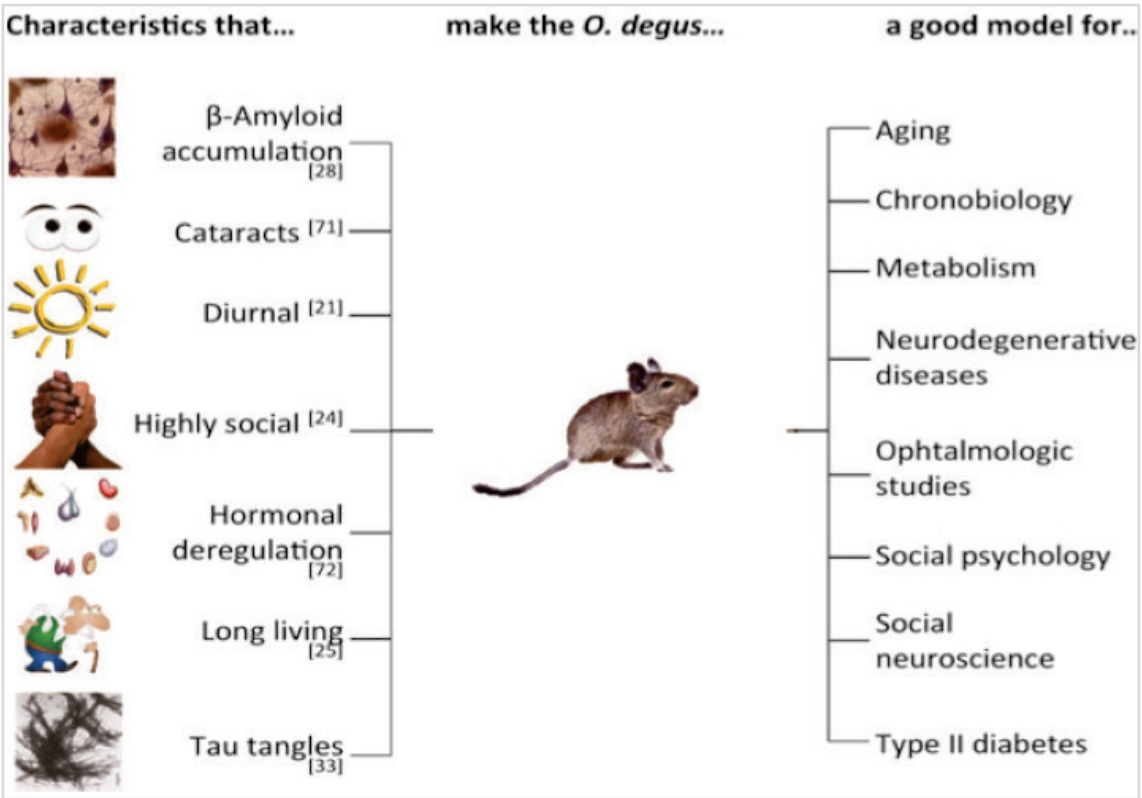

Figura 12. *Octodon degus* como modelo para estudio de enfermedades neurodegenerativas y deterioro cognitivo asociado a la edad. *(Figura de artículo de revisión, Tarragon y cols., 2013)*

II. Hipótesis y objetivos

II. Hipótesis y objetivos

La principal hipótesis sobre la que edificamos este trabajo de tesis, fue la de comprobar la idoneidad del *O. degus* como modelo para el estudio del envejecimiento y enfermedades asociadas, como son las neurodegenerativas, y más en concreto la EA. Por este motivo, en la mayoría de los estudios quisimos ver los efectos deletéreos de la privación de sueño, y por otro lado el efecto restaurador de la TMS pero a dos edades distintas, para evaluar el efecto de la edad.

Los objetivos específicos de este trabajo de tesis los enumeramos:

1.- Evaluar el posible papel terapéutico de la TMS en la función cognitiva de *Octodon degus* jóvenes, tras la privación de sueño, mediante la evaluación de la memoria de trabajo y espacial con los test conductuales de Radial Arm Maze, Barnes Maze y Novel Object Recognition.

2.- Una vez comprobada la idoneidad de esta herramienta en este modelo, se estudió la aplicación de TMS en *Octodon degus* de distintas edades: tras la privación de sueño, analizando la memoria de trabajo y espacial con los paradigmas conductuales anteriormente mencionados.

3.- Explorar si la acentuación del deterioro cognitivo asociado a la edad y los niveles de cortisol, como marcador de estrés, pueden mejorar con la modulación de factores medioambientales como es la práctica de ejercicio físico voluntario.

4.- Estudiar el efecto de la memantina, como fármaco empleado en la EA, para nuestro modelo animal de déficit cognitivo inducido por privación de sueño.

5.- Estudiar la posible diferencia en la actividad enzimática tanto de AChE como de BuChE asociada a la edad y a la práctica de actividad física voluntaria, en extractos de hipocampo de *Octodon degus*.

III. Compendio de artículos

III.Compendio de artículos

Esta tesis doctoral es un compendio de trabajos publicados. Los artículos que constituyen el cuerpo de esta tesis son:

III.I Artículos originales:

III.I.I "Cognitive Impairment After Sleep Deprivation Rescued by Transcranial Magnetic Stimulation Application in *Octodon degus*". *Neurotoxicicty Research*. **2015** 28(4): 361-371. PMID: 26194615.**Estrada C**, López D, Conesa A, Fernández-Gómez FJ, Gonzalez-Cuello A, Toledo F, Tunez I, Blin O, Bordet R, Richardson JC, Fernandez-Villalba E, Herrero MT.

III.I.II "Transcranial magnetic stimulation and aging: Effects on spatial learning and memory after sleep deprivation in *Octodon degus*". *Neurobiology of Learning Memory*. **2015** 125: 274-281. PMID: 26463507.**Estrada C**, Fernández-Gómez FJ, López D, Gonzalez-Cuello A, Tunez I, Toledo F, Blin O, Bordet R, Richardson JC, Fernandez-Villalba E, Herrero MT.

III.I.III"Transcranial Magnetic Stimulation on Rodent Models".*CNS & Neurological Disorders Drug targets*. **2016** 15(7):756-764. PMID: 27063016.**Estrada C**, Tarragon E, Kelley JB, Lopez D, Gil-Martínez AL, Villalba EF, Herrero MT.

III.I.IV Lim CK, Fernández-Gomez FJ, Braidy N, **Estrada C**, Costa C, Costa S, Bessede A, Fernandez-Villalba E, Zinger A, Herrero MT, Guillemin GJ. "Involvement of the kynurenine pathway in the pathogenesis of Parkinson's disease".*Progress in Neurobiology*. **2016**. [Epub ahead of print]. PMID: 27072742.

Artículos originales:

III.I.I**Estrada C**, López D, Conesa A, Fernández-Gómez FJ, Gonzalez-Cuello A, Toledo F, Tunez I, Blin O, Bordet R, Richardson JC, Fernandez-Villalba E, Herrero MT."Cognitive Impairment After Sleep Deprivation Rescued by Transcranial Magnetic Stimulation Application in *Octodon degus*". *Neurotoxicicty Research*. **2015** 28(4): 361-371.
PMID: 26194615. DOI: 10.1007/s12640-015-9544-x

URL: http://www.neurotherapeuticsgateway.net/ArticlePage.aspx?DOI=10.1007/s12640-015-9544-x
Factor de Impacto = 3.14 (1º quartil)

Abstract

Sleep is indispensable for maintaining regular daily life activities and is of fundamental physiological importance for cognitive performance. Sleep deprivation (SD) may affect learning capacity and the ability to form new memories, particularly with regard to hippocampus-dependent tasks. Transcranial magnetic stimulation (TMS) is a non-invasive procedure of electromagnetic induction that generates electric currents, activating nearby nerve cells in the stimulated cortical area. Several studies have looked into the potential therapeutic use of TMS. The present study was designed to evaluate how TMS could improve learning and memory functions following SD in *Octodon degus*. Thirty juvenile (18 months old) females were divided into three groups (control, acute, and chronic TMS treatment-with and without SD). TMS-treated groups were placed in plastic cylindrical cages designed to keep them immobile, while receiving head magnetic stimulation. SD was achieved by gently handling the animals to keep them awake during the night. Behavioral tests included radial arm maze (RAM), Barnes maze (BM), and novel object recognition. When TMS treatment was applied over several days, there was significant improvement of cognitive performance after SD, with no side effects. A single TMS session reduced the number of errors for the RAM test and improved latency and reduced errors for the BM test, which both evaluate spatial memory. Moreover, chronic TMS treatment brings about a significant improvement in both spatial and working memories.

III.I.II**Estrada C**, Fernández-Gómez FJ, López D, Gonzalez-Cuello A, Tunez I, Toledo F, Blin O, Bordet R, Richardson JC, Fernandez-Villalba E, Herrero MT.“Transcranial magnetic stimulation and aging: Effects on spatial learning and memory after sleep deprivation in *Octodon degus*”.*Neurobiology of Learning Memory*. **2015** 125: 274-281.

PMID: 26463507. DOI: 10.1016/j.nlm.2015.09.011.
URL: http://www.sciencedirect.com/science/article/pii/S107474271500180X
Factor de Impacto = 3.43 (1º quartil)

Abstract

The benefits of neuromodulatory procedures as a possible therapeutic application for cognitive rehabilitation have increased with the progress made in non-invasive modes of brain stimulation in aged-related disorders. Transcranial magnetic stimulation (TMS) is a non-invasive method used to examine multiple facets of the human brain and to ameliorate the impairment in cognition caused by Alzheimer’s disease (AD). The present study was designed to evaluate how a chronic TMS treatment could improve learning and memory functions after sleep deprivation (SD) in old *Octodon degus*. SD was executed by gently handling to keep the animals awake throughout the night. Thirty young and twenty-four old O. degus females were divided in six groups (control, acute and chronic TMS treatment). Behavioral tests included; Radial Arm Maze (RAM), Barnes Maze (BM) and Novel Object Recognition (NOR). Although learning and memory functions improved in young animals with only one session of TMS treatment, a significant improvement in cognitive performance was seen in old animals after 4 and 7days of TMS, depending on the task that was performed. No side effects were observed following, which showed therapeutic potential for improving age-related cognitive performance.

III.I.III**Estrada C**, Tarragon E, Kelley JB, Lopez D, Gil-Martínez AL, Villalba EF, Herrero MT."Transcranial Magnetic Stimulation on Rodent Models".*CNS & Neurological Disorders Drug targets*. **2016** 15(7):756-764.
PMID: 27063016. DOI: 10.2174/1871527315666160518125341

URL: http://www.eurekaselect.com/142305
Factor de Impacto = 2.5 (1º quartil)

Abstract

Transcranial magnetic stimulation (TMS) is a non-invasive method that can be used as an interventional technique to investigate causality in the brain-behavior relationship, through depolarization or hyperpolarization in the neurons of the brain. Different techniques of TMS can be used to investigate causality in the brain-behavior relationship. The behavioral effects induced by TMS are complex since it has been shown that the performance in the same cognitive task can be either facilitated or inhibited depending on the area being stimulated. To date, most studies involving TMS are focused mainly on the facilitation properties of this technique. It is used to treat a wide-range of neurological and psychiatric conditions such as depression, ischemia, Alzheimer's disease, or motor impairment. Interestingly, TMS can be used to induce a virtual lesion that could provide a valuable and much needed model of cognitive impairments in early preclinical development. This review describes the existing TMS paradigms in rodents and the major challenges that were encountered by the researchers as the method was translated from clinical to preclinical applications. In summary, the existing knowledge gained in animal research emphasizes the necessity to investigate new TMS paradigms in the preclinical setting and its effects.

III.I.IVLim CK, Fernández-Gomez FJ, Braidy N, **Estrada C**, Costa C, Costa S, Bessede A, Fernandez-Villalba E, Zinger A, Herrero MT, Guillemin GJ. "Involvement of the kynurenine pathway in the pathogenesis of Parkinson's disease".*Progress in Neurobiology*. **2017**. [Epub ahead of print].
PMID: 27072742. DOI: 10.1016/j.pneurobio.2015.12.009

URL: http://www.sciencedirect.com/science/article/pii/S0301008215300551
Factor de Impacto = 13.2 (1º quartil)

Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by loss of dopaminergic neurons and localized neuroinflammation occurring in the midbrain several years before the actual onset of symptoms. Neuroinflammation leads to microglia activation and release of a large number of proinflammatory mediators. The kynurenine pathway (KP) of tryptophan catabolism is one of the major regulators of the immune response and is also likely to be implicated in the inflammatory and neurotoxic events in Parkinsonism. Several neuroactive compounds are produced through the KP that can be either a neurotoxic, neuroprotective or immunomodulator. Among these metabolites kynurenic acid (KYNA), produced by astrocytes, is considered as neuroprotective whereas quinolinic acid (QUIN), released by activated microglia, can activate the N-methyl-d-aspartate (NMDA) receptor-signalling pathway, leading to excitotoxicity and amplify the inflammatory response. Previous studies have shown that NMDA antagonists can ease symptoms and exert a neuroprotective effect in PD both in vivo and in vitro. There are to date several lines of evidence linking some of the KP intermediates and the neuropathogenesis of PD. Moreover, it is likely that some of the KP metabolites could be used as prognostic biomarkers and that pharmacological modulators of the KP enzymes could represent a new therapeutic strategy for PD.

IV.Discusión

IV.Discusión

Este trabajo examina el impacto del tratamiento de TMS en la recuperación de la memoria espacial y de trabajo después del deterioro cognitivo transitorio provocado por la privación de sueño en *O. degus* de dos edades: jóvenes y añosos. Además, explora el efecto de la actividad física voluntaria sobre los niveles de cortisol, los dominios cognitivos tanto en un estado de sueño normal como en condiciones de privación de sueño, y las diferencias en la actividad enzimática de AChE y BuChE considerando su papel en la EA y otras demencias (Ramsay y cols., 2016).

Modelos conductuales

Se han utilizado multitud de paradigmas validados a lo largo de los años con el fin de evaluar la memoria y el aprendizaje en los distintos modelos animales usados en investigación (Sharma y cols., 2010). Uno de los más utilizados es el RAM, descrito por Olton y Samuelson en 1976 (Olton y Samuelton, 1976). A lo largo de los años, ha sido versionado con el objetivo de ir mejorando algunos aspectos de la versión inicial, o modificado para aumentar la validez del laberinto y ser más específico en la evaluación de distintos tipos de memoria. En 1983, Jarrard diseñó una variante que permitía evaluar tanto la referencia espacial como la memoria de trabajo, modelo que ha sido utilizado hasta hoy en día para la realización de nuestros estudios (Jarrard, 1983). Al reforzar cuatro de los ocho brazos del laberinto con un pellet de comida como recompensa, nos permite analizar la memoria de trabajo (al repetir una visita a un brazo durante una sesión) y la memoria de referencia (al visitar un brazo no reforzado). Otro de los test utilizados en estos estudios es el BM. Se trata de un paradigma de exploración de la memoria de referencia espacial (Seeger y cols., 2004) muy utilizado en la investigación básica de situaciones que reproducen deterioros cognitivos en modelos animales (Stewart y cols., 2011). Además, cabe destacar que ha sido uno de los pocos paradigmas validado en este modelo para la evaluación de la memoria de referencia y de trabajo (Popovic y cols., 2010). La inclusión de paradigmas como NOR nos aporta más información relacionada con la evaluación de la memoria espacial basada en el comportamiento espontáneo de los animales ante una novedad, es decir, no

está basada en el reforzamiento como puede ser encontrar una vía de escape o un pellet de comida, sino en la tendencia natural de estos roedores por explorar entornos novedosos (Ennaceur y Delacour, 1988).

Estos tests nos permiten conocer el papel del hipocampo, no sólo en la memoria de trabajo y espacial, sino que también en la memoria de reconocimiento (Barker y Warburton, 2011), y el papel de la corteza perirrinal en el reconocimiento de objetos puesto que tiene un papel importante en el reconocimiento visual de los objetos complejos (Nardone y cols., 2012). De hecho, se ha demostradoque en ratas con lesiones hipocampales, tenían intacto el reconocimiento de objeto (Mumby y cols., 2002).

Privación de sueño como inductor de deterioro cognitivo

Muchos estudios han confirmado el deterioro cognitivo que provoca la privación de sueño en varios modelos animales, implicando un efecto negativo en la capacidad para retener información y perturbando la consolidación de la memoria (Meerlo y cols., 2009, Prince y Abel, 2013). De hecho, la privación de sueño ya ha sido probada con anterioridad en nuestro modelo (Kas y Edgar, 1999), y se ha demostrado el deterioro cognitivo transitorio que provoca (Tarragon y cols., 2013). A lo largo de los estudios que hemos llevado a cabo, se ha privado de sueño a *O. degus* jóvenes y añosos mediante *gente-handling*, con el fin de provocar el deterioro cognitivo transitorio que este paradigma provoca. De esta manera, hemos podido evaluar el efecto de los distintos tratamientos con TMS como nueva terapia ante posibles alteraciones cognitivas (Puntos III.I.I, III.I.II). Relacionando sueño y cognición, los resultados obtenidos con la aplicación de una única sesión de TMS en animales jóvenes confirmaron que los grupos de sueño normal realizaron mejor las tareas de memoria espacial que los grupos que fueron sometidos a la privación de sueño (Punto III.I.I, Figura 2,3). Estos resultados son coherentes con otros que demuestran que la privación de sueño en animales tiene efectos adversos en la memoria, independientemente de cualquier otra situación estresante (Zagaar y cols., 2012, Aleisa y cols., 2011, Tarragon y cols., 2013).

La privación de sueño puede provocarse de dos formas diferentes, una de ellas podría ser mediante la inducción de un estado de agitación elevado,

causado por altos niveles de estrés o por la exposición a entornos poco familiares (McDermott y cols., 2006, Kopp y cols., 2006), o bien impidiendo que el animal duerma mediante una estimulación suave (Schwarts y Mong, 2011). Los mecanismos por los cuales este procedimiento es capaz de producir trastornos transitorios en la memoria no se conocen en su totalidad, aunque se han sugerido varias hipótesis (Meerlo y cols., 2009). No se puede obviar que el procedimiento de SD utilizado a lo largo de este trabajo con animales, muestra diferencias con el utilizado en humanos, pero éste presenta un modelo traslacional muy potente, que permite testar mejoras cognitivas en la clínica (Colavito y cols., 2013).

Aplicación de TMS y recuperación del deterioro cognitivo

Se ha demostrado un efecto positivo del tratamiento con TMS en las memoria espacial y aprendizaje evaluado con los test cognitivos (Punto III.I.I, Figuras 2,3; Punto III.I.II, Figuras 1,2,3). Los animales sometidos a la SD tuvieron una mejora cognitiva cuando los grupos eran tratados tanto de forma aguda (una sesión de TMS antes del test conductual) como de forma crónica con sesiones de TMS (dos sesiones diarias de TMS a lo largo de siete días, coincidiendo con los períodos de entrenamiento de los test). La mejoría en el rendimiento cognitivo fue igualmente observada en los grupos de animales con sueño normal que se sometieron al tratamiento con TMS cuando fueron comparados con los animales controles. Estos hallazgos coinciden con los resultados publicados anteriormente tanto en medicina humana (Pascual-Leone y cols., 1998) como en animales(Vázquez-García y cols., 2004). Igualmente, se asemejan con estudios que demuestran las alteraciones neurofisiológicas y comportamentales tras la exposición a estimulaciones extremadamente bajas (ELF-MF) (Capone y cols., 2009).

A pesar de manifestarse un efecto positivo tras el tratamiento con TMS, los mecanismos fisiológicos responsables de los resultados beneficiosos de esta aplicación magnética están pobremente dilucidados. Se ha descrito que después de un tratamiento crónico con ELF-MF, se manifestó un impacto directo sobre la memoria de reconocimiento social y espacial,se demostró que la frecuencia, intensidad, duración y número de pulsos de la exposición parece

jugar un papel importante (He y cols., 2011). Por lo tanto, esta habilidad que presenta la TMS para neutralizar la disfunción cerebral, parece ser una de sus presumibles aplicaciones en la rehabilitación cognitiva (Nardone y cols., 2012). Además, se ha observado que un pulso simple de TMS puede producir diferencias en la respuesta cortical, dependiendo de la activación de la corteza cerebral en el momento de la aplicación del mismo (Silvanto y Pascual-Leone, 2008). Por todo esto, a pesar de las modificaciones fisiológicas que ocurren en el sistema nervioso (SN) durante el tiempo de exposición, sus efectos conllevan a una más efectiva transmisión de las señales neuronales, incrementando la capacidad de mitigar la disfunción cognitiva inducida por la SD (Martinez-Cancino y cols., 2016).

La realización de los tests RAM, BM y NOR en los *O. degus* jóvenes demostró que con la aplicación de una sola sesión de TMS, se manifiesta una mejoría en variables específicas de los paradigmas conductuales utilizados para evaluar la memoria espacial (Punto III.I.I, Figuras 2,3). Cuando los animales jóvenes fueron tratados con siete días de TMS, mostraron un descenso significativo en el tiempo total del test, y en los RME y WME el día del test. Además, el tiempo total que los animales tardaron en realizar el test fue significativamente más bajo que los animales que recibieron una única sesión (Punto III.I.I, Figuras 2,3). Esto confirmaría como se ha publicado anteriormente, que la dosis utilizada es fisiológicamente relevante a nivel terapéutico sin ser nociva (Post y cols., 1999). Es más, en el test NOR, el índice de reconocimiento fue mayor en los animales que recibieron más sesiones que en los que sólo recibieron una (Punto III.I.I, Figura 4). En el análisis de la memoria de trabajo, los resultados obtenidos ponen de manifiesto que unasola sesión de TMS no es suficiente para contrarrestar los efectos de la SD, pero que un tratamiento crónico con TMS mejora el deterioro cognitivo transitorio.

Cada vez se entienden mejor los mecanismos por los cuales la TMS ejerce su efecto en los animales (Wang y cols., 1996, Medina-Fernandez y cols., 2017), además de haber verificado que su aplicación es segura (Russel y cols., 1994, Post y cols., 1999, Schutter, 2011). Es por eso que el tratamiento con TMS es capaz de modular la excitabilidad cortical (Pascual-Leone y cols.,

1998), y representa una técnica que induce corrientes eléctricas en el cerebro, una función que puede ser aplicada para cambiar la actividad cortical, acceder a la excitabilidad de la corteza o interrumpir la actividad cerebral (Cowey, 2005, Silvanto y Pascual-Leone, 2008). Sin embargo, hay algunas discrepancias en cuanto a si la aplicación de TMS puede causar inhibición o activación de la excitabilidad cortical. De hecho, distintos estudios han manifestado que esta técnica mejora los síntomas motores y afectivos en pacientes con depresión y enfermedad de Párkinson (Anderkova y Rektorova, 2014, Lefaucheur y cols., 2014, Kamble y cols., 2014). Algunos de los resultados obtenidos en modelos experimentales de depresión, EPo Enfermedad de Huntington, muestran que el tratamiento con TMS provoca un aumento considerable de los niveles de dopamina en el cerebro (Tasset y cols., 2013, Heumann y cols., 2014).

TMS como terapias para mejorar el rendimiento cognitivo en el envejecimiento

Está ampliamente establecido que el envejecimiento fisiológico va acompañado de una disminución de las capacidades cognitivas y estos problemas se deben a la neurodegeneración(Yang y cols., 2014). Como consecuencia del paso del tiempo, se producen disminuciones en múltiples dominios de las funciones cognitivas, incluyéndose la memoria a corto y largo plazo, la velocidad motora o la atención y función ejecutiva (Lyketsos y cols., 2002). La gran mayoría de los estudios realizados con animales que abordan las consecuencias del envejecimiento sobre la función cerebral, se han centrado en la memoria, y en concreto en la memoria espacial, de la que se sabe que es dependiente del hipocampo (Voineskos y cols., 2013). Las alteraciones en el sueño pueden provocar desórdenes cognitivos, como ya ha sido mencionado, por lo que estudiamos el efecto del tratamiento con TMS aguda y crónica en *O. degus* añosos, tras inducirles el deterioro cognitivo temporal provocado por la SD. Nuestros resultados indicaron que una única sesión de TMS no era suficiente para contrarrestar el efecto negativo en la memoria provocado por la SD, pero el tratamiento crónico si lo fue. Los grupos de animales añosos que fueron tratados con TMS de forma crónica realizaron los test conductuales mejor que los grupos SD sin tratar (Punto III.I.II, Figuras

1, 2, 3, 4). Esto indica que la exposición a TMS tiene un efecto muy positivo en la memoria espacial. En los test de BM y RAM, los grupos añosos realizaron los test en un tiempo total significativamente más bajo, al igual que el número de errores cometido también fue menor (Punto III.I.II, Figuras 1, 2, 3). Estos resultados coinciden tanto con los publicados por Vázquez-García y cols., donde un tratamiento basado en 2 horas diarias de ELF MF durante 9 días mejoraba la memoria de reconocimiento social (Vázquez-García y cols., 2004), como con los de Wang y cols.,(Wang y cols., 2015). Éstos estudiaron el efecto del tratamiento crónico con altas frecuencias de TMS repetitiva (rTMS) en ratones añosos demostrando que los animales no tratados presentaban un deterioro cognitivo y que el tratamiento durante 14 días consecutivos con rTMS mejoraba el rendimiento de los animales evaluados con NOR. Además, también demostraron que las neuronas piramidales CA1 del hipocampo tenían un potencial de membrana significativamente hiperpolarizado y una disminución significativa del número de potenciales de acción después de inducir las corrientes despolarizantes. Estos datos sugieren que la exposición a rTMS podrían mejorar el deterioro cognitivo asociado a la edad, a la vez que regularía la excitabilidad neuronal (Wang y cols., 2015). Otros estudios relacionados con la aplicación de rTMS, pero en modelos transgénicos para la EA con dificultades espaciales y cognitivas, muestran tras su aplicación una mejoría de las mismas (Tan y cols., 2013) y se regula el proceso de regeneración de neuronas del hipocampo cultivadas *in vitro* (Ma y cols., 2013). El efecto potencial de TMS para compensar el daño funcional es una futura aplicación prometedora en la rehabilitación cognitiva por su capacidad de mejorar las funciones cerebrales.

Envejecimiento y retina

El análisis oftalmológico constituye una vía única de exploración directa del SN. Y teniendo en cuenta que uno de los problemas más comunes asociados a la edad, es la degeneración de la retina, es por ello que hemos estudiado el *O. degus*, como modelo adecuado para comprender este proceso (Punto III.I.III). Este roedor diurno tiene una visión dicromática, por lo que su estructura retiniana es similar a la de los seres humanos en muchos aspectos y se puede

considerar un buen modelo para estudiar el envejecimiento retiniano. Además, la retina es una de las áreas más afectadas por la degeneración dependiente de la edad. Tras el estudio y caracterización de los tipos de neuronas retinales en *O. degus* jóvenes (6 meses), *O. degus* adultos (12 meses) y *O. degus* añosos (36 meses),observamos que la estructura tisular estaba ligeramente debilitada a nivel microscópico y había una elevada expresión GFAP en las células gliales de Müller (Punto III.I.III, Figura 2). A nivel ultraestructural, las alteraciones del epitelio pigmentario de la retina y la degeneración de las células fotoreceptoras, especialmente de los conos, fue evidente,características también comunes al humano, ratón y rata en el deterioro de la retina por la edad. La neuroretina junto con el epitelio pigmentario de la retina forman una unidad funcional del sistema visual. Dicho epitelio durante el envejecimiento, experimenta una serie de cambios bien caracterizados, incluyendo el aumento del número de cuerpos residuales y la acumulación de depósitos basales (Garron, 1963, Guymer y cols., 1999). En los animales añosos, se observó una degeneración del epitelio pigmentario de la retina, probablemente liderado por niveles alterados de oxigeno tisular y contribuyó a la pérdida de células fotorreceptoras (DiLoreto y cols., 2006). El proceso de degeneración es complejo e implica la acumulación de depósitos. La pérdida de células pigmentarias conlleva a la formación de áreas hipopigmentadas y al desarrollo de áreas hiperpigmentadas. Este estado puede progresar a un modo de proliferación neovascular caracterizado por el crecimiento de vasos coroidales o a una atrofia caracterizada por el daño del epitelio pigmentario y de la retina neural (Limaye y Mahmood, 1987). Las modificaciones estructurales en la retina interior puede ser una consecuencia del paso de la edad. Liets y cols., demostraron que los procesos aberrantes en las neuronas bipolares (bastones) son consecuencia del envejecimiento (Liets y cols., 2006). Estos procesos aberrantes normalmente establecen sinapsis ectópicas, y a medida que avanza la degeneración de las neuronas bipolares (conos), muestran una retracción precoz y pérdida de dentritas (Cuenca y cols., 2005). En el grupo más añoso del estudio, se observó que las dentritas de los conos ya no eran rectas y cepilladas, pero aparecían aplanadas en contraste con la retina normal, donde

las funciones de los bastones penetran en la capa nuclear externa, como ocurre en los grupos de *O. degus* jóvenes.

Estos resultados mostraron que: i) los bastones son más sensibles que los conos a la pérdida de células asociada a la edad, ii) los cambios estructurales en la membrana basal de las células epiteliales del pigmento pueden ser uno de los signos iniciales del proceso degenerativo, iii) las características de las proteínas sinápticas especialmente de las sinapsis de la lamela son las más afectadas, y iv) la retina del *O. degus* puede ser un modelo adecuado para estudiar el envejecimiento retiniano.

Neurotransmisión, tratamientos farmacológicos y estrés en *O. degus*

Se estima que el número de personas con demencia senil aumentará en los próximos años entre la población de edad avanzada (Sosa-Ortiz y cols., 2012). Los tratamientos farmacológicos que actualmente se están utilizando para las enfermedades neurodegenerativas, no son completos puesto que ni curan ni previenen en su totalidad las enfermedades. En concreto con la EA, los medicamentos que se utilizan para su tratamiento sólo alivian los síntomas clínicos de la enfermedad, pero no la curan, por lo que principalmente tienen una acción paliativa y no curativa. No obstante, como las alternativas actuales no son totalmente capaces de cubrir los síntomas de la enfermedad, hace imprescindible una mayor investigación preclínica.

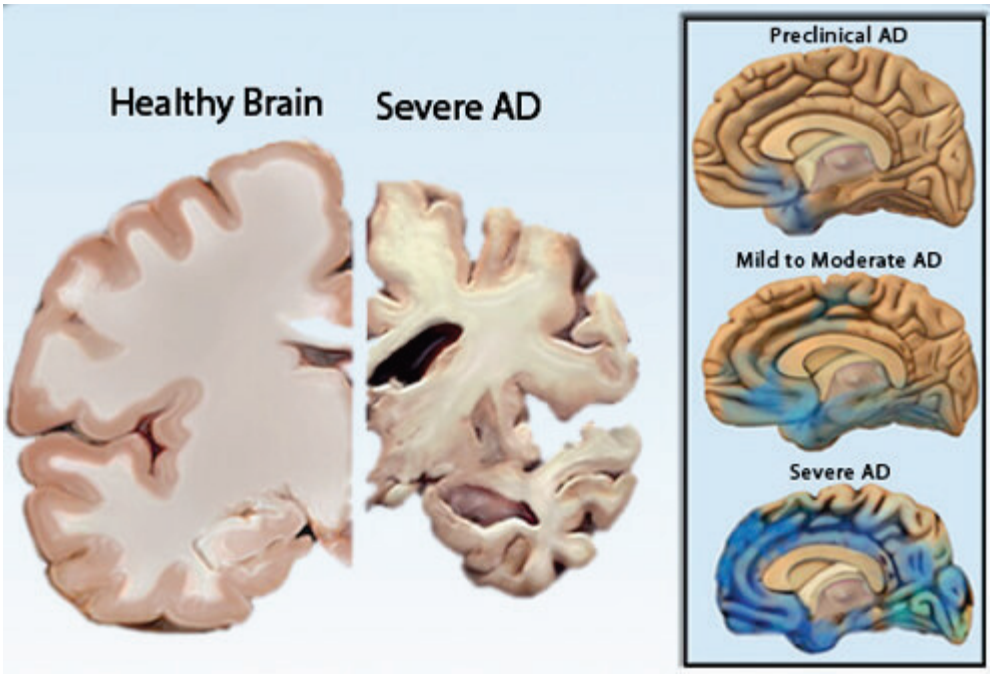

Figura 13. Degeneración del sistema límbico progresivo (desde estadios preclínicos a demencia grave). Sección transversal de un cerebrosano (izquierda) versus un cerebro con enfermedad de Alzheimer avanzada (derecha) *(Imagen de William C. Shiel). AD = Alzheimer disease.*

Los fármacos para combatir la demencia se consideran eficaces si logran estabilizar o retrasar la progresión de los síntomas de la enfermedad. En este sentido, el deterioro cognitivo en pacientes con EA parece estar directamente relacionado con alteraciones en la transmisión colinérgica. En este sentido, la mayor parte de la farmacología actual tiene como diana terapéutica la inhibición de las colinesterasas, con lo que se busca disminuir la actividad de la enzima AChE, aumentando así la concentración del neurotransmisor ACh, haciendo que la transmisión sináptica aumente, mejorando de esta manera los procesos cognitivos, mejorando la calidad de vida de los pacientes (Viegas y cols. 2005). Es por esto, que se estudiamos la posible diferencia de actividad enzimática AChE y BuChE en extractos de hipocampo en función de la edad y de la práctica de actividad física voluntaria en el *O. degus* (Punto III.I.VI). La determinación de la actividad de la enzima AChE en los animales resultó en una disminución de la actividad en los *O. degus* sometidos a ejercicio físico voluntario con respecto a los que no practicaban ejercicio, tanto en los jóvenes en la fracción S1, como en jóvenes y añosos en la S2 (Punto III.I.VI, Figura 1). Mientras que la medición de la actividad enzimática BuChE en ambas fracciones permaneció inalterada, no mostrando cambios significativos en

ninguno de los grupos (Punto III.I.VI, Figura 1). Este último resultado es especialmente curioso, si lo contrastamos con el aumento de la actividad BuChE, descrito en otro modelo de envejecimiento como es el SAMP8 (Fernández-Gómez y cols., 2008, Fernández-Gómez y cols., 2010), que a diferencia de lo que ocurre en*O. degus*, sí se han descrito mutaciones (Takeda y cols., 1997, Akiguchi y cols., 2017).

Otra clase de fármacos empleados en el tratamiento de la EA es la memantina. La memantina es un antagonista de los receptores NMDA que gracias a su singular mecanismo de acción, restablece la transmisión neuronal glutaminérgica fisiológica y evita los efectos excitotóxicos producidos por los valores tónicos patológicamente elevados del glutamato sináptico, que pueden dar lugar a una disfunción neuronal.Es por esto, que estudiamos la capacidad de la memantina para prevenir los efectos negativos de la privación de sueño en la memoria en *O. degus* jóvenes y añosos (Punto III.I.IV, Figura 4, 5). Los resultados obtenidos apoyan la idea de la memantina como agente neuroprotector, y son coherentes con estudios clínicos donde pacientes en estado avanzado de EA tratados con este fármaco, mejoran significativamente la memoria de reconocimiento y estado funcional general (Collingridge y cols., 2013).

Teniendo en cuenta que el *O. degus* es un roedor social y que se ha aceptado como un modelo animal experimental en los últimos años, por una de sus características de ser un modelo espontaneo de desórdenes neurológicos como ocurre en la EA (Tarragon y cols., 2013, Rivera y cols., 2016), se considera un candidato prometedor no solo para mejorar el entendimiento de los mecanismos de plasticidad cerebral, sino que también para el diseño de nuevas estrategias rehabilitadoras y para probar drogas en desarrollo para pacientes con enfermedades neurodegenerativas.

El ejercicio previene y mejora de los síntomas de ansiedad y depresión (Larson y cols., 2006, Peluso y cols., 2005), mejora las funciones cognitivas (Kramer y cols., 2006, Larson y cols., 2006) y aumenta la neuroplasticidad dando lugar a una mejora cognitiva tanto en humanos (Voelcker-Rehage y Niemann, 2013), como en animales (Ryan y Kelly, 2016). Debido a estos beneficios, decidimos estudiar la interacción entre estrés, ejercicio, edad y el

deterioro cognitivo transitorio provocado por SD en *O. degus* jóvenes y añosos. Este estudio mostró que la práctica de ejercicio mejoraba la memoria espacial y el aprendizaje y que además era capaz de contrarrestar el deterioro cognitivo transitorio en los grupos SD.Estos resultados se evaluaron mediante el test BM y el de RAM. A pesar de que los resultados del RAM fueron en el mismo sentido que el BM, no se muestran en el artículo por las reminiscencias que tuvieron los revisores para la publicación del mismo, al contrario de lo que sucedió con los trabajos de TMS. Complementariamente, se determinaronlos niveles plasmáticos de cortisol en los cuatro grupos experimentales. Lapráctica de ejercicio disminuyó los valores de cortisol tanto en condiciones normales de sueño como en la condición SD (Punto III.I.V, Figura 3). Curiosamente, no se observó un aumento en el nivel de cortisol en los grupos control de SD, puesto que los grupos control jóvenes y añosos lo presentaban igualmente por encima de los niveles basales. Por lo tanto, los niveles de cortisol en *O. degus* control no presentaban diferencias significativas, a pesar de ser ligeramente más altos en los grupos de jóvenes. Estos datos pueden indicarnos que debido al hábitat en el que se encuentran los animales, como el aislamiento en jaulas, podría ser uno de los factores que incrementen los niveles de estrés al tratarse de un roedor social, y que vive en comunidad (Rivera y cols., 2016). De hecho, han sido publicados cambios en la concentración plasmática del cortisol después del aislamiento social crónico (Dronjak y cols., 2004). Los grupos que practicaron ejercicio presentaron un descenso significativo de los niveles plasmáticos de cortisol. Este resultado podría corresponderse a la combinación de la práctica regular de ejercicio con la inserción de una nueva actividad en la jaula del animal, que aliviaría el empobrecimiento ambiental (Saland y Rodefer, 2011). Además, se ha demostrado que el enriquecimiento ambiental en roedores adultos reduce los niveles de cortisol a diferentes niveles de estrés (Peña y cols., 2009, Gregoire y cols., 2014), aumenta los niveles de receptores de glucocorticoides en el hipocampo, lo que facilitaría la recuperación a niveles basales de cortisol en condiciones de estrés (Garrido y col., 2011). Por lo tanto, consideramos el enriquecimiento ambiental como un factor positivo que podría contrarrestar algunos de los efectos del estrés en el cuidado del envejecimiento del cerebro en roedores (Harati y cols., 2013, Mora, 2013).

Limitaciones del estudio

Uno de los grandes inconvenientes que hemos tenido a lo largo de la realización de este trabajo, es el hecho de que, a día de hoy, apenas hay secuenciados genes para esta especie de *O. degus*. Se han secuenciado los genes de insulina, glucagón y el péptido ß-amiloide (Nishi y Steiner, 1990, Inestrosa y cols., 2005). Este aspecto limita enormemente no sólo el uso de técnicas de biología molecular, sino también la especificidad de los anticuerpos disponibles en el mercado y por lo tanto la probabilidad de obtener falsos positivos o negativos. Sin embargo, la comunidad científica no es ajena a este inconveniente, y en un artículo publicado en este mismo año, ya se ha realizado un RNA-seq del cerebro de estos animales. En dicho trabajo se realiza la comparación entre el transcriptoma del *O. degus* y el transcriptoma humano, consolidando este modelo como una herramienta eficaz para el estudio de la EA(Altimiras y cols., 2017).

V. Conclusions

V.Conclusions

1. TMS treatment may have a great therapeutic potential in cognitive impairment. Acute TMS treatment significantly improve spatial memory after SD insult in *O. degus*, while chronic TMS is required to additionally recover the working memory.

2. Chronic TMS treatment has a therapeutic potential effect in both young and old *O. degus*.However, acute treatment is insufficient in old animals meanwhile is enough in the young ones. Old *O.degus* requires until 7 TMS applications to recover in comparison to 1 needed for the young ones.

3. The administration of memantine at 10 mg/kg prior to the SD challenge, showed the suitability of the *O. degus* model for the study of aging-related disorders such as in Alzheimer´s disease.

4. Aging-changes observed in *O. degus´*retina are closely far similar to the ones described in human than other rodents, enhancing the suitability of this model for aging-related disorders

5. Voluntary physical activity improved spatial memory functions and decreased cortisol level in no-SD animals, highlighting the benefits of sleep hygiene in the lifestyle. The insertion of exercise programs in addition to pharmacological tool might promote memory recovery functions in the prognosis of neurodegenerative disorders, such as Alzheimer´s disease.

6. The study of both ChEs activities AChE and BuChE in *O. degus* after 45 days performing voluntary physical exercise, revealed that the aging process affect differently at each enzyme after SD insult. While there is no changes in BuChE activity, in AChE there is a decrease in young animals just in fraction S1, and this reduction occurs at both ages in the supernatant S2 after exercise stimuli.

VI.English summary

VI.English summary

Age-related cognitive deficiencies are extremely common and represent a considerable health risk in humans. The deterioration of cognition is one of the principal properties of the physiological consequences of aging. Although the initial effect of a degeneration process does not fundamentally suggest memory damage, age-related disturbances regularly emerge with diverse levels of cognitive dysfunction. Among these kind of disorders, Alzheimer´s disease (AD) is of particular concern because of its predominance and drastic consequences. It is a progressive neurological disease which is the mark of an extreme accumulation of neurofibrillary tangles and the deposition of amyloid peptides in the cortical parenchyma and hippocampal area. It has long been accepted that sleep disturbances may impair normal physiological functions including the immune system, thermoregulation, tissue restoration and energy conservation. Sleep orchestrates brain plasticity during maturation, which enables learning and memory processes, neurogenesis and adult consolidation of hippocampal integrity and types of memories. The paradigm of sleep deprivation (SD) during the paradoxical sleep window has been widely used to induce memory impairments in animal models. SD produces an efficient transient cognitive impairment in both human and animals, pointing out that SD prior to learning may influence memory processes by limiting the ability of neuronal networks to process new information. In this sense, SD could be a non-invasive alternative to reproduce AD cognitive deficits.

Preclinical research using animal models for the study of age-related disorders is crucial for the development and improvement of pharmacological strategies.

O. degus is a diurnal rodent that provides an excellent opportunity for exploring the mechanisms underlying late developmental changes in the nervous system, and therefore, the behavioral and cognitive outcomes resulting from such changes. These animals develop spontaneously several physiopathological conditions has recently been identified as a very valuable animal model for research in several medical fields, especially those concerned with neurodegenerative diseases in which risk is associated with aging.

The retina is sensitive to age-dependent degeneration, and it has been studied in the *O. degus* (Szabadfi et al., 2015). Its retinal structure is similar to that in humans in many respects, therefore, it is well suited to study retinal aging. The retina is arguably the best understood part of the vertebrate central nervous system with regard to its cellular patterning, circuitry, and function. Retinal neurons can be further subdivided into approximately 70 distinct functional subtypes for many of which markers are available to identify the aging-specific alterations. The visual system of *O. degus* is also comparable to the human visual system. It shows robust responses to both photic and non-photic circadian Zeitgebers (Goel et al., 1999; Jacobs et al., 2003).*O. degus* have the potential for dichromatic color vision on the basis of green-sensitive M cones and UV-sensitive S cones, the most common type of mammalian color vision (Jacobs, 1993, Chávez et al., 2003, Palacios-Muñoz et al., 2014). *O. degus* is also well suited model for studing eye pathology, because they have an increase susceptibility to cataract development and aging (Worgul and Rothstein, 1975, Brown and Donnelly, 2001, Peichl et al., 2005). It has been published a complex retinal characterization of *O. degus* at histological, ultrastructural and immunohistochemical levels during aging focused on the elements of the vertical pathway (photoreceptors to bipolar to ganglion cells). Since *O. degus´*retina is more similar to the human retina that to the retina of other rodents, this description will provide a strong manipulations and neuroprotective agents can be studied.

Currently approved treatments only provide temporary and modest improvement in cognitive impairment through cholinergic and antiglutamatergic mechanisms, without altering the natural course or the ultimate outcome of the disease. Disease-modifying treatments with the ability to slow or interrupt early pathologic changes, prevent disease progression, and alter the natural course and outcome of AD are desperately needed, and have been a goal on multiple fronts of modern research (Rosenberg, 2005, Walker et al., 2005).

A number of studies have found that changes in post-synaptic cholinergic-muscarinic receptors could be associated with alterations in the activity of acetylcolinesterase (AChE), an enzyme involved in the metabolism of acetylcholine (ACh), a principal neurotransmitter involved in cholinergic

neurotransmission (Ansari et al., 2012, Chandravanshi et al., 2014, Lee et al., 2009). The molecular structure and function of AChE are now known in detail, and this knowledge has facilitated the understanding of cognitive decline. Alteration in AChE activity has been associated with cognitive and neurobehavioral deficits observed in patients with neurodegenerative diseases (Taylor, 1996, Anand and Singh, 2013). It seems to be related to impaired cholinergic transmission that involves the synthesis of ACh by choline acetyltransferase (ChAT), release of ACh, binding to postsynaptic receptors (AChR), rapid withdrawal of ACh by cholinesterases (ChEs), and reuptake of choline in the presynaptic cell by high-affinity choline transporters (ChT). Although synaptic ACh is mostly hydrolyzed by AChE, the enzyme can be replaced functionally by butyrylcholinesterase (BuChE) when AChE is absent, or greatly reduced by AChE inhibitors (Li et al., 2000). Cholinesterase inhibitors prevent the breakdown of acetylcholine and therefore boost cholinergic neurotransmission in forebrain regions, and this is thought to contribute to their clinical benefits. Donepezil is a selective reversible inhibitor of acetylcholinesterase. Rivastigmine is an inhibitor of both acetylcholinesterase and butylcholinesterase. Galantamine can stimulate nicotinic acetylcholine receptors, in addition to inhibition of cholinesterase activity (Lanctôt et al., 2009).

One of the studies that has been presented is focused on memantine, a partial NMDA receptor antagonist. It has proved its beneficial effects in cognition and its usefulness as a treatment for memory loss. In the last years, memantine has been added to the clinic drug repertoire to battle cognitive decline symptoms, especially those observed in AD. There is evidence that this drug is able to improve recognition and spatial memories in animals. Moreover, clinical evidence indicates an improvement on the cognitive and behavioral symptoms in patients following chronic treatment. It has been demonstrated that the administration of memantine prior to the SD stimuli at the dose of 10 mg/kg, is an effective agent in preventing the transient cognitive impairments that SD causes not only in young but mostly in old *O. degus*, opening new possibilities for the use of this drug in an elderly population. Moreover, there was not seen

side effects after the administration of the drug, which is coherent with studies conducted in other species and similar dose ranges.

On the other hand, pharmacological treatments have limited effectiveness, and in some occasions, could present side effects. A new technique of non-invasive brain stimulation is Transcraneal Magnetic Stimulation (TMS), which is gradually becoming recognized as a useful tool in cognitive neuroscience. TMS permits non-invasive modulation of cortical neural activity and may be a useful way to study neurophysiology and plasticity in order to explore neural function. TMS has been examined in therapeutic trials as a treatment tool and has been found to be both safe and tolerable. It can modulate brain function and can be used to examine multiple facets of the human brain, including motor function and the amelioration of the cognitive impairment. It has been evaluated the plausible therapeutic role of TMS in cognitive function in young *O. degus*. In order to establish the accurancy of TMS in this rodent and the suitability for posterior applications during aging-associated diseases such as neurodegenerative disorders (Ardiles et al., 2012), adult *O. degus* were evaluated under the SD paradigm. TMS treatment was assayed by three different behavioral studies, one working memory analysis (Novel Object Recognition; NOR) and two hippocampal-dependent test [Radial Arm Test (RAM) and Barnes Maze (BM)] due to the hippocampus involvement in episodic, spatial and semantic human memory (Spiers and Bendor, 2014). It was demonstrated a positive effect of exposure to TMS on spatial learning and memory skills under all the cognitive test evaluated. Sleep-deprived animals showed enhanced cogntion when groups were treated with an acute or chronic (1 or 7 days, respectively) TMS sessions. Interestingly, these beneficial outcomes were also achieved with the animals under normal sleep conditions in comparison with the untreated ones. Additionally, it has been demonstrated that applying a magnetic pulse over a cortical zone of the brain has no consequence at all on the normal response, but magnetic stimulation interferes with task performance even when the treatment is triggered during cognitive recruitment (Pascual-Leone 2002, Cowey 2005). This potential ability of TMS to neutralize a brain dysfunction is one presumable application in cognitive rehabilitation (Nardone et al. 2012). Furthermore, it has been shown that a single TMS pulse

can produce significant differences in the cortical response depending on how activated the cortex is at the time the pulse is applied (Silvanto and Pascual-Leone 2008). For this reason, despite physiological modifications occurring in the nervous system during the exposure time, the late effect leads to a more efficient transmission of neural signals, increasing the capacity to mitigate the cognitive dysfunction induced by SD, as evidence by the BM and RAM data in sleep-deprived *O. degus*.TMS is a low-cost, versatile and suitable neurorehabilitative tool which can be use to restore memory performance. The technique has a significant role to play in increasing our comprehension of brain plasticity mechanisms and in the design of novel rehabilitation methodologies for patients with neurodegenerative diseases.

One more alternative to enhance cognitive function is regular exercise. Abundant data suggests that physical activity reduces the risk of various diseases, including those associated with compromised cognition and brain function. Exercise protects the brain from the adverse effects of aging. Recent studies have revealed that exercise-induces adaptations in skeletal muscle can initiate various signaling mechanisms that can lead to physiologic changes in both muscle and neuronal networks. Nowadays, it is accepted that the nervous system has evolved feedback mechanisms with peripheral structures such as muscles or joints to ultimately influence brain function and adaptability. The functional consequences of these adaptations in the hippocampus can be manipulated by the type, amount and duration of exercise. For instance, regular aerobic exercise leads to improve performance in spatial memory tasks, increased endogenous neurotrophin levels, which in turn, can enhance hippocampal plasticity and its signaling cascade. Many human studies indicate that exercise aids in the treatment and prevention of depression- and anxiety-related disorders, and the cognitive amelioration in asymptomatic as well as in senescence people during elderly. Surprisingly physical exercise has not been commonly inserted in mental health programs. Probably due to the fact that there are some studies describing the lack of positive effects, and even others showing negative outcomes, or physical limitations of the patients. Neurotrophins such as brain-derived neurotrophic factors (BDNF) and nerve growth factor have been shown to facilitate plasticity. An increased of protective

neurotrophins are associated with higher levels of exercise in animal and human studies, and these physiological effects may have a positive impact on cognition in the aging brain. Epidemiological studies suggest that physical activity may prevent cognitive decline in the elderly, the ocurrence of dementia and the risk of AD. In rodents, voluntary exercise prevents cognitive decline during aging. A study with THY-Tau22 transgenic model exhibiting progressive and parallel development of Tau pathology and memory impairments in absence of motor deficits, demonstrated that voluntary exercise exerts a preventive effect on the development of Tau pathology and its pathophysiological consequences (Belarbi et al., 2011). We evaluated the effects of voluntary exercise on spatial learning, memory and anxiety levels in young vs old *O. degus* undergoing SD condition. Since there are not so many studies related with the combined influence of SD and exercise on hippocampus-dependent memory and learning, *O. degus* underwent voluntary exercise for 45 days prior to SD insult. Our behavioral data revealed that voluntary exercise was enough to counteract the transient cognitive impairment caused by SD in young and old *O. degus.* We showed how voluntary exercise has different effects in SD and no-SD conditions. Thus, voluntary physical activity improved spatial memory functions and decrease cortisol level in no-SD animals, highlighting the benefits of sleep hygiene in the lifestyle. Furthermore, exercise reduces cortisol levels what could be used as an accessible nonpharmacological approach option for stress patients, which might be used as a therapy in combination with pharmacologic protocols. Our ability to capitalize on physical activity as a lifestyle change for the improved brain health critically depends on a better understanding of neurobiological mechanisms through which physical activity protects and restores the brain.

VII. Abreviaturas

EA: Enfermedad de Alzheimer
SNC: Sistema Nervioso Central
OMS: Organización Mundial de la salud
NMDA: N-Metil-D-Aspartato
MCP: Memoria a corto plazo
MLP: Memoria a largo plazo
FM: Fórceps menor
ATR: Radiación talámica anterior
MD: Sistema de demanda múltiple
BA 10: Área de *Brodmann*
NREM: Sueño son movimientos oculares rápidos
REM: Sueño con movimientos oculares rápidos
EEG: Electroencefalografía
SD: Privación de sueño
BDNF: Factor neurotrófico de origen cerebral
VEGF: Factor vascular de crecimiento endotelial
TMS: Estimulación Magnética Transcraneal
O. degus: *Octodon degus*
IAPP: Polipéptido amiloide de los islotes
EP: Enfermedad de Parkinson
ACh: Acetilcolina
ChAT: Acetiltransferasa
AChRs: Receptores postsinápticos
ChEs: Colinesterasas
ChT: Transportador de colina de alta afinidad
AChE: Acetilcolinesterasa
BuChE: Butirilcolinesterasa
ATCh: Sustratos acetiltiocolina
BuTCh: Butiriltiocolina
DTNB: Ácido 5,5 0-ditio-bis-2-nitrobenzoico
Iso-OMPA: Tetraisopropil pirofosforamida

Brij 96: Detergente polioxietilen10-oleil éter

UA: Unidades Arbitrarias

S1: Sobrenadante con las colinesterasas débilmente unidas a las membranas

S2: Sobrenadante con las colinesterasas fuertemente unidas a las membranas

RAM: Laberinto radial

BM: Laberinto Barnes

NOR: Reconocimiento de Nuevo Objeto

RME: Errores de referencia

WME: Errores de trabajo

ELF-MF: Estimulaciones Magnéticas extremadamente bajas

rTMS: Estimulación Magnética Transcraneal Repetitiva

LTP: Potenciación a largo plazo

GFAP: Proteína gliofibrilar ácida

SN: Sistema Nervioso

VIII.Bibliografía

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IX. Anexos

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IX.I “Retinal aging in the diurnal Chilean rodent (*Octodon degus*): histological, ultrastructural and neurochemical alterations of the vertical information processing pathway”.*Frontiers in Cellular Neuroscience*. **2015** 9:126. PMID: 25954153.Szabadfi K, **Estrada C**, Fernandez-Villalba E, Tarragon E, Setalo G Jr, Izura V, Reglodi D, Tamas A, Gabriel R, Herrero MT.

IX.II “Memantine prevents reference and working memory impairment caused by sleep deprivation in both young and aged *Octodon degus”*.
Neuropharmacology. **2014** 85:206-214. PMID: 24878242.Tarragon E, Lopez D, **Estrada C**, Gonzalez-Cuello A, Ros CM, Lamberty Y, Pifferi F, Cella M, Canovi M, Guiso G, Gobbi M, Fernández-Villalba E, Blin O, Bordet R, Richardson JC, Herrero MT.

IX.III “Inflammation in Parkinson's disease: role of glucocorticoids”.*Frontiers in Neuroanatomy*. **2015**2;9:32. PMID: 25883554.Herrero MT, **Estrada C**, Maatouk L, Vyas S.

IX.IV Thalamus: Anatomy. Brain Mapping: An Encyclopedic Reference (BRNM) Chapter: 00216 Elsevier 2015Herrero MT, Insausti R, **Estrada C.**

IX.V"Effects of voluntary exercise in memory impairment, stress and aging in Octodon degus"**Estrada C,** Fernández-Gómez FJ, Cuenca-Bermejo L, Gil-Martínez AL, Fernández-Villalba E, Herrero MT. Enviado

IX.VI Resultadosrecientes. Estudiodelaactividadcolinesterasaen*O. degus*

IX.I Szabadfi K, **Estrada C**, Fernandez-Villalba E, Tarragon E, Setalo G Jr, Izura V, Reglodi D, Tamas A, Gabriel R, Herrero MT.“Retinal aging in the diurnal Chilean rodent (*Octodon degus*): histological, ultrastructural and neurochemical alterations of the vertical information processing pathway”.*Frontiers in Cellular Neuroscience*. **2015** 9:126.

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Abstract

The retina is sensitive to age-dependent degeneration. To find suitable animal models to understand and map this process has particular importance. The degu (*Octodon degus*) is a diurnal rodent with dichromatic color vision. Its retinal structure is similar to that in humans in many respects, therefore, it is well suited to study retinal aging. Histological, cell type-specific and ultrastructural alterations were examined in 6-, 12- and 36-months old degus. The characteristic layers of the retina were present at all ages, but slightly loosened tissue structure could be observed in 36-month-old animals both at light and electron microscopic levels. Elevated Glial fibrillary acidic protein (GFAP) expression was observed in Müller glial cells in aging retinas. The number of rod bipolar cells and the ganglion cells was reduced in the aging specimens, while that of cone bipolar cells remained unchanged. Other age-related differences were detected at ultrastructural level: alteration of the retinal pigment epithelium and degenerated photoreceptor cells were evident. Ribbon synapses were sparse and often differed in morphology from those in the young animals. These results support our hypothesis that (i) the rod pathway seems to be more sensitive than the cone pathway to age-related cell loss; (ii) structural changes in the basement membrane of pigment epithelial cells can be one of the early signs of degenerative processes; (iii) the loss of synaptic proteins especially from those of the ribbon synapses are characteristic; and (iv) the degu retina may be a suitable model for studying retinal aging.

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Retinal aging in the diurnal Chilean rodent (*Octodon degus*): histological, ultrastructural and neurochemical alterations of the vertical information processing pathway

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The retina is sensitive to age-dependent degeneration. To find suitable animal models to understand and map this process has particular importance. The degu (*Octodon degus*) is a diurnal rodent with dichromatic color vision. Its retinal structure is similar to that in humans in many respects, therefore, it is well suited to study retinal aging. Histological, cell type-specific and ultrastructural alterations were examined in 6-, 12- and 36-months old degus. The characteristic layers of the retina were present at all ages, but slightly loosened tissue structure could be observed in 36-month-old animals both at light and electron microscopic levels. Elevated Glial fibrillary acidic protein (GFAP) expression was observed in Müller glial cells in aging retinas. The number of rod bipolar cells and the ganglion cells was reduced in the aging specimens, while that of cone bipolar cells remained unchanged. Other age-related differences were detected at ultrastructural level: alteration of the retinal pigment epithelium and degenerated photoreceptor cells were evident. Ribbon synapses were sparse and often differed in morphology from those in the young animals. These results support our hypothesis that (i) the rod pathway seems to be more sensitive than the cone pathway to age-related cell loss; (ii) structural changes in the basement membrane of pigment epithelial cells can be one of the early signs of degenerative processes; (iii) the loss of synaptic proteins especially from those of the ribbon synapses are characteristic; and (iv) the degu retina may be a suitable model for studying retinal aging.

Keywords: ***Octodon degus*, aging, retina, vertical pathway, ultrastructure, rod bipolar cells, synaptic proteins**

Introduction

The vertebrate retina, like other parts of the central nervous system, is subjected to degenerative changes caused by aging. The retina is also the site of diseases for which age is

a major risk factor, including macular degeneration and glaucoma (Jackson and Owsley, 2003). The retina is arguably the best understood part of the vertebrate central nervous system with regard to its cellular patterning, circuitry, and function. It is composed of five major neuron types: photoreceptors, interneurons (horizontal, bipolar, and amacrine cells), and retinal ganglion cells (RGCs) that integrate visual information and send it to the brain (Sanes and Zipursky, 2010). Retinal neurons can be further subdivided into approximately 70 distinct functional subtypes (Masland, 2001) for many of which markers are available to identify the aging-specific alterations.

Age-related complications have been demonstrated in several mammalian species, including monkeys, cats, sheep, rats, mice and *Octodon degus* (degu). This latter species presents several advantages for studying different pathological conditions. The degu is a diurnal, highly visual South American hystricomorph rodent native to Chile, which in old age expresses cognitive deficits, anxiety (Popović et al., 2009) and unstable circadian rhythms of low amplitude (Vivanco et al., 2007). Particularly notable is that the animals develop spontaneous Alzheimer-like pathology and show signs of significant white matter disruption, diabetes and cancer in aging (Inestrosa et al., 2005; Ardiles et al., 2012, 2013), resembling several aspects of pathological human aging (van Groen et al., 2011).

The visual system of degus is also comparable to the human visual system. It shows robust responses to both photic and non-photic circadian Zeitgebers (Goel et al., 1999; Jacobs et al., 2003). Degus have the potential for dichromatic color vision on the basis of green-sensitive M cones and UV-sensitive (in the near UV) S cones, the most common type of mammalian color vision (Jacobs, 1993; Chávez et al., 2003; Palacios-Muñoz et al., 2014). In degus, the retinal projection is primarily contralateral, with a small ipsilateral component (Fite and Janusonis, 2001). Degu is also well suited model for studying eye pathology, because they have an increased susceptibility to cataract development and aging (Worgul and Rothstein, 1975; Brown and Donnelly, 2001; Peichl et al., 2005). However, no data are available on retinal aging in degu. In other rodents (rats and mice), retinas show some aging alterations. For example, the total retinal area expands while RGC dendritic arbors shrink with age, thus, each RGC covers a decreased fraction of the visual field in old animals. Amacrine and bipolar cells also exhibit age-related structural changes, some of which may contribute to reduced visual function (Samuel et al., 2011). Neuronal loss with age is characteristic for some, but not all species, leading to thinning of the cellular and synaptic layers (Miller et al., 1984; Limaye and Mahmood, 1987; Morrison et al., 1990; Gao and Hollyfield, 1992; Kim et al., 1996; Samuel et al., 2011). Ultrastructural changes have also been revealed in the neural, vascular and epithelial components. Even more prominent changes can be observed in the retinal pigment epithelial (RPE) layer than in the neuroretina during the early phases of aging. Signs include increased number of basal infoldings, phagolysosomes and lipofuscin deposits. In aged rat retina, organelle atrophy and whirling extensions of the basal membrane into the cytoplasm are characteristic in the RPE cells (DiLoreto et al., 2006). However, it is not known at present if these changes are also characteristic to degus. In spite of the similarities between human and degu retinas (Cuenca et al., 2010), surprisingly little is known about the degu retina and its retinal aging.

Therefore, the aim of the present study was to perform a complex retinal characterization of degu at histological, ultrastructural and immunohistochemical levels during aging focused on the elements of the vertical pathway (photoreceptors to bipolar to ganglion cells). Since degu retina is more similar to the human retina than to the retina of other rodents, this description will provide a strong foundation for future studies where experimental manipulations and/or neuroprotective agents can be studied.

Materials and Methods

Animals

A total of 28 female degus (body weight 180–270 g) of 6 ($n = 8$), 12 ($n = 8$), and 36-months of age ($n = 12$) were used. This latter group is considered as aging (but not old) group. Degus were housed individually in opaque glass cages (40 × 25 × 25 cm) at the animal facilities of the University of Murcia. Throughout the study, the experimental room was maintained under controlled temperature (21 ± 1°C) and 12 h light/dark cycle (lights on at 7:00 a.m. and off at 19:00 p.m.). The floors of the cages were covered with wood shavings that were changed once a week. Food and water were provided *ad libitum* by placing 120 g food pellets (Harlan Tekland Global Diet®, Harlan Laboratories, USA) per day and water bottles on a grid located on the top of the tank. The water in the tank was changed daily. All experiments were performed in accordance with relevant regulatory standards, experimental guidelines and procedures complied with the European Community Council Directive (2010/63/UE) and the ethical committee of the University of Murcia.

Histological and Electron Microscopic Analysis

Animals were anesthetized with Isofluorane (Isoba® vet, USA), administered with a continuous flow vaporizer (MSS3, Medical Supplies and Services, England, UK), and then sacrificed by decapitation. Both eyes were immediately removed and distinctly disposed according to histological or electron microscopic procedure to be performed.

For histology eyes were fixed in 4% paraformaldehyde (PFA; Merck, Hungary) dissolved in 0.1M phosphate buffer (PB; Spektrum3D, Hungary). The eyecups were dissected and embedded in epoxy resin (Durcupan ACM resin; Sigma-Aldrich, Hungary) as we previously described (Szabadfi et al., 2012). Sections were cut at 2 μm, stained with toluidine blue (Sigma-Aldrich, Hungary), and examined in a Nikon Eclipse 80i microscope. Measurements were taken with the SPOT Basic program. Central retinal areas within 1 and 2 mm from the optic disc were used for measurements ($n = 2$–5 measurements from one tissue block). The following parameters were measured: (i) cross-section of the retina from the outer limiting membrane (OLM) to the inner limiting

TABLE 1 | Antibodies used in immunohistochemical experiments.

Primary antibodies	Company	Raised in	Dilution	Secondary antibodies	Company	Dilution
Anti-Brn3a	Santa Cruz, Hungary	Mouse	1:50	Alexa Fluor 488	Invitrogen, USA	1:1000
Anti-GFAP	Sigma-Aldrich, Hungary	Rabbit	1:500	Alexa Fluor 568	Invitrogen, USA	1:1000
PNA	Vector Labs, USA	-	1:500	-	-	-
Anti-CtBP2	BD Transduction, USA	Mouse	1:20000	Alexa Fluor 488	Invitrogen, USA	1:1000
Anti-Bassoon	AbCam, Hungary	Rabbit	1:500	Alexa Fluor 568	Invitrogen, USA	1:1000
Anti-Chx10	Thermo Scientific, Hungary	Sheep	1:100	Alexa Fluor 488	Life Technologies, Hungary	1:1000
Anti-VGLUT1	AbCam, Hungary	Rabbit	1:500	Alexa Fluor 568	Invitrogen, USA	1:1000
Anti-PKCα	Sigma-Aldrich, Hungary	Mouse	1:250	Cy 3.5	AbCam, Hungary	1:1000
Alexa Fluor 488	Invitrogen, USA	1:1000				

Abbreviations: CtBP2—C-terminal Binding Protein 2; GFAP—glial fibrillary acidic protein; PKCα—protein kinase Cα; PNA—peanut agglutinin-conjugated with FITC; VGLUT1—vesicular glutamate transporter 1.

membrane (ILM); (ii) the width of individual retinal layers. Statistical comparisons were made using one-way ANOVA test followed by Tukey-B posthoc analysis. Data were presented as mean ± SEM (GraphPadPrism5.0).

Electron microscopy was performed on eyes fixed with 4% PFA supplemented with 1% glutaraldehyde dissolved in 0.1M PB. After washing in PB, tissue samples were treated with 1% OsO_4 in PB, dehydrated through ascending ethanol series and embedded in Durcupan ACM resin (Sigma-Aldrich, Hungary). Sections were cut at 70 nm in Reichert Ultracut S and counterstained with Reynold's lead citrate. Samples were examined and photographed in a JEOL 1200EX electron microscope.

Immunohistochemistry

Eyes were dissected immediately after sacrifice in ice-cold phosphate buffer with saline (PBS) and fixed in 4% PFA at room temperature. Tissues were then washed in PBS and cryoprotected in 20% sucrose at 4°C. For cryostat sectioning, retinas were embedded in tissue-freezing medium (Shandon Cryomatrix, USA), cut in a cryostat (Leica, Germany) at 10 μm radially. Sections were mounted on subbed slides. Primary antibodies and peanut agglutinin-conjugated with FITC (PNA) were used overnight at room temperature (**Table 1**). Next day the sections were incubated for 2 h at room temperature with the corresponding secondary fluorescent antibodies in the dark, then coverslipped using Fluoromount-G (Southern Biotech, USA). For the colocalization study, we used 10 μm cryostat sections simultaneously with antibodies to Chx10 and protein kinase Cα (PKCα); C-terminal Binding Protein 2 (CtBP2) and Bassoon; PKCα and Bassoon; PKCα and postsynaptic density 95 protein (PSD95/SAP90); and PKCα and vesicular glutamate transporter 1 (VGLUT1), respectively. These were detected with corresponding secondary antibodies (**Table 1**); nuclei were counterstained with DAPI (4′, 6-diamidino-2-phenylindole; 1:10000), then coverslipped using Fluoromount-G (Southern Biotech, USA). For control experiments, primary antibodies were omitted, and cross-reactivity of the non-corresponding secondary antibodies with the primaries was also checked. Photographs were taken with Nikon Eclipse 80i Microscope (Nikon, Japan) and Fluoview FV-1000 Laser Confocal Scanning Microscope (Olympus, Japan) and further processed with Adobe Photoshop 7.0 program. Images were adjusted for contrast only, aligned, arranged, and labeled using the functions of the above program.

The number of RGCs (Brn3a-positive cells; Xiang et al., 1995; Nadal-Nicolás et al., 2009) ± SEM was measured in 100 μm ganglion cell layer (GCL) length. The cells expressing both Chx10 and PKCα were scored as rod bipolar cells, and cells expressing Chx10 but not PKCα were scored as cone bipolar cells as followed the protocol of Morrow et al. (2008). The number of all bipolar cells and rod bipolar and cone bipolar cells were counted in 100 μm^2 area of INL. Statistical comparisons were made using one-way ANOVA test followed by Tukey-B posthoc analysis. Data were presented as mean ± SEM (GraphPadPrism5.0).

Results

The baseline characterization of 6-, 12- and 36-month-old degu retinas was initially done by routine histology.

Descriptive Morphological and Morphometric Analysis

The characteristic layers of the mammalian retina were well distinguishable in degu: photoreceptor layer (PL), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL) and GCL (GCL; **Figure 1**). The typical cells of the mammalian retina (photoreceptor cell bodies and outer segments (OS) of cones and rods, bipolar cells, different types of amacrine cells, horizontal cells, displaced amacrine cells, ganglion cells and Müller glial cells) were also well visible at all ages.

There were only minor differences between the three groups (**Figures 1A–C**). A loose retinal structure could be observed in aging degu retinas (**Figure 1C**). This observation was manifested in a significantly increased OLM–ILM distance and IPL thickness in 36-month-old degu retinas (**Figure 1D**). However, the thickness of other layers did not change significantly in the aging retinas (**Figure 1D**; $^{\#\#}p < 0.001$ vs. 6-month-old, $^{\#\#\#}p < 0.001$ vs. 12-month-old degu retinas). The number of RGCs (Brn3a-positive cells)/100 μm retina length was significantly decreased in the 36-month-old degu retina ($^{**}p < 0.001$ vs. 6- and 12-month-old degu retinas; **Figure 1E**) compared to the 6- and 12-month-old retinas.

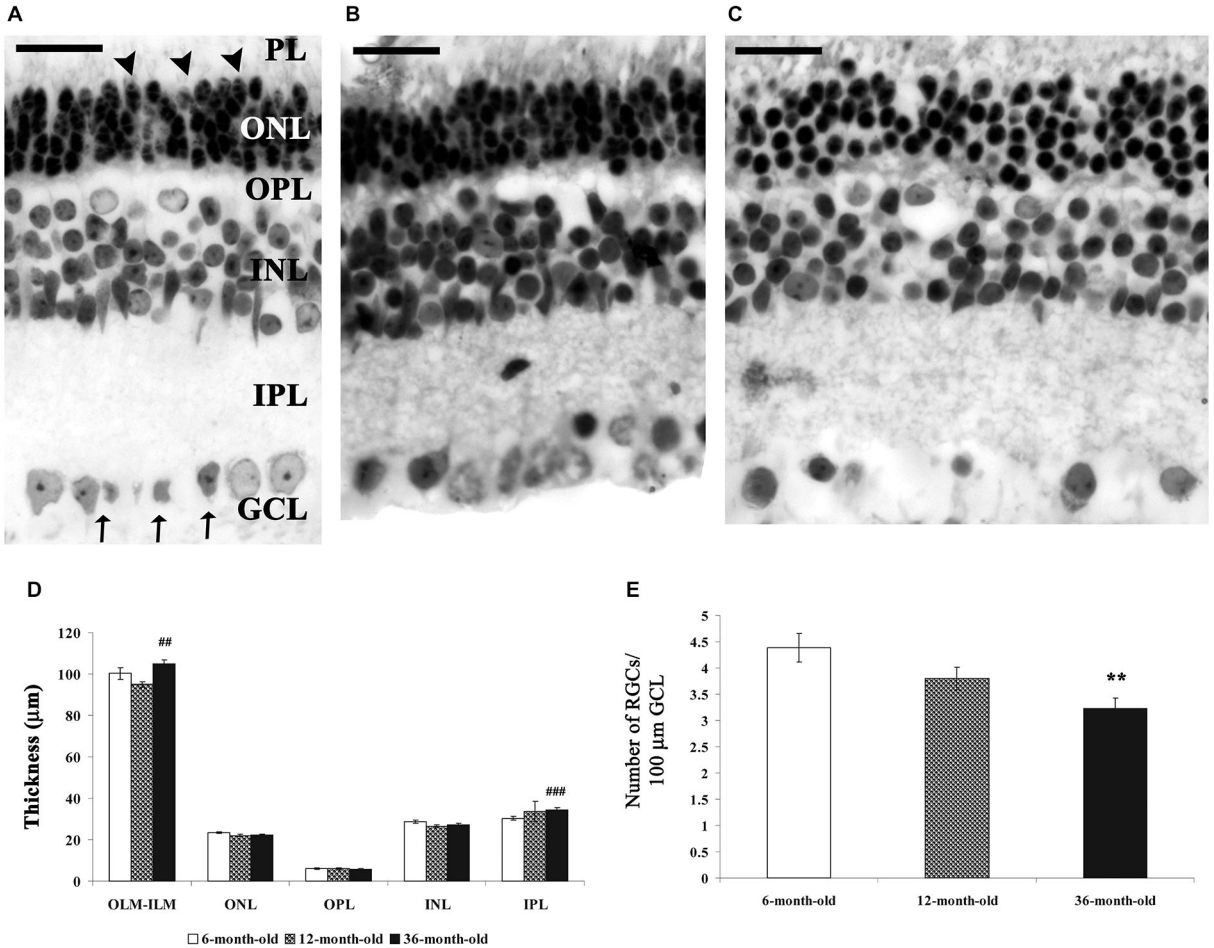

FIGURE 1 | Morphological and morphometric analysis of the 6-, 12- and 36-month-old representative degu retina sections stained with toluidin-blue. The characteristic layers of the mammalian retina were well visible in all groups, major morphological differences could not be observed between the three groups **(A–C)**. However, the thickness of inner plexiform layer (IPL) **(D)** and the whole retina (OLM-inner limiting membrane (ILM), **(D)** significantly increased, while the RGCs (Brn3a-positive cells) number in 100 μm ganglion cell layer (GCL) length significantly decreased in the 36-month-old degus retinas compared to the 6- and 12-month-old animals **(E)**. Data are presented as mean ± SEM. $^{**}p < 0.001$ vs. 6- and 12-month-old; $^{\#\#}p < 0.001$ vs. 12-month-old; $^{\#\#\#}p < 0.0001$ vs. 6-month-old degu retinas. Abbreviations: photoreceptor layer (PL); outer nuclear layer (ONL)—outer nuclear layer (ONL); outer plexiform layer (OPL)—outer plexiform layer (OPL); INL—inner nuclear layer; IPL—inner plexiform layer (IPL); GCL—ganglion cell layer (GCL); OLM—outer limiting membrane (OLM) (arrowheads); ILM—ILM (arrows); RGCs—retinal ganglion cells. Scale bar: 20 μm.

Glial Cells and The Structure of Outer Retina

Glial fibrillary acidic protein (GFAP)-positivity was selectively localized to endfeet of Müller cells (**Figures 2A–D**). Müller glial cells respond rapidly to any alterations of the retinal microenvironment by elevated expression of GFAP as a specific metabolic stress signal in the mammalian retina (Cuenca et al., 2014). Increased GFAP immunoreactivity was observed in the entire width of the 36-month-old degu retina in few, but not all, Müller glial cells (**Figures 2C,D**), compared to 6- (**Figure 2A**) and 12-month-old (**Figure 2B**) degu retinas.

Basement membrane proliferation of the RPE in the areas of age-related retinal peripheral degeneration was observed in the 36-month-old degus retinas. The thickening of Bruch's membrane and fibrosis of the choriocapillary were evident. The somas of the RPE cells were pressed toward this thinner basement membrane in the 36-month-old retinas, suggesting an initial age-related alteration of the RPE. Early peripheral changes of the RPE included increased basal infoldings, phagolysosomes and lipofuscin deposits, as well as atrophy and whirling extensions of the basement membrane into the cytoplasm (**Figure 3C**). The relation between RPE and the OS of the photoreceptors seemed intact in all groups (**Figures 3A–C**).

We observed that the cones composed a nest between the rods in all age groups, in accordance with the cone-dominant retinal structure of the degu (Cuenca et al., 2010). Most of these cone-nests were located near the OLM (**Figures 3D–F**). In the 36-month-old group, we detected altered photoreceptor ratio for the favor of cones suggesting that rods are more sensitive to

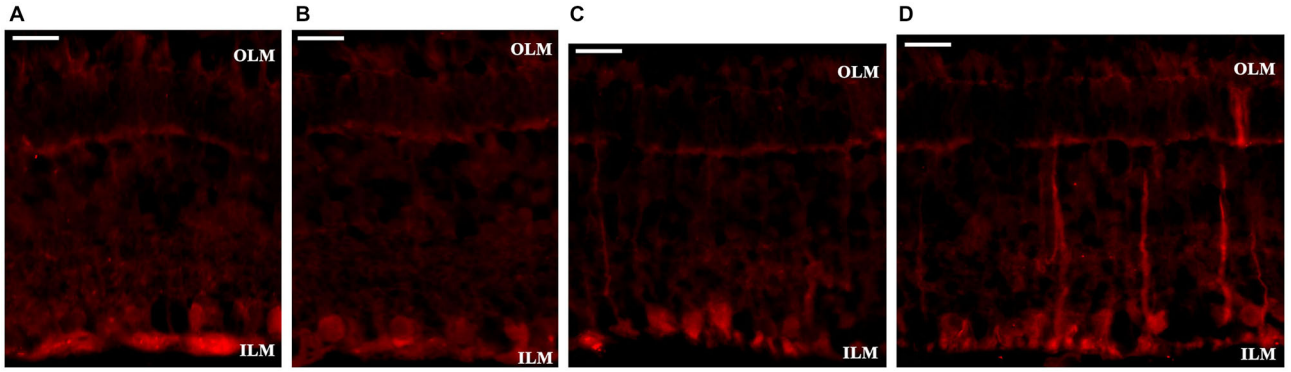

FIGURE 2 | Glial fibrillary acidic protein (GFAP) labeling of Müller glial cells. GFAP immunostaining was observed in the endfeet of Müller glial cells in 6- and 12-month-old degu retinas **(A,B)**. In the 36-month-old degu retinas the GFAP immunoreactivity is very strong within their endfeet at the vitreoretinal border. Other processes of Müller cells in some places span from the ILM to the OLM **(D)**, but not in all retinal locations **(C)**. Abbreviations: GFAP—Glial fibrillary acidic protein (GFAP); OLM—outer limiting membrane; ILM—inner limiting membrane. Scale bar: 20 μm.

aging. We also observed degenerating rods in the ONL of the 36-month-old group (**Figure 3F**). PNA is used to label the OS and the terminals of cone photoreceptors in the OPL. No differences could be observed in the OSs between the three different age groups (**Figures 3G–I**) and the number of cone terminals (data not shown), which further supports the selective loss of rods at 36-months.

We found ribbon synapses in the OPL and observed differences between the rod and the cone terminals. The rod terminals (RT) were more electron dense and cup-shaped spherules, while cone terminals were bowl-shaped, less dense pedicles (**Figures 4A,B**). The only difference between the three groups was the reduced cytoplasmic density of the RT with older age. As a consequence we could not easily distinguish the rod and the cone terminals in the 36-month-old groups (**Figure 4C**).

Photoreceptors transmit their signals at ribbon synapses in the OPL, the first synaptic region in the retina, whereas bipolar cells make their ribbon synaptic contacts in the IPL. A large number of regularly aligned synaptic vesicles were tethered to the ribbon. The rod photoreceptor ribbon synapses had horseshoe-shaped structure in the OPL, however, the cone photoreceptor (OPL) and bipolar cell ribbons (IPL) had a dot-like appearance at light microscopic level. We analyzed the structure of the OPL with retina-specific ribbon synapse markers presynaptic CtBP2 and Bassoon labeling. The rod ribbon synaptic profiles were marked with horseshoe-shaped ribbons by CtBP2 and continuous distribution of punctate staining by Bassoon protein (**Figures 4D,E**). Near the horseshoe-shaped ribbons, some degenerated synaptic structures (fragmented ribbons) could be observed in the OPL of 36-month-old degu retinas (**Figure 4F**). The localization of Bassoon was similar in the three groups (**Figures 4D–F**).

Bipolar Cells and Ultrastructure of the Synaptic Layers

The pan-bipolar cell marker Chx10 reveals the organization of bipolar cells in the INL of the retina (Elshatory et al., 2007). We found that anti-Chx10 antibodies stained all bipolar cells in all groups (**Figures 5A,E,I**). The presence of PKCα was detected in the rod bipolar cell population. The labeled structures were the cell bodies of the INL, the dendrites in the OPL and cell processes extending into the IPL, close to the GCL. There were no alterations detected in the arborization and pattern of the cone and rod bipolar cells between the 6- and 12-month-old groups (**Figures 5A–L**). In the 36-month-old retinas empty cell body-like shapes could be observed in the bipolar cell area of INL (**Figures 5I,J,L**).

Differences could be observed in the total bipolar and rod bipolar cell numbers ($^{*}p < 0.05$ vs. 6- and $^{\#}p < 0.05$ vs. 12-month-old groups and $^{***}p < 0.0001$ vs. 6- and $^{\#\#\#}p < 0.0001$ vs. 12-month-old groups, respectively). The most prominent difference was at the age of 36 months with a significant reduction in these parameters (**Figures 6A,B**). However, the number of cone bipolar cells did not differ between the three groups (**Figure 6C**). These alterations resulted in an increased cone/rod bipolar cell ratio in the 36-month-old degu retina.

The dendritic field of rod bipolar cells was also altered. The progression of the degeneration was manifested by the retraction of rod bipolar cell dendrites. These dendrites became flatter and the loss of dendritic branches above the cell bodies was evident. In the 36-month-old group the PKCα positive dendrites were sparsely distributed (**Figures 7E,F**) compared to the 6- and 12-month-old groups. The pattern of PKCα positive dendrites was dense and each cell had a huge arbor in these latter two groups (**Figures 7A–D**). In normal retina the fine processes of rod bipolar cells penetrate the ONL. The dendritic trees appear brushy and candelabrum-like as we showed in the case of 6-month-old degus (**Figures 7A,B**). In 36-month-old degu retinas the rod bipolar cell dendrites were no longer erect and brushy, but appeared flattened (**Figures 7C,F**). The Bassoon-staining seemed to be unchanged during aging (**Figures 7A,C,E**). Glutamatergic photoreceptors affect the physiological properties of bipolar cells in the mammalian retina. To visualize the spatial pattern of glutamatergic input to the

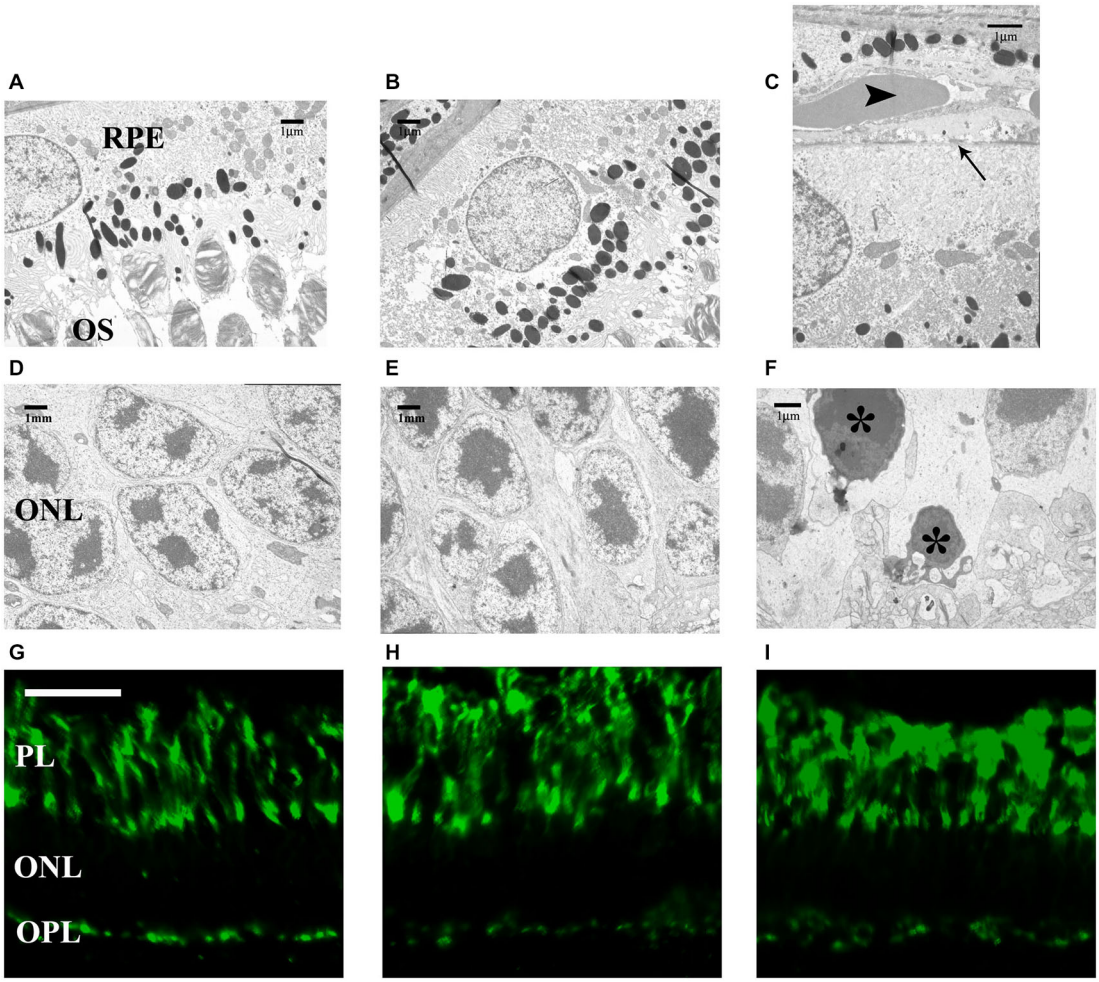

FIGURE 3 | Electron microphotographs of pigment epithelial cells, their connection to the outer segments (OS) (A–C) and somas of photoreceptors (D–F). Labeling of cone photoreceptor OS and terminals **(G–I)** in 6-, 12- and 36-month-old degu retinas. Ultrastructure of a retinal pigment epithelial (RPE) cell is visible above the photoreceptors OS **(A–C)**, however, the somas of the RPE cells are compressed against the basement membrane (arrow) in the 36-month-old retinas (arrowhead: blood vessel; **C**). The somas of photoreceptors seem intact in all groups **(D–F)** but some degenerative photoreceptor cell bodies and terminals appear in the 36-month-old degu retinas (asterisks; **F**). Both the cone OS and terminals show PNA in 6-month-old degu retinas, no differences could be observed in the OS and number of cone photoreceptor terminals between the three groups **(G–I)**. Abbreviations: RPE—retinal pigment epithelium; OS—outer segments (OS) of photoreceptors; ONL—outer nuclear layer; PL—photoreceptor layer; PNA—peanut agglutinin conjugated with FITC; OPL—outer plexiform layer. Scale bars are 20 µm in **(G–I)** and scale bars are indicated in all electron microscopic images **(A–F)**.

bipolar cells in the OPL of degu retina, PSD95 was used to label these synapses. PSD95 puncta were regularly spaced along the membranes of the bipolar cell dendrites. The spatial distribution of PSD95 labeling of the bipolar cells were similar in the 6- and 12-month-old groups (**Figures 7B,D**). However, in the 36-month-old group the pattern of PSD95 was altered, only a few puncta were detected on the somas of rod bipolar cells and labeling was not shown along the non-brushy dendritic arbor (**Figure 7F**).

VGLUT1 was detected in the OPL and throughout the laminae of the IPL, a distribution consistent with the expected synaptic localization of the protein (Brandstätter et al., 1999). In degus, the coarse structure of the retina was not significantly affected, however, the axon terminals of rods and rod bipolar cells showed dramatic alterations in VGLUT1 expression in the 36-month-old group (**Figures 8C,F**). These degu retinas showed loss of most of the rod outputs to their bipolars (**Figures 8C,F**) compared to 6- (**Figures 8A,D**) and 12-month-old (**Figures 8B,E**) degu retinas. The axon terminals of the rod bipolar cells showed some alterations in the 36-month-old group, such as decreased staining both for PKCα and VGLUT1 in the innermost IPL. The loss of PKCα immunoreactivity in the axons and axon terminals of rod bipolar cells was also evident and their terminals were further reduced in size and density (**Figure 8F**) compared to 6- (**Figure 8D**) and 12-month-old (**Figure 8E**) retinas.

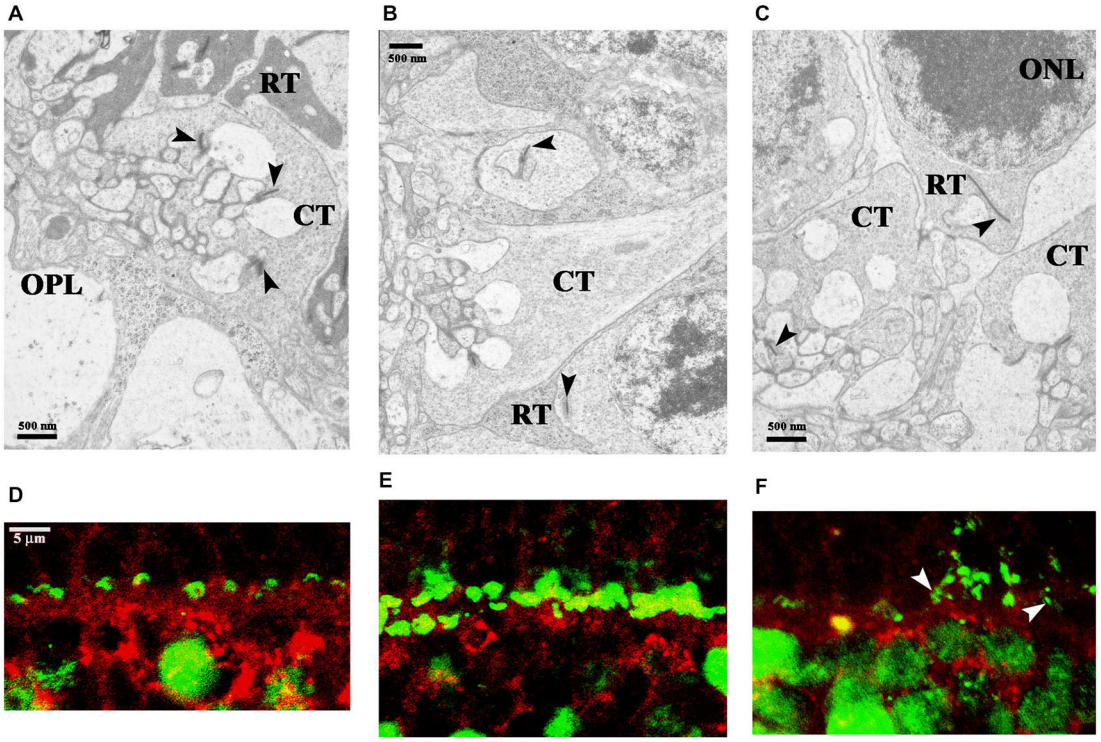

FIGURE 4 | Electron microphotographs of representative retinal locations in OPL (A–C) and ribbon synaptic markers in the OPL (D–F). Ribbon synapses (arrowheads) in the OPL. At the fine structural level, the major difference between the three groups is the reduced cytoplasmic density of the rod terminals (RT) in aging animals **(A–C)**. The synaptic profiles marked with horseshoe-shaped rod ribbons by CtBP2 (green) and Bassoon (red) labeling **(D–F)**. Near the horseshoe-shaped ribbons, fragmented ribbons (white arrowheads) could also be observed in 36-month-old degu retinas **(F)**. Abbreviations: CT—cone terminals; ONL—outer nuclear layer; OPL—outer plexiform layer; RT—rod terminals; CtBP2—C-terminal Binding Protein 2. Scale bars are indicated in the images **(A–C)** and 5 μm in **(D–F)**.

The IPL structure was well retained (**Figures 9A–F**) even at 36 months of age (**Figures 9D–F**). Both ribbon and conventional synapses were visible, synaptic vesicles were regularly distributed. Sometimes a few swollen neural profiles (lacking synaptic vesicles) were seen along with space-filling glial protrusions which can be clearly identified in the electron microscope. The structural elements of the GCL, nerve fiber layer (NFL) and ILM in general did not show major signs of degeneration in any of the groups (**Figures 9G–J**). The NFL fibers were embedded into the large endfeet of Müller glial cells (**Figures 9G,I,J**).

Discussion

Progressive and irreversible functional decay during aging is characterized by region-specific neuron loss (Lossi et al., 2005). The retina is a potentially sensitive target of age-dependent degeneration. In this paper, we report a comparison of the major retinal neuronal types of the vertical pathway in young adult (6-months-old), adult (12-months-old) and aging (36-months-old) degus. There are several remarkable differences along with numerous unchanged features in the different age groups.

In our study we focused on the elements of the vertical information processing pathway with the addition of the RPE and the Müller glial cells. In the retinas of 36-month-old animals we observed a slightly loosened tissue structure both at light and electron microscopic levels, elevated GFAP expression in Müller glial cells and reduced number of rod bipolar cells and RGCs. Other age-related differences were detected at ultrastructural level: alteration of the retinal RPE and degenerated photoreceptor cells, especially rods, was evident. Ribbon synapses in the OPL were sparse and often fragmented (**Figure 10**).

We revealed well-defined alterations in degus that are also characteristic in human, mouse and rat retinal aging. The neuroretina together with RPE cells form a functional unit of the visual system. The RPE usually bears very long sheet-like apical microvilli that project into a complex matrix. During aging the RPE undergoes a number of well characterized changes, including increase in the number of residual bodies and accumulation of basal deposits (Garron, 1963; Guymer et al., 1999). We observed degeneration of the RPE/Bruch's/choriocapillary complex in 36-month-old degu retinas, possibly leading to altered tissue oxygen levels and contributing to photoreceptor cell loss (DiLoreto et al., 2006). The degeneration process is complex and involves the accumulation of deposits, RPE cell loss leads to formation of hypopigmented areas, and the development of hyperpigmented areas. This stage can progress to a proliferative neovascular

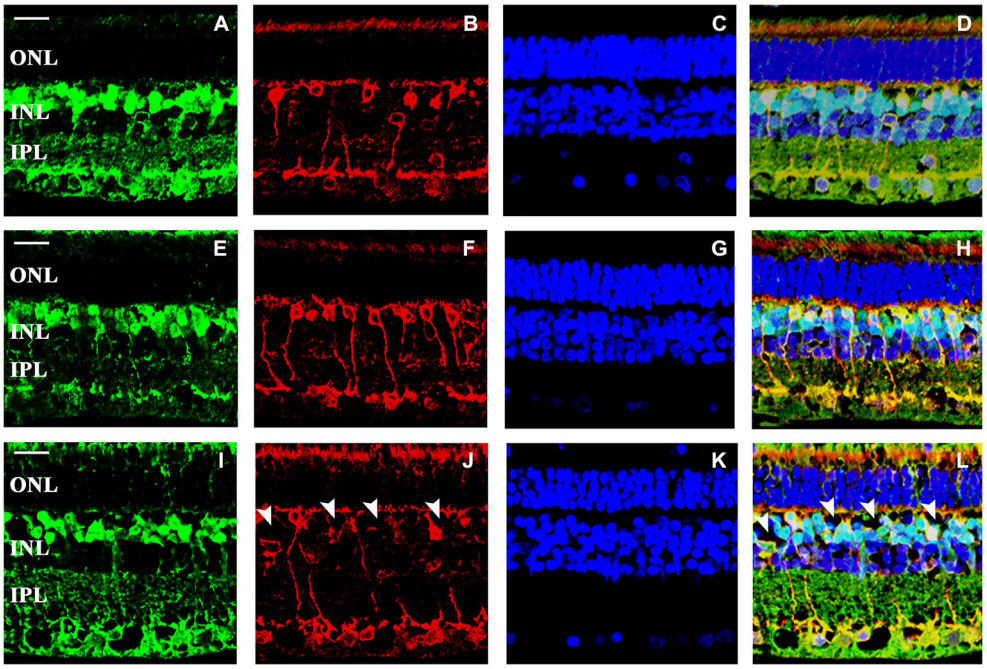

FIGURE 5 | Representative retinal sections stained with pan-bipolar and rod bipolar cell markers. Pan-bipolar cell marker (Chx10) labeled the somas and terminals of all bipolar cells (green—**A,E,I**; light blue—**D,H,L**), while protein kinase Cα (PKCα) labeled the somas, dendrites and axon terminals of rod bipolar cells (red—**B,F,J**; yellow, white—**D,H,L**). DAPI staining labels the nuclei of all retinal cells (dark blue—**C,G,K**). The staining pattern was similar in all groups **(A–L)**, except that empty cell body shapes (arrowheads) could be observed among the bipolar cells in the 36-month-old degus retina **(I–L)**. Abbreviations: ONL—outer nuclear layer; INL—inner nuclear layer; IPL—inner plexiform layer; PKCα—protein kinase Cα (PKCα). Scale bar: 20 μm.

(wet or exudative) form of degeneration characterized by the growth of choroidal vessels (choroidal neovascularization) or to a geographic form of atrophy characterized by damage of the RPE and of the neural retina (Limaye and Mahmood, 1987). Some signs of this process could already be seen at 36-months of age and we predict that these changes will become dominant at later ages. The observed elevated cone/rod bipolar cell ratio in the 36-month-old degu group indicates that the elements of cone pathway were more resistant to the age-related degeneration than that of rods. This parallels well with the observation that in the human retinas there is a decrease of approximately 54% of the total rod photoreceptor density between the fourth and ninth decades of life (Gao and Hollyfield, 1992; Aggarwal et al., 2007) whereas cone density remains essentially unchanged (Curcio et al., 1993). The decrease in rod density ultimately triggered the associated decline of neurons connected to them (Gao and Hollyfield, 1992; Aggarwal et al., 2007).

Photoreceptors transfer the visual signals to the post-receptorial retinal network; malfunctioning of this process due to degeneration of rods, rod bipolar cells, or ribbon synapses will lead to impaired vision. During human rod degeneration, surviving rods, horizontal and amacrine cells similarly extend anomalous neurits throughout the retina (Li et al., 1995; Fariss et al., 2000). Photoreceptor degeneration-dependent modifications in the synaptic machinery connecting photoreceptors with second-order neurons are evident: altered connectivity of rods and rod bipolar cells as well as horizontal cells affects retinal circuity. The normal pairing of presynaptic and postsynaptic markers are lost. The synaptic markers associated with photoreceptors and processes of bipolar and horizontal cells show abnormalities prior to significant photoreceptor loss (Cuenca et al., 2005). We report here age-dependent structural changes at the ribbon synapses in the synaptic terminals of rod photoreceptor and rod bipolar cells, which conforms well with these observations.

In contrast to the observed initial decline of ribbon synapses and rod bipolar cells density, the loss of synaptic sites was not complete in the aging degu retina, since Bassoon staining persisted marking the functional integrity of the arciform density. Currently we do not know whether the rods or the rod bipolars are lost first. In other pathological conditions, for example in a rat model of hyperoxia, the loss of bipolar dendrites takes place before photoreceptor death (Dorfman et al., 2011).

Putative structural modifications of the inner retina can be a consequence of aging. Liets et al. (2006) have indeed shown aberrant processes in rod bipolar neurons as a consequence of aging. Aberrant processes establish normally structured synapses ectopically (Terzibasi et al., 2009). As the degeneration of rod bipolar cells progresses they display early retraction and loss of dendrites (Cuenca et al., 2005). In 36-month-old degu retinas the rod bipolar cell dendrites were no longer erect and brushy but appeared flattened in contrast to the normal retina, where the fine

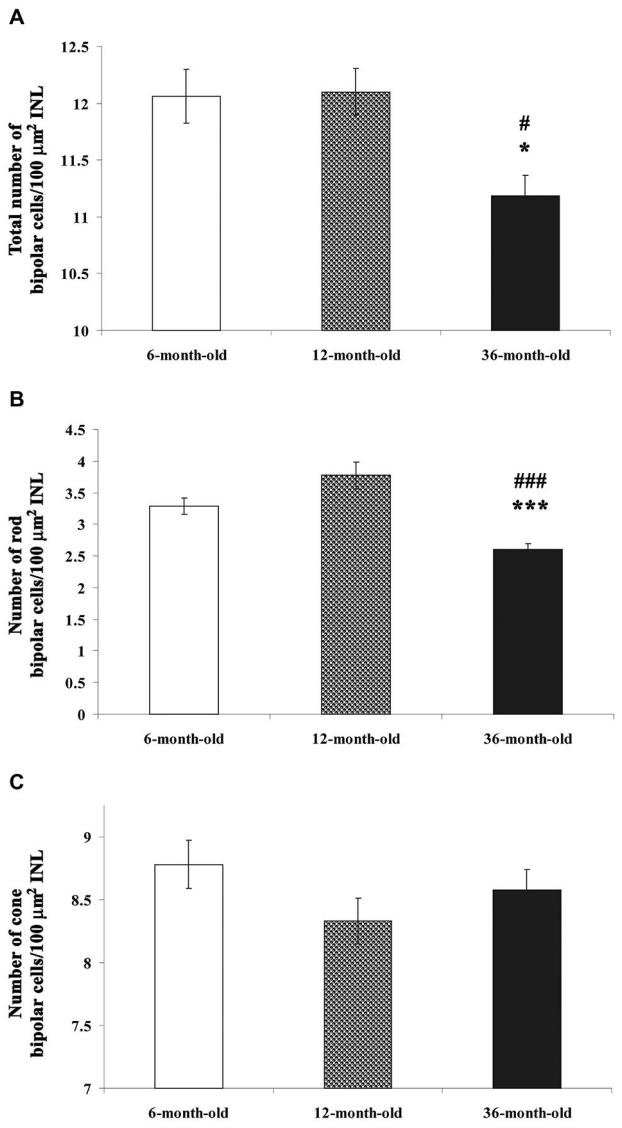

FIGURE 6 | Quantification of all bipolar cells (A), only rod (B) and cone bipolar cells (C) in each group. The cell number of all bipolar cells and rod bipolar cells/100 μm^2 INL was significantly decreased in 36-month-old degu retinas (**A,B**; $^{*}p < 0.05$; $^{***}p < 0.0001$ vs. 6-month-old; $^{\#}p < 0.05$; $^{\#\#\#}p < 0.0001$ vs. 12-month-old degu retinas). No differences could be observed in the cone bipolar cell number between the three groups **(C)**.

processes of rod bipolar cells penetrate the ONL, like those in the 6-month-old degus.

The PSD95 is a part of the dense structure attached to the postsynaptic membrane opposed to the presynaptic active zone to ensure normal synaptic transmission. The structural alterations in aged learning-impaired rats correlate with altered content of PSD proteins that are critically involved in normal synaptic function. The alterations in synaptic protein content resulted in reduced synaptic function (Nyffeler et al., 2007; Takada et al., 2008). Immunofluorescence for PSD95 was most prominent in the retina, the dendrites in the OPL opposed to the rod spherules and cone pedicles were strongly labeled (Koulen et al., 1998). The spatial distribution of PSD95 labeling on the bipolar cell dendrites was altered in the 36-month-old group: only a few puncta were detected, furthermore, labeling was not shown nearby and along the non-brushy dendritic arbors of rod bipolar cells, indicative of rod degeneration. At the same time, however, cones remained unaltered as it was proven by PNA-labeling. The specialization of rod and cone bipolar cells involves the differential expression of proteins involved in glutamatergic signaling. Hanna and Calkins (2007) described that 26 rod bipolar cells expressed at least one AMPA glutamate receptor subunit gene in monkey retina. This infers the presence of those scaffolding proteins (including PSD95) that are related to the ionotropic glutamate receptors (Hanna and Calkins, 2007). It is known that ischemia induces severe progressive inner retinal degeneration and down-regulation of synaptic proteins, such as PSD95 and synaptophysin (Guo et al., 2014). Although PSD95 is a very important protein in the retinal synaptic transmission in both the OPL and IPL, there is no information on its alteration in retinal aging. The decreased PSD95 expression in aging degu retinas suggested that the synthesis of PSD95 could be altered.

VGLUT1 was localized to photoreceptor and bipolar cell terminals, which is consistent with the function of photoreceptors and bipolar cells in vertical excitatory transmission with glutamate release in mammalian retina (Gong et al., 2006). As a result of photoreceptor degeneration VGLUT1-immunostaining was decreased in the OPL. Remodeling of bipolar cells during aging also affects their axon terminals. These reduced axon terminals of the rod bipolar cells showed reduced VGLUT1-staining and the shape and structure of terminals was also altered in 36-month-old degu retinas. Response of VGLUTs to diverse stimuli is altered with aging, for example after transient global cerebral ischemia in the rat brain (Llorente et al., 2013) or ischemia and excitotoxicity in the retina (Atlasz et al., 2008, 2010). Similarly to our findings, decreased VGLUT1-immunostaining was observed in aging ventral cochlear nuclei, possibly associated with age-related hearing loss (Alvarado et al., 2014).

These above observations suggest that both the input and the output synapses of the rod bipolar cells were affected in the aging degu retina. In contrast, the cone pathway appeared mostly unchanged in the aging degu retinas. It is possible that this only reflects the different time course of the degeneration of rods and cones in the aging process. Rod bipolar cells and rods disappear first, therefore, the secondary degeneration within the rod pathway is expected to occur earlier. This observed alteration in aging has serious consequences in the light of the fact that one rod bipolar cell makes synapses with multiple RT via their dendritic arbor (Wässle and Boycott, 1991). With advancing age, the reported significant decrease of the rods coincides with considerable reduction in the density of rod bipolar cells in humans (Gao and Hollyfield, 1992; Aggarwal et al., 2007), similarly to degus. Not all retinal cells are equally vulnerable to the effects of advancing age (Roufail and Rees, 1997). Marked differences in the 36-month-old degus were partly cell type specific, such as elevated GFAP expression in the Müller

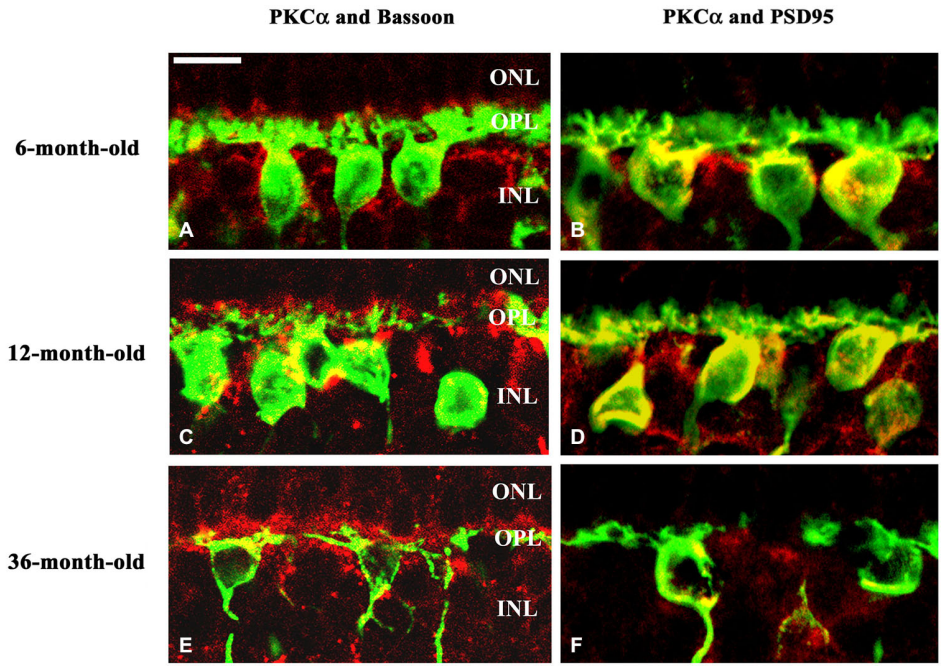

FIGURE 7 | Spatial relationship of the dendritic arbor of rod bipolar cells and photoreceptor terminals. Double immunostaining with PKCα (green) and Bassoon (red) antibodies **(A,C,E)**. The dendrites of rod bipolar cells were longer and more brushy in the 6- and 12-month-old degu retinas **(A,C)**, than in the 36-month-old retinas **(E)**. Double immunolabeling with PKCα (green) and PSD95 (red) antibodies shows the related alterations in the rod synaptic spherules and in the dendritic field of rod bipolar cells. PSD95 immunofluorescence is prominent in the OPL, where the axon terminals of rods, the so-called rod spherules, are labeled intensively in the 6- and 12-month-old degu retinas **(B,D)**. Decreased PSD95 labeling in the reduced dendritic arbors of rod bipolar cells was shown in 36-month-old aging degu retina **(F)**. Abbreviations: ONL—outer nuclear layer; OPL—outer plexiform layer; INL—inner nuclear layer; PKCα—protein kinase Cα; PSD95—postsynaptic density 95 protein. Scale bar: 10 μm.

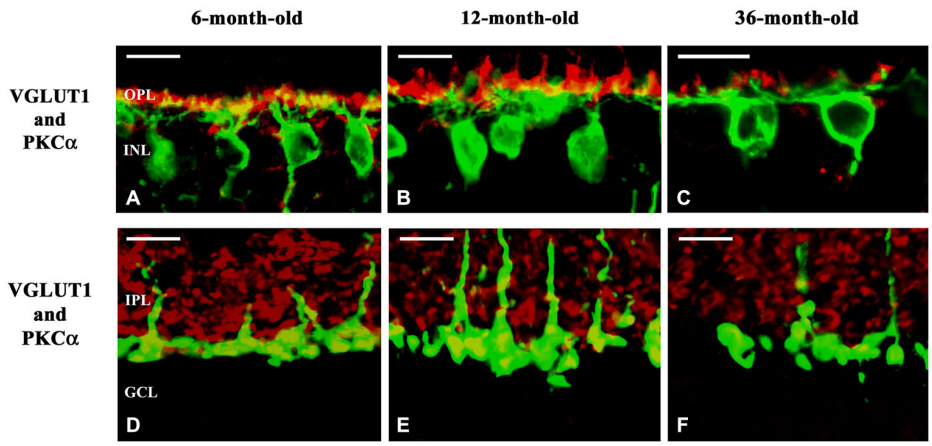

FIGURE 8 | Confocal microscopy of degu retinal sections immunolabeled with VGLUT1 (red) and PKCα (green). Subcellular localization of VGLUT1 in the outer **(A–C)** and inner plexiform layers **(D–F)** of 6- **(A,D)**, 12- **(B,E)** and 36-month-old **(C,F)** degu retinas. VGLUT1-immunopositive structures were found in photoreceptor terminals in close apposition to PKCα-positive rod bipolar cell dendrites in the OPL of 6- and 12-month-old degu retinas **(A,B)**. Altogether with the decreased dendritic arbor of the rod bipolar cells the VGLUT1-expression in photoreceptors also decreased in 36-month-old degu retinas **(C)**. VGLUT1-positive large puncta were co-localized with PKCα in the inner part of the IPL indicating VGLUT1 expression in axon terminals of rod bipolar cell in 6- and 12-month-old degu retinas **(D,E)**. In the 36-month-old degu retinas both the arbor of axon terminals of rod bipolar cells and their VGLUT1 expression decreased **(F)**. Abbreviations: OPL—outer plexiform layer; INL—inner nuclear layer; IPL—inner plexiform layer; GCL—ganglion cell layer; PKCα—protein kinase Cα; VGLUT1—vesicular glutamate transporter 1. Scale bar: 10 μm.

glial cells, photoreceptors (especially rods) and rod bipolar cell loss with the alteration in their synaptic profile (ribbon synapse and altered dendritic trees: no longer erect and brushy). In contrast the bipolar cells of mice show arbor-specific alteration;

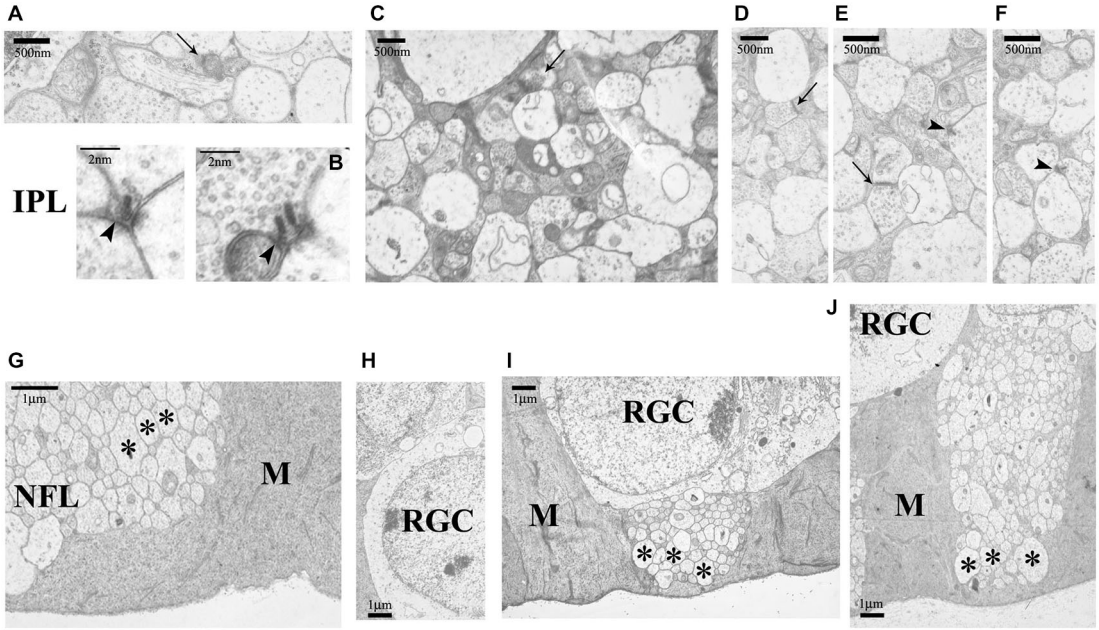

FIGURE 9 | Ultrastructure of IPL, GCL and nerve fiber layer (NFL) in the 6-, 12- and 36-month-old degu retinas. In the IPL conventional (arrows) and ribbon synapses (arrowheads) were also present in all groups (6-month-old: **(A,B)**; 12-month-old: **(C)**; 36-month-old: **(D–F)**). The NFL is intact in all age groups (6-month-old: **(G,H)**; 12-month-old: **(I)**; 36-month-old: **(J)**; asterisks: nerve fibers). Abbreviations: IPL—inner plexiform layer; M—Müller glial cell; NFL—nerve fiber layer (NFL); RGC—retinal ganglion cell. Scale bars are indicated in the pictures.

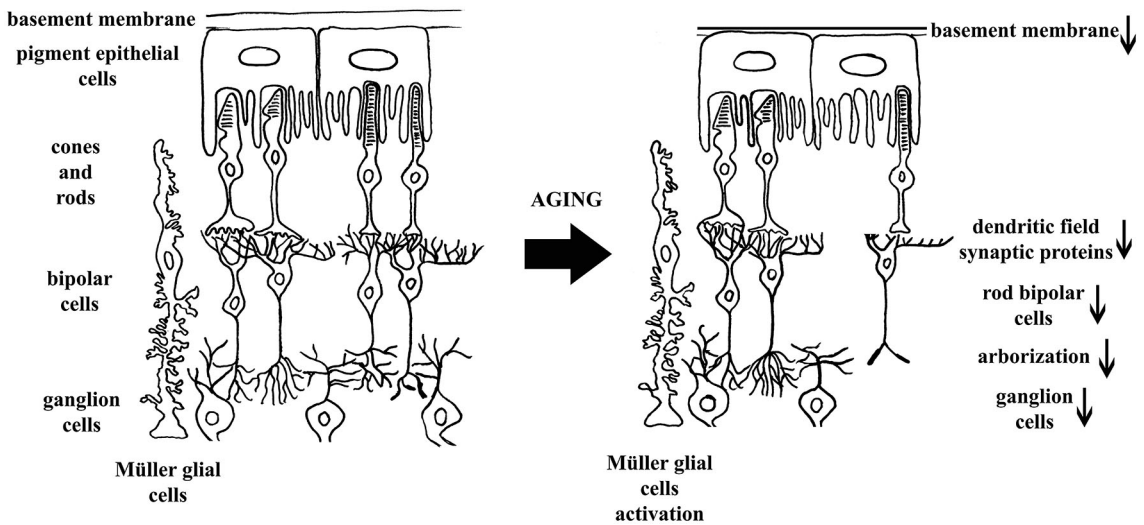

FIGURE 10 | Schematic representation of concluded structural and cellular changes during retinal aging in 36-month-old degu retina. In the left panel major structural elements of the vertical processing pathway are indicated while in the right panel the alterations caused by aging retina are emphasized. The direction of changes is indicated with arrows.

their dendrites are sprouted but remain stable (Samuel et al., 2011).

Furthermore, the number of RGCs, the output neurons of the retina, was altered in degus. The prominent loss of RGC axons in the optic nerve as described across mammalian species must translate *ipso facto* to the corresponding decline in RGC bodies in the retina, whether RGC bodies in the retina are also susceptible to age-related loss (Calkins, 2013). The number of RGC bodies in the rat and mouse retina does not change with age though the retina itself enlarges and RGC shrink with a concomitant decrease in the density of IPL synapses (Harman et al., 2003; Samuel et al., 2011). In contrast, only the human retina appears to

progress to actual RGC body loss with age; perhaps progression in normal aging depends on the actual extent of the lifetime (Calkins, 2013). Degu retina in this respect behaves like human retina, making it a suitable model for examining ganglion cell loss mechanisms. Altogether, these data reveal selective age-related alterations in the neural circuitry in the degu retina. These changes in the degu retina seem more closely related to those observed in human retinal aging than alterations observed in other rodents, such as rats and mice. Animal models of retinal aging have usually employed nocturnal species (e.g., rats and mice), however, to better approximate the human retinal changes during aging, the diurnal rodent, *Octodon degus* is more useful, since the (i) rod/cone ratio is similar to the human ratio; (ii) the diurnal behavior is charactheristic to this species and its activity pattern resembles that of the human; (iii) its lifespan is considerably longer than those of the other experimental models, therefore the age-related changes can be better monitored and compared to those described in the human retina; and (iv) other, age-related diseases (e.g., cataract, diabetes, Alzheimer's disease, cancer) often appear spontaneously in this species (Jacobs, 1993; Brown and Donnelly, 2001; Chávez et al., 2003; Peichl et al., 2005; Ardiles et al., 2013; Palacios-Muñoz et al., 2014).

The proper functioning of the nervous system (including that of the retina) depends on the underlying structure of neural networks. Any loss of the pre- and/or postsynaptic profiles of the retinal neurons causes changes in their morphology and function. As a consequence, these retained neurons may be capable to establish new synaptic contacts (ectopic synapses, unusual synaptic arrangements), so the retina may undergo marked remodeling. In the aging degu retina, the synaptic rearrangements/alterations in the OPL and potentially concomitant synaptic alterations in the IPL efficacy would reduce transmission, while the loss of function of the RPE cells would alter the homeostasis of the retinas. The neuronal elements of the vertical pathway such as rod bipolar and ganglion cells were seen affected already at 36-month of age. We think that these structural changes become more obvious, will reach other cells and will more seriously affect the synaptic complexes with advancing age.

The proven similarities between the degus and the human retina in their structure and aging processes offer a possibility to develop potential treatments and therapies for retinal age-related alterations and diseases.

Authors Contribution

KS researched data and wrote, edited and reviewed the manuscript; CE, EF-V, ET, VI and GS Jr. researched data; RG and MTH researched data, reviewed and edited the manuscript; DR and AT reviewed the manuscript.

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IX.II Tarragon E, Lopez D, **Estrada C**, Gonzalez-Cuello A, Ros CM, Lamberty Y, Pifferi F, Cella M, Canovi M, Guiso G, Gobbi M, Fernández-Villalba E, Blin O, Bordet R, Richardson JC, Herrero MT.“Memantine prevents reference and working memory impairment caused by sleep deprivation in both young and aged *Octodon degus”*.*Neuropharmacology*. **2014** 85:206-214.
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Abstract

Memory loss is one of the key features of cognitive impairment in either aging, Mild Cognitive Impairment (MCI) or dementia. Pharmacological treatments for memory loss are today focused on addressing symptomatology. One of these approved compounds is memantine, a partial NMDA receptor antagonist that has proved its beneficial effects in cognition. The *Octodon degus* (O. degus) has been recently proposed as a potential model relevant for neurodegenerative diseases. However, there are no previous studies investigating the effect of pharmacological treatments for age-related cognitive impairment in this rodent. In this work we aimed to evaluate the effect of memantine on sleep deprivation (SD)-induced memory impairment in young and old O. degus. Young and old animals were trained in different behavioral paradigms validated for memory evaluation, and randomly assigned to a control (CTL, n=14) or an SD (n=14) condition, and treated with vehicle or memantine (10-mg/Kg i.p.) before the SD started. We demonstrate that SD impairs memory in both young and old animals, although the effect in the old group was significantly more severe ($P<0.05$). Memantine pretreatment was able to prevent the cognitive impairment caused by SD in both age groups, while it had no negative effect on CTL animals. The positive effect of memantine in counteracting the negative effect of SD on the retrieval process even in the aged O. degus further supports the translational potential of both the challenge and the species, and will enable a better understanding of the behavioral features of memantine effects, especially related with reference and working memories.

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Memantine prevents reference and working memory impairment caused by sleep deprivation in both young and aged *Octodon degus*

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ABSTRACT

Memory loss is one of the key features of cognitive impairment in either aging, Mild Cognitive Impairment (MCI) or dementia. Pharmacological treatments for memory loss are today focused on addressing symptomatology. One of these approved compounds is memantine, a partial NMDA receptor antagonist that has proved its beneficial effects in cognition. The *Octodon degus* (*O. degus*) has been recently proposed as a potential model relevant for neurodegenerative diseases. However, there are no previous studies investigating the effect of pharmacological treatments for age-related cognitive impairment in this rodent. In this work we aimed to evaluate the effect of memantine on sleep deprivation (SD)-induced memory impairment in young and old *O. degus*. Young and old animals were trained in different behavioral paradigms validated for memory evaluation, and randomly assigned to a control (CTL, $n = 14$) or an SD ($n = 14$) condition, and treated with vehicle or memantine (10-mg/Kg i.p.) before the SD started. We demonstrate that SD impairs memory in both young and old animals, although the effect in the old group was significantly more severe ($P < 0.05$). Memantine pretreatment was able to prevent the cognitive impairment caused by SD in both age groups, while it had no negative effect on CTL animals. The positive effect of memantine in counteracting the negative effect of SD on the retrieval process even in the aged *O. degus* further supports the translational potential of both the challenge and the species, and will enable a better understanding of the behavioral features of memantine effects, especially related with reference and working memories.

1. Introduction

Regardless of cause, i.e. dementia or physiological aging, age-related cognitive impairment directly affects several psychological domains crucial for daily functioning, such as attention and memory (Masdeu et al., 2012). However, as certain functional loss is expected to occur in aging, it also may be indicative for other more severe conditions to come. Small but consistent differences in the behavioral and physiological outcome may distinguish these

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impairments. Among these different profiles, Mild Cognitive Impairment (MCI) is especially worth considering because its appearance frequently anticipates the development of Alzheimer's disease (AD) (Lee et al., 2012; Vos et al., 2013). One of the principal challenges to prevent this functional loss is to elucidate the interactions between pathophysiological features that underlie the cognitive decline (Laursen et al., 2003; Reitz, 2012). Current pharmacotherapy of AD lacks strategies that totally prevent or cure the disease. Instead, the main focus of the approved pharmacological treatment is on alleviating the clinical symptoms associated with AD (Francis et al., 2010).

The importance of the glutamatergic N-methyl-D-aspartate (NMDA) receptor in memory and learning processes is well recognized (Myhrer, 2003). Moreover, given the role of NMDA-mediated excitotoxicity in disease, it has been suggested that these receptors underlie some of the age-related changes in neurotransmission that lead to an impaired signaling process (Wenk, 2006; Parsons et al., 2007). It is not surprising then that glutamatergic neurotransmission has been one of the main targets in the development of different pharmacological strategies for AD symptomatology (Danysz et al., 1997; Minkeviciene et al., 2004; Borre et al., 2012). Memantine, a low-affinity non-competitive NMDA receptor antagonist is the only glutamatergic drug approved for the treatment of moderate-to-severe AD cognitive symptomatology (Ballard et al., 2011). However, there is some controversy regarding this drug, as some studies in rats find no enhancing cognitive effects, or even a memantine-induced cognitive deficit (Creeley et al., 2006; Quan et al., 2011).

Among the paradigms used to induce transient cognitive impairment, sleep deprivation (SD) is a consolidated one of them having some advantages (Alhaider et al., 2011; McEwen and Chattarji, 2004). It has been widely demonstrated that this procedure effectively induces temporary cognitive deficits analogous to those shown by patients with AD-like dementia (Aleisa et al., 2011; Alzoubi et al., 2012). Preclinical research using animal models for the study of age-related disorders is crucial for the development and improvement of pharmacological strategies. However, the traditional animal models of age-related cognitive impairment used in pharmacotherapy research exhibit questionable translational value. Despite the priceless knowledge that these models have provided to the study of AD, the vast majorities need genetic and/or pharmacological manipulation to reach the inherent pathophysiological state of Alzheimer's (Götz et al., 2004; Braidy et al., 2012), which it remains an open issue in ecological terms. The *Octodon degus* (*O. degus*) is a diurnal rodent recently proposed as a putative model for aging disorders (i.e. age-related cognitive decline), and more specifically for neurodegenerative diseases because of its physiopathological characteristics (Braidy et al., 2012; Tarragon et al., 2013). For instance, this rodent spontaneously develops histopathological hallmarks reminiscent of AD (such as β-amyloid depositions and hyperphosphorylated tau tangles), approximately at three or four years of age (Inestrosa et al., 2005; Ardiles et al., 2012) and is insulin resistant, features that are both common in the clinical manifestations of AD patients. Taking into account that the *O. degus*' life span may reach more than 9 years (Lee, 2004), it makes this rodent a valuable alternative to standard rats or triple-transgenic mouse models.

To our knowledge, there is no previous literature comparing the combination effect of age and pharmacotherapy for cognitive decline in this novel model. Moreover, the studies in aged long-living animals within this theoretical and experimental framework are remarkably scarce. Thus, because of the advantages this rodent beholds as a model of neurodegenerative disease, we explored the effect of a marketed symptomatic agent on the different cognitive domains affected in AD and other age-related disorders.

2. Material and methods

2.1. Animals

For the behavioral experiments, 28 female adult (16 young of 18 months; 12 old of 46–48 months) *O. degus* (180–270 g) were provided by the animal facilities from the University of Alicante. For biochemical experiments, 8 female adult (24 months) *O. degus* were provided by the same facilities. *O. degus* were transported and housed individually in opaque glass cages (40 × 25 × 25 cm) at the animal facilities of the University of Murcia and housed under a maintained temperature of 21 ± 1 °C and a 12 h light/dark cycle (lights on at 7 a.m.). The floors of the cages were covered with wood shavings that were changed once a week. During this habituation period, each animal received water *ad libitum* and 120 g food pellets (Harlan Tekland Global Diet®, Harlan laboratories) per day. The "Three R's principle" was carefully applied in this study, following the most current suggestions from the European Community Council Directive for animal care and experimentation as regards the number of animals to be used in preclinical studies. All experimental procedures complied with the European Community Council Directive (2010/63/UE) and the ethical committee of the University of Murcia.

2.2. Drugs and solutions

Memantine hydrochloride was obtained from Sigma–Aldrich (Madrid, Spain) and stored at room temperature. Memantine was diluted in physiological saline and administered intraperitoneally (i.p.).

2.3. Analysis of memantine levels in plasma

Given the lack of information regarding the pharmacokinetic (PK) properties of memantine in *O. degus*, the dose of memantine to be administered in these experiments was selected under the assumption that the PK of memantine in *O. degus* is similar to the one shown by other rodents. We allometrically extrapolated memantine PK parameters in *O. degus* from human parameters (Kornhuber et al., 2007), i.e. clearance and volume of distribution were linked to body weight using the following equations:

$$CL_{O.\ degus} = CL_{human} \times BW^{0.75}$$

$$V_{O.\ degus} = V_{human} \times BW$$

Where CL is the clearance, *V* the volume of distribution and BW the body weight of the *O. degus*.

For the analysis of the pharmacokinetic response of memantine 8 animals were injected with memantine (10 mg/kg i.p.) and then anaesthetized with Isoflurane (Isoba® vet, USA), administered with a continuous flow vaporiser (MSS3, Medical Supplies & Services, England, UK). Blood was extracted from the saphenous vein after 15 and 30 min, and 1, 2, 4, 8 and 24 h after the administration of memantine. Given the rapid recovery from Isoflurane, animals were anaesthetized before each extraction time point. Blood samples were collected in tubes containing heparin, gently shaken by inversion and kept in ice. Plasma was obtained after blood centrifugation at 10,000 × *g* for 5 min at 4 °C, immediately frozen on dry ice and kept at −80 °C until analysis. Memantine was extracted from plasma samples by liquid–liquid extraction procedure. Aliquots of 50 μL were added with internal standard (IS, amantadine) and 10 μL of 25% ammonium hydroxide. Samples were extracted two times with 8 volumes of n-hexane, shaked for 15 min and centrifuged at 5000 × g for 5 min. The combined extracts were dried under a gentle nitrogen stream at room temperature, and samples were finally reconstituted in methanol to be injected in the HPLC-MS/MS system maintained at 6 °C. The recovery of memantine with this procedure was 89 ± 8%.

The HPLC system consisted of an Alliance separation module 2695 coupled with a Micromass Quattro Micro triple quadrupole mass spectrometer (Waters) controlled by Mass Lynx software 4.1. The mass spectrometer operated in positive ion and multiple reaction monitoring (MRM) mode, measuring the fragmentation products of molecular ions of substances. The instrument is equipped with an electrospray ionization interface and uses argon as collision gas. Source and desolvation temperatures were set at 100 and 300 °C respectively. Samples were analyzed with the ion spray needle operating at 4.5 kV, the cone voltage at 28 V and the collision energy at 20 eV. The principal ion transition 180.2 > 163.0 was selected for memantine quantification, while the transition 152.0 > 135.1 was selected for IS. Chromatographic separation was achieved on a Waters XTerra MS C18 column (100 × 2.1 mm, 3.5 μm) coupled with an XTerra C18 cartridge, held at 30 °C. The mobile phases consisted of water (MP-A) and MeOH:water 80:20 (MP-B), both containing 0.05% formic acid. The HPLC system was set up to operate at a flow rate of 0.2 mL/min at the following linear gradient: from 0% to 100% MP-B in 10 min, at 100% MP-B for 6 min, from 100% to 0% MP-B in 1 min (total run time 37 min). The retention times were 14.2 min for IS and 15.1 min for memantine. Analytes were quantified by reference to calibration curves run at the beginning of each series of assays. Each calibration curve was linear (R^2 > 0.99) over the concentration range of 0.3–3000 ng/mL plasma, and no interfering peaks were observed in blank extracts. The quality of analytical results was checked by assaying quality control samples in every series of assays, always within 20% error.

2.4. Sleep deprivation

The SD procedure used in this study consisted of interrupting the sleeping cycle of the animal with a soft tactile stimulus and or with a gentle jostling of the home cage whenever the animal showed signs of sleep (more than 1 min of inactivity). The procedure began with the lights-off period (7 p.m.) and lasted for 12 h, thus encompassing the entire sleeping cycle. At 7 a.m., the lights were turned on again, thereby starting the diurnal phase. Animals were subjected to a single session of SD before the test, and were allowed to recover their sleeping routine (15 days) before the next session.

2.5. Radial arm maze (RAM)

Eight dark dull glass horizontal arms (57 × 11 cm) were placed radially around a central platform. To prevent the animal moving from one arm to another without returning to the central platform, partial walls (17–2 cm high; 15 cm long) were located at the entrance and along each arm, respectively. Similarly to other works using this paradigm in this rodent model (Kumazawa-Manita et al., 2013), all animals were habituated to the paradigm before the training and test sessions. In the habituation period (day 0), animals were moved from the colony and placed on the central platform. Then, they were allowed to explore the maze freely for 15 min. Following the habituation, the animals were trained for 1 session per day on 7 consecutive days. One piece of reinforcer (food pellet) was randomly placed at the end of four arms and the animal was allowed to freely explore the maze. Arms baited remained the same for each animal during the training phase. A training session ended after one of the following criteria was reached: a) 10 min had passed, b) the animal entered the 8 arms, or c) 2 min passed since the last arm entrance. An "entry" was defined as the animal introducing its entire body (except for the tail) into the arm. The total number of arm entries was recorded for later analysis. Between sessions, the maze was wiped clean with 70% ethanol to prevent the animal from using odor cues to solve the task. On the test day, animals were removed from the maze after entering the four arms that were previously baited during the training sessions. Performance level was determined by analyzing a) reference memory errors (entering an arm that had no reinforcer during the training period); and b) working memory errors (counting an error as an entry in a previously visited arm in the same session).

2.6. Barnes maze (BM)

The procedure was divided into 3 phases: habituation, training and test phases, performed in a similar way as previous works that used this paradigm in this rodent model (Popović et al., 2010). Briefly, following the habituation phase, animals were trained for 7 days, and exposed to a memory-retrieval session a week later the final training session (Test phase). Both training and test sessions consisted of 4 consecutive 4 min trials, separated by a 5 min resting phase in the animal home cage. At the beginning of each trial, the animal was confined for 30 s in the start box in the center of the maze. If the animal did not enter the escape box within the allotted time, it was manually picked up and placed in the escape box, where it remained undisturbed for 2 min. The surfaces of the maze platform and start box were thoroughly cleaned with alcohol between trials. The response parameters measured and subsequently analyzed were: a) reference memory errors (every first visit of a non-escape hole in each trial); and b) working memory errors (repeated visits to the same non-escape hole in the same trial).

2.7. Novel object recognition (NOR)

For this test we modified a previous object recognition protocol used in the *O. degus* (Popović et al., 2009). Briefly, animals were moved in their home cages to the procedure room and remained undisturbed for 30 min. After this period, animals were exposed to a 10 min familiarization assay and then tested in 2 consecutive 5 min assays, with a 1-h inter-trial interval. For the familiarization trial, 2 toy objects were randomly named as "Object A" and "Object B". The objects were placed in the corners of the home cage and the animal was allowed to freely explore the field for 10 min. Following this period, objects were removed from the cage and wiped with 70% ethanol to remove odors. One hour after the familiarization assay, the animals were tested in a "novel location recognition" (NLR) test in which one of the familiar objects (Object B) was moved to an adjacent unoccupied corner. After 5 min, the objects were removed and cleaned with ethanol. One hour later, the *O. degus* were tested in a "novel object recognition" (NOR) test in which one of the familiar objects (Object B) was replaced by a different, but similar, object. Familiarization and testing times were recorded, and the time spent exploring each object was later measured. Here, 'exploration' was defined as approaching to within 1–3 cm of the object. To quantitate NLR and NOR, a recognition index (RI) was calculated as $RI = T_B/T_B + T_A$

2.8. Experimental design

Since the *O. degus* is considered a diurnal rodent, all of the experiments were performed in the daytime; between 7 a.m. and 10 a.m. Animals were left undisturbed for 7 days prior to the start of the behavioral experiments. On the following week, the *O. degus* underwent handling procedures (5 min per day) to become accustomed to experimenters manipulation. In the context of the RAM test, one week before the training started animals were gradually food-deprived (at 80% of their free-feeding weight). This decision was made to avoid sweetened reinforcers because of the high risk that *O. degus* develop diabetes (Okamoto et al., 2009).

Both young and old animals alternatively underwent the RAM and the BM training and testing (Fig. 1). For both paradigms, the first test after training (days 8, 17, 26, and 41, respectively for RAM and BM) was performed under normal sleep condition (No SD), whereas sleep deprivation (SD) was conducted before the second test (days 9, 18, 33 and 48, respectively for RAM and BM). Animals were randomly administered vehicle (saline) or memantine at 10 mg/kg (i.p.) 15 min before the SD period started. As NOLR does not require a training phase, the old group was tested two days following the young group. After a period of four weeks in which animals were undisturbed, the same experimental procedure was repeated and counterbalanced, so animals that were previously injected with vehicle would now receive memantine and vice versa. Thus, we collected data from four different experimental groups: vehicle/No SD condition (control group, $n = 14$); vehicle/SD ($n = 14$); memantine 10 mg/kg/No SD ($n = 13$); and memantine 10 mg/kg/SD ($n = 13$). One animal from the young group was eliminated because it died during memantine administration. The estrous cycle was not taken into consideration for the design, as there is controversy on the effects of this cycle in memory of female rodents (Stackman et al., 1997; Hornung et al., 2007).

2.9. Statistical analysis

In the RAM and BM tests, the total number of reference memory and working memory errors across all sessions were considered to be the dependent variables. In the BM, values are expressed as the mean of the four assays included within a session. In the NOR test, the recognition index was analyzed. To examine the learning of the different age groups in the RAM and BM, a Two-Way ANOVA with repeated measures was performed. To analyze the effect of sleep deprivation and memantine treatment, a Two-Way ANOVA was performed as required. Tukey and Dunnett's post hoc comparisons were used when appropriate. Normal distribution was confirmed by a fitting test of the data. All statistical analyses were performed using the Statistica 9.0 (StarSoft, Tulsa, OK) software package.

3. Results

3.1. Plasma levels of memantine after i.p. treatment

Plasma levels of memantine, measured at different time points after a single i.p. injection of 10 mg/kg, are shown in Fig 2. The highest concentration (about 500 ng/mL) was measured at the first time point considered (15 min) with a decrease thereafter and an estimated half-life of about 6.5 h. Levels in the range of 200–500 ng/mL are present during the sleep-deprivation period.

3.2. Learning of the behavioral tasks RAM and BM

A two way ANOVA within subjects was conducted to analyze the task acquisition level at the end of the training sessions. The analysis showed a significant effect of training in both reference memory errors [$F(6,156) = 32.64$; $P < 0.01$] and working memory errors [$F(6,156) = 44.12$; $P < 0.01$] committed in the RAM, compared with day 1 of exposition. Both measures are represented (Fig. 3A and B). In the BM, the analysis also indicated a significant reduction in the number of reference memory errors [$F(6,156) = 27.14$; $P < 0.01$] (Fig. 3C) and in the number of working memory errors [$F(6,156) = 13.23$; $P < 0.01$] (Fig. 3D), compared with day 1 of exposition. A Dunnett's post hoc test indicated that there were no differences in the learning between young and old animals.

3.3. Effect of memantine on memory impairment induced by sleep deprivation in young and old animals

3.3.1. Effect of memantine on sleep deprivation in young and old animals measured by the RAM

A Three-Way ANOVA (Dose × Age × Condition) was conducted to analyze the effect of memantine on age and SD. Results on reference memory errors showed a significant effect of Dose [$F(1,44) = 63.78$; $P < 0.01$], Age [$F(1,44) = 20.01$; $P < 0.01$], and Condition [$F(1,44) = 20.81$; $P < 0.01$]. The analysis also revealed an interaction effect between Dose and Condition [$F(1,44) = 42.77$;

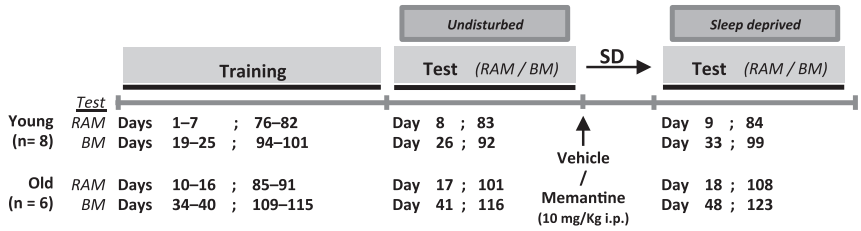

Fig. 1. Design of experimental phases 1 and 2. In phase 1, young and old animals were trained alternatively in the RAM and BM (young RAM, old RAM; young BM, old BM; young NOR; old NOR), and randomly assigned to one of the two sleep conditions before the test. In phase 2, the same training + test scheme was followed, although the animals were administered either vehicle or memantine (10 mg/kg, i.p.). A four-week resting period was introduced between phases.

$P < 0.01$], and between Age and Condition [$F(1,44) = 5.10$; $P < 0.05$]. A more detailed examination showed significant differences between age groups under SD condition compared to the No SD condition when animals were injected with vehicle ($P < 0.01$). A comparison between age groups treated with vehicle indicated that old animals committed more errors than the young ones under SD condition ($P < 0.05$). When animals were treated with 10 mg/kg of memantine no differences were found between age groups. However, both young ($P < 0.01$) and old ($P < 0.01$) animals performed better compared with the groups treated with vehicle under SD condition (Fig. 4A).

As regard with the working memory errors, analysis indicated a significant effect of Dose [$F(1,44) = 15.17$; $P < 0.01$], Age [$F(1,44) = 12.30$; $P < 0.01$], and Condition [$F(1,44) = 30.77$; $P < 0.01$]. An interaction effect between Dose and Condition [$F(1,44) = 30.54$; $P < 0.01$], and between Dose, Age and Condition [$F(1,44) = 6.09$; $P < 0.05$] was also found. A post hoc Tukey test revealed significant differences. Animals injected with vehicle committed more errors under the SD condition ($P < 0.01$). When treated with 10 mg/kg memantine, old animals under SD performed significantly better compared to vehicle treated controls ($P < 0.01$). No differences were found either between young and old animals or sleep conditions when 10 mg/kg of memantine was administered (Fig. 4B). Effect of time was neither found with respect to reference memory or working memory errors.

3.3.2. *Effect of memantine on sleep deprivation in young and old animals measured by the BM*

A similar Three-Way ANOVA (Dose × Age × Condition) was conducted to analyze the memory errors. The analysis of the reference memory errors showed a significant effect of Age [$F_{(1,44)} = 18.58$; $P < 0.01$], Dose [$F(1,44) = 9.40$; $P < 0.01$] and Condition [$F(1,44) = 52.42$; $P < 0.01$]. The ANOVA also revealed an interaction effect between Dose and Age [$F(1,44) = 19.89$; $P < 0.01$], and Dose and Condition [$F(1,44) = 19.68$; $P < 0.01$]. A further analysis exposed significant differences. When animals were treated with vehicle under SD, animals committed more errors compared with the No SD condition ($P < 0.01$). Also, under this condition, old animals performed significantly worse than young animals ($P < 0.01$). When animals were treated with 10 mg/kg memantine, the number of errors was significantly reduced with respect to the similar age groups and sleep condition in the vehicle treatment ($P < 0.05$, $P < 0.01$). No differences were found between sleep condition and age groups when animals were treated with 10 mg/kg of memantine (Fig. 4C).

Working memory errors were analyzed likewise. The ANOVA showed a significant effect of Age [$F(1,44) = 10.76$; $P < 0.01$], Dose [$F(1,44) = 35.96$; $P < 0.01$] and Condition [$F(1,44) = 23.72$; $P < 0.01$]. The analysis also revealed an interaction effect between Dose and Age [$F(1,44) = 10.05$; $P < 0.01$], and Dose and Condition [$F(1,44) = 39.15$; $P < 0.01$]. Tukey test indicated significant differences in both young ($P < 0.01$) and old ($P < 0.01$) groups under the SD condition compared to the No SD condition when animals were treated with vehicle. Moreover, under SD, with after vehicle treatment old animals committed more errors than young animals ($P < 0.05$). Also, both young and old animals performed significantly better under SD condition when memantine was administered, comparing to vehicle treatment. No differences were found between age groups and conditions when animals were treated with 10 mg/kg of memantine (Fig. 4D). Effect of time was neither found with respect to reference memory or working memory errors.

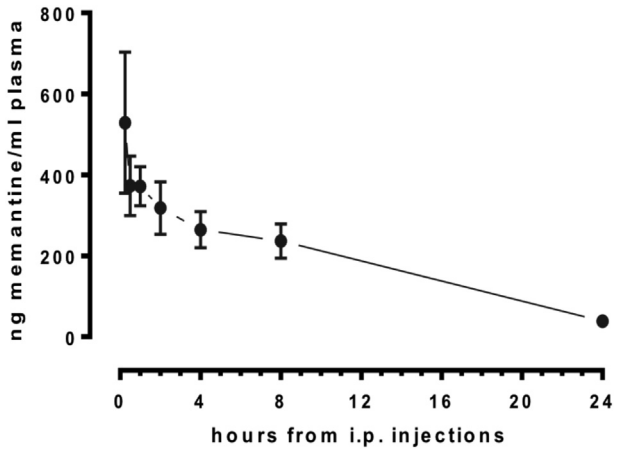

Fig. 2. Plasma levels of memantine at different time points after an acute intraperitoneal (i.p.) administration of 10 mg/kg. Each point in the graph represents the mean ± SD of four octodons.

3.3.3. *Effect of memantine on sleep deprivation in young and old animals measured by the NOR test*

The analysis of the effect of memantine measured by the NOR test indicated no significant effect of Dose, Age, or Condition in the familiarization phase, taking the RI as the dependent variable (Fig. 5A). In the NLR trial, analysis revealed a significant effect of Dose [$F(1,44) = 19.26$; $P < 0.01$] and Condition [$F(1,44) = 28.87$; $P < 0.01$]. Also, ANOVA showed an interaction effect between Dose and Condition [$F(1,44) = 25.76$; $P < 0.01$]. A Tukey post hoc test informed of significant differences in young ($P < 0.01$) and old ($P < 0.01$) animals under the SD condition (Fig. 5B). Differences within the same age group were found when compared with the doses of vehicle and 10 mg/kg of memantine under SD ($P < 0.01$). No differences were found after the administration of 10 mg/kg memantine. The object recognition assay was similarly analyzed. The ANOVA analysis showed a significant effect of Dose [$F(1,44) = 14.66$; $P < 0.01$] and Condition [$F(1,44) = 27.24$; $P < 0.01$]. Also, ANOVA showed an interaction effect between Dose and Condition [$F(1,44) = 14.47$; $P < 0.01$]. More extensive analyses

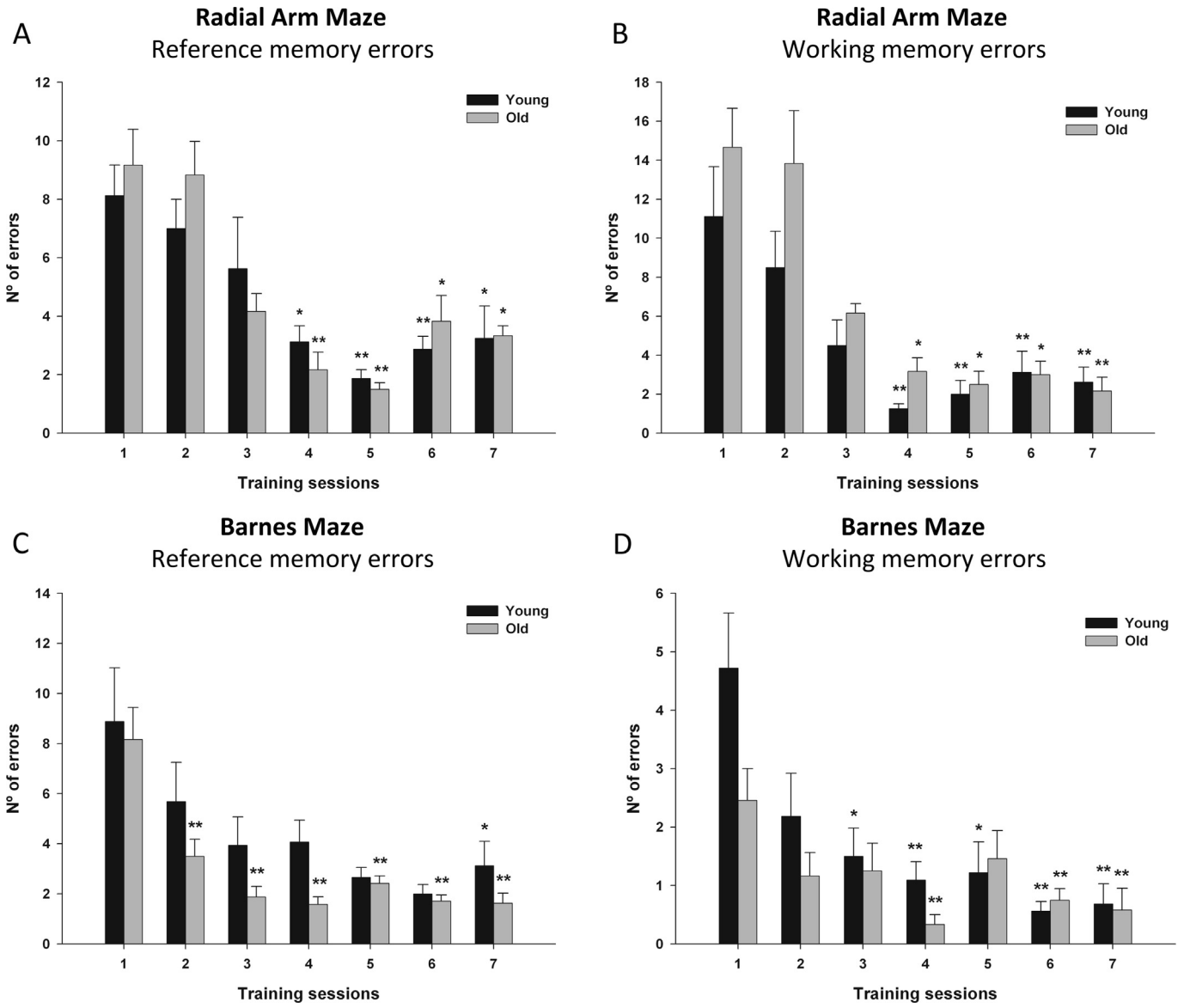

Fig. 3. Learning curve in the RAM and BM. Number of reference memory errors and working memory errors in the RAM (A and B) and in the BM (C and D) committed by young ($n = 8$) and old ($n = 6$) *O. degus* through the 7-day training phase of both paradigms. Results are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared to day 1 of exposition.

revealed that the RI of both sleep deprived young and old animals is significantly lower when compared with the No SD condition following administration of vehicle (Fig. 5C). Furthermore, animals that were treated with 10 mg/kg of memantine showed differences with the animals treated with vehicle as regard to the RI under SD ($P < 0.05$, $P < 0.01$). Differences between age groups and sleep conditions were not significant when a dose of 10 mg/kg memantine was administered.

4. Discussion

This work aims to explore the effects of memantine on the cognitive impairment induced by sleep deprivation in a rodent model of *O. degus* of two different group ages (young and old). Using the pharmacokinetic parameters described above, we calculated the doses that would approximately produce the same exposure of memantine in *O. degus* as observed in humans. We then compared the extrapolated doses with the doses of memantine that were previously reported in other rodents: in experiments with mice, 1 mg/kg, orally administered, memantine is considered a low dose, whilst 30 mg/kg is deemed a high dose (Yiğit et al., 2011). In rats, doses ranges between 0.2 and 25 mg/kg intravenously are reported (Chen et al., 2012; Kornhuber and Quack, 1995), covering both pharmacological and toxicological relevant ranges. Combining this information with allometric extrapolations from human PK, a dose of 10 mg/kg was selected (unpublished results). Intraperitoneal administration was chosen to avoid the variability in absorption associated with oral administration. It has been described that the therapeutic schedules, indicated by several preclinical studies, are those producing a steady-state plasma drug level of around 200 ng/mL (Minkeviciene et al., 2004; Zajaczkowski et al., 1996; Periclou et al., 2006). In rats, these levels are obtained after acute intraperitoneal dose of 5.0 mg/kg of memantine, producing plasma Cmax of approximately 200 ng/mL (Danysz et al., 1997). Thus, the data obtained in the *O. degus* are quite similar to those previously obtained in rats, and represent a reasonable rationale for the choice

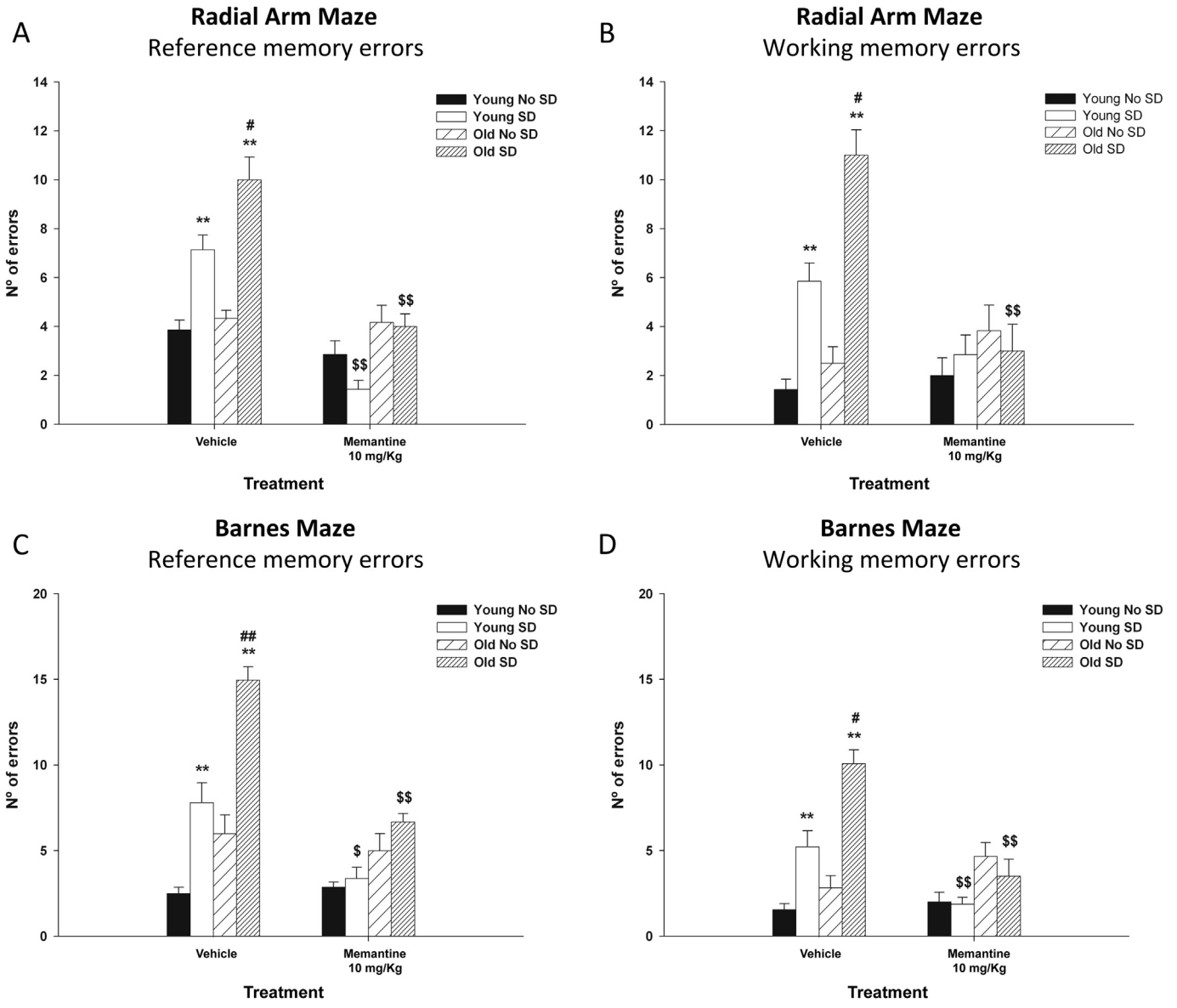

Fig. 4. Effect of memantine on age and sleep deprivation measured by the RAM and the BM. Number of reference memory errors and working memory errors in the RAM (A and B), and in the BM (C and D; as the mean of the four trials) committed by young and old *O. degus* under No SD and SD conditions, treated (i.p.) with either vehicle or 10 mg/kg of memantine before SD ($n = 13$ per group). Results are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared to No SD condition; $\#P < 0.05$, $\#\#P < 0.01$, significantly different compared to the young group; \$ $P < 0.05$, \$\$ $P < 0.01$, significantly different from the group treated with vehicle.

of the dose. In fact, the pharmacokinetic curve estimates levels in the range of 200 ng/mL in the period relevant for the behavioral tests (12–15 h later), with memantine being administered 12 h before the testing. In clinical practice, the steady-state plasma levels of memantine are lower but not excessive, being about 100 ng/mL (Zajaczkowski et al., 1996; ter Horst et al., 2013). The first set of behavioral experiments were focused on the exploration of the effects of memantine on the cognitive impairment induced by total sleep deprivation in a rodent model of female *O. degus* of two different group ages (young and old — 18 and 48 months old).

There is some controversy in the effect of the estrous cycle on the learning and memory of female rodents (Stackman et al., 1997; Hornung et al., 2007). Despite the evidence of the effect of estrous cycle on behavior some authors suggest otherwise, specifically as regards memory performance (Hornung et al., 2007). For instance, ter Horst and collaborators report differences in the strategy but not in the performance in female mice in a circular board hole paradigm, very similar to the BM used in this study (Stackman et al., 1997). Hence, given these evidences we did not take in consideration this variable for the design and performance of the experiments. The training phase of RAM and BM show that both young and old animals progressively reduce the number of memory errors through the consecutive sessions. Results on the test sessions show that the animals that underwent SD committed significantly more errors in both the RAM and in the BM. Alternatively, animals with a normal sleep cycle performed similarly to the previous days of training. This suggests that under normal sleep conditions, the access to the maze-related information is intact in these animals. It is worth mentioning that under No SD young and old animals behave similarly, but SD appears to be more severe in the old *O. degus*. Both reference and working memory errors are significantly higher in the old group than the young animals under this sleep condition. This supports previous reports of an age effect on retrieval after sleep deprivation procedures, especially REM sleep

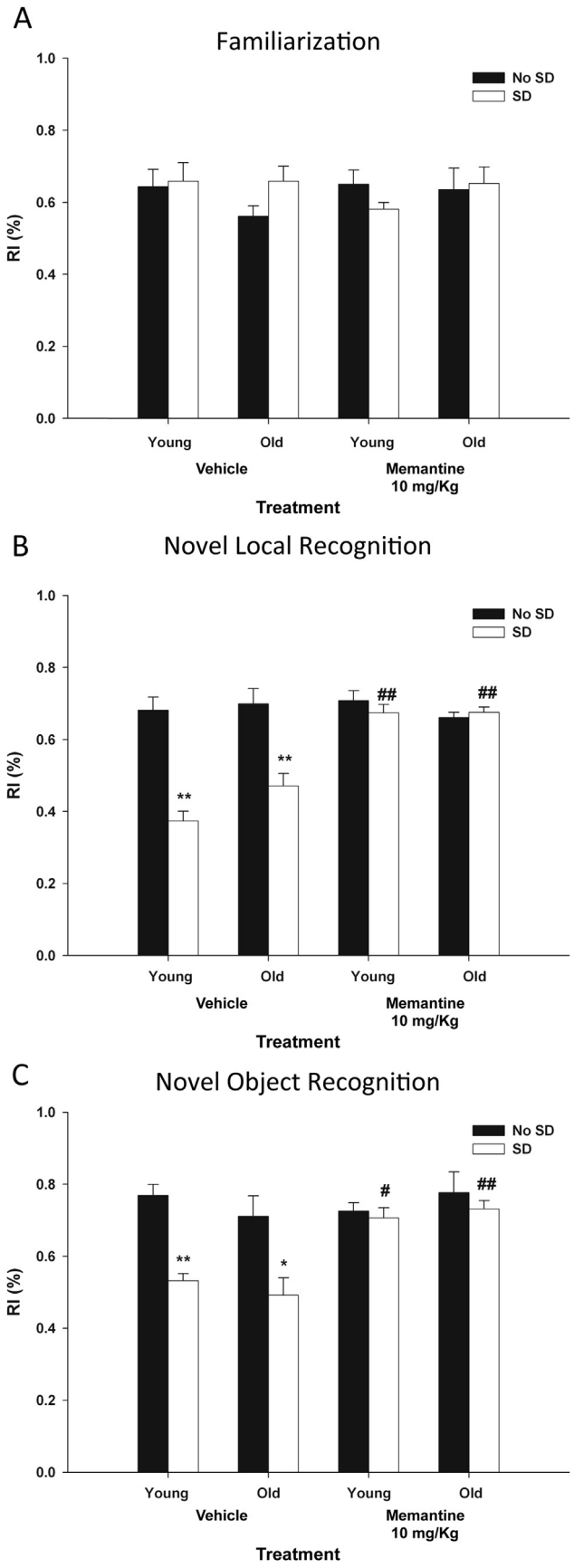

Fig. 5. Effect of memantine on age and sleep deprivation measured by the NLOR test. A) Familiarization trial, B) "local recognition" trial, and C) "object recognition" trial in young and old *O. degus* under No SD and SD conditions, treated (i.p.) with either vehicle or 10 mg/kg of memantine on the test day ($n = 13$ per group). Results are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared to No SD condition; $\#P < 0.05$, $\#\#P < 0.01$, significantly different from the group treated with vehicle.

restriction (Tucker et al., 2011; Scholtzova et al., 2008). However, in contrast to the RAM and the BM, the NOR test does not show a difference in age groups when the performance under SD is analyzed. Chronic sleep deprivation has a very different effect than acute sleep deprivation as to what cognition is concerned. Indeed, animals were sleep-deprived more than once in this work. However, the different sessions of acute sleep deprivation occurred with several days apart, allowing the animal to recover from the consequences of sleep loss and recovering its normal sleeping routine.

The second set of experiments aimed to evaluate the effect of memantine on the effect of age and the cognitive impairment caused by SD. The results in the different paradigms show an overall improvement in both young and old animals' performance when they were treated with memantine (10 mg/kg i.p.) previous to the SD procedure. The selected dose of memantine prevented development of cognitive impairment both in young and old animals. Taking these values as measures of reference, working, and recognition memories, our results further support a wide range of studies that report beneficial effects of this drug on memory improvement in both animal models (Ihalainen et al., 2011; Agüera-Ortiz, 2010) and AD patients (Fernandes-Santos et al., 2012), supporting further trials with this drug in other models and conditions.

It is worth mentioning that in general, the old *O. degus* behave similarly to the young ones in the three paradigms used here for the evaluation of learning and memory. Both young and old animals correctly acquire and perform the tasks' requirements, with no significant differences between the two groups of age. Our results are coherent with previous studies (Vecsey et al., 2012; Descamps and Cespuglio, 2010), and indicate that SD impairs memory retrieval regardless of age. However, it seems that this condition has a more significant effect on cognition in aged animals, which is consistent with previous clinical and preclinical data showing memory deficits in those elder subjects with sleep disruption problems (Crowley, 2011; Pilz et al., 2011). There is evidence that aged subjects perform worse in different memory tasks in comparison with young subjects (Ming and song, 2005). Particularly, as regard to the object recognition in the *O. degus*, it has been demonstrated that aged animals are not able to recognize the novel object under normal sleep conditions (Ardiles et al., 2012). However, this disparity with our own results may be due to methodological differences. While Ardiles et al. perform their experiments in an open field and no familiarization/habituation period, our experiment is carried out in an environment that the animal likely assesses as familiar. The familiarity of this environment possibly allows the animal to focus only in the novelty related to the objects. Also, it may be also possible that the open field might induce stress to the animal, acting as a confounding factor. Another possible explanation for this difference is that the animals used in this work are not as old as the animals used by Ardiles and co-workers.

Although the mechanism of SD-induced impairment is still unknown, different factors have been pointed to as a possible mechanism for the effects of SD, such as stress and a variety of alterations in neurotransmission (Meerlo et al., 2009; Walker et al., 2004). The consequences of SD for the formation, expression and retrieval of memories have been previously described in different animal models (Alhaider et al., 2011; Aleisa et al., 2011; Pietá Dias et al., 2007). Particularly, data from our own laboratory

demonstrates that this procedure is able to produce memory impairment in the *O. degus*, when SD is conducted previous to the evaluation of spatial and working memories. In this sense, there is evidence supporting that chronic SD may cause atrophy of neurons in the hippocampus and in other cerebral structures involved in cognitive domains such as memory or attention (McEwen and Chattarji, 2004), which is coherent with results from previous works.

In the last years, memantine has been added to the clinic drug repertoire to battle cognitive decline symptoms, especially those observed in AD. There is evidence that this drug is able to improve recognition (Peskind et al., 2006) and spatial (Minkeviciene et al., 2004) memories in rats. Moreover, clinical evidence indicates an improvement on the cognitive and behavioral symptoms in patients following chronic treatment (Schulz et al., 2011; Collingridge et al., 2013). Furthermore, some authors have attributed to memantine a cognitive enhancing effect (Parsons et al., 1999). There is evidence that the antagonism that memantine exerts effectively inhibits the hyperfunction of NMDA receptors, still allowing a normal range of Ca_2^+ flux and ensuring a non-pathologic cell activity (Fitzjohn et al., 2008; Slutsky et al., 2010; Danysz and Parsons, 2003). Our data support the efficacy of SD as a paradigm of cognitive impairment in young and old *O. degus*, from which memantine effectively exerts a protective effect. It is interesting that not only the youngest group of animals shows an improvement in their performance, but memantine can also prevent the deficit shown by old animals that are sleep deprived. This suggests that memantine may have not only an effect on the maintenance of the cognitive function in young subjects with cognitive deficit, but also in an older population that shows a more severe deficit [59]. This result supports the memantine prescription in patients whose cognitive condition has deteriorated further, eg. mild-moderate patients.

In summary, our results indicate that memantine, administered prior to the SD challenge at the designated dose (10 mg/kg), is an effective agent in preventing the transient cognitive impairments that SD causes not only in young but mostly in old *O. degus*, opening new possibilities for the use of this drug in an elderly population. Moreover, under the condition used, we did not observe any side-effect after the *O. degus* were dosed with memantine, which is coherent with studies conducted in other species and similar dose ranges (Yiğit et al., 2011; Zajaczkowski et al., 1996; ter Horst et al., 2013). Nevertheless, in order to better comprehend these advances, further studies exploring other translational approaches and more histopathological studies involving the *O. degus* should be performed. However, the validation of the positive effect of this compound in this promising model will enable a better understanding of the mechanism of action and the behavioral outcome of memantine, from which progress in the study and comprehension of AD will directly benefit. Finally, this represents an opportunity to test other drugs with potential therapeutic effect, especially in old *O. degus*.

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IX.IIIHerrero MT, **Estrada C**, Maatouk L, Vyas S.“Inflammation in Parkinson's disease: role of glucocorticoids”.*Frontiers in Neuroanatomy*. **2015**2;9:32. PMID: 25883554. DOI: 10.3389/fnana.2015.00032
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Abstract

Chronic inflammation is a major characteristic feature of Parkinson's disease (PD). Studies in PD patients show evidence of augmented levels of potent pro-inflammatory molecules e.g., TNF-α, iNOS, IL-1β whereas in experimental Parkinsonism it has been consistently demonstrated that dopaminergic neurons are particularly vulnerable to activated glia releasing these toxic factors. Recent genetic studies point to the role of immune system in the etiology of PD, thus in combination with environmental factors, both peripheral and CNS-mediated immune responses could play important roles in onset and progression of PD. Whereas microglia, astrocytes and infiltrating T cells are known to mediate chronic inflammation, the roles of other immune-competent cells are less well understood. Inflammation is a tightly controlled process. One major effector system of regulation is HPA axis. Glucocorticoids (GCs) released from adrenal glands upon stimulation of HPA axis, in response to either cell injury or presence of pathogen, activate their receptor, GR. GR regulates inflammation both through direct transcriptional action on target genes and by indirectly inhibiting transcriptional activities of transcriptional factors such as NF-κB, AP-1 or interferon regulatory factors. In PD patients, the HPA axis is unbalanced and the cortisol levels are significantly increased, implying a deregulation of GR function in immune cells. In experimental Parkinsonism, the activation of microglial GR has a crucial effect in diminishing microglial cell activation and reducing dopaminergic degeneration. Moreover, GCs are also known to regulate human brain vasculature as well as blood brain barrier (BBB) permeability, any dysfunction in their actions may influence infiltration of cytotoxic molecules resulting in increased vulnerability of dopamine neurons in PD. Overall, deregulation of glucocorticoid receptor actions is likely important in dopamine neuron degeneration through establishment of chronic inflammation.

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REVIEW
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Inflammation in Parkinson's disease: role of glucocorticoids

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Chronic inflammation is a major characteristic feature of Parkinson's disease (PD). Studies in PD patients show evidence of augmented levels of potent pro-inflammatory molecules e.g., TNF-α, iNOS, IL-1β whereas in experimental Parkinsonism it has been consistently demonstrated that dopaminergic neurons are particularly vulnerable to activated glia releasing these toxic factors. Recent genetic studies point to the role of immune system in the etiology of PD, thus in combination with environmental factors, both peripheral and CNS-mediated immune responses could play important roles in onset and progression of PD. Whereas microglia, astrocytes and infiltrating T cells are known to mediate chronic inflammation, the roles of other immune-competent cells are less well understood. Inflammation is a tightly controlled process. One major effector system of regulation is HPA axis. Glucocorticoids (GCs) released from adrenal glands upon stimulation of HPA axis, in response to either cell injury or presence of pathogen, activate their receptor, GR. GR regulates inflammation both through direct transcriptional action on target genes and by indirectly inhibiting transcriptional activities of transcriptional factors such as NF-κB, AP-1 or interferon regulatory factors. In PD patients, the HPA axis is unbalanced and the cortisol levels are significantly increased, implying a deregulation of GR function in immune cells. In experimental Parkinsonism, the activation of microglial GR has a crucial effect in diminishing microglial cell activation and reducing dopaminergic degeneration. Moreover, GCs are also known to regulate human brain vasculature as well as blood brain barrier (BBB) permeability, any dysfunction in their actions may influence infiltration of cytotoxic molecules resulting in increased vulnerability of dopamine neurons in PD. Overall, deregulation of glucocorticoid receptor actions is likely important in dopamine neuron degeneration through establishment of chronic inflammation.

Keywords: glucocorticoid receptor, Parkinson's disease (PD), neuroinflammation, neurodegeneration, microglia

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Introduction

Parkinson's disease (PD) is a common age-related neurodegenerative disorder characterized by cardinal motor symptoms that include bradykinesia with resting tremor, rigidity and gait disturbance. These motor symptoms become evident when already 70–80% of nigrostriatal terminals have degenerated. At present, therapeutic treatments, for example, levodopa, mostly address motor symptoms. However, a wide spectrum of non-motor clinical

features such as REM (Rapid eye movement) sleep disturbances, autonomic dysfunction, depression, anxiety, cognitive impairment or falling are associated with PD, and are moreover debilitating and unresponsive to dopamine-related treatments. Thus, PD is a complex systemic disorder with non-motor symptoms often preceding motor symptoms and worsening with disease progression (Berg et al., 2014; Goldman and Postuma, 2014). PD has an age-adjusted incidence of 13.5–13.9 per 100.000 person-years, and a prevalence of 315 per 100,000 individuals (the second most common worldwide). As PD affects predominantly older people, its prevalence increases with age from 428 at 60–69 years to 1,903 per 100.000 in 80 years old people (Abdullah et al., 2014; Pringsheim et al., 2014).

The major neuropathological hallmarks of PD are progressive degeneration of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc); presence of proteinaceous inclusions called Lewy bodies (LBs) and chronic inflammation. However, the initial causes and underlying mechanisms pertaining to these neuropathological features in the majority of patients, classified as sporadic PD, remain unknown. Recent studies indicate that combined genetic, environmental factors and aging confer risk for developing sporadic PD rather than genetic or environmental factor individually. Approximately 5–10% of PD patients present the familial form of the disease with either autosomal dominant or recessive mode of inheritance. Epidemiological analysis confirm that up to 40% of PD patients with age at onset of less than 30 years and 17% of those with age at onset of less than 50 years will probably present the familial form of the disease. At least 18 loci as well as 12 genes with Mendelian inheritance and highly penetrant mutations causing rare monogenic forms have been identified. The genetic discovery of point mutations, duplication or triplication of *SNCA* (synuclein) gene coding for α-synuclein protein (reviewed in Goedert et al., 2013) with demonstration by Spillantini et al. (1997) that α-synuclein is a major component of LBs, led Braak et al. (2003) to staging PD according to appearance of α-synuclein containing LBs and Lewy neuritis with disease severity. Accordingly to Braak's hypothesis PD progresses in neuronally-connected ascending manner to dorsal motor nucleus of the glossopharyngeal and vagus nerves likely from gut and/or olfactory mucosa (stage 1 and 2), then from lower brain stem to midbrain including nigral regions (stage 3 and 4) and lastly to the neocortical regions (stage 5 and 6). However, it has been suggested that LB pathology alone is not sufficient and that associated neuronal loss leads to Parkinsonism (Buchman et al., 2012).

In last few years, large genetic and genome-wide association (GWA) studies together with meta-analyses have led to significant and rapid advances in the genetic basis of sporadic PD, with realization both for its wide implication and complexity (Lill et al., 2012; Clarimón and Kulisevsky, 2013). There is great expectation for further insights with advent of new DNA sequencing technologies (exome and whole-genome sequencing) and the NeuroX genotyping platform (Nalls et al., 2015). These studies have identified at least 20 susceptibility loci as risk for sporadic PD (Nalls et al., 2014). As yet, the true significance of many of these loci is still unknown. Of interest, susceptibility related to *SNCA* and *LRRK2* (Leucine rich repeat kinase 2) loci consistently observed are also genes that have been identified in the monogenic, autosomal dominant familial PD patients. Occurrence of somatic mosaicism has been also hypothesized in the etiology of some cases of PD (Kim and Jeon, 2014). Cases of somatic mosaicism in many central nervous system (CNS) disorders have been reported, for example, somatic mutation in the *presenilin-1* gene associated with Alzheimer's disease (Beck et al., 2004), in *SPG4/SPAST* (spastic paraplegia4/spastin) causing spastic paraplegia (Depienne et al., 2007) or *MECP2* (methyl CpG binding protein) resulting in Rett syndrome (Topçu et al., 2002). Thus far, no cases of PD with somatic mosaicism are known, as well, in this regard results of study on *SNCA* somatic mutations by Proukakis et al. (2014) were negative.

Summing up: (a) although at present there is a rapid progress and evolution in technology to unravel genetic basis of PD, most PD risk is not understood; (b) the pathogenicity arising from several of identified gene mutations remains to be determined; (c) highly penetrant gene mutations (as *DJ-1, LRRK2, Parkin, PINK1* (PTEN-induced putative kinase 1), and *SNCA*) cause rare monogenic forms of the disease; (d) somatic mosaicism could shed light on the heterogeneity of PD; and (e) additive mechanisms is suggested in risk for PD, increasing with the number of risk alleles carried by a single subject e.g., in HLA (human leucocyte antigen) region (Hill-Burns et al., 2011).

There is strong epidemiological evidence to show that aging is a single most important risk factor for PD, with increase in incidence between fifth to eight decades. Modifications that occur in specific brain regions during aging, such as increased oxidative and nitrative stress, changes in glial functions, dysfunction of proteasomes and lysosomes and altered α-synuclein protein are also manifestations of PD (Collier et al., 2011; Kieburtz and Wunderle, 2013). Environmental toxins identified as risk for PD are herbicides (e.g., paraquat or rotenone), heavy metals such as manganese and lead, nanoparticles as air pollutants, head trauma or well water. Thus, for example, it was shown that people exposed to pesticides and harboring Cytochrome P450 2D6 (CYP2D6) genotype with poor metabolic capacity for xenobiotics are at increased risk for developing PD (Elbaz et al., 2004). Epidemiologic link also exists between rotenone and PD. Rotenone is a powerful inhibitor of mitochondrial complex 1 and interestingly complex I deficiency is found in PD. The role of viral infections as risk factor has been evoked ever since the famous and controversial von Economo's encephalitis lethargica pandemic suspected to be caused by H1N1 (Hemagglutinin1 neuraminidase1) influenza virus where patients exhibited Parkinsonism symptoms (Ravenholt and Foege, 1982). Recently, animals infected with highly pathogenic H5N1 virus showed clear motor deficits as well human cases with encephalitis have been reported (Jang et al., 2009). Epigenetic modifiers could be potential mediators of environmental factors (Portela and Esteller, 2010). Aberrant epigenetic modifications include changes in gene functions or gene expression but without changing the DNA sequence: non-coding RNA-mediated changes of gene expression, DNA methylation or post-transcriptional

modifications and acetylation of histones (Nalls et al., 2014). For instance, the methylation of the Tumor Necrosis Factor alpha (TNF-α) promoter is significantly decreased in the SNpc of PD patients compared with controls or with the methylation in the cortex (Pieper et al., 2008) suggesting increased susceptibility of dopamine neurons to TNF-α mediated inflammation (Barcia et al., 2005, 2011).

Thus, aging together with genetic susceptibility and cumulative environmental factors such as air pollutants, pesticides, infections or exposure to heavy metals likely have a role in the development of idiopathic PD.

Immune System and the Etiology of Parkinson's Disease

All of the above environmental factors together with multiple cellular changes occurring during aging can impact immune functions. There is now a growing realization, particularly from genetic studies, that immune system is most likely involved in the etiology as well as early phases of PD, thus the inflammatory component of the disease may simply not be a consequence of neuronal dysfunction and neurodegeneration. In GWA studies, a number of susceptibility loci that have been identified as strong risk factors, are related to both innate and adaptive immune functions, for example, *HLA-DQB1*, *LRRK2*, *GPNMB* (glycoprotein NMB), or *BST-1* (bone marrow stromal cell antigen) (Liu et al., 2011; Pihlstrøm et al., 2013). In this regard, *LRRK2*, *Parkin*, *PLA2G6* (phospholipase A2, group VI), *DJ-1* and *SNCA* genes mutated in both familial and idiopathic PD are also known to function in microglia and astrocytes (Russo et al., 2014). Interestingly, several studies have identified risk of PD with polymorphisms present in the promoter regions of *IL-1β* and *TNF-α* genes that augment the expression of these genes and whose protein products have potent pro-inflammatory activity (Wahner et al., 2007). Moreover, polymorphisms reported in other pro-inflammatory genes e.g., CD14, *HLA-DBQ1*, *HLA-DRA*, HLA-DRB1, *HLA-DRB5* can also increase the risk for PD (Ahmed et al., 2012). In the analysis of potential markers of motor and cognitive progression, SNPrs 6482992 of clarin3 (CLRN3) was described as the best predictor of cognitive deterioration (Chung et al., 2012) whereas SNPrs 10958605 as involved in neuroinflammatory pathways (Cappellano et al., 2013). The implication of early involvement of immune system is also reinforced by epidemiological studies showing a prolonged use of NSAIDs (Nonsteroidal anti-inflammatory) particularly ibruprofen subsequently lowers the risk of PD (Rees et al., 2011).

Neuroinflammation in PD

As a progressive neurodegenerative disorder, PD is a multifactorial complex disease most likely evolving because of the genetic and environmental risk factors, as well as cellular alterations and aging. Inflammatory component in PD not only encompasses deregulation of inflammatory pathways resulting from genetic vulnerability but also immune alterations associated with aging and with primary activation of glia in the face of neuronal injury. Aging affects the functions of immune system, resulting in so-called "immune senescence". Specifically, advancing age has been associated with chronic mild inflammation in the SNpc, thereby rendering dopaminergic neurons vulnerable to degeneration (Kanaan et al., 2010). Increasing evidence points to the role of active peripheral inflammation in PD that can contribute to the initiation and/or the progression of the disease by, for example, exacerbating and synergizing with the discordant central inflammatory response to drive dopaminergic neurodegeneration. Combination of aging, heritable risk factors and exposure to environmental agents has been suggested as potential host-pathogen specific pathophysiologic elements that can cause deregulation of both innate and adaptive immune system responses (Kanaan et al., 2008; Chao et al., 2014). Thus, in both sporadic and familial PD, immune activation occurring at multiple levels would play an important role in PD pathology.

Evidence of an on-going neuroinflammation in affected brain regions in PD stems from analyses of pro-inflammatory cytokines (Interferon gamma, IFN-γ; TNF-α; Interleukin-6, IL-6; or Interleukin-1β, IL-1β) showing their accumulation in both cerebrospinal fluid and post-mortem brain (Mogi et al., 1994; Dobbs et al., 1999; Reale et al., 2009a,b). Recently, it has been demonstrated that the serum levels of IL-6 and the chemokine ligand 5 (CCL5) also known as Regulated on Activation, Normal T cell Expressed and Secreted (RANTES) were significantly increased in PD patients, and importantly, RANTES levels correlated with the severity and duration of the disease (Tang et al., 2014). Furthermore, the augmentation of iNOS (Inducible nitric oxide synthase) observed in SN and striatum of PD (Hunot et al., 1996) suggests that the toxicity originating both from cytokines/chemokines and inflammation-derived oxidative stress could contribute to dopaminergic neuronal degeneration and progression of the disease (Orr et al., 2005; Wilms et al., 2007). Numerous studies in experimental PD models indicate that dopamine neurons are particularly vulnerable to both oxidative stress and inflammatory attack (McGeer and McGeer, 2008; Pott Godoy et al., 2008). Interestingly, in this regard, Lipopolysaccharide (LPS)-activated microglia in the vicinity of dopamine neurons in SN induce degeneration of these neurons whilst sparing GABAergic and serotonergic neurons, suggesting a selective dopamine neuron vulnerability to inflammation (Liu and Bing, 2011).

Inflammation and immune-related responses may be viewed not only as determinant factors in disease progression but also as pathogenic processes in the onset of both familial and sporadic PD (Halliday and Stevens, 2011; Chao et al., 2014; Dzamko et al., 2014). On this point, presence of activated microglia, visualized by PET (positron emission tomography) analysis using radioligand ^{11}C-PK-11195, was recently reported in the SN and putamen of PD patients diagnosed within a year from clinical onset (Iannaccone et al., 2013). This, together with study of Ouchi et al. (Ouchi et al., 2005) suggests a microglial-mediated inflammatory process in early stage of PD. Several lines of evidence also point to relevant actions of different PD-linked gene mutations e.g., *SNCA* or *LRRK2* in stimulating inflammatory responses through activation of microglia and astrocytes thereby participating directly in chronic

PD progression (Gillardon et al., 2012; Moehle et al., 2012; Harms et al., 2013). Both central and peripheral inflammation occurs in the prodromal stage of PD, which thus sustains disease progression (Dzamko et al., 2014; Su and Federoff, 2014). Overall, accumulation of pathological α-synuclein in PD brain leads to neurodegeneration with T-cell infiltration, microglial activation and increased production of inflammatory cytokines and chemokines (Harms et al., 2013). The detection of T lymphocytes and activated microglia in the SN of Parkinsonian patients is striking because systemic immune cells have to penetrate several barriers in order to reach the brain parenchyma.

The CNS was considered as an immunologically privileged site because of the lack of lymphatic vessels, the absence of classical major histocompatibility complex (MHC) positive antigen presenting cells, and the presence of barriers as the tanycytic barrier around the circumventricular organs or the neurovascular unit of the BBB. The latter is composed of endothelial cells, pericytes and astrocytes and associated strong, tight junctions prevent the entry of immune cells into the brain parenchyma. BBB is a metabolic and physical barrier that separates the CNS from the peripheral circulation, actively allowing the transports of nutrients to the brain but limiting passive diffusion of blood-borne solutes. However, in aging and in PD, a BBB disruption has been described with loss of the barrier permeability leading to secondary leukocyte migration within the brain parenchyma, reactive gliosis and damage to neurons (Stolp and Dziegielewska, 2009; Cabezas et al., 2014). BBB dysfunction in PD favors an invasion of immune cells (and/or peripheral mediators and factors as toxins or elements of adaptive immunity) into the brain parenchyma that provokes a progressive and self-perpetuating degenerative process (Monahan et al., 2008). Additionally, it has been demonstrated that PD patients have increased permeability of the intestinal epithelial barrier (Forsyth et al., 2011) as well as chronic enteric/colonic inflammation (Devos et al., 2013). As proposed by Braak et al. (2003), an environmental pathogen can cross the monolayer of polarized epithelial cells (the intestinal epithelial barrier) (Sharkey and Savidge, 2014) and enter into the terminal axons of the submucosal plexus spreading to the medulla oblongata via the vagal preganglionic innervation of the gut (Hawkes et al., 2009). Moreover, brain injuries or systemic infections can induce systemic inflammatory responses that easily communicate with brain. Both Alzheimer's disease and PD have been associated with both the HLA region (Ahmed et al., 2012; Wissemann et al., 2013), and with the production of autoantibodies (Maetzler et al., 2014) suggesting putative genetic susceptibility to inflammation that could initiate the neuronal dysfunction.

Microglia are the resident innate immune cells in the brain. Being only 5–15% of the total cells of the brain, microglia functions include tissue repair and cellular homeostasis after neuronal injury. Activated microglia produce neurotoxic molecules, for example, pro-inflammatory cytokines, chemokines, complement proteins or nitric oxide. Additionally, activated microglia acquire phagocytic properties and develop neuro-immune interactions involving the expression of surface molecules as CD200/CD200R, CD47/CD172a, CX3C chemokine ligand 1 and its receptor (CX3CL1/CX3CR) and the complement regulatory proteins, complement components C1q and C3 in order to eliminate cellular debris and damaged neurons by gliapses (Barcia et al., 2012). However, microglial responses can have neuroprotective as well as harmful consequences mainly if there is a continuous exposure to a pro-inflammatory environment with a persistent release of inflammatory mediators (Bardou et al., 2014) as activated microglia can still persist even years after the toxic insults (Barcia et al., 2004; Jackson-Lewis and Smeyne, 2005; Block et al., 2007). In fact, if as a defense mechanism of the organism, an inflammatory response starts and continues without control, a chronic persistent inflammation environment in the brain can result in tissue destruction and progressive neurodegeneration.

Glucocorticoids, Inflammation and Parkinson's Disease

Inflammation is normally a tightly regulated process that acts to prevent pathogen invasion as well as cellular injury, whilst at the same time enabling tissue repair. Several endogenous mechanisms act to regulate the immune cell functions, which are involved in triggering an inflammatory process. Among them, the steroid hormone, glucocorticoid, is a known major regulator of immune system and inflammation. Glucocorticoids (GCs) are one of the most potent and effective anti-inflammatory agents in clinical use ever since the isolation of cortisone and its clinical application in the early 1950s by the Nobel Prize winners Hench, Kendall and Reichstein (Hench et al., 1950; Reichstein, 1951).

GCs (cortisol in humans and corticosterone in rodents) are endogenous steroid hormones synthesized in adrenal glands and secreted into systemic circulation. The GC secretion occurs in ultradian pulsatile manner (Hellman et al., 1970; Veldhuis et al., 1989) and over-riding this pattern is acute GC rise in response to a stressor (psychogenic or physical e.g., tissue injury or pathogen invasion) whereby increased levels of GCs exert important adaptive actions in multiple tissues to restore homeostasis (Young et al., 2004; McEwen, 2007). Both ultradian/circadian and stress-evoked GC secretion is tightly controlled by various negative feedback mechanisms affecting each component of HPA axis, notably synthesis and release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus and adrenocorticotropic hormone (ACTH) from anterior pituitary. Any change in negative feedback loops will affect HPA axis, resulting in altered ultradian/circadian rhythm of GC release often with abnormally high basal GC levels, which in turn could lead to GC resistance.

Measurement of plasma cortisol in idiopathic PD patients has consistently shown significantly elevated levels compared to age-matched control subjects, and as well correlating with impulsive behaviors (Bellomo et al., 1991; Stypula et al., 1996; Hartmann et al., 1997; Charlett et al., 1998; Djamshidian et al., 2011; Ros-Bernal et al., 2011). The high cortisol levels seem unrelated to L-DOPA treatment or disease duration (Müller et al., 2007). Elevated cortisol levels are observed in many other neurodegenerative diseases including Alzheimer disease (Huang

et al., 2009). In PD, however, the normally quiescent nocturnal cortisol secretory pattern is particularly affected (Hartmann et al., 1997) raising the question as to whether the circadian control of HPA axis by suprachiasmatic nucleus is altered. The underlying causes of HPA deregulation and whether or how it impacts PD pathology is presently not well understood. However, presence of LBs in both adrenal glands and hypothalamus in PD has been reported (Wakabayashi and Takahashi, 1997; Braak et al., 2006), which may imply a role of α-synuclein pathology in HPA axis deregulation. In addition to neuronal networks regulating HPA axis through feed back loops, cytokines liberated by peripheral immune cells can also stimulate HPA axis in several ways. Potent inflammatory cytokines (TNF-α, Il-1β and Il-6) can induce release of GCs by directly stimulating CRH synthesizing neurons of PVN or indirectly by stimulating production of prostaglandin E2 synthesis in perivascular cells (Ericsson et al., 1994; Kang et al., 2006; Serrats et al., 2010). In addition, IL-6 was shown to directly act on anterior pituitary cells as well as in adrenal glands, via its receptor, to stimulate the synthesis of ACTH and GC respectively (Zarković et al., 2008). Thus, deregulated immune responses with elevated levels of pro-inflammatory cytokins may lead to chronic activation of HPA axis.

Once secreted, GCs act on diverse physiological processes ranging from metabolism, immune responses to cognition and behavior. Their therapeutic potential, however, has limitations as chronic use with sustained high levels of GCs can result in serious side effects such as diabetes, obesity, dyslipidemia, hypertension, osteoporosis or behavioral anomalies. GCs clearly exert anti-inflammatory actions especially in an inflammatory setting, however, a number of recent studies indicate that they also exert pro-inflammatory responses, which are cell-type dependent. Thus, in response to acute stress resulting in increased GC levels, high levels of pro-inflammatory mediators such as IL-1β were found (Dhabhar, 2002; O'Connor et al., 2003; Sorrells et al., 2007). In a microarray study by Galon et al. (2002) on human mononuclear cells, dexamethasone treatment was found to induce the expression of several innate-immune related genes in addition to down-regulation of pro-inflammatory genes. It is believed that this opposing action of GCs "prepares" the immune system to respond rapidly to harmful stimulus and subsequently GCs act to down-regulate the immune response to restore homeostasis.

Glucocorticoid Regulation of Inflammation through GR

In brain, GC signaling is mediated by almost ubiquitously expressed GRs (GRs) as well as mineralocorticoid receptors (MR) that have restricted expression in neurons. However, it should be noted that MR is also expressed in glia (Sierra et al., 2008). GR, a prototype member of nuclear receptor superfamily (designated as NR3C1 in nomenclature of nuclear receptor family) is a ligand-activated transcription factor, it can also exert non-genomic actions (Groeneweg et al., 2011). GR is a modular protein with an N-terminal transactivation domain, a C-terminal ligand binding domain (LBD) and a central Zinc fingers-containing DNA-binding domain (DBD) that recognizes a specific DNA sequence. The LBD is the high affinity binding site for cortisol and other ligands. In humans, two major isoforms of GR, hGRα and hGRβ arising from alternative splicing have been described (Zhou and Cidlowski, 2005) and they differ in their C-terminal ligand-binding domain such that hGRβ cannot bind to endogenous or synthetic GCs. Experimental evidence indicates that hGRβ is expressed at low levels and it antagonizes the transcriptional activity of hGRα thus acting as dominant negative inhibitor of hGRα. However, recent genome-wide microarray studies indicate that hGRβ also regulates gene transcription (Kino et al., 2009). Interestingly, reduction in hGRα:hGRβ ratio has been associated with behavioral and mood disorders such as depression and schizophrenia (Perlman et al., 2004; Matsubara et al., 2006). In addition, alternative translational initiation sites generating 8 different GR proteins both in mouse and humans have been described (Oakley and Cidlowski, 2013).

Recent evidence shows that pulsatile pattern of GC secretion is crucial to proper GR transcriptional activity, thus loss of GC oscillatory pattern can result in continuous transcription with abnormal protein accumulation or GR targeting inappropriate genes leading to undesirable outcomes. GR is normally inert in the cytoplasm, in association with complex of proteins including heat-shock chaperones (HSP90, HSP70, HSP40, HSP23) and immunophilins such as FKBP51 (FK506 binding protein51), FKBP52, CP44 and PP5 (Grad and Picard, 2007). GC binding to this complex results in conformational change in GR exposing a nuclear localization signal resulting in importin-mediated translocation through the nuclear pore to the nucleoplasm.

In the nucleus, GR regulates transcription of its target genes in multiple, complex ways as well in highly cell- and context-specific manner. The transcriptional activity of GR has been especially studied with respect to its actions on metabolism and regulation of immune responses in the periphery. Multitude of studies indicates that GCs through GR influence each stage of inflammatory response i.e., from initiation, effector to resolution phases of an inflammatory reaction. Inflammatory response is triggered by specific receptors in immune cells and in this regard toll-like receptors (TLRs) activation and intracellular signaling cascade is thus far best characterized (Kawai and Akira, 2010) resulting in activation of transcriptional factors such as Nuclear factor kappa B (NF-κB), Activator Protein 1 (AP-1) or interferon response factors (IRFs). Each TLR family member (from 1–13 in mouse; all expressed in microglia) recognizes specific molecular signature present in either pathogens (PAMPs-Pathogen Associated Molecular Patterns) or molecules released by injured cells called DAMPs (Damage-associated Molecular Patterns). GR is reported to regulate key components of TLR signaling e.g., transforming growth factor beta-activated kinase 1 (TAK1; Bhattacharyya et al., 2010).

The GR regulation of inflammation is a result of both its transcriptional stimulatory and repressive activity. Classically, GR stimulates transcription of genes that act to inhibit inflammation and conversely it inhibits transcription of pro-inflammatory genes. GR stimulates transcription as homodimer binding to specific cognate DNA sequence,

GAGAACAnnnTGTTCT, called Glucocorticoid Responsive Elements (GREs) present in the promoter regions of its target genes. This transcriptional activity of GR requires the presence of chromatin modifiers (e.g., Nuclear receptor coactivator NCoA1), basal transcriptional machinery and co-factors (CREB binding protein CBP, p300) (Rosenfeld and Glass, 2001). This mode of transcriptional activity has been notably described for genes coding for proteins of metabolic pathways such as glucose-6-phosphotase, fatty acid synthase or tyrosine aminotransferase as well as anti-inflammatory genes e.g., (Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB-α), MAPK phosphatase (MPK-1), IL-4, IL-10 or annexin-1 (De Bosscher et al., 2003). GR can also bind to negative GREs (nGREs) to repress transcription, thus among the genes identified containing nGREs are CRH as well as ACTH receptor in adrenal glands (Dostert and Heinzel, 2004; Surjit et al., 2011). Importantly, with regards to inflammation, GR can also inhibit transcription by tethering (i.e., through protein-protein interactions) or modulating the activity of other transcriptional factors, for example NF-κB, AP1 or IRF (Chinenov et al., 2013). This action mediated by GR monomers has been particularly studied in peripheral immune cells involving inhibition of expression of powerful pro-inflammatory genes as well as resolution of inflammation. The cross talk between AP1, NF-κB and GR is well documented (De Bosscher et al., 2003). As it has pertinence in the effects of GR observed in microglia it will be briefly reiterated here.

AP1 is comprised of heterodimers of c-Fos (C-Fos, FosB, Fra1 and 2), Jun (c-N, B-Jun, D-Jun) as well as ATF (Activating transcription factor) families of transcription factors, which controls expression of many cytokines. AP1 activity is stimulated by MAPK cascade resulting in activation of c-Jun-N terminal kinase (JNK), which phosphorylates c-Jun. GR regulates AP1 activity by associating with Jun-Fos complex at AP1 DNA elements in promoter regions of genes, inducing a conformational change in the complex that is not functional (Diamond et al., 1990). Additionally, GR also stimulates transcription of MAPK-phosphatase 1 (MKP-1) by binding to GRE elements present in its promoter region (as mentioned above) resulting in MKP-1 termination of JNK phosphorylation activity on c-Jun.

NF-κB signaling in positive immune regulation has been thoroughly characterized especially in the periphery (Karin and Greten, 2005). NF-κB comprises of RelA (p65), RelB, c-Rel, NF-κB1 (p50/p105 subunits) and NF-κB2 (p50/p100) proteins and the transcriptionally active dimers identified are: p65/p50 (classic NF-κB), p65/p65, p65/c-Rel, RelB/p50, RelB/p52 and, of note, Rel domains of these proteins bind to DNA. The p65/p50 NF-κB protein is normally sequestered in the cytoplasm by IκB family of proteins. NF-κB translocates to nucleus following phosphorylation of IκB by IKK kinases followed by rapid degradation of IκB. Importantly, phosphorylation of the p65 subunit is important for NF-κB activation. This involves phosphorylation of Serine 27Kuro6 of p65 by protein kinase A (PKA) catalytic subunit in complex with NF-κB and IκB as well as by nuclear localized MAPK-activated mitogen- and stress-activated protein kinase 1 (MSK1) in the nucleus. Interestingly GR was shown to decrease the nuclear pool of MSK1 thus down regulating NF-κB activity (Beck et al., 2008). With regards to its interaction with NF-κB, it was shown that upon GR activation by GC, GR is acetylated. In the nucleus, GR is deacetylated by histone deacetylase (HDAC2) (Ito et al., 2006) before it can physically bind to p65 subunit of NF-κB, functioning as transcriptional antagonist. Another manner by which GR can terminate NF-κB activation is by directly stimulating transcription of IκB-α as mentioned above.

Innate Immune Regulation by GRs in Microglia during Dopamine Neurodegeneration

In the CNS the role of endogenous GCs in regulating expression of pro-inflammatory cytokines such as IL-1β, TNF-α or IL-6 was shown originally following peripheral administration of LPS in adrenalectomized mice (Goujon et al., 1996). The finding that HPA axis is reactive to CNS inflammation triggered by an intrastriatal LPS injection was revealed through prior challenge with systemic LPS that resulted in rise in systemic corticosterone levels with concomitant and significant reductions in proinflammatory TNF-α, Monocyte chemoattractant protein MCP-1, IκBα transcripts in lesioned striatal/cortical region (Nadeau and Rivest, 2002). Interestingly in this paradigm, LPS does not trigger neuronal degeneration. However, neuronal death was observed by prior treatment with GR antagonist RU486 suggesting that GCs acting through GR prevent neuronal degeneration (Nadeau and Rivest, 2003). Recently, the role played by microglial GR in regulating neuronal survival in this intrastriatal model of LPS was shown conclusively in mice with selective inactivation of GR in microglia/macrophages, $GR^{LysMCre}$ mice (Carrillo-de Sauvage et al., 2013). Inflammation triggered by low dose of LPS (1–2 μg) injection has negligible effect on striatal or cortical neurons (Carrillo-de Sauvage et al., 2013), however the same dose of LPS injection in substantia nigra causes specific loss of dopamine neurons (Castaño et al., 2002) indicating a selective vulnerability of dopamine neurons to microglial inflammatory response mediated by LPS-activated TLR4. However, the fact that endogenous GCs activating GRs in microglia are neuroprotective during LPS-induced inflammation in cortex/striatum but not in midbrain substantia nigra implies that their actions in microglia during TLR4 activation may be region-specific. In this regard, recently the concept of microglial heterogeneity with respect to their functional capabilities, for example LPS/TLR4 signaling, has been evoked (Noh et al., 2014).

Nigral dopamine neurodegeneration triggered by MPTP is significantly reduced by pharmacological treatments with GC agonists e.g., corticosterone that artificially increase GCs above endogenous levels, conversely adrenalectomy augments dopamine neuronal loss (Kurkowska-Jastrzebska et al., 2004; Sugama et al., 2009; Ros-Bernal et al., 2011) indicating that high levels of GCs present during MPTP intoxication protect dopamine neurons. Immuno-labeling of GR revealed its localization mainly in the nucleus of microglia and its quantification was carried out in substantia nigra and striatum

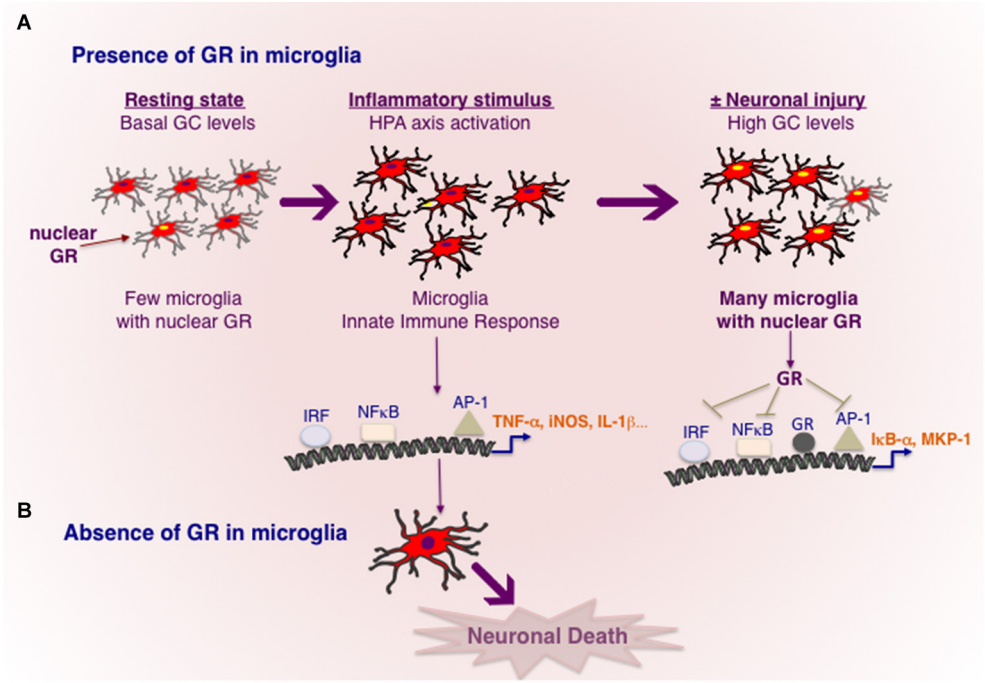

FIGURE 1 | Transcriptional regulation of inflammation by GR in microglia. **(A)** In resting, healthy state without any cellular injury or pathogen invasion, GR is inactive in most microglia. Toxins e.g., MPTP or powerful inflammogen LPS would rapidly trigger innate immune response in microglia. In the case of LPS, TLR4 present in microglia results in activation of major transcription factors NF-κB, IRF and AP-1, known to orchestrate an inflammatory response. Cellular injury or pro-inflammatory cytokines would activate HPA axis and increased GC secretion. High circulating GC levels results in GR activation in microglia, which act to repress the transcriptional activity of NF-κB, IRF and AP-1 and also stimulating the expression of genes such as IκB-α and or MKP-1 known to inhibit NF-κB and AP-1 respectively. Neuronal injury and death are prevented. **(B)** In the absence of GR activity in microglia, microglia remain in activated state causing neuronal death.

in saline and MPTP injected mice. The results showed that number of microglia with nuclear GR augmented from 35% in resting state to 70–80% 3 days after MPTP injections, which then declined to almost normal levels after 3 weeks. Measurement of endogenous corticosterone levels showed a three-fold rise 1 day after MPTP (Ros-Bernal et al., 2011). Importantly, these results indicate that GR activation during endogenous rise in corticosterone levels is progressive concurring loss of dopamine neurons (**Figure 1**). However increasing GC levels by corticosterone treatment results in significant neuroprotection likely because GR activation in microglia is rapid enough to counteract the inflammatory response mounted by activated glia.

The precise actions of GR in microglia during dopamine neurodegeneration were studied using $GR^{LysMCre}$ mice (Ros-Bernal et al., 2011). Functionally, absence of GR in microglia/macrophages resulted in significant dopamine neuronal loss in two paradigms of MPTP intoxication: (a) acute toxicity (4 injections/day) which is accompanied by intense microglial and astroglial activation of short duration; and (b) sub-chronic treatment (1 injection for 5 days) the loss of dopamine neurons is less, and morphologically, microglial activation is less apparent. In microglial GR mutant mice, both MPTP paradigms augmented microglial activation i.e., number hypertrophied microglia, compared to controls. Additionally, in sub-chronic paradigm, GR was found to prolong the duration of activation. Several molecules released by degenerating dopamine neurons can potentially trigger morphological and functional changes in microglia i.e., its activation status or its mobility (e.g., Matrix metalloprotease MMP-9, α-synuclein, Annese et al., 2015), however how the primary signals emitted from degenerating dopamine neurons trigger microglial activation is not well elucidated. With regards to regulation of inflammation, microglial GR was found to modulate 3 classes of inflammatory genes: (a) increasing expression of pro-inflammatory molecules in particular, TNF-α, iNOS, Intercellular adhesion molecule (ICAM); (b) anti-inflammatory genes e.g., MKP-1 (as described above for inhibiting AP1 transcriptional activity) and IL-1R2 which is a decoy receptor for IL-1 receptor1; (c) inflammatory caspases, i.e., caspases 1 and 4 as well as TLR3, TLR4, TLR9 and MyD88. The inflammatory caspases and TLRs are core components of innate immunity important for stimulating the transcriptional activity of AP1, NF-κB and IRF and thereby expression of plethora of inflammatory mediators. These findings indicate that GR not only inhibits the molecules like TNF-α known to execute the inflammatory reaction but also prevent excessive expression of upstream activators that initiate an inflammatory reaction.

Nuclear expression of p65 subunit of NF-κB, indicative of transcriptional activity NF-κB, was observed in microglia of SNpc in PD patients as well as in mice treated with MPTP.

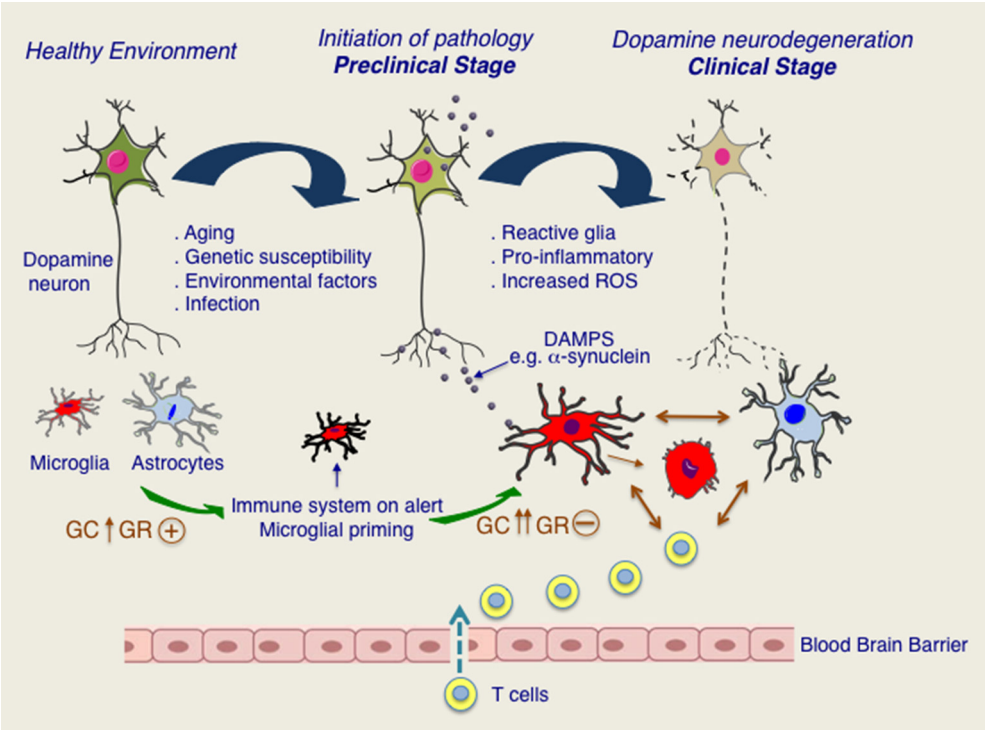

FIGURE 2 | Putative roles of glucocorticoids (GC) and glucocorticoid receptor (GR) in progression to chronic inflammation and dopamine neurodegeneration. In healthy state, microglia and astroglia surrounding are quiescent. Aging as well as other stressors such as infections or PD-related genetic and environmental factors would put immune system on alert and possibly also stimulating HPA axis. Activation of HPA axis results in increase in GC levels and activation of GR. In pre-clinical stage, secretion of DAMPS, such as pathological form of α-synuclein would activate immune system as well as HPA axis. Persistent activation of HPA axis results in loss of its regulation and chronically high GC levels. Chronic GCs are known to result in GR dysfunction in immune cells. Microglia and astroglia remain activated creating a pro-inflammatory environment and augmenting oxidative stress. Disruption in blood brain barrier resulting in T cell infiltration further promotes glial activation. Dopamine degeneration is progressively increased leading to clinical manifestation of PD.

Moreover inhibiting NF-κB in mice significantly protected dopamine neurons against MPTP toxicity (Ghosh et al., 2007). Thus sustained transcriptional activity of NF-κB is likely involved in chronic activation of microglia in PD. Interestingly, GR was found to associate with p65 subunit of NF-κB in microglial cultures, as well in luciferase reporter assays GR inhibited its transcriptional activity (Ros-Bernal et al., 2011; Carrillo-de Sauvage et al., 2013). *In vivo*, Serine 276 phosphorylation of P65 subunit of NF-κB, indicative of its activation, was sustained in MPTP-lesioned SNpc and striatum of mutant GR microglial mice (Ros-Bernal et al., 2011).

The halting of inflammation is central to immune response. A failure to limit the amplitude and duration of this process as well as initiate a resolution phase can lead to chronic inflammatory state. In addition to GR, other members of nuclear receptor family e.g., Peroxisome proliferator activated receptor gamma (PPAR-γ), Liver X receptor (LXR), Estrogen receptor ER-β, Nuclear receptor NR4A family (Nurr 77, Nurr1) are expressed in microglia, thus they can also control microglial activation. In this regard, Nurr1 inhibition was found to increase NF-κB activity in glia resulting in exaggerated expression of pro-inflammatory mediators and increased loss of dopamine neurons following LPS injection in substantia nigra (Saijo et al., 2009).

Regarding GCs, it is possible that in PD, GR functions in immune cells are compromised because of chronically elevated levels of cortisol. The putative scenario of dysfunction of GR signaling in PD is illustrated in **Figure 2**. Different stressors such as aging, infections, environmental and genetic susceptibility factors would activate HPA axis resulting in augmentation of circulating GC levels and activation of GR. In parallel, activation of peripheral immune system would result in increased circulating levels of pro-inflammatory molecules e.g., Il-1β known to activate HPA axis and also to induce microglial priming such that any subsequent insult exacerbates microglial inflammatory phenotype. Persistent activation of HPA axis with chronically high cortisol levels would compromise GR functions (Dejager et al., 2014). Further studies are needed to understand how GR activity is affected in microglia during chronically active HPA axis, as is the case for PD patients and whether GR inflammatory function is affected in PD. In addition, it would be important to understand the redundant and non-redundant functions of GR with closely related nuclear receptor members such as Nurr1 for envisaging therapeutic potentials in PD.

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Abbreviations

ACTH, Adrenocorticotropic hormone; AP1, Activator protein 1; ATF, Activating transcription factor; BBB, Blood brain barrier; BST-1, Bone marrow stromal cell antigen; C1Q, Complement component 1q; C3, Complement component 3; CBP, CREB Binding protein; CCL5, Chemokine ligand 5; CD14, cluster of differentiation 14; CD200, Cluster of Differentiation 200; CD47/CD172A, Cluster of Differentiation 47; CLRN3, Clarin3; CNS, Central nervous system; CRH, Corticotropin-releasing hormone; CX3CL1, Chemokine (C-X3-C motif) ligand 1; CX3CR1, CX3C chemokine receptor 1; CYP2D6, Cytochrome P450 2D6; DAMP, Damage-associated molecular pattern; DBD, DNA-binding domain; ER-β, Estrogen Receptor β; FKBP51, FK506 binding protein; GC, Glucocorticoids; GPNMB, Glycoprotein NMB gene; GR, Glucocorticoid receptor; GRE, Glucocorticoid response element; H1N1, Hemagglutinin neuraminidase; HDAC2, Histone deacetylase 2; HLA, Human leucocyte antigen; HPA, Hypothalamic-pituitary-adrenal; HSP90, Heat shock protein 90; ICAM, Intercellular adhesion molecule; IFN-γ, Interferon γ; IκBa, Nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor; IKK, IκB kinase; IL-1β, Interleukin-1β; IL-6, Interleukin-6; iNOS, Inducible nitric oxide synthase; IRF, Interferon response factor; JNK, c-Jun N-terminal kinase; LB, Lewy bodies; LBD, Ligand-binding domain; LPS, Lipopolysaccharide; LRRK2, Leucine rich repeat kinase 2; LXR, Liver X receptor; MAPK, Mitogen-activated protein kinase; MCP1, Monocyte chemoattractant protein-1; MECP2, Methyl CpG binding protein 2; MHC, Major histocompatibility complex; MKP1, MAPK phosphatase 1; MMP9, Matrix metalloprotease 9; MPTP, 1-methyl, 4-phenyl, 1, 2, 3, 6-tetrahydropyridine; MR, Mineralocorticoid receptor; MSK1, Mitogen- and stress-activated protein kinase-1; MYD88, Myeloid differentiation primary response gene 88; NCOA1, Nuclear receptor coactivator 1; NF-κβ, Nuclear factor κ-light-chain-enhancer of activated β cells; NR3C1, Nuclear Receptor Subfamily 3, Group C, Member 1; NR4A, Nuclear receptor 4A; NSAID, Nonsteroidal anti-inflammatory drugs; P300, E1A binding protein p300; PAMP, Pathogen-associated molecular patterns; PD, Parkinson's disease; PET, Positron emission tomography; PINK1, PTEN-induced putative kinase 1; PKA, Protein kinase A; PLA2G6, Phospholipase A2, group VI; PPAR-γ, Peroxisome proliferator-activated receptor γ; PVN, Paraventricular nucleus of hypothalamus; RANTES, Regulated on activation, normal T cell expressed and secreted- CCL5; REM, Rapid eye movement (REM) sleep; SN, Substantia nigra; SNCA, Synuclein, α; SNP, Single Nucleotide Polymorphism; SPG4/SPAST, Spastic paraplegia-4/spastin; TAK1, Transforming growth factor activated kinase-1; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor-α.

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IX.IV Herrero MT, Insausti R, Estrada C. Thalamus: Anatomy. Brain Mapping: An Encyclopedic Reference (BRNM) Elsevier 2015

Abstract

The human thalamus is a nuclearcomplex located in the diencephalon. Anatomically, the thalamus can be subdivided in nuclear groups with respect to the internal medullary lamina, the anterior, medial, lateral, intralaminar, and posterior nuclei, and to the external medullary lamina, the reticular nucleus (RN). The thalamic nuclei can be grouped in six functional classes: (i) the RN, (ii) the specific sensory nuclei, (iii) the effector nuclei, (iv) the limbic nuclei, (v) the intralaminar nuclei, and (vi) the associative nuclei. Thalamic nuclei receive glutamatergic modulators mainly from the lower sublamina of cortical layer VI and receive denser projections from brain stem modulators (cholinergic, dopaminergic, noradrenergic, and serotonergic). A peculiarity that characterizes thalamic nuclei is the complexity of their projections: almost all the thalamic nuclei project to one or to a few well-defined cortical areas. Multiple cortical areas receive afferents from a single thalamic nucleus and send back information to different thalamic nuclei providing positive and reciprocal feedback and, if necessary, suppressing irrelevant information. One of the main sources of information about thalamic function is due to diseases of the cerebrovascular system. There are four Au7 important arterial territories in which lesions develop the main four thalamic vascular syndromes: (i) tuberothalamic artery, (ii) paramedian artery, (iii) inferolateral artery, and (iv) posterior choroidal artery. The thalamus is an important relay center subserving sensory and motor mechanisms, arousal, cortical synchrony, emotion, cognition, and memory. However, not only thalamic neurons are relays of information, but also they have a role in controlling executive networks and in regulating complex behaviors, such as behavioral flexibility and reward-directed behavior.

Thalamus: Anatomy

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Glossary

Allothalamic region It is divided into (i) the paraventricular area or midline nuclei, (ii) the center median–parafascicular complex (isolated by a continuous capsule), and (iii) the intralaminar nuclei within the lamina medialis.
Burst mode also called 'oscillatory mode' Neurons have an intrinsic rhythmicity and are tonically hyperpolarized.
External medullary lamina Bundle of myelinated nerve fibers laterally around the dorsal thalamus that separates the ventral and lateral thalamus from the subthalamic nucleus and the reticular nucleus.
Internal medullary lamina (IML) 'Y'-shaped bundle of myelinated nerve fibers that course through the thalamus along its rostrocaudal axis.
Isothalamic region It constitutes the bulk of the thalamus. The IML divides the principal thalamic nuclei into two major parts: a medial group on one side and a ventrolateral group on the other side. The anterior part of the IML splits into two lamellae and surrounds the anterior group of thalamic nuclei.
Situs Topographical locations that may or may not correspond to cytoarchitectonic subdivisions.
Thalamic drivers Thalamic afferents that target proximal dendrites with relatively large synaptic boutons, and its function is thought to be the faithful transmission of the spike message relayed by thalamic cells to postsynaptic structures.
Thalamic modulators Thalamic afferents that target distal dendrites and influence spike transmission by adjusting the cellular and synaptic mechanisms underlying spike generation.
Thalamic nucleus The intersection of a thalamocortical source space with one territory.
Thalamic region A gross topographical division corresponding to the nuclei included on it. The dorsal thalamus has two regions: the allothalamic and the isothalamic regions.
Thalamic space (this term is not in use nowadays) The source space was the thalamic space where neurons project to a given cortical target, and then, the thalamic nucleus was defined as the intersection of a thalamocortical space with one territory.
Thalamic territory The cerebral space filled by afferent endings from one source (when having a distinct topography in a region, a given territory makes a 'subregion').
Tonic mode When the neurons respond to depolarization and hyperpolarization.

Abbreviations

Anterior nuclear complex

AD	Anterodorsal nucleus (anterior group)
AM	Anteromedial nucleus (anterior group)
AV	Anteroventral nucleus (anterior group)
LD	Laterodorsal nucleus (anterior group)

Medial–midline group

MDmc	Mediodorsal nucleus, magnocellular (midline group)
MDpc	Mediodorsal nucleus, parvocellular (midline group)
Pt	Paratenial nucleus (midline group)
PV	Paraventricular nucleus (midline group)
Re	Nucleus reuniens (midline group)

Lateral group

LP	Lateral posterior nucleus (lateral dorsal group)
VA	Ventral anterior nucleus (lateral ventral group)
VL	Ventral lateral nucleus (lateral ventral group)
VM	Ventral medial nucleus (lateral ventral group)
VP	Ventral posterior nucleus (lateral ventral group)
VPL	Ventral posterolateral nucleus
VPM	Ventral posteromedial nucleus
VPpo	Ventral posterior nucleus
VPI	Ventral posterior inferior nucleus

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Intralaminar group

CL Central lateral nucleus (intralaminar group – anterior/rostral)
CM Centromedian nucleus (intralaminar group – posterior/caudal)
PCn Paracentral nucleus (intralaminar group – anterior/rostral)
Pf Parafascicular nucleus (intralaminar group – posterior/caudal)

Posterior group

Pul Pulvinar complex (posterior group)
PM Pulvinar inferior/medial nucleus (posterior group)

Metathalamus

LGN Lateral geniculate nucleus (metathalamus)
MGN Medial geniculate nucleus (metathalamus)
RN Reticular nucleus

The Thalamus

The diencephalon, which forms the central core of the brain, is at the dorsal end of the brain stem. Inside the diencephalon, the dorsal thalamus (just called the thalamus) is its largest component; however, the human thalamus is small when compared to the rest of the encephalon (Tuohy et al., 2004). The thalamus is a midline bilateral structure with two symmetrical halves separated by the third ventricle but connected by the *interthalamic adhesion* (*massa intermedia*), a flattened gray band (Morel, 2007). Then, the medial surface of the thalamus corresponds to the upper part of the lateral wall of the third ventricle. From a radiological point of view, thalamic segmentation defines the anterior boundary of the thalamus as the first slice caudal to the anterior commissure: at rostral slices, the thalamus starts behind the *columnae fornicis* and the mammillary bodies, and at the caudal slices, the thalamus is the region below the fornix. The geniculate nuclei (both medial and lateral) were included in the outline at levels where the lateral geniculate nucleus (LGN) was attached to the corpus of the thalamus and not separated from it by a white matter tract. Ventrally, the thalamus is located close to the hypothalamus and the mesencephalon. The hypothalamus lies ventral to the thalamus and is more anteriorly situated relative to the thalamus; therefore, in coronal sections, the mammillary bodies (the caudal part of the hypothalamus) are at the same plane of section compared with the anterior complex of the thalamus. At all levels, the thalamus is bordered medially by the third ventricle, dorsally by the lateral ventricles, and laterally by the internal capsule on both sides (**Figure 1(a)** and **1(b)**). The thalamus has an average volume of around 9 cm^3 in males and 8 cm^3 in females being around 5.7 cm in length, 2.5 cm in height, and about 2 cm in width. It has been estimated that there are about 10 million thalamic neurons in each hemisphere.

The thalamus, the major relay to the cerebral cortex, is not only the part of the nervous system where sensory inputs (except olfaction) can be modulated but also the relay for limbic pathways and cerebellar and basal ganglia inputs to the cerebral cortex (Zhang et al., 2010). Moreover, the thalamus is also implicated in numerous systems and functions as in alertness, arousal, memory, and the ability to accomplish tasks of fast information processing. The thalamus dispenses specific alerting or engagement response to the different domains of function to which it contributes. With the exception of the reticular nucleus (RN), the other nuclei of the thalamus project to the cerebral cortex and receive reciprocal connections from the same cortical areas that in turn can modulate the thalamic functions filtering its thalamic inputs.

Ontogeny of the Thalamus

The ontogeny of the nervous system can be envisioned as a tube (neural tube) that results from the closure of a furrow that is formed in the dorsal part of the embryo (neuroectoderm). The furrow, once closed to form a tube, dilates into several cerebral vesicles, which will derive in the main divisions of the central nervous system. The correspondence between neural tube vesicles and fully developed central nervous system is as follows: the telencephalon (the cerebral cortex), diencephalon (the thalamus and hypothalamus), mesencephalon (the mesencephalon), and rhombencephalon (the cerebellum, pons, and medulla). The remainder of the neural tube becomes the spinal cord. The diencephalic vesicle separates into a dorsal and a ventral part by the thalamohypothalamic sulcus. The classical partition of the diencephalon corresponded to Herrick's columnar model; however, the new prosomeric model (Puelles & Rubinstein, 1993) indicates that the rostral forebrain (including the telencephalon and hypothalamus) represents a complex protosegment not divided into prosomeres, whereas the caudal forebrain is divided into three prosomeres (P1, P2, and P3) and the thalamus corresponds to P2 (**Figure 1(c)**). The caudal population of thalamic progenitors in P2 gives rise to all thalamic nuclei that relay sensory information from the periphery to primary sensory regions of the neocortex via thalamocortical axons (Vue, Aaker, Taniguchi, et al., 2007).

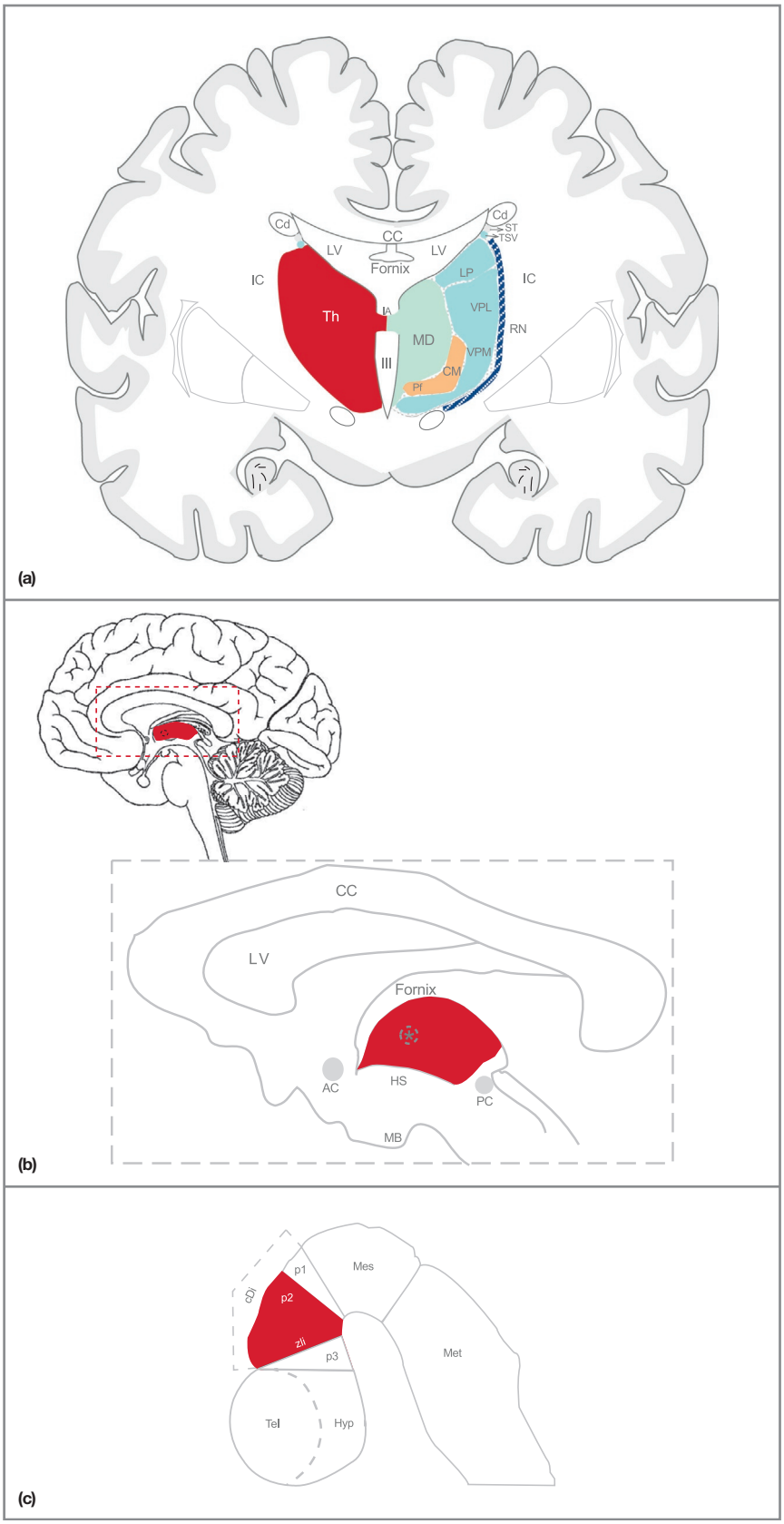

Figure 1 The thalamus is an oval-shaped region located in the center of the brain. (a) Coronal cross section of the brain showing the thalamic medial surface that forms part of the lateral wall of the third ventricle (III) and is usually connected to the opposite thalamus by the gray matter interthalamic connection (interthalamic adhesion) and the thalamic lateral limits, the internal capsule, and the caudate nucleus; please note the lateral border of the superior surface of the thalamus is limited by the stria terminalis (gray) and overlying thalamostriate vein (blue), which separate thalamic nuclei from the body of the caudate nucleus. Additionally, the external medullary lamina, white matter, separates the main body of the thalamus

Continued

Anatomical General Topography and Thalamic Nuclei

The thalamus differentiates into a series of neuronal groups that can be classified according to a number of landmarks that determine their relative position in the thalamus (see Table 1 for a classical description). The principal terms in the nomenclature are the anteroposterior, mediolateral, and dorsoventral subdivisions, but, in fact, there are three types of thalamic nuclei in relation to its function: specific, nonspecific, and association nuclei. However, as the specific partitions of the different nuclei and 'territories' differ from author to author to name the thalamic nuclei, there nomenclature is still very confusing. In general, there has been a rigid conception of the 'thalamic nucleus' combining cytoarchitectonic techniques, comparative anatomy, and cortical connections (with underexploitation of subcortical afferents), and it has been established according to rational and historically grounded rules.

In this article, we basically follow the terminology of Hirai and Jones (1989) that is important in relation to functional studies, but we include as well other terms used in the literature. From a morphological point of view, the thalamus is divided into three regions (anterior, lateral, and medial) that are anatomically defined by a 'Y'-shaped bundle of myelinated nerve fibers termed the internal medullary lamina (IML) that courses through the thalamus along its rostrocaudal axis (Figure 2). The anterior pole of the thalamus is between the arms of the rostral part of the IML, and it is made up of the anterior nuclear (AN) complex, of which the anterodorsal (AD), anteromedial (AM), and the anteroventral (AV) nuclei are the most representative. Thalamic nuclei medially located to the IML receive the name of midline thalamic nuclei and include the mediodorsal nucleus (or dorsal medial nucleus) (MD) with two subnuclei (magno- and parvocellular, medial and laterally located, respectively) and the midline structures (the paratenial, paraventricular, nucleus reuniens, and rhomboid, beneath the wall of the third ventricle). The nuclei external to the IML form a lateral group of neuronal ensembles bounded by the RN of the thalamus (a thin shell of neurons covering the entire lateral aspect of the thalamus and separated from the thalamus by the external medullary lamina, EML). The lateral group is subdivided into ventral and dorsal tiers, each of which contains different subnuclei. The lateral ventral tier contains the ventral anterior (VA), the ventral lateral (VL), the ventral medial (VM), and the ventral posterior (VP) nuclei. This last one is divided into the ventral posterolateral (VPL) nuclei and ventral posteromedial (VPM) and the ventral posterior (VPpo) and ventral posterior inferior (VPI) nuclei. The lateral dorsal (LD) tier contains, from rostral to caudal, the LD nucleus (included in the anterior group), the lateral posterior (LP) nucleus, and, finally, the thalamus thickens and expands posteriorly to constitute the pulvinar complex (Pul) (also considered as posterior thalamus, and it is only well represented in human and nonhuman primates). The metathalamus is located at the posterior part of the pulvinar with two distinct groups forming the relay for hearing (the medial geniculate nucleus, MGN) and vision (LGN). Furthermore, within the IML are the intralaminar nuclei, among those being rostrally the central lateral (CL) and paracentral nuclei (PCn) and caudally the centromedian (CM) and parafascicular (Pf) nuclei (Figure 2). Further subdivisions are recognized, in particular in the human thalamus. Thus, the thalamus contains no less than 40 different nuclei, some of which can be related to specific sensory inputs, while others are more related to other functional systems such as the limbic system or the reticular formation (Krauth et al., 2010).

General Topography from the Functional Point of View

Every nucleus in the dorsal thalamus receives subcortical inputs and projects to the cerebral cortex. Ramon y Cajal was the first to demonstrate thalamocortical fibers, their terminations in the cortex, and the feedback provided by corticothalamic fibers (Ramon y Cajal, 1900, 1903). Thalamic nuclei also project to the amygdala, the hippocampal formation, the striatum, and other basal telencephalic areas. In relation to these functional projections, thalamic nuclei could be divided into those that project to the cerebral cortex and those that project to the cortex and striatum (as the intralaminar nuclei) (Jones, 2007). Moreover, those projecting to the cortex include (i) the specific sensory relay nuclei (the VP, the medial geniculate, and the lateral geniculate nuclei) projecting to the layer IV of its corresponding cortical area (and with both afferents and efferents topographically organized) and (ii) the thalamic nuclei projecting upon several cortical areas in a diffuse manner, whose projections end in one or more of several layers (mainly layers I and VI). However, from a general functional point of view, the thalamic nuclei can be grouped in six functional classes (Schmahmann & Pandya, 2008): (i) the RN, (ii) the specific sensory nuclei, (iii) the effector nuclei, (iv) the limbic nuclei, (v) the intralaminar nuclei, and (vi) associative nuclei.

(i) The *reticular nucleus* (RN) (*situs perithalamicus*) was described by Kölliker as the 'Gitterkern' or lattice nucleus (because of the fibrous latticework characteristic of the area) (Kölliker, 1896). The RN surrounds much of the thalamus like an eggshell and can be divided into different sectors topographically connected with different thalamic

Figure 1 **Cont'd** from the reticular nucleus. (b) Sagittal section showing the rostral limits, the anterior commissure (AC), and the caudal limits, the posterior commissure (PC). It can be noted that dorsally, the thalamus is bounded by the fornix (and subarachnoid space in the form of the transverse cerebral fissure) and ventrally is limited by the hypothalamic sulcus (HS, sulcus of Monro) of the third ventricle (this sulcus in the lateral wall of third ventricle extends from the upper end of midbrain aqueduct posteriorly to the interventricular foramen anteriorly). (c) Cartoon of the prosomeric model showing the three prosomeres' part of the caudal forebrain, being P2 (in gray) the segment origin of the thalamus (modified from Epstein, 2012). AC, anterior commissure; CC, corpus callosum; Cd, caudate nucleus; cDi, caudal diencephalon; CM, centromedian nucleus; HS, hypothalamic sulcus; Hyp, hypothalamus; IA, interthalamic adhesion; IC, internal capsule; LP, lateral posterior nucleus; LV, lateral ventricle; MD, mediodorsal nucleus; Mes, mesencephalon; Met, metencephalon; PC, posterior commissure; Pf, parafascicular nucleus; RN, reticular nucleus; ST, stria terminalis; Tel, telencephalon; Th, thalamus; TSV, thalamostriate vein; VPL, ventral posterior lateral nucleus; VPM, ventral posteromedial nucleus; III, third ventricle. Note: The interthalamic adhesion (mediodorsal neurons) is found in 70–80% of humans and its anteroposterior diameter is about 1 cm in length.

Table 1 Diencephalic elements in hierarchical order

	Abbreviations	*Comments*
I. Diencephalic nonthalamic elements		
I.A. Perithalamus, PTh (*ventral thalamus*, *thalamus ventralis*)		
i. *Nucleus perithalamicus*	PTh	
Situs perithalamicus	PTh	N. reticular, RN
Situs incertus	PTh ZI	Zona incerta, Zi
Situs pregeniculatus	PTh Pr	*N. pregeniculatus or prepeduncularis*
I.B. Epithalamus, Hb	Hb	Habenula, Hb
i. *Nucleus habenulae lateralis*	HbL (Hlmc)	
ii. *Nucleus habenulae medialis*	HbM (Hlpc)	
II. Thalamus, Th (dorsal thalamus)		
II.A. Allothalamus (neuronal types different from those of the isothalamus)		
i. *Regio paraventricularis* (paramediana)	E	Midline nuclei + interthalamic *adhesion* (*massa intermedia*)
ii. *Regio centralis*	C	Center median–parafascicular complex
Nucleus centralis medius	CMe	N. centromedian, CM
Nucleus centralis parafascicularis	CPf	N. parafascicular, Pf
Nucleus centralis paralateralis	CPl	
iii. *Regio intralaminaris*	Il	Intralaminar nuclei (in true sense of term)
Nucleus intralaminaris oralis	Ilo	N. paracentral, PCn
Nucleus intralaminaris caudalis	Ilc	N. centralis lateral, CL
Nucleus intralaminaris posterior	Ilp	*N. posterioris*, lLa
iv. *Nucleus limitans*	Li	
II.B. Isothalamus (made up of bushy thalamocortical projection neurons + microneurons)		
II.B.1. (*Superregio*) *regio superior*	S	
Nucleus anterior	A	*N. anterior principalis* (anteroventral, AV + anteromedial, AM)
Nucleus anterodorsalis	AD	*N. anterior accesorius*, Aa (anterodorsal, AD)
Nucleus superficialis	S	N. laterodorsal, LD
II.B.2. *Superregio medioposterior*		
i. *Regio medialis*	M	Main part of the mediodorsal nucleus, MD
Nucleus medialis (in human)	M	
Nucleus medialis medialis	MM	N. MD magnocellular, MDmc
Nucleus medialis lateralis	ML	N. MD parvocelular, MDpc
ii. *Regio posterior*	P	Main part of the pulvinar, Pul
Nucleus posterior		
Situs medialis	PuM	N. pulvinar medial (inferior), PM
Situs lateralis	PuL	N. pulvinar lateral, PL
Situs oralis	PuO	N. pulvinar oral, PO
Situs oralis dorsalis	PuOD	N. lateral posterior, LP
II.B.3. *Superregio basalis*		
i. *Regio basalis*	B	
Nucleus subgeniculatus		Spinothalamic fibers
ii. *Regio Intergeniculata*	Ig	
Nucleus intergeniculatus		Tectal thalamus
II.B.4. Superregio inferolateralis		Sensory and motor thalamus
i. *Regio lateralis*	L	Lateral mass + paralaminar areas of the MD
Sensorimotor thalamus		
a. Motor thalamus		
a.1. *Subregio oralis*	LO	Nigral and pallidal thalamus[a]
Nucleus lateralis oralis	LO	N. ventral anterior, VA (VOL + DO)
Situs principales	LOl	VOL (VOe, VLo)
Situs dorsalis	LOd	DO (DOe, VA dorsolateral)
Situs ventralis	LOv	VOV (VLm, lateral)[b]
a.2. *Subregio dorsalis*	LR	Nigral and pallidal thalamus[a]
Nucleus lateralis rostralis	LR	N. ventral medial, VM (VOM + LPo)
Situs polaris	LRPo	
Situs perifascicularis	LRmc	
Situs ventralis medialis	LRpm	
Situs paralaminaris rostralis	PlO	
Nucleus lateralis rostralis pars medialis	LRM	Nigral and amygdalar thalamus
a.3. *Subregio intermedia*	LI	Cerebellar thalamus
Nucleus lateralis intermedius lateralis	LIL	N. ventral lateral, VL

(*Continued*)

Table 1 (Continued)

	Abbreviations	*Comments*
Nucleus lateralis intermedius mediodorsalis	LIM	*N. ventralis intermedius* (Vim) + Dl
Situs ventralis medialis	LIMm	Vim[c]
Situs dorsalis	LIMd	Dl
Situs postremus	LIMps	
Situs paralaminaris intermedius	pll	
b. Sensorial thalamus		
b.1. *Subregio caudalis*		Lemniscal thalamus
Nucleus ventralis caudalis lateralis	LCL	N. ventral posterolateral, VPL
Nucleus ventralis caudalis medialis	LCM	N. ventral posteromedial, VPM
b.2 *Subregio arcuata*		Gustatory thalamus
Nucleus lateralis arcuatus	LArc	
		Spinothalamic thalamus
		N. ventral posterior inferior, VPI
		N. ventral posterior, VPpo
ii. *Regio geniculata*	G	
Nucleus geniculatus medialis	Gm	Auditory thalamus, MGN
Nucleus geniculatus lateralis	Gl	Visual thalamus, LGN

The dorsal thalamus (II) is divided into allo- (II.A) and isothalamus (II.B).

[d]"There is no polar subdivision without lower afferents in front of the pallidal and nigral territories and thus no reason for isolating a nucleus lateralis polaris or a polar VA" (Percheron et al., 1996).

[b]*Nucleus lateralis oralis*, *situs ventralis* (LOv) = *nucleus ventralis oralis posterior*, *Vop* (Hassler, 1959) = nucleus ventral lateral ventral (Hirai & Jones, 1989). Pallidal connections.

[c]*Nucleus lateralis intermedius mediodorsalis*, *situs ventralis medialis* (LIMm) = *N. ventralis intermedius* (Vim) (Hassler, 1959) = nucleus ventral lateral posterior (ventral part) (Hirai & Jones, 1989). Cerebellar connections.

Source: Modified with permission from Herrero, M. T., Barcia, C., Navarro, & J. M. (2002). Functional anatomy of thalamus and basal ganglia. *Child's Nervous System*, *18*, 386–404; previously modified from Percheron, G., François, C., Talbi. B., Yelnik, J., & Fénelon, G. (1996). The primate motor thalamus. *Brain Research Reviews*, *22*, 93–181.

nuclei and with different functions (arousal, hearing, emotional, movement, sight or touch). The RN is the only thalamic nucleus that does not project to the cerebral cortex but in this nucleus blend afferents from cortical areas, from the globus pallidus, and from dorsal thalamic nuclei. In fact, most inputs to other thalamic nuclei are collaterals of fibers passing through this nucleus modulating the flow of information from other nuclei to the cortex. Its neurons are GABAergic (inhibitory) and contribute to the synchrony and rhythms of thalamic activity with a relevant importance in consciousness, sleep, and epilepsy. These GABAergic cells are innervated by collateral branches of thalamocortical and corticothalamic fibers (with connectivity with both the thalamic nuclei and its associated cortical area) (Jones, 2002a, 2002b).

(ii) The *specific sensory thalamic nuclei* include the ventroposterior nuclei (lateral, medial, and inferior; VPL, VPM, and VPI, respectively) and the geniculate nuclei (medial and lateral; MGN and LGN, respectively). The topographical organization of the afferents to the somatosensory thalamic nuclei is contralateral (however, some nuclei have both contralateral and ipsilateral connections (as the gustatory system, Iannilli, Singh, Schuster, Gerber, & Hummel, 2012)). The ventroposterior nuclei receive topographically organized projections from the dorsal column (medial lemniscus) and the spinothalamic and the trigeminothalamic tracts: VPL nucleus controls body and limbs, and VPM nucleus the head, neck, and gustatory relays. VPL and VPM nuclei are somatotopically interconnected with primary unimodal somatosensory parietal cortices (**Figures 2** and **3**, **Table 2**). The gustatory information, which is important in taste detection and recognition, is controlled by the parvicellular/paucicellular part of the medial tip of the VPM nucleus, close to the midline. VPI nucleus concentrates spinothalamic tract afferents instead of dorsal column–medial lemniscus ones. The spinothalamic relay for pain and temperature sensation in humans terminates in the posterior portion of the VPM (VPpo) nucleus, and lesions to this area would be the neural substrate for thalamic pain syndromes (Craig, 2003). VPI and VPpo nuclei are important regions for discriminative processing and perception of painful stimuli. The somatosensory thalamus has anteroposteriorly oriented rods with a somatotopic distribution. Each rod (a compilations of neurons) relays sensory information to one specific cortical column. It have been described two types of rods: nociceptive and non-nociceptive (Apkarian, Shi, Brüggemann, & Airapetian, 2000). The MGN can be divided into three main subdivisions, the ventral, dorsal, and medial (Bartlett, 2013). The MGN has a role in both elementary audition and higher-level auditory processing; it receives projections from the inferior colliculus and sends its axons to the primary and association auditory temporal cortices (**Figures 2** and **3**, **Table 2**). The LGN comprises six histologically distinct layers that receive organized and topographic projections from the retina (retinotopic mapping) as well as from visual cortical areas. Optic tract axons originating in the contralateral nasal retina innervate layers 1, 4, and 6 of the LGN, and axons from ipsilateral temporal retina innervate layers 2, 3, and 5 (Chen, Zhu, Thulborn, & Ugurbil, 1999). LGN neurons send its axons topographically to the primary and

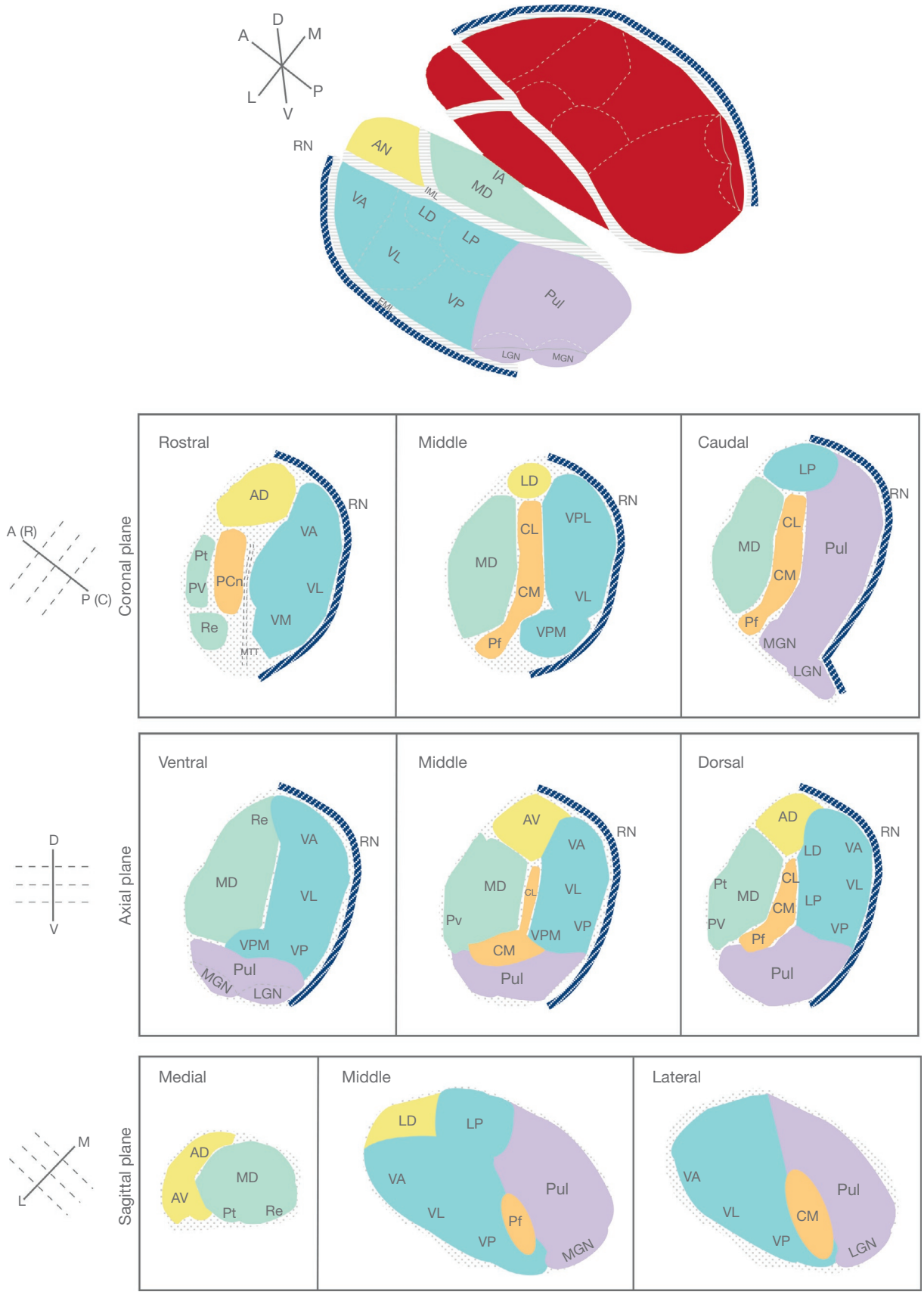

Figure 2 At the top: Divisions of the thalamus. Nuclei: yellow, anterior group; blue, lateral group; purple, posterior group; green, midline group; orange, intralaminar group. Underneath: anatomy and subdivisions of the thalamus nuclei analyzed by means of coronal, axial, and sagittal planes.

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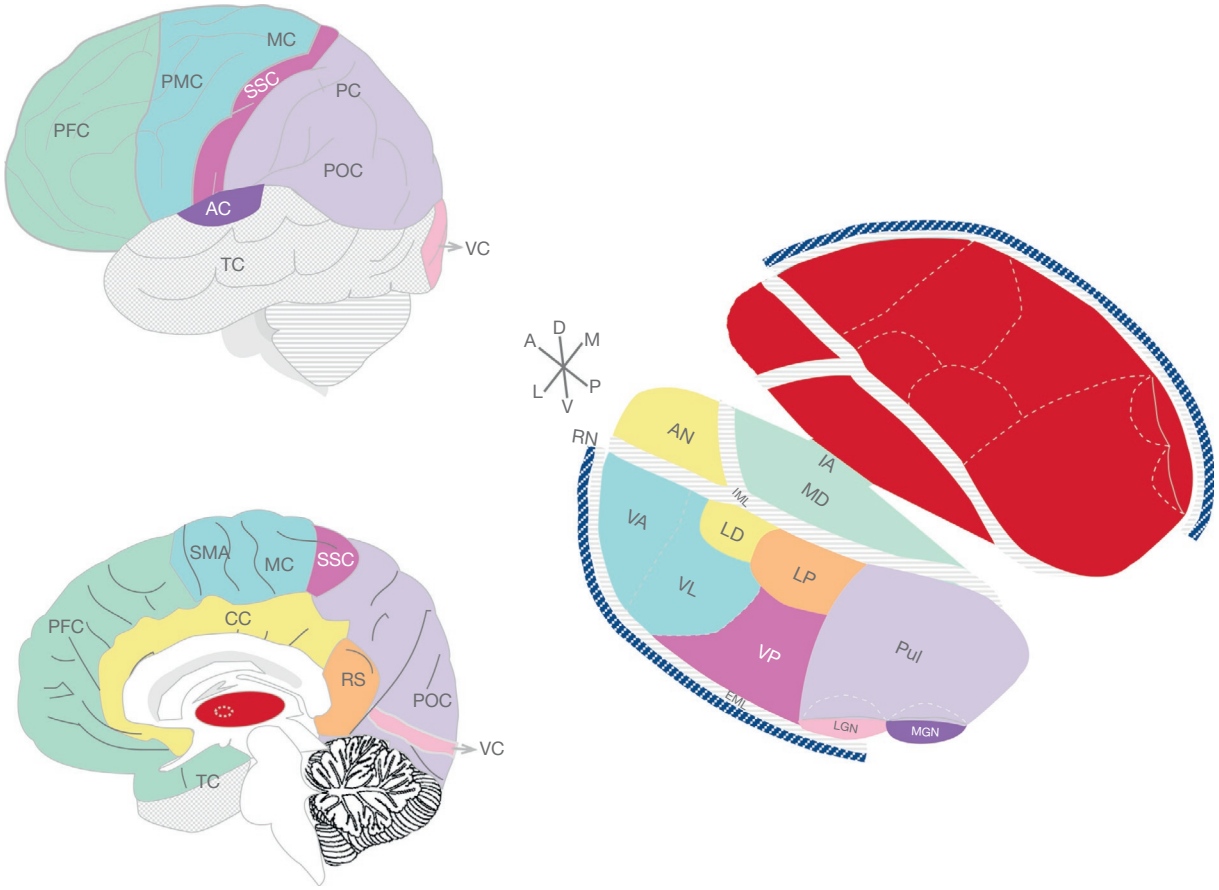

Figure 3 Topography of projections of the thalamic nuclei. (a) Cerebral cortex, lateral view of the right hemisphere; (b) cerebral cortex, sagittal view of the left hemisphere; (c) thalamic nuclei. Please see the code of colors similar in the left thalamic nuclei and its corresponding cortical areas (Behrens et al., 2003). A, anterior; AC, auditory cortex; AN, anterior nuclear complex; CC, cingulate cortex; D, dorsal; EML, external medullary lamina; IA, interthalamic adhesion; IML, internal medullary lamina; L, lateral; LD, laterodorsal nucleus; LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; M, medial; MC, motor cortex; MD, mediodorsal nucleus; MGN, medial geniculate nucleus; PC, parietal cortex; PFC, prefrontal cortex; PMC, premotor cortex; P, posterior; POC, parieto-occipital cortex; Pul, pulvinar complex; RN, reticular nucleus; RS, retrospenial cortex; SMA, supplementary motor area; SSC, somatosensory cortex; TC, temporal cortex; V, ventral; VA, ventral anterior nucleus; VC, visual cortex; VL, ventral lateral nucleus; VP, ventral posterior nucleus.

secondary occipital visual cortices (Schneider, Richter, & Kastner, 2004; **Figure 2**, **Table 2**).

(iii) The *thalamic effector nuclei* are the motor nuclei, which include the VA, the VM, and the VL nuclei, which are covering the entire anterior part of the lateral region of the thalamus. The motor thalamus is the center of integration of networks and not just a relay of information to the cortex; then, these nuclei underlie the ability to modulate behaviors concerning movement and aspects of language (Haber & Calzavara, 2009). The thalamic effector nuclei receive neuronal afferents from the basal ganglia (the substantia nigra *pars reticulata* and medial globus pallidus by the *thalamic fasciculus*) and from the cerebellum (by the dentatorubrothalamic tract); then, the named motor thalamus is made up of three topographically distinct territories: nigral, pallidal, and cerebellar. The thalamic cerebellar territory is located caudally in front of the somesthetic ventral posterior nucleus. These nuclei are implicated in the control of movement because they are strategically located between motor areas of the cerebral cortex and motor-related subcortical structures, such as the cerebellum and basal ganglia; in fact, these nuclei are a part of the segregated but integrated cortico– striatal–thalamo–cortical loops (the functional entities for motor and sensory processes as well as for higher cognitive functions like cognitive and emotional processes, which also include the dorsomedial, intralaminar, and anterior thalamic nuclei) (**Figure 3**; Metzger et al., 2013).

Figure 2 **Cont'd** A, anterior; AD, anterodorsal nucleus; AV, anteroventral nucleus; C, caudal; CL, central lateral nucleus; CM, centromedian nucleus; D, dorsal; EML, external medullary lamina; IA, interthalamic adhesion; IML, internal medullary lamina; L, lateral; LD, laterodorsal nucleus; LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; M, medial; MD, mediodorsal nucleus; MGN, medial geniculate nucleus; MTT, mammillothalamic tract; P, posterior; PCn, paracentral nucleus; Pf, parafascicular nucleus; Pt, paratenial nucleus; Pul, pulvinar complex; PV, paraventricular nucleus; R, rostral; Re, nucleus reuniens; RN, reticular nucleus; V, ventral; VA, ventral anterior nucleus; VL, ventral lateral nucleus; VM, ventral medial nucleus; VP, ventral posterior nucleus; VPL, ventral posterior lateral nucleus; VPM, ventral posteromedial nucleus.

Table 2 Afferent and efferent projections of the thalamic nuclei

Nucleus	*Afferents*	*Efferents*
Anterior nuclear complex		
Anterodorsal, anteromedial, and anteroventral nuclei Laterodorsal nucleus	Mammillary complex (from hippocampus) Cingulate cortex	Cingulate cortex
Medial–midline group		
	Prefrontal cortex Amygdala, olfactory cortex Basal ganglia	Medial and lateral prefrontal cortices Orbitofrontal cortex
Lateral group		
Lateral posterior nucleus	Posterior cingulate cortex Posterior parietal region Extrastriate occipital areas	Posterior cingulate cortex Extrastriate occipital cortices Posterior parietal cortex
Ventral anterior nucleus	Substantia nigra pars reticulata and medial globus pallidus Premotor cortex	Premotor cortical cortex Dorsolateral prefrontal cortex
Ventral lateral nucleus	Deep cerebellar nuclei	Motor cortex, premotor cortex, supplementary motor area
Ventral posterior nucleus		
Ventral posterolateral nucleus	Lemniscus medialis (body and limbs)	Somatosensory cortex
Ventral posteromedial nucleus	Lemniscus medialis and trigeminal lemniscus (head, neck, and gustatory relays)	Somatosensory cortex
Ventral posterior nucleus	Spinothalamic relays (pain)	Somatosensory cortex
Intralaminar nuclei		
	Spinal cord, periaqueductal gray matter, reticular formation, cerebellum	Anterior: cortical area of eye movement Posterior: striatum
Posterior group – pulvinar complex		
	Superior colliculus Secondary somatosensory cortices	Hippocampal formation; parahippocampal, prefrontal, cingular, posterior parietal temporal, and occipital cortices Insula
Metathalamus		
Medial geniculate nucleus	Inferior colliculus	Auditory temporal cortex
Lateral geniculate nucleus	Retina (optic tract)	Visual occipital cortex
Reticular nucleus		
	Thalamocortical projections Corticothalamic projections Globus pallidus Dorsal thalamic nuclei	Thalamic nuclei

- The VA nucleus has two parts: (i) the magnocellular part, VAmc, which receives nigral inputs, and (ii) the parvocellular part, VApc, which receives neuronal projections from the internal globus pallidus. The VA nucleus has a role in the initiation and planning of movement, having reciprocal interconnections with dorsal premotor cortical areas and with the caudal dorsolateral prefrontal cortex (DLPFC); also, it receives nonreciprocal afferents from medial prefrontal areas (Figures 2 and 3, Table 2).
- The VM nucleus, receiving afferents from the substantia nigra *pars reticulata*, is a point of convergence for several components of the extrapyramidal motor system. The nigrothalamic projection is topographic: medial and lateral areas of the *pars reticulata* connect to medial and lateral parts of the VM nucleus, respectively. The medial VM nucleus projects to the medial prefrontal cortex, and the lateral VM nucleus projects to the lateral prefrontal cortex, which is sensorimotor (Figures 2 and 3, Table 2). The function of the medial VM nucleus is engaged in 'behavioral inhibition,' while the function of the lateral VM nucleus is related to motoric slowing or loss of motivational attention.
- The VL nucleus sends neuronal output to the frontal primary motor cortex (M1) and premotor cortex and supplementary motor area (SMA) (Figures 2 and 3, Table 2). It can be distinguish two sectors: (a) The VP one is related to the motor cortex and helps to the coordination and planning of movement, playing a role in the learning of movement (its lesion causes ataxia); and (b) the dorsal sector projects to the posterior parietal cortex, to the prefrontal cortex, and to the superior temporal cortex. The dorsal sector of the VL nucleus is related with articulation of words and language and the encoding and retrieval of verbal and nonverbal information. The *nucleus ventralis oralis posterior* (Vop) receives the main output from motor regions of medial globus pallidus, sending projections to the SMA and DLPFC and then to M1 (Figures 2 and 3, Table 2). The *nucleus ventralis intermedius*, Vim (ventral LP nucleus), receives afferents from the contralateral cerebellum and is the main

surgery target for treating tremor (Hyam, Owen, Kringelbach, et al., 2012; Schatelbrand & Bailey, 1959).

(iv) The *thalamic limbic nuclei* (Although nowadays the concept of 'limbic system' (related to emotion and memory) is diffuse and variable, it was initially related to the cortex that surrounds the thalamus, the '*grand lobe limbique*' of Broca and the Papez's circuit. This circuit starts in the subiculum of the hippocampal formation projecting (fimbria–fornix system) to the hypothalamic mammillary complex that projects (mammillothalamic tract) to the AN complex of the thalamus that sends its projections to the cingulate cortex.) related to the limbic system (limbic system used in its broadest sense) are located at the anterior half part of the thalamus: the rostral pole with the AN complex, the LD nucleus, and the magnocellular part of the dorsomedial nucleus. Its function in general is related with memory, learning, mood, emotional experiences, and motivation (Child & Benarroch, 2012). The anterior complex is made up of the AD, AM, and AV nuclei and the LD nucleus, which is considered a caudal extension of the anterior complex. This group receives the projection from the mammillary complex, being the AD nucleus the nucleus that receives bilateral projections, while the projections to the AM and AV nuclei are only ipsilateral. It has been established that it is only the posterior cingulate cortex that receives afferents from the AN complex and the LD of the thalamus, while anterior cingulate cortex does receive afferents from the midline thalamic nuclei. The anterior complex is reciprocally connected with the hippocampal formation (memory-related functions) through the mammillary nuclei. The mammillary nuclei receive the postcommissural fornix and project to the AN complex through the mammillothalamic tract. Other functional activities of the AN complex are related to the prefrontal cortex, with which it has extensive connections, as well with parts of the cingulate cortex (The anterior cingulate cortex receives afferents as well from the MD thalamic nucleus. In fact, there are two functional components in the cingulate cortex: an anterior cingulate cortex territory under the dependence of the magnocellular part of the MD nucleus and a posterior cingulate cortex dependent on the thalamic AN complex.) (**Figures 2** and **3**, **Table 2**).

(v) The *intralaminar thalamic nuclei* can be classified into an anterior and a posterior group. The anterior includes the PCn and CL nuclei. The posterior group is integrated by the CM and the Pf nuclei. The last two nuclei are the most representative of the group and are related mainly to the striatum and basal ganglia being the CM nucleus implicated in motor functions and the Pf nucleus in emotional and cognitive circuits/loops. The intralaminar nuclei have reciprocal connections with cortical areas and receive afferents from the spinal cord, the brain stem, and the cerebellum. In fact, the midline nuclei receive nonspecific projections from the periaqueductal gray matter processing the motivational and affective components of pain. Moreover, due to its cortical connections, the intralaminar nuclei can form important hubs in frontal, parietal, and temporal networks (Saalman, 2014; **Figures 2** and **3**, **Table 2**): (a) The anterior intralaminar nuclei (PCn and CL nuclei) are part of the oculomotor thalamus acting during spontaneous eye movements and are active in arousal regulation probably synchronizing ensembles of cortical neurons in different circuits; and (b) the posterior intralaminar (CM and Pf) nuclei showed preferential connectivity with subcortical structures (with the exception of the anterior insula) and provided information about behaviorally relevant sensory events to the striatum and, therefore, can modulate cortical synchrony by direct inputs to the cortex or through thalamostriatal inputs to cortico–thalamo–cortical loops (**Figure 3**).

(vi) The *associative thalamic nuclei* are those nuclei that have no peripheral afferents, have no links with the primary sensorimotor cortices, but are strongly interconnected with cortical association regions. The associative nuclei are the MD nucleus, the LP nucleus, and the Pul.

- The mediodorsal nucleus (MD). Its size in humans is much larger relative to the nonhuman primate and constitutes an easy target for volumetric and functional studies (Mitchell & Chakraborty, 2013). It presents well-defined connections with the lateral, orbital, and medial prefrontal cortex in nonhuman primates as well as in humans. The territories projecting to the prefrontal cortex form adjacent bands running in a dorsolateral to ventromedial direction. The magnocellular division (MDmc), which is the most medially located band, is connected with the medial frontal and orbitofrontal cortices and functionally included in the limbic territories (**Figures 2** and **3**, **Table 2**); the adjacent, parvocellular band (MDpc) is related to the dorsolateral frontal cortex, and the most lateral band (paralaminar) is specifically related to the frontal eye fields. MD function is important for the understanding of different symptoms of thalamic syndromes. The nucleus reuniens provides afferents to the entorhinal cortex and is reciprocally connected with the prefrontal cortex. Other heterogeneous midline nuclei (corresponding to the allothalamus, regio paraventricularis) are the paratenial, paraventricular, paracentral, and rhomboidal nuclei. Therefore, a great number of small volume individual nuclei are concentrated in a small extension of brain tissue; for instance, midline infarcts of the thalamus usually damage several nuclei, thus making it difficult to assign specific function to particular nuclei.
- The lateral posterior (LP) nucleus is interconnected with the posterior cingulate cortex, the posterior parietal region, the lateral and dorsal extrastriate occipital areas, and the parahippocampal region. Being able to integrate multimodal sensory information, it is engaged in goal direction reaching, spatial functions, and conceptual and analytical thinking.
- The pulvinar complex (Pul) is the most conspicuous part of the thalamus. It presents extensive connections with the hippocampal formation; the parahippocampal, prefrontal, cingular, posterior parietal, and temporal cortices; and the insula. These connections sustain various functions such as memory, attention, spatial neglect, and visuospatial information. The anterior extension of the nucleus named anterior pulvinar nucleus (*nucleus pulvinar oralis*, PO) has been pointed

as one important part of the brain related to the appreciation of the pain. It is interconnected with the secondary somatosensory cortical as well as with cortical association areas in the inferior parietal region (Figures 2 and 3, Table 2). The lateral pulvinar (PL) is specially linked with the posterior parietal region, with the superior temporal cortex, and with dorsal extrastriate occipital areas, playing a role in the integration of somatosensory, auditory, and visual information. The inferior/medial pulvinar (PM) nucleus group lies medial to the medial geniculate nucleus and it is interconnected with temporal and occipital areas concerned with visuomotor responses as visual motion and visual discrimination.

Thalamic Neurons and Afferents: Neurotransmitters, Neuromodulators, and Function

Thalamic nuclei have the ability to filter signals, and to do so, each thalamic projection neuron has an intrinsic rhythmicity. All are in one of two basic physiological states: tonic mode and burst mode. In tonic mode, thalamic neurons respond to depolarization and hyperpolarization. In burst mode, thalamic neurons are tonically hyperpolarized (for instance, during sleep) and do not communicate specific information. But if a novel stimulus is presented (by afferent fibers), the immediate change of the physiological mode (from burst to tonic) can alert the cortical areas. These changes are possible because thalamic relay neurons (in all thalamic nuclei and in all species) have a symmetrical, bushy dendritic field (originally described by Kölliker, 1890) (Jones, 2009). These neurons are excitatory having glutamate as neurotransmitter (with the exception of the RN in which projection neurons are all GABAergic). There are two classes of relay cells: calbindin- and parvalbumin-positive cells. The parvalbumin neurons are topographically concentrated in certain nuclei, and the calbindin ones define a matrix in the whole thalamus and project diffusely to the cortex. This fact provides a substrate for binding together activities of multiple cortical areas that receive focused input from single thalamic nuclei. Moreover, in all thalamic nuclei exist many inhibitory (GABAergic and peptidergic) interneurons, and its function is very important in controlling the transmission of signals through the thalamus.

In general, thalamic afferents can be divided into drivers and modulators (Varela, 2014). Thalamic drivers target proximal dendrites with relatively large synaptic boutons, and its function is thought to be the faithful transmission of the spike message relayed by thalamic cells to postsynaptic structures. Thalamic modulators target distal dendrites and influence spike transmission by adjusting the cellular and synaptic mechanisms underlying spike generation. Then, they are thought to fine-tune the message relayed by thalamic cells and control its probability of transmission. Thalamic modulators are heterogeneous in regard to their origin, the neurotransmitter they use, and the effect on thalamic cells. The activity of thalamic nuclei is changed by thalamic modulators as cholinergic, serotonergic, dopaminergic, and noradrenergic afferents from the brain stem; histaminergic afferents from the hypothalamus; and glutamatergic afferents from the cortical areas. Corticothalamic fibers interact with RN cells and relay cells through glutamatergic receptors (NMDA, AMPA, and metabotropic). The presence of specific and diffuse corticothalamic projections may serve to promote coherent activity of large populations of cortical and thalamic neurons in perception, attention, and conscious awareness (Jones, 2002a). Additionally, thalamic nuclei receive inhibitory projections from the RN. The interactions between RN cells and relay cells are mediated by GABAergic inhibitory receptors (GABA-A and GABA-B). There are intrinsic thalamic circuitries (generated and maintained by corticothalamic projections) that interact with GABAergic cells of the RN and glutamatergic relay cells. These intrinsic circuits underlie rhythmic activities of neurons in the thalamo–cortico–thalamic network (associated with changes in the conscious state). Electrical synapses are a major feature of the inhibitory circuitry in the thalamocortical system, mainly in the RN. The interaction between inhibitory neurons can induce rhythmic action potentials that are synchronized within a millisecond precision, being the substrate of temporal and spatial coordination of the complex neural activity in the thalamocortical projections (The electrical synapses are active in the inhibitory circuitry of the thalamocortical system. Electrical synapses have gap junction channels interconnecting neurons. Gap junction channels (made of specific proteins connexins) allow ionic current and small organic molecules to pass directly between cells, usually with symmetrical ease (Cruikshank, Landisman, Mancilla, & Connors, 2005).

Glutamatergic afferents from cortical layer VI

30–50% of the pyramidal cells in layer VI of cortical areas project to the thalamus with a high degree of topographic precision. This is a very important afferent because layer VI neurons receive input from all cortical layers and its information could serve to integrate processed cortical information with the direct input from the thalamus.

Cholinergic afferents

Cholinergic systems have been broadly involved in state regulation (sleep–wake cycle and attention) and may contribute to state-dependent changes in information routing in the neocortex; brain stem cholinergic inputs can modulate cortical activity through the thalamus. Cholinergic neurons projecting to the thalamus have collaterals to more than one thalamic nucleus as well as to other nonthalamic regions. The main sources of cholinergic innervation are the laterodorsal tegmental nucleus (LDTN) and the pedunculopontine tegmental nucleus (PPN). The MD, LP, VA, ventral lateral, LD, and posterior nuclei receive fibers from the LDTN and the intralaminar nuclei, and the CL nuclei receive afferent fibers bilaterally from the PPN. The CM nucleus receives from both cholinergic areas. Additionally, the PPN projects both to the LGN and to the superior colliculus.

Serotonin innervation

Serotonergic projections from medial and lateral divisions of the midbrain are implicated in the control of the sleep–wake cycle as well as in emotional disorders like depression. In the thalamus, serotonergic afferents target preferentially the midline, anterior, and intralaminar nuclei (which are strongly interconnected with cortical executive areas), where they have heterogeneous effects on membrane potential and could evoke changes in firing mode throughout the sleep–wake cycle.

Noradrenaline (and galanin) projections

Noradrenergic afferents come from the locus coeruleus (LC). The LP nucleus, the Pul, and the somatosensory thalamus are densely innervated by the LC. Thalamic noradrenaline modulation is important in executive and motor disorders as well as in sensory responses.

Dopaminergic modulation

The thalamus does not receive strong dopaminergic innervation from the *substantia nigra*, but it gets dopamine afferents from the ventral tegmental area, the hypothalamus, the zona incerta, the periaqueductal gray, and the lateral parabrachial nucleus. The densest projections are to the midline thalamus, and the lowest densities are found in sensory first-order nuclei. Dopaminergic terminals are often near thalamic terminals at their targets (e.g., the neocortex and striatum); this means that at least some of the thalamic dopaminergic modulation may occur at the terminal site rather than at the soma.

Histamine

The histaminergic projection arises from the hypothalamic tuberomammillary neurons (which are only active during wakefulness and their degree of activation correlates with alertness levels). This modulator can be involved in attentional levels and in the regulation of general changes of activity across states of vigilance through the sleep–wake cycle. Histaminergic presynaptic receptors (H3 and H4) could be important in the modulation of thalamostriatal terminals.

Thalamic Arterial Supply

One of the main sources of information about thalamic functions is due to diseases of the cerebrovascular system, comparing the clinical symptoms with the location of the lesion on *in vivo* neuroimaging or on pathological examination of the brain (Schmahmann, 2003). The symptoms can reveal the functional and behavioral roles of different thalamic nuclei, but it is exceptional to have 'clean' lesions (the infarcts usually affect to vascular territories, which are not specific of single nuclei) (Schmahmann & Pandya, 2008). In fact, vascular lesions involve larger regions implicating few anatomical nuclei; however, the functions of one (or more) region(s) depending on the personal variation of the vascular anatomy of each individual frequently prevail. The thalamic arteries vary between individuals (the number of arteries and its position, the origin, or the nuclei supplied by each branch). For instance, the tuberothalamic artery is absent in approximately one-third of the population (its territory is supplied by the paramedian artery). Thalamic infarction or hemorrhage of each of the different arterial territories develop the main four thalamic vascular syndromes: (i) the tuberothalamic artery, which is a branch of the carotid artery; (ii) the paramedian artery, which is branch of the basilar artery; (iii) the inferolateral artery, which is a branch of the posterior cerebral artery; and (iv) the posterior choroidal artery, which is a branch of the mesencephalic artery (the mesencephalic artery is the P1 region of the posterior cerebral artery) (**Figure 4**). For more details about the vascular supply of thalamic nuclei and the main clinical features after focal thalamic infarctions, please see **Tables 3** and **4** (modified from Schmahmann & Pandya, 2008).

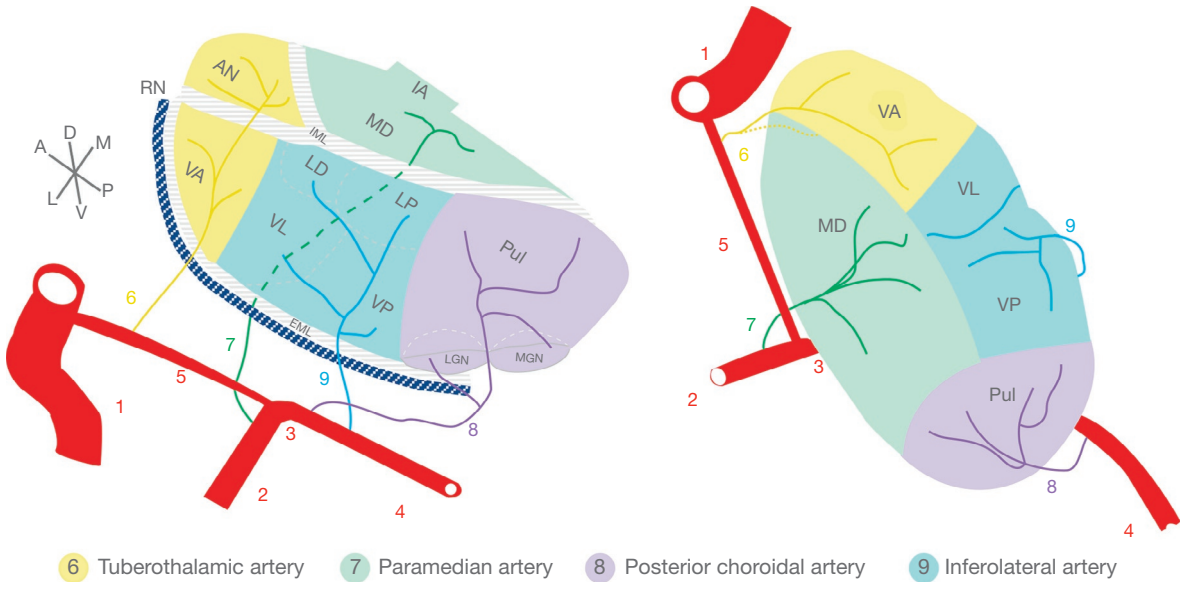

Figure 4 Thalamic vascularization. A, anterior; AN, anterior nuclear complex; D, dorsal; EML, external medullary lamina; IA, interthalamic adhesion; IML, internal medullary lamina; L, lateral; LD, laterodorsal nucleus; LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; M, medial; MD, mediodorsal nucleus; MGN, medial geniculate nucleus; P, posterior; Pul, pulvinar complex; V, ventral; VA, ventral anterior nucleus; VL, ventral lateral nucleus; VP, ventral posterior nucleus. The tuberothalamic artery (6, in yellow) is a branch of the carotid artery (1); the paramedian artery (7, in green) is a branch of the basilar artery (2); the posterior choroidal artery (8, in purple) is a branch of the mesencephalic artery (3) (the P1 region of the posterior cerebral artery); the inferolateral artery (9, in blue) is a branch of the posterior cerebral artery (4). The posterior communicant artery (5) is located between the carotid and the basilar arteries. Modified from Schmahmann, J. D., & Pandya, D. N. (2008). Disconnection syndromes of basal ganglia, thalamus, and cerebrocerebellar systems. *Cortex*, *44*, 1037–1066.

Table 3 Thalamic arterial supply

Thalamic arterial supply

			Inferolateral artery			*Posterior choroidal artery*	
Four major thalamic arteries	*Tuberothalamic artery*	*Paramedian artery*	*Principal inferolateral branch*	*Medial branch*	*Inferolateral pulvinar branches*	*Lateral branches*	*Medial branches*
Nuclei irrigated	RN AD, AM, AV MD (ventral pole) IL VA, VL (rostral pole)	MD, PV CL, CM, Pf VPM LD Pul (ventromedial)	VP VL (ventral part)	MGN	LD Pul (rostral and lateral)	LGN LD LP Pul (inferolateral)	MGN CM, Pf Pul
Tracts irrigated	IML (ventral) VAP MTT	IML (dorsal)					

AD, anterodorsal nucleus; AM, anteromedial nucleus; AV, anteroventral nucleus; CL, central lateral nucleus; CM, centromedian nucleus; IL, intralaminar nuclei; IML, internal medullary lamina; LD, laterodorsal nucleus; LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; MD, mediodorsal nucleus; MGN, medial geniculate nucleus; MTT, mammillothalamic tract; Pf, parafascicular nucleus; Pul, pulvinar complex; PV, paraventricular nucleus; RN, reticular nucleus; VA, ventral anterior nucleus; VAP, ventral amygdalofugal pathway; VL, ventral lateral nucleus; VP, ventral posterior nucleus; VPM, ventral posteromedial nucleus. The tuberothalamic artery is a branch of the carotid artery; the paramedian artery is a branch of the basilar artery; the inferolateral artery is a branch of the posterior cerebral artery; the posterior choroidal artery is a branch of the mesencephalic artery (the P1 region of the posterior cerebral artery).

Source: Modified from Schmahmann, J. D., & Pandya, D. N. (2008). Disconnection syndromes of basal ganglia, thalamus, and cerebrocerebellar systems. *Cortex*, *44*, 1037–1066.

Table 4 Clinical features of focal infarction

Artery and territory			*Clinical features of focal infarction*
Tuberothalamic artery	RN AD, AM, AV MD (ventral pole) IL VA, VL (rostral pole) Tracts irrigated	IML VAP MTT	● *Confusion*, *memory*, *emotion*, *behavior* ● Executive failure, acalculia, apraxia ● Fluctuating arousal and orientation ● Impaired learning, memory, autobiographical memory ● Personality changes, perseveration, apathy, abulia ● Superimposition of temporally unrelated information ● True to hemisphere: – Language → if VL involved on left side – Hemispatial neglect → if right-sided involved
Paramedian artery	MD, PV CL, CM, Pf VPM LD Pul (ventromedial) Tracts irrigated	IML	● *Confusion*, *memory*, *language*, *behavior* ● Altered social skills and personality (including apathy, aggression, and agitation) ● Decreased arousal (coma vigil if bilateral) ● Impaired learning and memory, confabulation, temporal disorientation ● Poor autobiographical memory ● True to hemisphere: – Aphasia → if left-sided – Spatial deficits → if right-sided
Inferolateral artery	Principal inferolateral branch	VP complex	● Variable elements of a triad: hemisensory loss, hemiataxia, and hemiparesis ● Sensory loss (variable extent, all modalities)
		VA, VL, VM	● Hemiataxia, hemiparesis, dystonia, tremor ● Postlesion pain syndrome (Dejerine–Roussy) → if right hemisphere predominant
	Medial branch	MGN	● Auditory consequences
	Inferolateral pulvinar branches	Pul (rostral and lateral) LD	● Behavioral impairment
Posterior choroidal artery	Lateral branches	Pul (inferolateral), LGN LD, LP	● Visual field loss (hemianopsia and quadrantanopsia)
	Medial branches	Pul, MGN CM, Pf	● Aphasia ● Memory impairment ● Variable sensory loss ● Weakness

AD, anterodorsal nucleus; AM, anteromedial nucleus; AV, anteroventral nucleus; CL, central lateral nucleus; CM, centromedian nucleus; IL, intralaminar nuclei; IML, internal medullary lamina; LD, laterodorsal nucleus; LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; MD, mediodorsal nucleus; MGN, medial geniculate nucleus; MTT, mamilothalamic tract; Pf, parafascicular nucleus; Pul, pulvinar complex; PV, paraventricular nucleus; RN, reticular nucleus; VA, ventral anterior nucleus; VAP, ventral amygdalofugal pathway; VL, ventral lateral nucleus; VP, ventral posterior nucleus; VPM, ventral posteromedial nucleus.

Source: Modified from Schmahmann, J. D., & Pandya, D. N. (2008). Disconnection syndromes of basal ganglia, thalamus, and cerebrocerebellar systems. *Cortex*, *44*, 1037–1066.

The thalamus not only relays peripheral information but also plays a central role in cortical function. The role of thalamic neurons is more complex than just sending information to the cortical areas; the thalamic nuclei, with complex cell and circuit properties, have dynamic roles controlling the flow of all information, allowing the target cortical areas to be updated at all time. The role of the RN is essential on this because inputs coming, for example, to the MD nucleus from the periphery, or from intrinsic brain structures, excite relay neurons; the collaterals of these neurons' cortically projecting axons excite the RN cells projecting back to the same nucleus, thus forming an inhibitory feedback connection to the relay cells. Additionally, fibers returning to the thalamus from the cortical area to which the MD nucleus projects excite, by collaterals, cells in the same sector of the RN, and its projection cells into the MD nucleus provide an inhibitory feedforward to the relay cells. This bidirectional circuitry holds the key to understanding certain aspects of the capacity of the cortex to induce and/or maintain thalamocortical synchrony (Jones, 2002b). However, we still do not know the diverse thalamic circuitries and how those control the information flowing to the cortex. In the last years, with the new *in vivo* techniques, novel thalamic functions have been described, but this is just the tip of the iceberg, and we are far from understanding the complex role of all these small but very important thalamic nuclei.

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III.II Artículos de Revisión:

III.II.ITarragon E, Lopez D, **Estrada C**, Ana GC, Schenker E, Pifferi F, Bordet R, Richardson JC, Herrero MT.*Octodon degus*: a model for the cognitive impairment associated with Alzheimer's disease.*CNS Neuroscience & Therapeutics*. **2013** 19(9):643-648.

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Abstract

Octodon degus (O. degus) is a diurnal rodent that spontaneously develops several physiopathological conditions, analogous in many cases to those experienced by humans. In light of this, O. degus has recently been identified as a very valuable animal model for research in several medical fields, especially those concerned with neurodegenerative diseases in which risk is associated with aging. Octodon degus spontaneously develops β-amyloid deposits analogous to those observed in some cases of Alzheimer's disease (AD). Moreover, these deposits are thought to be the key feature for AD diagnosis, and one of the suggested causes of cell loss and cognitive deficit. This review aims to bring together information to support O. degus as a valuable model for the study of AD.

CNS Neuroscience & Therapeutics

REVIEW

CNS Neuroscience & Therapeutics

Octodon degus: A Model for the Cognitive Impairment Associated with Alzheimer's Disease

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Keywords
Alzheimer disease; Amyloid beta-protein; Memory; Mild Cognitive Impairment; *Octodon degus*.

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SUMMARY

Octodon degus (*O. degus*) is a diurnal rodent that spontaneously develops several physiopathological conditions, analogous in many cases to those experienced by humans. In light of this, *O. degus* has recently been identified as a very valuable animal model for research in several medical fields, especially those concerned with neurodegenerative diseases in which risk is associated with aging. *Octodon degus* spontaneously develops β-amyloid deposits analogous to those observed in some cases of Alzheimer's disease (AD). Moreover, these deposits are thought to be the key feature for AD diagnosis, and one of the suggested causes of cell loss and cognitive deficit. This review aims to bring together information to support *O. degus* as a valuable model for the study of AD.

Introduction

One of the major areas of interest in the field of neuroscience is the study of age-related brain pathologies. Understanding the origin of such pathologies, as well as how they progress through different cellular mechanisms and how this process finally affects cognitive and behavioral processes is essential for developing therapies and intervention strategies. Among the vast variety of brain pathologies, Alzheimer's disease (AD) deserves special attention. Being a neurodegenerative disease, the symptoms do not appear spontaneously, but, unlike in the case of a psychosis or amnesia, gradually. The pathology progresses relentlessly until the symptoms are manifest and the memory function, as well as other cognitive domains such as orientation, problem solving, or even changes in personality, become apparent. In most cases, patients are no longer able to take care of themselves and require full-time care [1].

Basic Research in Alzheimer's Disease

There are currently no approved disease-modifying treatments that are able to halt or slow down the pathology in AD or other common neurodegenerative disease. There are, however, vast ranges of different pharmacological and psychological therapies in development stages that aim to slow down the advance of functional loss. However, it is clear that we need to understand more about the cause of this disease and its natural progression if we are to understand when and how to treat it.

There are several approaches to the study of AD, including those based on cellular models [2–4]. Nonetheless, although these models may be very useful for unraveling the molecular mechanisms that underlie the symptomatology, there is a great gap between the conclusions deduced from them and the clinical outcome that this disease displays. On the other hand, the use of animal species may contribute not only to understanding cellular and pathophys-

iological characteristics of Alzheimer's, but also to reproduce the cognitive deficits shown in patients. In our mind, this could seem a more ecological and appropriate approach and one more suited for a better appreciation of the different features of the illness. In this sense, we could say that animal models are good for their capacity to imitate both pathophysiological conditions and behavioral outcome (if any). Therefore, it is fundamental for such models to be able to measure cognitive and behavioral function in an accurately and reliably way.

A number of animal models have been generated in an attempt to reproduce AD pathology. Most of these models have used rodents, and there have been some promising advances [5–7]. Several studies have demonstrated that Alzheimer pathology markers are absent in wild-type rodents, making it necessary to generate transgenic animals overexpressing human amyloid precursor protein (β-APP) harboring familial AD mutations [8–10] or to perform intracerebral injections of Aβ aggregates [11,12] to achieve homologous states of the disease.

Despite the wide range of animal models that are currently used in the study of behavioral and physiopathology features of AD [13], rodents are the most utilized. In the last decades, for instance, the number of transgenic models that have been developed has remarkably increased, widening the alternatives and targeting those characteristics that most significantly are identified within this neurodegenerative disease [14]. As the Aβ cascade is the main hypothesis for the AD, the achievement of models that lead to the development of such characteristics is a milestone for the advance in understanding this pathology.

In this sense, following the Aβ hypothesis, transgenic models are mainly derived from different branches that overexpress three hallmarks identified as regard the AD: APP protein, presenilin 1 and 2, and tau protein [15], aiming to develop the characteristic amyloid accumulation and neurofibrillary tangles (NFTs) [16]. The major advantage of these models is that they succeed in reproducing a similar pathophysiological and behavioral outcome that is observed among patients with AD [17,18]. However, although transgenic models have proved their value for the study of AD, they raise important restrictions.

In the first place, to our view, the most important limitation these models present is the need for genetic and/or pharmacological manipulation to reach the inherent pathophysiological state of Alzheimer's. For instance, it is known that patients with AD show a significant neuron loss [19], and this feature has to be implanted in the mouse because even transgenic models show no such loss without manipulation [20]. Another important similarity between human and rodent pathology that these models lack of is the anatomical distribution of the senile plaques and NFT accumulation [17]. In humans, neuronal death derived from these two properties has been primarily located in the prefrontal and parietal cortices (mainly hippocampus) [16]. However, this allocation has not been achieved with the different models available. Taking this into account, the availability of a model that may cover these limitations would be undoubtedly appreciated (Table 1).

In recent years, a rodent endogenous to Chile, the *Octodon degus* (*O. degus*) has gained prominence as a valued model for many different diseases, including those related with neurodegeneration, as this animal may develop naturally several symptoms that can be linked to a similar number of pathological conditions (Figure 1). Because of its particular diurnal cycle, it has frequently

Table 1 Advantages and disadvantages of the *Octodon degus* with respect to other very commonly used rodent models for AD [10,13]

Model	Line	Advantages	Disadvantages
TAU transgenic mice [72,73]	✓PrP	Accumulation of hyperphosphorylated tau	External manipulation
	✓mThy1.2	Intracellular tau tangles	Phenotype not representative of AD
	✓R406W	Cognitive impairment	Tau positive astrocytes, not common in AD
	✓V337M	Neural plasticity impairment	Regional expression of tau different in what is observed in AD
APP transgenic mice [74,75]	✓APP23	Aβ deposits	No tau pathology
	✓PS2APP	Senile plaques immunoreactive for hyperphosphorylated tau	Lack of neuronal and synaptic loss
		Cognitive impairment	External manipulation
Triple transgenic mice [6,10,76]	✓3xTg-AD	Aβ deposits	Lack of neuronal and synaptic loss
	✓Tg2576	Mutant PS1, PS2, and ApoE	External manipulation
		Senile plaques	
		Neurofibrillary tangles	
		Cognitive impairment	
Octodon degus [13,28,29,33]	–	Complete physiological phenotype	Interindividual variability
		Age-related cognitive decline	Breeding
		Aβ deposits	
		Hyperphosphorylated tau	
		Extensive neuronal loss associated with age	
		Impaired neural transmission	
		Gliosis and Inflammation	

AD, Alzheimer's disease; APP, amyloid precursor protein.

been used in circadian studies [21,22]. It is also a highly social rodent, which explains its role in social and neuroaffective research [23,24]. However, over the last few years, the participation of degus in the study of neurodegeneration has suggested that this area of research is the most promising application of this model. This diurnal caviomorph rodent lives up to 7 years average in captivity [25], making it *per se* an interesting model for use in longitudinal studies, including those related in the neuropsychobiology of aging, and AD.

Octodon-Human Aβ Aggregates Similarities

Among the different hypotheses raised to explain the origin and evolution of AD, the most widely held is that which stresses the importance of cholinergic neurodegeneration and the appearance of two principal markers: the NFTs formed through the dysfunctional hyperphosphorylation of tau protein, and the deposition of Aβ aggregates, which are thought to be the trigger for neuronal death [26]. However, the relationship between these two elements is not clear, although several hypotheses have attempted to link them [26,27].

A few years ago, Inestrosa et al. [28] demonstrated that *O. degus* naturally develops characteristic histopathological hallmarks reminiscent to those typically found in patients with AD. The discovery showed that this rodent, in its natural environment, might produce plaques in different brain areas [29], including hippocampus and frontal cortex, both of which are severely affected in patients with AD [16]. Moreover, immunohistochemical and genetic analyses performed on the *O. degus* revealed a high degree of similarity between human deposits and the Aβ precursor protein (Aβ-PP). Also, RT-PCR analysis showed the *O. degus* and human Aβ peptide sequence to be 97.5% homologous [28]. This animal presents only one amino acid substitution with respect to the human, which presents an advantage to other models (rats, for example, present in their Aβ sequence three amino acid substitution; Figure 2). In this sense, differently to transgenic animals, there is no need to overexpress this human APP to generate significant levels of amyloid protein, which will help to avoid the overexpression of APP. This is an important question, as it has been postulated as to why there is a limited neuronal loss in the APP transgenic models and is one of the main advantages of the *O. degus* as a model in preclinical research of this pathology. AD models usually reproduce the pathological hallmarks of familial AD cases,

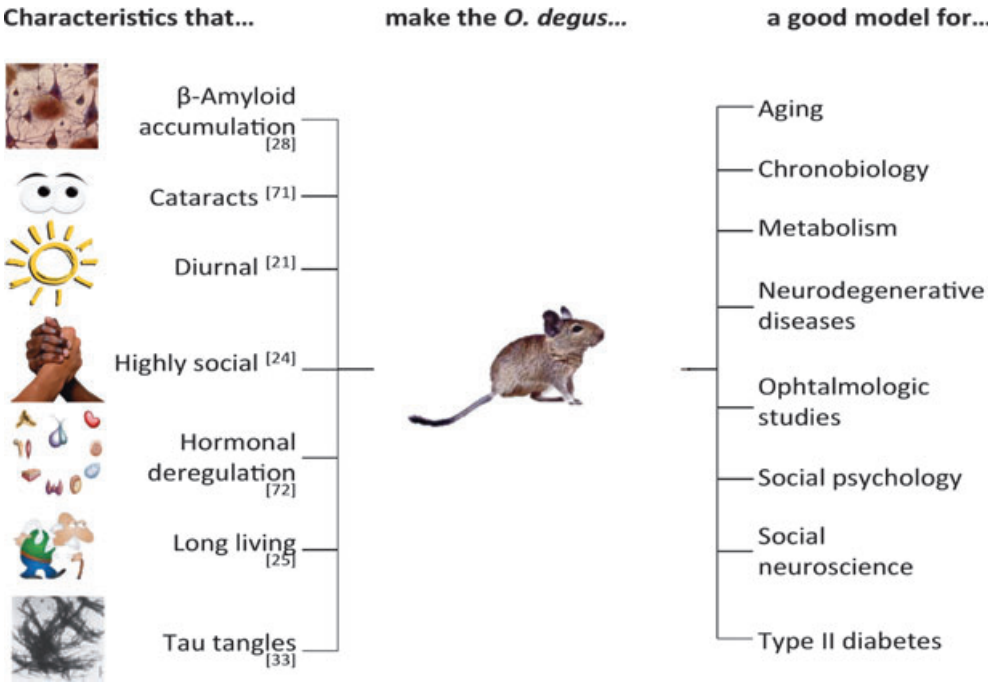

Figure 1 Characteristics of *Octodon degus*. Brief description of several characteristics that naturally develop in the *O. degus* making it useful as an animal model in several fields. Numbers in brackets are for the correspondent reference in the bibliography.

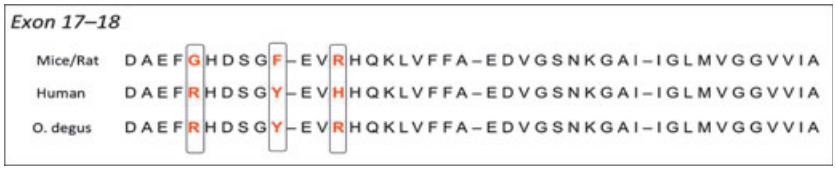

Figure 2 Amino acid Aβ sequence. Differences and similarities between mice/rat, human, and *Octodon degus* amino acid Aβ sequence. Differently from the mice/rat, the *O. degus* is only one amino acid different from the human sequence [28,29].

which represent around 5% of total cases of AD [16]. The initiating pathogenic mechanisms for the appearance of sporadic AD are not fully described, and due to the spontaneous growth of such markers in the *O. degus*, it could be advantageous to use this animal within an experimental context.

Nevertheless, as promising as this animal might be, it still needs to satisfy certain requirements before it can be used as an appropriate model. In this sense, it is worth mentioning that the histopathological changes occurring in *O. degus* brains are only observed in aged animals [28,29] and have never been detected in young animals so far. Similar comments may be made regarding tau, which suggests that amyloid and tau deposition are age dependent, as they are in patients with AD [30,31] and in some of the more successful transgenic mice studied to date [10]. Nevertheless, differently from transgenic models that require mutated form of tau [32], this mutation is neither present in humans nor in the *O. degus,* thus arising as a more suitable alternative given that similarly to what occurs in humans.

Another interesting analogy concerns the cholinergic system. The cerebral cortex of some human and non-human primates contains acetylcholine (AChE)-rich pyramidal neurons, which have been seen to decline in numbers during the progression of AD, a decrease claimed to be partly responsible for the memory deficits in Alzheimer patients [15,19]. *Octodon degus* apparently shares the same AChE-rich neurons that are found in the cerebral cortex of adult humans. Moreover, the high degree of homology (97.5%) between the human and *O. degus* in Aβ sequence and the tau structure, possibly triggered by the Aβ found in these animals, suggests that both play a major role in the appearance of AD markers in this rodent, including the presence of extra- and intracellular amyloid deposits and NFTs [10,28,31].

Octodon degus, What Does It Offer?

We have already mentioned the histological advantages that this model presents for AD research. However, without a cognitive counterpart, assessment of this model is not complete. As mentioned above, one of the key features of an animal model for AD should be its ability to mimic the cognitive and behavioral response in the different domains affected by this illness.

It has been recently demonstrated that the age-progressive accumulation of Aβ oligomers and phosphorylated tau proteins in *O. degus* from 12 to 36 months negatively correlated with their performance in spatial and object recognition memory measured by two different behavioral paradigms: the Object Recognition Memory task and a spatial T-Maze. In this work, Ardiles et al. demonstrate that memory performance declines in an age-dependent manner, as aged animals made fewer correct choices in the arms of the T-Maze, and the time spent exploring the novel objects was significantly reduced in the object recognition task. Interestingly, the synaptic strength in the old *O. degus* was reduced compared with the young ones, and the postsynaptic transmission was also impaired [33].

As memory impairment is the first manifestation of AD symptoms and the most prominent of observable consequences, one of the requirements that *O. degus* should fulfill is that it should discriminate in different cognitive tests and different memory deficits classically impaired in AD. Moreover, this should be achieved in response to the different challenges that are used to induce cognitive impairment, one of the most widespread of which is sleep deprivation (SD).

Sleep deprivation has been widely documented as one of the challenges that most effectively induce transient cognitive impairment [34–37] in animals [38–40] and humans [41]. SD has also been studied in the *O. degus* [42] (Figure 3). This condition affects the formation, expression, and retrieval of memories [36,37] and produces a deficient consolidation in both procedural and declarative memories [34,43]. Evaluating memory impairment caused by this challenge in the *O. degus* is especially interesting, given their phase inversion capacity [22]. Sleep-wake deregulation is commonly seen in AD [44,45] and is displayed as agitation, disrupted sleep, or breathing difficulties [44]. Sleep studies performed on *O. degus* have demonstrated that, despite being diurnal, this animal is able to switch from diurnal to nocturnal phase behavior in a few days [46]. Together with all the AD-like hallmarks displayed by this rodent, this chronobiological characteristic adds value to the *O. degus* as an attractive model of the cognitive decline and behavioral outcome observed in age-related neurodegenerative diseases and also confirms SD as an appropriate methodological choice. With this method, researchers would be able to induce transitory memory deficits in both young and old animals to further compare the impact of such procedure and the effect of aging and histopathological hallmarks formation on the behavioral outcome.

The most noteworthy feature of AD is memory loss, but it is not the only one. Besides the well-known deficit in problem solving [47,48] and spatial orientation [48,49], patients with AD also present a wide range of psychological affectations such as stress [50] and anxiety [51,52], as well as different systemic impairments [53]. In this sense, it has been demonstrated that the *O. degus* may develop atherosclerosis, a pathological states frequently concomitant with AD [54]. It has been demonstrated that this rodent is able to develop an atherosclerosis condition directly derived from

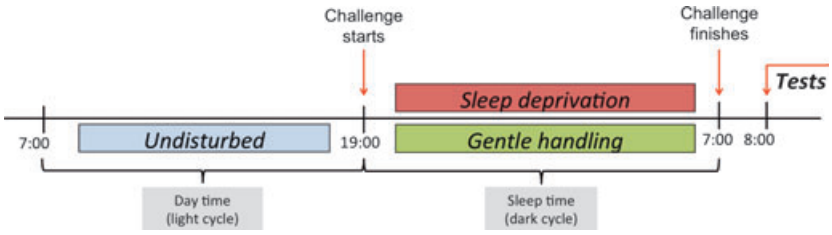

Figure 3 Procedural scheme of the sleep deprivation (SD) induced by gentle handling. Adapted to the normal diurnal activity of the *Octodon degus*, SD challenge starts at 7 p.m. in a 12/12-h light and dark cycle. Gentle handling is a non-stressful way of preventing the animal from sleeping. After the procedure, the behavioral test takes place [42].

a rich-cholesterol diet, together with a lipoprotein metabolism similar to humans [55]. This combined with the presence of hyperglycemia strongly correlates with the appearance of type 2 diabetes [56]. Interestingly, aged *O. degus* also share with humans these pathological conditions [55].

Despite the fact that many studies have shared inconsistent results concerning age-related changes in anxiety in different rodent models [57,58], there is evidence of a significant age-related effect in *O. degus* (young and old adults) in the open-field test and dark and light test, two widely validated procedures to assess anxiety in rodent models [59]. Popovic et al. [60] explored the relationship between age and anxiety and demonstrated that the older group spent less time in the center of the open field compared with the young adult group and that the latter group were more willing to spend more time in the light than the older ones [61], suggesting that anxiety may increase with age in these animals.

Another AD-like symptom that is apparent in the *O. degus* is related to their social life. It is known that in many cases of AD, patients tend to develop social problems mainly for two reasons: the dementia associated with the disorder and the stress caused to caregivers [62,63]. *Octodon degus* is generally described as a very social rodent with a highly complex social behavior [23,24]. However, as in AD, this animal is also subjected to problematic interactions with other members of its colony when stressful events occur [64]. In this sense, Poeggel et al. [65] reported severe behavioral deficits and neural alterations in the frontal cortex, which also shown to be affected in patients with AD [63,66].

It is also common to find patients who have difficulty in manipulating complex objects, or performing fine motor movements [67,68]. To date, the range of possibilities to test this particular deficit is scarce and is mainly confined to non-human primates. However, to the best of our knowledge, there is no literature covering manipulative and fine motor problems in rodents. Thus, it is worth mentioning that *O. degus* is the only rodent to date which has been demonstrated to be sensitive to training in object manipulation toward obtaining a reward [69]. The authors of this work were able to train five animals to retrieve a food reward located in a platform that could only be reached using one of the different tools to which they were given access. Animals learned the task with the same efficiency as shown by non-human primates in similar conditions [70], demonstrating an increasingly understanding of tool usage, not only regarding the physical properties of the tool, but also its functional attributes [69].

Further Directions

We have reviewed a range of studies performed with the *O. degus*, a diurnal rodent native to Chile. The main interest in this animal in the field of cognition is that it has recently been proposed as a putative model for AD, principally because it presents two of the major histopathological markers for this disorder (β-Amyloid plaques and NFTs containing hyperphosphorylated tau). Several reports have also suggested that cognitive impairment in *O. degus* may be compared with that observed in humans. Therefore, this animal could represent one of the most promising models for the study of cognitive impairment associated with AD. While investigation with *O. degus* in the field of cognitive sciences is still in its early stages, we believe that the degus provides an excellent opportunity for exploring the mechanisms underlying late developmental changes in the nervous system, and therefore, the behavioral and cognitive outcomes resulting from such changes.

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Conflict of Interest

The authors declare no conflict of interest.

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IX.V**Estrada C,** Fernández-Gómez FJ, Cuenca-Bermejo L, Gil-Martínez AL, Mascarell I, Fernández-Villalba E, Herrero MT. "Effects of voluntary exercise in memory impairment, stress and aging in Octodon degus"Enviado

Abstract

Rationale: Sleep deprivation (SD) has been reported to induce transient cognitive impairment in domains that are commonly affected in dementia, including memory. Indeed, sleep disturbance has been proposed as an early marker for Alzheimer's disease (AD). Among the possible mechanisms through which SD exerts its effects on memory, triggers of stress such as cortisol might be included. SD emulates many aging-related modifications, including important memory dysfunctions. Although exercise is widely assumed to be beneficial for overall health, only recently has the research community focused its attention on its possible effects on brain functions such as cognition. Octodon degus (O. degus) is a recent rodent model considered suitable for the study of neurodegenerative diseases, since it spontaneously develops several histopathological hallmarks observed in AD.

Objectives: The study aimed to uncover the interaction between stress, exercise, age and transient memory impairments after SD insult.

Methods: Animals had free individual access to wheels to practice voluntary exercise. The Barnes Maze (BM) task was conducted with young and aged O. degus animals after combining voluntary exercise and either normal sleep or SD. Plasma cortisol levels were also measured after each condition.

Results: SD impaired hippocampus-dependent memory in both young and old animals, while cortisol levels did not significantly differ between non-SD and SD animals. However, voluntary exercise for 45 days improved the cognitive impairment caused by SD compared with the control conditions, and decreased plasma cortisol levels in animals of both age groups and in both conditions.

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Effects of voluntary exercise in memory impairment, stress and aging in Octodon degus

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Author contribution statement

CE researched data and wrote; LCB and ALGM researched data; FJFG, IM and EFV reviewed the manuscript; MTH planned the experimental design, edited and reviewed the manuscript.

Keywords

O. degus, Sleep Deprivation, stress, voluntary exercise, cortisol, memory impairment, Alzheimer's disease

Abstract

Word count: 291

Rationale: Sleep deprivation (SD) has been reported to induce transient cognitive impairment in domains commonly affected in dementia, including memory. Indeed, sleep disturbance has been proposed as an early marker for Alzheimer's disease (AD). Among the possible mechanisms through which SD exerts its effects on memory, stress triggers such as cortisol might be included. SD emulates many aging-related modifications, including important memory dysfunctions. Although exercise is widely assumed to be beneficial for overall health, only recently has the research community focused its attention on its possible effects on brain functions such as cognition. Octodon degus (O. degus) is a recent rodent model considered suitable for the study of neurodegenerative diseases, since it spontaneously develops several histopathological hallmarks observed in AD.
Objectives: The study aimed to uncover the interaction between stress, exercise, age and transient memory impairments after SD insult.
Methods: Animals had free individual access to wheels to practice voluntary exercise. The Barnes Maze (BM) task was conducted with young and aged O. degus animals after combining voluntary exercise and either normal sleep or SD. Plasma cortisol levels were also measured after each condition.
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European Community Council Directive (2010/63/UE)
The ethical committee of the University of Murcia

Effects of voluntary exercise in memory impairment, stress and aging in *Octodon degus*

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Abstract

Rationale: Sleep deprivation (SD) has been reported to induce transient cognitive impairment in domains commonly affected in dementia, including memory. Indeed, sleep disturbance has been proposed as an early marker for Alzheimer's disease (AD). Among the possible mechanisms through which SD exerts its effects on memory, stress triggers such as cortisol might be included. SD emulates many aging-related modifications, including important memory dysfunctions. Although exercise is widely assumed to be beneficial for overall health, only recently has the research community focused its attention on its possible effects on brain functions such as cognition. *Octodon degus* (*O. degus*) is a recent rodent model considered suitable for the study of neurodegenerative diseases, since it spontaneously develops several histopathological hallmarks observed in AD.

Objectives: The study aimed to uncover the interaction between stress, exercise, age and transient memory impairments after SD insult.

Methods: Animals had free individual access to wheels to practice voluntary exercise. The Barnes Maze (BM) task was conducted with young and aged *O. degus* animals after combining voluntary exercise and either normal sleep or SD. Plasma cortisol levels were also measured after each condition.

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Conclusions: SD induced transitory memory impairment in young and old *O. degus,* deregulating cortisol levels, which were higher in control conditions in both young and old animals. This stress effect was diminished, as revealed by the BM hippocampal test, when the animals performed voluntary exercise over a period of 45 days.

Keywords: *O. degus*, sleep deprivation, stress, voluntary exercise, cortisol, memory impairment, Alzheimer's disease.

Introduction

Age-related cognitive deficits are common in technologically advanced countries, where they constitute an important public health problem. Modern society is rapidly aging and, with, the prevalence of dementia is increasing. One of the main characteristics of the physiological aging process is cognitive decline and memory disturbance. Especially striking is the working and short-term memory deficit in social and residential contexts (1). Moreover, although memory loss does not necessarily involve the beginning of a neurodegenerative process, common problems associated with old age usually appear with different degrees of cognitive decline (1-3). Among these disorders, Alzheimer's disease (AD) is the first cause of dementia and so deserves special attention. There is still no efficient and lasting pharmacological treatment for this disorder, so it is essential to develop an early and reliable diagnosis tool in order to mitigate the prognosis (4, 5). In this regard, translational animal models constitute one of the most important sources of knowledge to help identify the first symptoms (6).

Adverse effects of sleep loss have been well described (7). At the neurological level, the disturbance of normal sleep causes cognitive impairment in both animals and humans (8) (9), possibly influencing non-pathological and pathological aging (10). Furthermore, increases in fragmented and disturbed sleep has been positively correlated with the aging process (11), suggesting a relationship between memory and sleep (12). It has been demonstrated that sleep deprivation (SD) induces memory deficits in animals and humans (13-16), and as a tool, has been used to ascertain the characteristics of sleep function in brain homeostasis. The exact mechanisms by which SD exerts pernicious effects are not totally understood, and different hypotheses have been proposed (17, 18). Because of the nature of the different methodologies used to induce this stress, it can be difficult to discriminate whether the effects are caused by stressor exposure over time or by the lack of sleep *per se* (19, 20). Thus, cortisol levels have been studied as a measure of stress on cognition during the aging process (21, 22). However, the involvement of cortisol on retrieval mechanisms is one of parallel lights and shadows (23), despite which it has been widely reported that increased cortisol levels are directly involved in physiological forms of the sleep function (24). In rodents, it has been reported that corticosterone levels often do not significantly change during or after SD stimuli (25-34). This observation may be due to the variation depending on the method, duration and experimental set-up (35).

Regular exercise and physical activity have been shown enormous benefits for human health, and for several years researchers have paid increasing attention to the role of exercise in relation to brain functions. For instance, many human studies have indicated that exercise aids in the treatment and prevention of depression- and anxiety-related disorders (36, 37), and in the cognitive amelioration in asymptomatic subjects as well as in senescence-related conditions (36, 38-40). Surprisingly physical exercise has not been commonly included in mental health programs (41), due to the existence of some studies describing the lack of positive effects (39, 42), and others which even describe negative outcomes (37), or simply because of the physical limitations of the patients. Similar studies have been performed in animals, where the relationship between exercise and anxiety levels has been examined with debatable results. Regular exercise improves cognitive functions in rats and is associated with an enhancement of memory and spatial learning (43-47), even in AD-like models, when it increases the release of neurotrophic factors (47).

The *Octodon degus (O. degus)* is a diurnal rodent ~~recently~~ proposed as a putative model for aging disorders (i.e. age-related cognitive decline), and more specifically for neurodegenerative diseases (48-50). For instance, this rodent model spontaneously develops histopathological hallmarks reminiscent of AD such as β-amyloid depositions and hyperphosphorylated tau tangles, at approximately three or four years of age (51, 52). These features, together with insulin resistance (53, 54), are common manifestation in the clinical prognosis of AD patients. Considering that the *O. degus* life span may reach more than 9 years (55), it makes this rodent a valuable alternative to standard AD-like models based on transgenic strategies.

The aim of the study was to evaluate the effects of voluntary exercise on spatial learning, memory and anxiety levels in young *versus* old *O. degus* exposed to SD condition, and how cortisol levels are related to age-dependent memory.

Material and Methods

1. Animals

Twelve healthy juveniles (female, twelve months-old) and twelve healthy old *O. degus* (female, aged forty to sixty months) weighing 200-270 g at the beginning of study were obtained from our colony. Both young and old animals were individually housed in an isolated room in plexiglas cages,the floors of which were covered with wood shavings that were changed once a week, with controlled temperature (23±1ºC) and humidity (60%) (50). Each cage was equipped with wheels as an environmental enrichment condition, which enabled the animals to perform exercise.
A 12:12 light/dark cycle (light on from 08.00 to 20.00 h) was provided by fluorescent lamps regulated by an electronic timer (DataMicro, Orbis), with a light intensity of 350–400 lx at cage level. The *O. degus* were fed *ad libitum* throughout the experiment using a commercial feed (Harlan complete feed for rodent maintenance). The experiments were performed during the light period (09.00–15.00 h) (68). Substantial efforts were made to minimize and refine the number of animals used, following the "three R's principle" of the European Community Council Directive for animal experimentation with respect to the total number of animals that were used in the preclinical study. All experimental procedures complied with the European Community Council Directive (2010/63/UE) and the ethical committee of the University of Murcia.

2. Sleep Deprivation

Sleep deprivation (SD) is considered one of the paradigms that most efficiently produces transient cognitive impairment (16, 56-58) in both animals and humans (14, 15, 59). It produces inadequate integration of both procedural and declarative memories (56). SD has also been used in the *O. degus* (60), where it consisted of interrupting the sleeping cycle of the animal with a soft tactile stimulus and or with a gentle jostling of the home cage whenever the animal showed signs of sleep (more than 1min of inactivity) (61). The behavioral test took place 12 hours after this SD procedure (60), which started at 19.00 h in the 12/12-h light and dark cycle. Animals were subjected to a single session of SD before the test, and were allowed to recover their sleeping routine (15 days) before the next session (50) (68).

3. Exercise protocol

Young and old animals had free access to a running wheel (see picture 1) which was connected to a digital counter for 45 days. Running activity was measured in revolutions per day and transformed into km/day based on the functional 28 cm circumference of the wheel. The running wheel was rotated by animal effort (44, 62).

4. Behavioral test

Before the behavioral experiments, the animals were left undisturbed for 7 days. Then, the following week, they underwent handling procedures (5 min per day) to become used to manipulation by the experimenters. Because *O. degus* is a diurnal rodent, all experiments were performed during the day (from 07:00 to 19:00 h).
An experimental room with the same noise and temperature conditions was used to perform all the behavioral tests, allowing the animals to adapt to the experimental room for 24 hours (50).

4.1 Room configuration

The experimental room was set up with visual clues such as different colors and shapes (triangle, rectangle, circle or a cross) placed surrounding the maze. Animals had other visual signs in the room (for example, a chair, a trash can or a computer) prepared by the experimenter. During the experiments, none of these clues was moved so that could be used by the animals as spatial reference points for locating the target hole or arms (68).

4.2 Barnes Maze

The Barnes Maze (BM) is a circular platform 160 cm in diameter raised 75 cm from the ground and surrounded by a 55 cm high plastic wall. The platform is made of white Plexiglas and has eighteen circular holes (8 cm in diameter). The task was divided into 3 phases: i) The habituation session, ii) The learning period and iii) The retention session. In the habituation session (i) the animals accustom themselves to the escape cage and platform, as explained in previous works (63, 64). In the learning period (ii), the animals were trained for seven consecutive days. Four trials of 4 minutes were carried out in each session, leaving the animals in their home cage for 5 minutes between each trial. In the trial; the animal was kept in the start box for 30 seconds in the center of the platform, and the following parameters were recorded: a) Time elapsed to the first visit to the escape hole; b) Decision time: time elapsing between beginning to explore the escape hole and entrance into the escape hole; c) Total time before escaping; d) Number of reference memory errors (RME); in each trial, every first visit of a non-escape hole in each trial was scored as an RME; and e) Number of working memory errors (WME), that is, repeated visits to the same non-escape hole in the same trial. Finally, the retention session (iii) was performed the day following the last day of the learning period. Animals were given one trial, and the same parameters as in the learning period were recorded (50) (64) (68). All animals had free access to wheel running during the behavioral test.

5. Determination of cortisol levels

Blood extraction for plasma cortisol measures was always performed at 8 a.m. Blood (200 to 300 µl) was collected from the saphenous veins of anaesthetized animals (isoflurane 3% mixed with oxygen for induction and 1.5% for maintenance) into pre-cooled plastic centrifuge tubes containing 0.01% ethylenediamine tetraacetic acid (EDTA) as anticoagulant. This was immediately centrifuged at 3600 rpm for 10 minutes at 4º C. Next, 100 to 150 µl of supernatant were collected and stored at -80º C until use. Cortisol levels were measured using a commercially available radioimmunoassay (Immunotechas® IM1841) following the manufacturer's instructions.

6. Statistical analysis

In the BM test, the total number of both RME and WME for all sessions, as a whole, were considered to be the dependent variables. Values were expressed as the mean of the four assays included within a session. To examine the average running distance and the learning of the different age groups in the BM, a two-way ANOVA with repeated measures was performed. To analyze the effect of sleep deprivation and exercise, a two-way ANOVA was performed as required. Plasma cortisol levels were analyzed with a two-way ANOVA (age x condition). Tukey's multiple comparison test was used. Normal distribution was confirmed by a test suitable for the data. All statistical analyses were performed using the Statistica 10 (StatSoft, Tulsa, OK) software package.

Results

Running activity during 45 days in young *vs* old *O. degus*

O. degus were housed singly in cages with running wheels for 45 days. Wheel revolutions were recorded to calculate the distance that the animals ran each day. Firstly, it was calculated the length of the wheel circumference ($L = \pi * d$), multiplied by the revolutions registered in the installed software. As shown in Figure 1, the highest average distance run per day by young *O. degus* in the study was 12470 m on day 14. Thus, there was a significant difference in total running distance in young groups compared to the old ones, whose maximum average distance per day was 6004 m during the 45 days of the study (Fig 1. * $p<0.05$).

Effect of voluntary exercise on spatial memory.

BM was performed in order to evaluate the effect of 45 days of voluntary exercise on cognition after the temporary impairment caused by SD. The paradigm used to evaluate spatial memory was based on escape time, WME and RME (50) (68). As can be seen from Figure 2, SD induced a substantial degree of cognitive impairment in both young and old animals, as measured by Total time, WME and RME values. This harmful effect of SD was improved by 45 days of voluntary exercise in both groups. Similar conclusions were offered by the two-way ANOVA analyses: the total time pointed to a significant effect of condition [$F(1, 43) = 5.631$; $p < 0.05$] and of the interaction between age and condition [$F(1, 43) = 12.584$; $p < 0.01$]. WME pointed to a significant effect of age [$F(1, 40) = 32.318$; $p < 0.001$], condition [$F(1, 40) = 28.892$; $p < 0.001$], and voluntary exercise [$F(1, 40) = 46.879$; $p < 0.001$], while RME suggested an effect of age [$F(1, 41) = 68.863$; $p < 0.001$], condition [$F(1, 41) = 29.723$; $p < 0.001$] and of the interaction between voluntary exercise and condition [$F(1,41) = 10.814$; $P < 0.01$].]

These results clearly reflect the importance of exercise in the recovery of cognitive capacity after SD.

Effect of voluntary exercise and sleep deprivation in cortisol levels

To evaluate the effect of exercise in *O. degus* at both ages, a plasma cortisol sampling protocol was followed. The experiment was initiated at 08.00 h when the light cycle started in all conditions. Figure 3 shows that 45 days of voluntary exercise significantly decreased cortisol levels in both groups (young and old; normal sleep and SD exercise groups). Curiously, there were no significant changes in plasma levels in the no-exercise groups. Analysis of the cortisol levels revealed a significant effect of exercise [$F(1,50) = 33.085$; $p < 0.001$] and an interaction effect between age and SD [$F(1,50) = 6.967$; $p < 0.05$], and between exercise and SD [$F(1,50) = 11.746$; $p < 0.01$ was also found. A Tukey test identified significant difference between the exercise groups under SD conditions with respect to normal sleep condition ($p < 0.01$).

Discussion

The current study shows that voluntary physical activity improves learning and memory dysfunctions induced by SD in young and old *O. degus*. As previously reported, sleep disturbance causes significant changes in behavioral markers such as in BM, Radial Arm Maze (RAM) and Novel Object Recognition (NOR) (49, 50 68). Moreover, this SD model has been widely demonstrated to induce transitory cognitive deficits similar to those shown by AD (13, 15). Preclinical studies´ using animal models to discover new molecular pathways in aging-related disorders is a key research point for the development of new pharmacological strategies. Despite the inestimable contribution of "traditional" animal models in pharmacotherapy research, current studies need to include not only pharmacological research but also genetic profiles and environmental factors to seek the pathophysiological etiology and evolution of neurodegenerative diseases such as AD (6, 48, 65). In accordance with these observations, *O. degus* has been used as a suitable model to study AD-like symptomatology by carrying out different behavioral paradigms (66) (49). Thus, the classic BM behavioral test to examine spatial learning and memory in rodents (67) was used to ascertain deficits in hippocampal-based spatial reference memory after SD insult, and in pharmacological and environmental enrichment conditions (50, 64, 68).

Since few studies have looked at the combined influence of SD and exercise on hippocampus-dependent memory and learning, the *O. degus* used here were given the opportunity to take voluntary exercise for 45 days prior to SD insult. Our behavioral data revealed that voluntary exercise was sufficient to counteract the transient cognitive impairment caused by SD in both young and old *O. degus*. On the test days, exercise groups spent less time finding the escape hole and, during the training phase, there was a gradual decrease in RME and WME as the number of training sessions increased in the animals at both ages. While exercise is known to have effective and powerful cognitive and neurogenic effects in the case of human health, it is increasingly considered as a promising non-pharmacological tool to slow down the progression of neurodegenerative disorders (69, 70). Several mechanisms have been described as being responsible for the beneficial effect of voluntary running, such as improvements in memory formation increasing hippocampal neurogenesis (71, 72) and consequently spatial learning and memory tasks (73, 74). Moreover, an RAM test showed that

exercise does not affect normal memory compared with control rats, but, rather, protects against the SD-associated impairment of long-term memory (75).

Analysis of the plasmatic levels of cortisol performed by ELISA revealed that voluntary physical activity decreased physiologically normal values in *O. degus* for both ages, under normal sleep and SD conditions. Interestingly, no increase in cortisol level in the SD control groups was observed, but, under normal sleep conditions, the exercise groups displayed a significant decrease in plasmatic glucocorticoid levels. Hence plasma cortisol levels after undisturbed sleep were not significantly different between young and aged animals in the control groups, despite being slightly higher in young groups. Our study indicates that in the control groups, SD animals appeared to have the same levels of cortisol as animals from normal sleep groups. Contrarily to that observed for cortisol levels, the SD control groups appeared to be more sensitive to SD insult in terms of spatial memory deficit, as deduced from the behavioral test.
Basal cortisol levels in *O. degus* are approximately 300-500 ng/ml (76). In this study cortisol levels were significantly higher than this in both normal sleep and SD young animals, so it can be hypothesized that higher basal levels of cortisol could make these animals more sensitive to impairments caused by sleep disruption. This observation agrees with the assumption that stress might strengthen the cognitive impairment caused by SD. Moreover, cortisol levels in normal sleep and SD groups of older animals were in the range established for *O. degus*, and there were no differences between the groups (normal sleep and SD animals). These observations may be an indication that *O. degus* has high plasmatic cortisol levels in both conditions due to the influence of habitat - their isolation in cages, for example. Indeed, in adult rodents, inconsistent changes in plasma concentration of glucocorticoids have been observed after chronic social isolation. Thus, some reports reveal that plasma cortisol levels increased in socially isolated animals (77, 78), while others found that levels were not altered (79-81) or even decreased after chronic stress (82). Our animals were individually housed in an isolated room in plexiglas cages where they can see and hear their companions, and, as mentioned, social isolation may be considered a stressful situation that can result in plasmatic cortisol alterations. Therefore, habitat type can affect group size and composition (83), which supports the premise that habitat type may mediate the cortisol response through differences in sociality (84). Previous studies have shown that the habitat composition of field, boulder or tree could interfere in the level of basal cortisol in *O. degus.* These rodents are social, plural-breeding mammals that live in groups ranging from one to twelve adult individuals (85) and social dynamics might influence the stress response (86).

Some reports suggest that when SD is accomplished by forced locomotion or techniques that induce movement *per se*, cortisol levels may increase (87). In this study, the glucocorticoid measurements were performed at the end of the deprivation period, but information regarding any alterations that may have occurred during the time-course is not available, so that it cannot be ruled out that cortisol levels may vary during SD by themselves. Our results suggest that young groups in these conditions may have high levels of cortisol as a result of the simple environment, isolation or any other stressful situation. No differences in basal cortisol levels between isolated animals and those housed in groups have been reported (76). Hence, the influence of social isolation on plasma cortisol levels remains unclear. Interestingly, despite the isolated housing of the animals, cortisol levels decreased in the animals taking voluntary exercise.

There is evidence for neuroprotective and neuroplastic effects of physical exercise on brain structures, as well as it has been shown that physical exercise is an effective component to manage stress because it diminishes serum cortisol levels in chronic mild stress (88). The results obtained for exercise could correspond to the combined effect of physical activity and the insertion of a novel activity, in the form of the running wheel, which alleviates environmental impoverishment (89). Indeed, environmental enrichment is an experimental model that has been seen to be effective in the attenuation of age-related-cognitive deficits in housed rodents as a result of modulations in brain plasticity (90-92). Investigations regarding the relationship between physical exercise and cognition performed in a double transgenic mouse model for AD, reported an improvement in cognition and memory after the combination of forced exercise (treadmill running three days/week) and spontaneous wheel running (93). In addition, environmental enhancement in young adult rodents reduces the response of plasma adrenocorticotropic hormone and cortisol to different stressors (94), increases the levels of glucocorticoid receptors in the hippocampus, thus facilitating a faster recovery of basal levels of cortisol under stress conditions (95), and reduces chronic stress and cortisol values (96). Therefore, environmental enrichment is thought to be a positive factor that could counteract some of the effects of stress on the aging brain.

The present study shows how voluntary exercise has different effects in SD and non-SD conditions. Voluntary physical activity improved spatial memory functions and decreased cortisol levels in non-SD animals, underlining the benefits of sleep hygiene and lifestyle. Furthermore, exercise reduces cortisol levels, which may represent an accessible non-pharmacologic approach option for stress patients, and might be used as a therapy in combination with pharmacologic protocols. Further research is necessary to identify the principal healthy and therapeutic aspect of exercise programs in memory functions for the prognosis of neurodegenerative disorders such as AD.

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Figure legends:

Figure 1. Voluntary running of young and old *O. degus* during 45 days. Curves represent the average distance run by young (blue plot) and old (red plot) animals each day. The results are expressed as mean ± SEM. *p < 0.05, represents significant differences between young and old animals for individual running days.

Figure 2. Effect of 45 days of voluntary exercise on spatial memory and SD in young and old *O. degus*, measured by the Barnes Maze (BM). (A-C) Histograms for young *O. degus* (A) total time (seconds) spent to escape. (B) Number of working errors. (C) Number of reference memory errors. (D-F) Columns represent (D) total time (seconds) spent to escape. (E) Number of working errors. (F) Number of reference memory errors for old O. *degus*. Results are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 represent significant and very significant differences compared with non-SD condition, $^{\#}p < 0.05$ shows significant differences between SD condition in control and exercise groups with same age.

Figure 3. Effect of voluntary exercise and sleep deprivation in cortisol levels. Cortisol levels of O. *degus* at both ages under the exercise condition. Results are expressed as mean ± SEM. *p < 0.05 represents significant differences compared with non-SD condition, $^{\#}p < 0.05$ represents significant differences between control and exercise groups of the same age.

Picture 1 (Supplementary material). The *O. degus* cage design was home-made (the running wheel was rotated by animal effort). All animals had free access to the running wheel which was connected to a digital counter for 45 days.

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Figure 1.JPEG

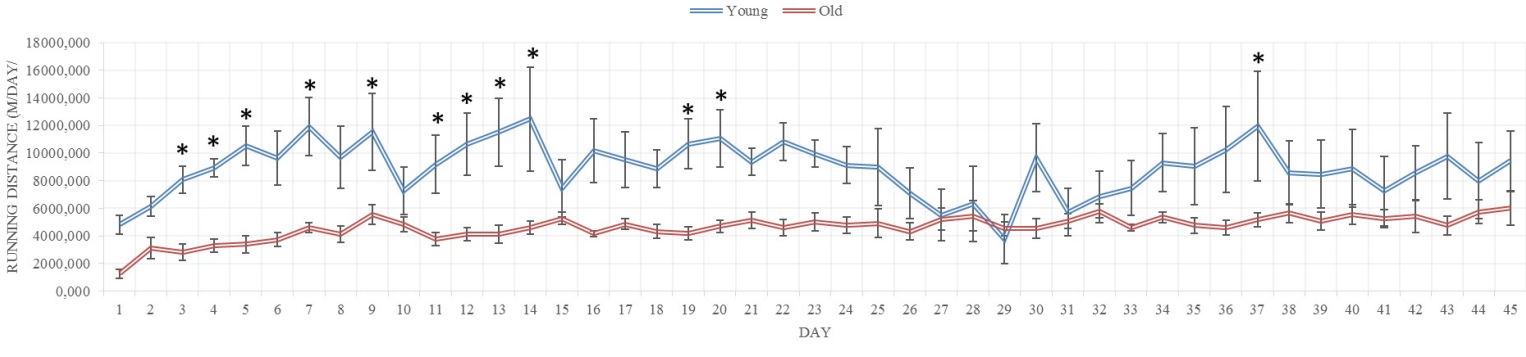
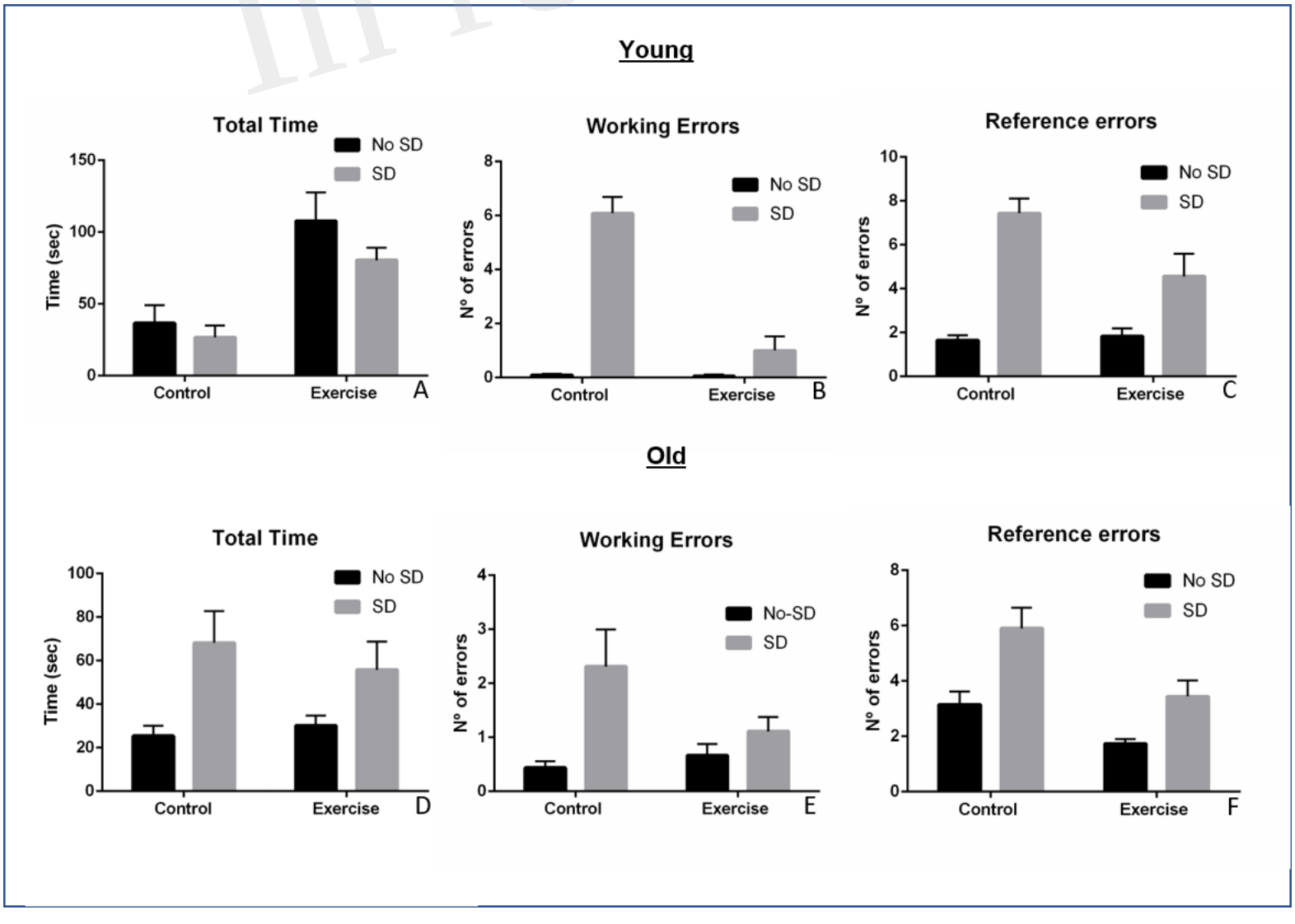


Figure 2.TIF



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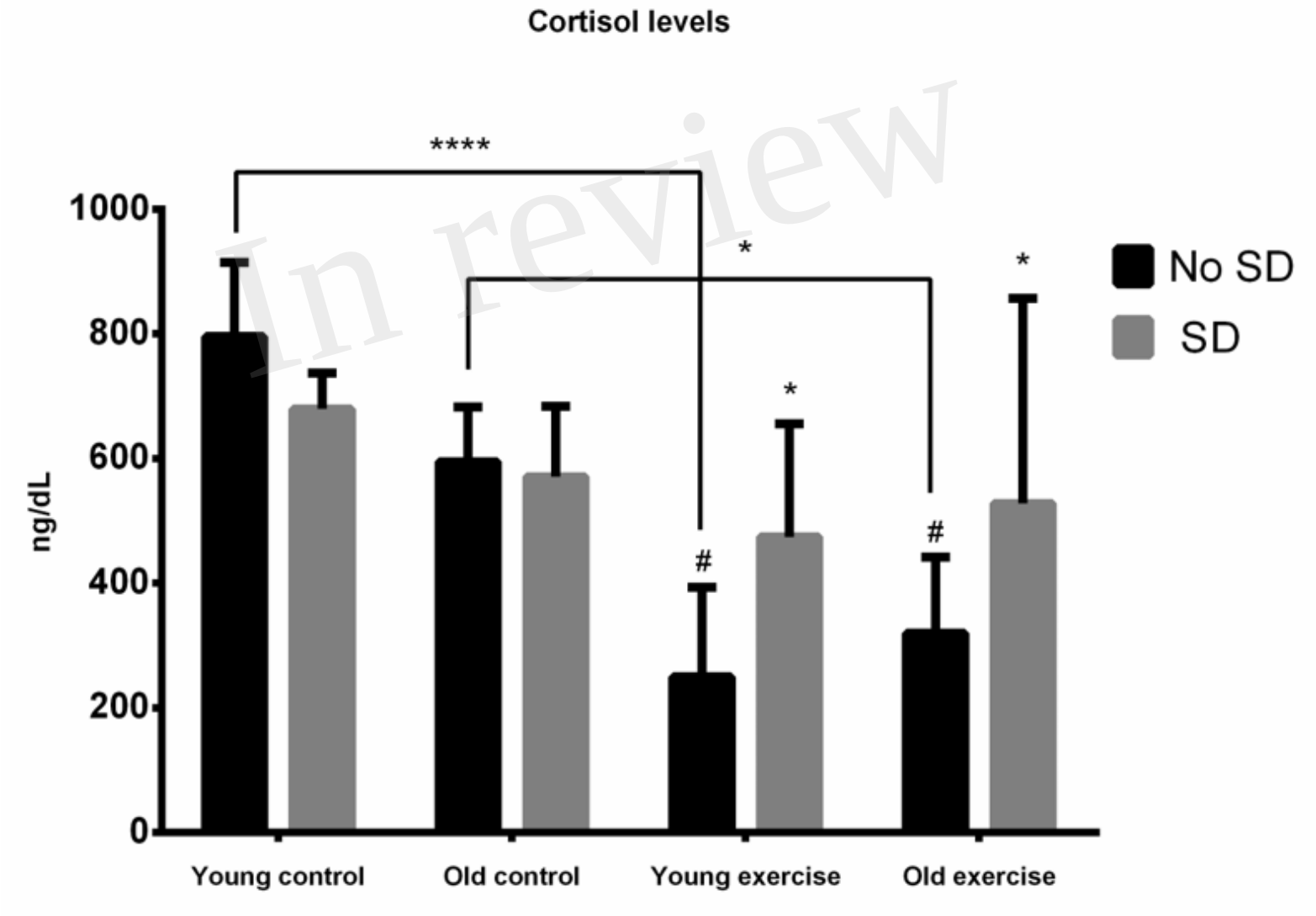

IX.VIResultados recientes: Estudiodelaactividadcolinesterasaen*O.degus*

Introducción

La enfermedad de Alzheimer constituye la primera causa de demencia a nivel mundial. El déficit cognitivo en pacientes de esta enfermedad, parece estar directamente relacionado con alteraciones en la transmisión colinérgica. La síntesis de acetilcolina (ACh) se lleva a cabo mediante la enzima acetiltransferasa (ChAT), se libera ACh en la hendidura sináptica y se une a los receptores postsinápticos (AChRs). Posteriormente es hidrolizada mediante las colinesterasas (ChEs), y se produce la recaptación de la colina mediante un transportador de colina de alta afinidad (ChT). A nivel clínico este hecho se pone de manifiesto si observamos que la mayoría de la farmacología actual tiene como diana terapéutica la inhibición de las ChEs, enzimas encargadas de la degradación de la ACh. En España podemos encontrar comercializados a donepezilo (Aricept®), rivastigmina (Exelon®, Prometax®) y galantamina (Reminyl®).

A pesar de que en la hendidura sináptica la hidrólisis de la ACh se lleva a cabo de manera mayoritaria por la acetilcolinesterasa (AChE) (EC 3.1.1.7), esta enzima puede ser perfectamente reemplazada desde un punto de vista funcional por la butirilcolinesterasa BuChE (EC 3.1.1.8). Esta duplicidad genética constituye uno de los ejemplos más evidentes de redundancia, y de su vital trascendencia a lo largo de la evolución, como demuestra el hecho de que se conserve hasta la actualidad, como se refleja en algunos trabajos (Li y cols., 2000). Además, numerosos estudios se están centrando en la investigación sobre esta segunda enzima, no sólo a nivel de actividad, sino también de expresión genética como posible diana farmacológica (Li y cols., 2017, Han y cols., 2015).

A nivel experimental tan solo existe un estudio realizado en *O. degus*, donde se pone de manifiesto el papel de la AChE (Inestrosa y cols., 2005). En este estudio histológico se compararon la densidad y tamaño de neuronas ricas en AChE en *O. degus*, cerebros humanos y en cerebros de ratas de la cepa Sprague–Dawley. En el córtex de *O. degus*, se observó un marcaje AChE en

las neuronas piramidales, mientras que en el córtex de rata no se detectó ningún marcaje. Como cabía esperar, en el tejido humano el marcaje fue positivo al igual que en el *O. degus*, aunque con algunas diferencias. Fundamentalmente en *O. degus*, estas neuronas se localizan exclusivamente en la capa IIIc, mientras que en el tejido humano se encontraban en las capas III y V, y aunque presentaban una morfología parecida, el tamaño era distinto, siendo mayor en humanos (Li y cols., 2000).

Esta falta de información sobre el papel de la ACh en el modelo de *O. degus*, y su relevancia tanto en la EA como en otros trastornos neurológicos (Giacobini, 2004, Fu y cols., 2005, Silveyra y cols., 2008), sólo se puede justificar por la relativa "novedad" que constituye este modelo, así como que muy pocos genes de esta especie se encuentren secuenciados actualmente.

Considerando el papel que tienen las ChEs en la EA y otras demencias (Diamant y cols.,2006), nos propusimos estudiar la posible diferencia en la actividad enzimática de tanto la AChE como BuChE bajo dos paradigmas, el primero de ellos la edad, y el segundo, bajo la influencia de factores ambientales, como es la realización voluntaria de ejercicio físico. Las medidas de actividad enzimática se llevaron a cabo en extractos de hipocampo, dada la importancia de esta zona en la etiopatogenia de la EA, atendiendo a las dos marcas histopatológicas fundamentales de la enfermedad, como son la deposición del péptido beta-amiloide y los agregados de la proteína tau (Luckiw y Bazan, 2000).

Material y método

Materiales

Los sustratos acetiltiocolina (ATCh), butiriltiocolina (BuTCh), el ácido 5,5 0-ditio-bis-2-nitrobenzoico (DTNB), los inhibidores 1,5-bis(4-alildimetilamoniofenil)-pentan-3-ona (BW284c51) y tetraisopropil pirofosforamida (Iso-OMPA), y el detergente polioxietilen10-oleil éter (Brij 96) proceden de SIGMA. El reactivo Pierce BCA Protein Assay Kit para la determinación de la cantidad de proteína se adquirió de Thermo Fisher Scientific.

Animales

Se utilizaron seis hembras por condición, a dos edades distintas (de un año de edad considerados como jóvenes, y entre cuarenta y sesenta meses los añosos), además de las condiciones control y ejercicio (ver sección material y método del artículo cortisol para mayor detalle). Los animales se mantuvieron en el estabulario siguiendo la legislación vigente y de acuerdo con el comité de ética para la experimentación animal de la Universidad de Murcia.

Extracción de ChEs de hipocampo

Previa anestesia con sevofluorano, los animales se sacrificaron mediante decapitación. Mediante un corte sagital por el cuerpo calloso, el cerebro se separó en dos mitades, una para inmunohistoquímica y la otra se lavó con el tampón salino HSB (HEPES-saline buffer), se diseccionó el hipocampo y posteriormente se congeló a -80ºC con la ayuda de isopentano. El extracto proteico de hipocampo se extrajo con el tampón HSB, guardando la proporción de 10 ml/g de tejido, conteniendo este tampón una mezcla de inhibidores de proteasas (Moral-Naranjo y cols., 1996). El extracto se centrifugó a 100,000xg durante una hora a 4ºC. Este primer sobrenadante llamado S1, contiene las ChEs correspondientes la fracción soluble o débilmente unidas a las membranas. El precipitado se resuspende en tampón HSB con detergente Brij 96 1% p/v y el cocktail de antiproteinasas. Se centrifuga de nuevo en las mismas condiciones, y en este sobrenadande denominado S2, se hallan las ChEs que se encontraban fuertemente ligadas a las membranas biológicas.

Determinación de la actividad ChE

La actividad enzimática de las ChEs se valoró mediante el método de Ellman (Moral-Naranjo et al., 1996); La actividad AChE se midió en presencia de 1 mM ATCh y 50 microM Iso-OMPA, y la BuChE con 1 mM BuTCh y 10 microM BW284c51. Cabe destacar que tanto Iso-OMPA como BW284c51 son inhibidores selectivos de las enzimas BuChE and AChE respectivamente, y que la presencia de ambos inhibidores en los ensayos tiene como finalidad mostrar la contribución de otras esterasas, aparte de las ChEs, en la posible hidrólisis

de los sustratos ATCh o BuTCh. Estos valores se toman como blanco para cada muestra, y se restan al valor obtenido en presencia de un solo inhibidor.

Una unidad de actividad ChE se define como la cantidad de enzima que hidroliza 1 micromol del sustrato preferente por hora a una temperatura de 37ºC. En nuestro caso, la actividad se expresa en unidades arbitrarias (U.A), donde una unidad corresponde a un incremento de absorbancia de 0.001 por microlitro de muestra por minuto, normalizado respecto al volumen total de la muestra. La determinación de proteína se realizó mediante el reactivo Pierce BCA.

Análisis estadístico

Para el estudio estadístico se utilizó el software Statistics® 10. Los resultados se expresan como la media de dos lecturas de actividad para cada enzima, realizadas de manera independiente. En primer lugar, se comprobó si cada uno de los cuatro grupos seguía una distribución normal mediante el test Saphiro-Wilks. Cada grupo de actividad enzimática (AChE y BuChE en las fracciones S1 y S2) constaba de un n=6. A continuación se aplicó el test de Barlett para ver si se cumplía la condición de homocedasticidad, en caso positivo se utilizó el test *T* de Student. En caso de que no cumpliese con la condición de homocedasticidad se aplicó el test no paramétrico de Mann-Whitney-Wilcoxon. Los resultados con un valor de $p < 0.05$ se consideraron significativos para ambos tests.

Resultados

Los niveles de actividad enzimática de AChE y BuChE en los sobrenadantes de S1 y S2 de *O. degus* jóvenes *vs* añosos se muestran en las Figuras 1 & 2. A pesar de que el porcentaje de actividad en el sobrenadante en ausencia de detergente (S1) es aproximadamente el 14% de la actividad AChE observada en el sobrenadante S2, este resultado apunta en la misma dirección que los datos previos observados en cerebro (Moral-Naranjo et al., 1996), donde se pone de manifiesto la fuerte unión de la enzima AChE a las membranas celulares del tejido cerebral. La falta de diferencias significativas entre la actividad total de las fracciones S1 y S2 entre *O. degus* jóvenes vs *O.*

degus añosos (Figura 1A y 1B), sugiere que el envejecimiento no ejerce ningún efecto sobre la actividad ni sobre la unión de esta enzima con las membranas biológicas. Estas observaciones están en la línea de los resultados publicados en otro modelo animal de envejecimiento como es el SAMP8 (Fernández-Gómez y cols.,2010).

Los niveles de actividad AChE en el sobrenadante S1 muestran que no hay diferencias significativas entre los *O. degus* jóvenes y añosos (p=0.064) (Figura 1A). Sin embargo, bajo el paradigma del ejercicio físico voluntario, se puede observar como sí existen diferencias estadísticas cuando los individuos jóvenes se someten a esta actividad física (p<0.05), mientras que en los animales añosos no hay variación, tan solo una tendencia (p=0.116) (Figura 1A).

En contraste con los resultados previos, en la fracción S2, se puede observar que a pesar de que los niveles de AChE son muy similares en individuos jóvenes y añosos (Figura 1B), el impacto de un factor medioambiental como puede ser la realización de una actividad física, afecta por igual tanto a los animales jóvenes (p<0.01) como a los añosos (p<0.05) (Figura 1B). Si bien, cabe destacar que este efecto es más rotundo en jóvenes (p<0.01) que en añosos (p<0.05), poniendo en relieve el papel que juega el envejecimiento.

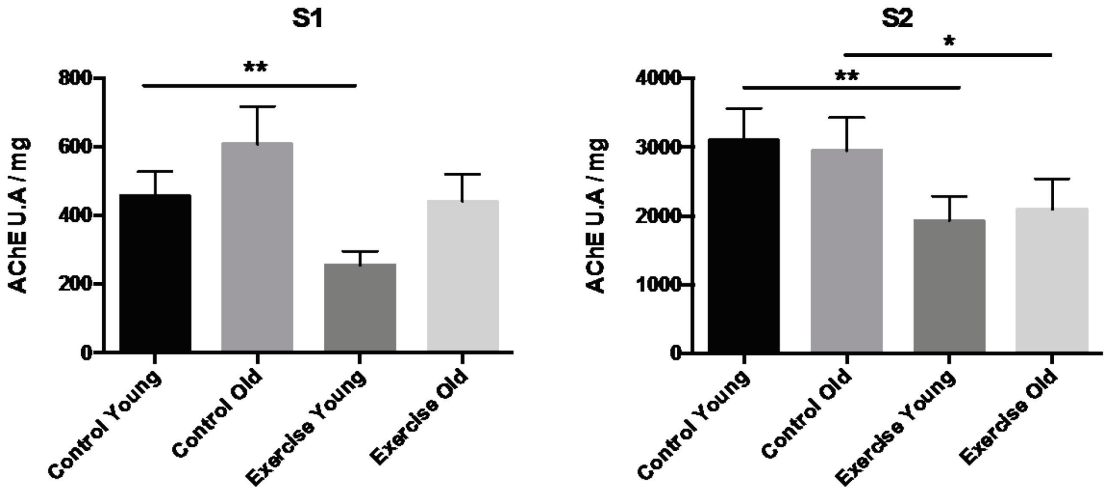

Figura 1: (A) Actividad AChE en los sobrenadantes S1 y (B)S2. Los resultados se representan como la media ± el error estándar, considerando significativos los valores de *p<0.05 y **p<0.01 (n = 6/ grupo).

La actividad de los extractos S1 y S2 de BuChE se encuentra en una ratio de 51 / 49% respectivamente (Figura 2A y B), lo que está en la línea de

estudios previos, donde se describe la escasa unión de esta enzima a las membranas celulares del tejido nervioso, en comparación con la AChE (Moral-Naranjo et al., 1996). El análisis estadístico de la actividad BuChE en el extracto S1 revela que el aumento de actividad en *O. degus* añosos *vs* jóvenes no es significativo (p=0.336) (Figura 2A). Además, al contrario de lo que sucede con la actividad AChE, la realización de ejercicio físico voluntario, disminuye si bien no de manera significativa, la actividad BuChE en animales jóvenes (p=0.053) y en añosos (p=0.132) de la fracción S1 (Figura 2A). Resultados similares se pueden observar en la fracción S2 (Figura 2B), donde tampoco se observan resultados estadísticamente relevantes.

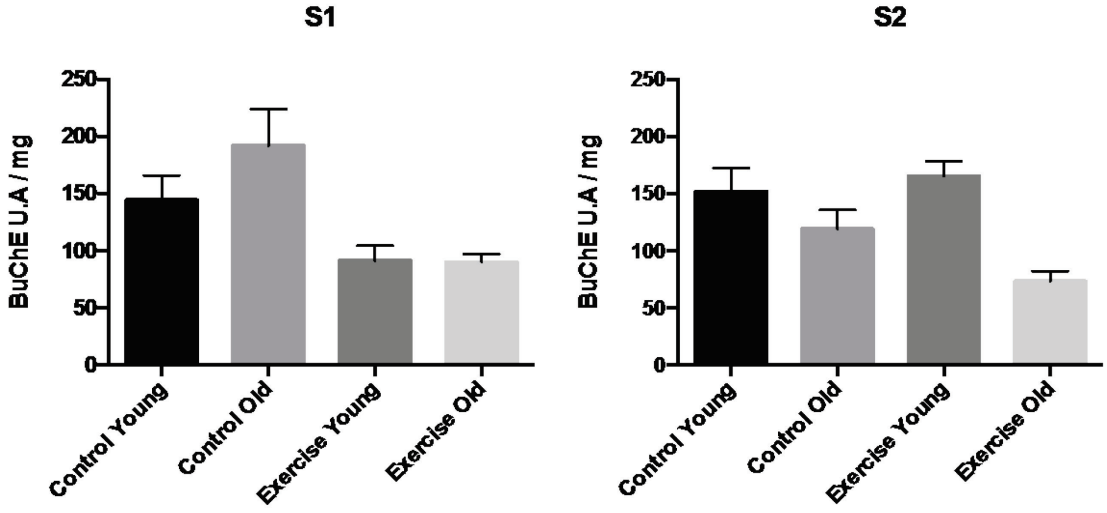

Figura 2: (A) Actividad BuChE en los sobrenadantes S1 y (B)S2. Los resultados se representan como la media ± el error estándar, considerando significativos si los hubiere, los valores de *p<0.05 (n = 6/ grupo).

Discusión

El estudio de nuevas dianas farmacológicas constituye una de las principales prioridades para el diseño de nuevos fármacos en enfermedades neurodegenerativas como es el caso de la EA. En este sentido, los fármacos anticolinesterásicos han demostrado ejercer una actividad beneficiosa, si bien sólo durante un tiempo limitado de aproximadamente dos años (O´Brien y cols., 2017, Eleti, 2016). La búsqueda de dianas terapéuticas comprende no sólo la aparición de nuevas vías, sino también la mejora de las ya existentes, y en el trabajo que nos ataña, la disección entre las actividades AChE y BuChE podría ser un nuevo objetivo. En nuestro estudio nos hemos centrado en el efecto de

la edad, asumiendo el envejecimiento como uno de los factores primordiales para el desarrollo de la EA (Niccoli y Partridqe, 2012), así como el impacto de factores medioambientales como es la realización de ejercicio físico moderado (Dorszewska y cols., 2016). En nuestro diseño experimental hemos utilizado *O. degus* a dos edades distintas, similares a las empleadas en otros trabajos para el estudio de la deposición del péptido β-amiloide y agregación de la proteína tau en este modelo (Ardiles y cols., 2012), así como para diversos tratamientos farmacológicos (Tarragon y cols., 2014, Estrada y cols., 2015). El tejido objeto de estudio fue el hipocampo, zona que ha sido identificada como una de las primeras afectas por la EA. De hecho, en las clasificaciones de la etiopatología realizada en base a los depósitos del péptido beta-amiloide (Braak y Braak, 1998), y a los agregados de la proteína tau (Delacourte y cols., 1999, Clavaquera y cols., 2009), sitúan a esta zona en los primeros estadíos. El paradigma del ejercicio físico voluntario se ha utilizado ampliamente en la literatura científica como factor medioambiental influenciable en la prognosis de la EA (Belarbi y cols., 2011, Andrieu y cols., 2017).

La determinación de la actividad de la enzima AChE aportó diferencias significativas tanto en el sobrenadante S1 como S2 (Figura 1A y 1B). Si bien en la fracción S1, estas diferencias afectaron sólo a las condiciones jóvenes control y jóvenes sometidos a ejercicio voluntario (Figura 1A), mientras que en la fracción S2, tanto los animales jóvenes como añosos que realizaban actividad física disminuyeron significativamente la actividad AChE (Figura 1B). Cabe destacar que la actividad correspondiente al sobrenadante S2 representa a las colinesterasas fuertemente ligadas a las membranas celulares, y es sólo aquí donde se observa el descenso de actividad en los animales añosos, a diferencia de los jóvenes que tras la actividad física disminuyen su actividad AChE en ambas fracciones.

Teniendo en cuenta que la fracción S2 constituye el 86% de la actividad enzimática total, es importante enfatizar que la bajada es más pronunciada en los individuos jóvenes ($p<0.01$) que en los añosos ($p<0.05$). Datos que están en concordancia con estudios previos, donde *O. degus* respondió de forma distinta en función de la edad tanto a tratamientos farmacológicos como es el caso de la memantina (Tarragon y cols., 2014), los tratados con estimulación

magnética transcraneal (Estrada y cols., 2015) y a los que se midieron los niveles de cortisol (datos no publicados, artículo en revisión). Estos resultados ponen de relieve el papel del envejecimiento en la susceptibilidad a los factores medioambientales considerados “protectores” en la transmisión colinérgica (Bohr, 2005).

Si bien el porcentaje de actividad BuChE en ambas fracciones permaneció ≈ 50% como se ha publicado previamente en cerebro (Moral-Naranjo y cols.,1996), la valoración de la actividad en los sobrenadantes S1 y S2 aportó divergencias interesantes con respecto a la AChE. Así, en la fracción S1 no se hallaron diferencias significativas entre los distintos grupos de estudio (Figura 2A), de la misma forma sucedió en la fracción S2 (Figura 2B). Estos resultados discrepan considerablemente, con el aumento de actividad BuChE descrita en los sobrenadantes S1 y S2 de los ratones SAMP8, donde la actividad enzimática incluso se duplicaba (Fernández-Gómez y cols., 2008, Fernández-Gómez y cols., 2010). Esta disonancia puede ser debida a que en el modelo de envejecimiento del SAMP8 existe una mutación mientras que el *O. degus*a día de hoy no se ha descrito ninguna.

Nuestros resultados muestran como el efecto beneficioso de la realización de ejercicio físico voluntario afecta de manera distinta a la actividad de las enzimas AChE y BuChE. Nuestro estudio pone de manifiesto la importancia del anclaje de estas moléculas de liberación de neurotransmisores en las membranas de las distintas zonas del cerebro y su relevancia en la transmisión colinérgica(García-Ayllon y cols., 2014, Campanari y cols., 2016). Recientemente se ha publicado la presencia de metales pesados en *O. degus* de hasta 36 meses, alterando la homeostasis de los metales de transición, afectando a la función lisosomal e incrementando el péptido β 42 (Braidy y cols., 2017), aunque el aumento de actividad AChE y presencia de metales pesados solo se ha descrito en ratones (Rani y cols., 2015).

A la luz de nuestros resultados experimentales podemos concluir que el impacto de los factores ambientales no influye por igual a las actividades AChE y BuChE, y que este efecto va en función del anclaje a las membranas celulares y a la edad. Estudios posteriores deberán centrarse en el papel de cada una de estas enzimas durante el envejecimiento, y la idoneidad de las

mismas como diana terapéutica en función de cuál sea el estado en el que se encuentre la EA y la terapia utilizada.