SISTEMAS NEURALES IMPLICADOS EN APRENDIZAJE AVERSIVO GUSTATIVO COMPLEJO: COMPARACIÓN CON OTROS TIPOS DE MEMORIA Y EFECTO DE LA EDAD

Neural systems involved in taste aversion learning: comparison with other types of memory and the effect of aging

TESIS DOCTORAL

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CERTIFICA:

Que el trabajo de investigación titulado "SISTEMAS NEURALES IMPLICADOS EN APRENDIZAJE AVERSIVO GUSTATIVO COMPLEJO: COMPARACIÓN CON OTROS TIPOS DE MEMORIA Y EFECTO DE LA EDAD" ha sido realizado por D. Fernando Gámiz Ruiz para optar al grado de Doctor Internacional en Psicología en el Departamento de Psicología Experimental y Fisiología del Comportamiento de la Facultad de Psicología de la Universidad de Granada, bajo su dirección. Reuniendo dicho trabajo los requisitos académicos, formales y de calidad necesarios para que pueda ser defendida públicamente.

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FINANCIACIÓN

El trabajo de tesis que vamos a presentar ha sido subvencionado por las siguientes becas y proyectos de investigación:

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SISTEMAS NEURALES IMPLICADOS EN APRENDIZAJE AVERSIVO GUSTATIVO COMPLEJO:

COMPARACIÓN CON OTROS TIPOS DE MEMORIA Y EFECTO DE LA EDAD

NEURAL SYSTEMS INVOLVED IN TASTE AVERSION LEARNING:

COMPARISON WITH OTHER TYPES OF MEMORY AND THE EFFECT OF AGING

"*Quien no haya experimentado la irresistible atracción de la ciencia,*

no podrá comprender su tiranía."Frankenstein. Mary Shelley

Es complicado saldar, en unas pocas palabras, todas las deudas que he contraído durante los años de elaboración de esta tesis pero más difícil sería ocultar mis sinceros agradecimientos a todos los que me han ayudado.

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RESUMEN

La presente tesis doctoral está dirigida a investigar los mecanismos cerebrales responsables del aprendizaje y la memoria en ratas combinando distintos niveles de análisis desde el molecular hasta el de sistemas con una aproximación de desarrollo y estableciendo comparaciones entre tareas de memoria gustativa, memoria de reconocimiento de objetos y memoria espacial. La tesis está formada por cuatro capítulos organizados en dos partes. Además se incluyen apartados generales dedicados a introducción, discusión, conclusiones y perspectivas de la tesis.

La parte primera contiene una exhaustiva revisión de los conocimientos previos sobre el papel de PKMζ en el mantenimiento de la memoria a largo plazo (Capítulo 1). En el capítulo 2 se demuestra que la actividad de PKMζ en la Amígdala basolateral es necesaria tanto para el mantenimiento de la memoria a largo plazo de una tarea de evitación activa, como en la modulación de la adquisición de aversiones gustativas condicionadas. Ello se interpreta como apoyo a la existencia de mecanismos moleculares compartidos entre distintos tipos de memoria y confirma el papel crítico de PKMζ en el mantenimiento de memorias dependientes de sistemas neurales disociables.

La segunda parte incluye estudios experimentales llevados a cabo en ratas de edad avanzada. El capítulo 3 contiene una amplia revisión de los efectos del envejecimiento sobre las capacidades de memoria gustativa, así como los hallazgos obtenidos en una serie de experimental dirigida a explorar el efecto de la inactivación reversible del Hipocampo dorsal mediante tetrodotoxina sobre la modulación de la memoria gustativa por parte del contexto temporal. Los resultados indican que las ratas naïve de edades avanzadas no muestran dicha modulación, pero que pueden ser reactivadas por las experiencias de aprendizaje discretas. La implicación del sistema hipocampal queda patente al administrar en el hipocampo dorsal infusiones bilaterales de TTX durante el condicionamiento en ratas con experiencia previa, ya que el efecto se invierte tal y como se ha descrito

en el caso de lesiones permanentes. Ello permite proponer el estudio de la memoria gustativa durante el envejecimiento como herramienta útil para aplicar una aproximación de sistemas a la investigación de la memoria.

En el capítulo 4 se presentan resultados que demuestran la existencia durante el envejecimiento de un deterioro en la capacidad de retención de memoria de reconocimiento de reconocimiento de objetos. El deterioro evidente con intervalos de retención de 24 horas cuando se utilizan objetos estándar, se exacerba, poniéndose de manifiesto con dilaciones de 1 hora al emplear objetos de alta complejidad pero no en función de la similitud o ambigüedad si se trata de formas elementales. Dado que este tipo de deterioro es similar al inducido por lesiones de la corteza perirhinal en ratas adultas, los resultados sugieren cambios selectivos de la función de dicha zona asociados al envejecimiento.

ABSTRACT

The present doctoral thesis was aimed to investigate the brain mechanisms of learning and memory in rats by combining different levels of analysis from the molecular to the systems and establishing comparison between taste, object recognition and spatial memory. The thesis contains four chapters organized in two parts. In addition a general introduction, discussion, conclusions and perspectives are included.

The first part contains an exhaustive review of the present knowledge on PKMζ role in long-term memory maintenance (Chapter 1). The results included in chapter 2 demonstrate that activity of PKMζ is required both for long-term retention of a learned active avoidance response and for modulating the acquisition of conditioned taste aversion.

The second part consists of experimental studies performed in aged rats. Chapter 3 contains a wide review of the age-related changes in taste memory abilities and findings drown by an experimental series exploring the effects of dorsal Hippocampus TTX inactivation on the taste memory modulation by the temporal context. The results indicate that naïve aged rats do not exhibit such modulation that can be reactivated by previous learning experiences. The fact that bilateral hipocampal inactivation by TTX reverse the effect of a time-of-day change as it has been reported in permanent lesion studies support a critical role of age-related hipocampal dysfunction. Therefore, taste memory in aged rats is proposed as a useful tool for applying a systems approach to memory research.

Chapter 4 presents results demonstrating age-related retention impairments object recognition memory. Such impairment is evident at 24 hours retention intervals using standard objects, but it is exacerbated, appearing at 1 hour delays, when increasing the complexity but not the similarity/ambiguity of elemental objects. Since similar deficits have been induced in perirhinal cortex damaged adult rats, these findings are interpreted in terms of a selective age-related functional changes in this brain area.

INTRODUCCIÓN

Las diversas aproximaciones al estudio de los mecanismos cerebrales responsables de la adquisición y el mantenimiento de la memoria aplicando diversos niveles de análisis ha dado lugar a conceptos tales como "consolidación celular" y consolidación de sistemas". Dichos conceptos están asentados en la teoría de la consolidación surgida a partir de los trabajos de Müller y Pilzecker a comienzos del siglo XX y las propuestas de Donald Hebb a mediados de siglo (para una revisión McGaugh, 2000). La teoría de la consolidación propone la estabilización del recuerdo como engrama o "huella" de memoria permanente gracias a una serie de fases en las que se encuentra en estado lábil desde su adquisición hasta su estabilización. Aunque la teoría de consolidación ha ido siendo modificada y enriquecida a lo largo de los años incluyendo en la actualidad fases adicionales de reconsolidación (Dudai, 2006; Rodriguez-Ortiz & Bermudez-Rattoni, 2007; Wang & Morris) sus presupuestos fundamentales continúan representando un fecundo marco conceptual para la investigación actual sobre los procesos neurales implicados en aprendizaje y memoria.

Con respecto a la denominada "consolidación celular" grandes avances se han realizado en las últimas décadas en la identificación de los mecanismos celulares y moleculares responsables de las primeras fases del proceso, gracias inicialmente a fructíferas líneas de investigación en invertebrados (Kandel & Squire, 2000) y al empleo de modelos de plasticidad sináptica en rodajas de Hipocampo

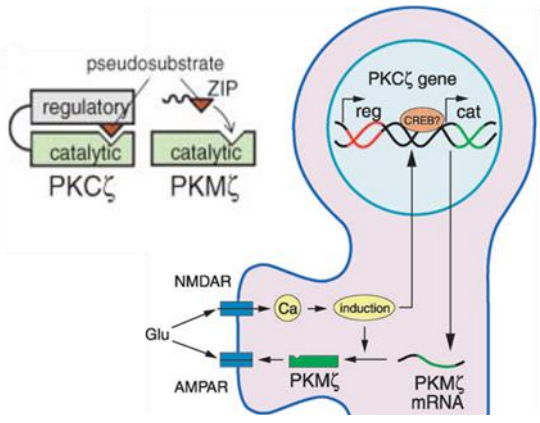

Fig. i.1 Modelo de la acción de PKMζ en el mantenimiento de la LTP*. La estimulación de los receptores NMDA induce la formación de PKMζ y la fosforilación de esta proteína potenciaría la formación y mantenimiento de receptores AMPA. Tomada de Hernandez et al. 2003*

(Cooke & Bliss, 2006). Una variedad de moléculas, entre las que destaca quinasas como PKC, han sido implicadas en diversas fases del proceso de consolidación, pero sólo recientemente se ha identificado su potencial implicación en el mantenimiento de la memoria a largo plazo o de manera permanente (Hernandez, et al., 2003; Ling, et al., 2002; Osten, Valsamis, Harris, & Sacktor, 1996; Sacktor, et al., 1993; Serrano, Yao, & Sacktor, 2005). Efectivamente, en el año 2006 el grupo dirigido por Todd Sacktor en colaboración con el grupo de André Fenton demostraron por primera vez el papel crítico de la proteína quinasa Mζ (PKMzeta), una isoforma atípica de PKC que presenta actividad persistente automantenida debido a carecer de dominio regulador (Fig. i.1) (Pastalkova, et al., 2006). Sus resultados aplicando un péptido inhibidor de PKMζ (ZIP) en el Hipocampo dorsal indicaron la posibilidad de eliminar tanto potenciación a largo plazo como recuerdos adquiridos en una tarea de evitación espacial 24 horas después de la adquisición. Un año después el mismo grupo en colaboración con el equipo dirigido por Yadin Dudai presentó resultados sorprendentes indicando la posibilidad de eliminar aversiones gustativas condicionadas semanas después de la adquisición mediante inyecciones de ZIP en la Corteza Insular (Shema, Sacktor, & Dudai, 2007). Desde entonces se ha confirmado el papel crítico de PKMζ en el mantenimiento de una gran variedad de recuerdos consolidados, aunque también han surgido nuevas cuestiones acerca de la generalidad de su función en memorias adquiridas con diversas tareas de aprendizaje y su posible participación en procesos relevantes durante la adquisición, tal como la detección de la novedad (Moncada & Viola, 2008).

En lo que se refiere a la denominada “consolidación de sistemas” se acepta que las diversas fases de adquisición y consolidación de los recuerdos pueden tener lugar en diversas áreas cerebrales, dependiendo los circuitos cerebrales involucrados del tipo de memoria de que se trate. Desde que Scoville y Milner (1957) describieron pacientes amnésicos con lesiones del lóbulo temporal medial que afectaban al hipocampo, capaces de adquirir y mantener otros tipos de memorias, se han sucedido propuestas que distinguen entre tipos de memoria a largo plazo que dependen de circuitos cerebrales disociables. Estas propuestas han contribuido de forma decisiva al desarrollo de la visión actual del aprendizaje y la memoria que contempla múltiples sistemas neuronales independientes aunque

pueden interaccionar entre sí. En este sentido son predominantes las visiones dicotómicas como la propuesta por Larry Squire (2004) que distingue entre memoria declarativa y no declarativa, distinción que se corresponde en cierta medida con otras tales como memoria explícita versus implícita (Fig. i.2).

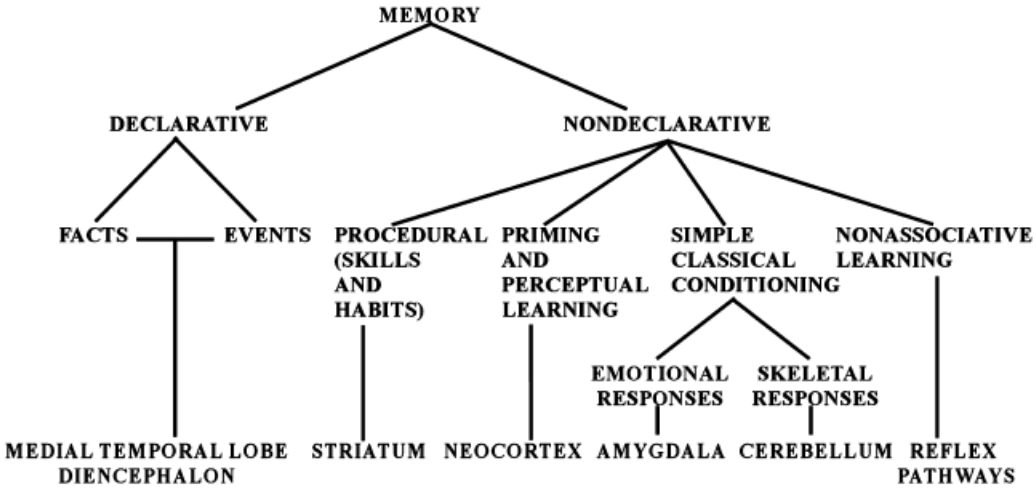

Fig i.2 Clasificación de los sistemas de memoria a largo plazo en mamíferos*. Cada uno de los tipos de memoria declarativa y no declarativa han sido asociados con diferentes estructuras cerebrales. Tomada de Squire 2004.*

La memoria declarativa o explícita que depende de la integridad del sistema hipocampo-cortical permite la adquisición de recuerdos acerca de episodios vividos (memoria episódica) y conocimientos (memoria semántica). Se trata de recuerdos conscientes en el caso del ser humano con otras características que pueden ser estudiadas en modelos animales. Por un lado, parecen depender de complejas representaciones elaboradas gracias a la detección de relaciones entre múltiples estímulos. Por otro lado, estas representaciones pueden recuperarse de modo flexible en diversas circunstancias. En este sentido, es frecuente el empleo de tareas de aprendizaje que requieran memoria del contexto, tales como las basadas en representaciones espaciales flexibles.

La organización anatómico-funcional del hipocampo como lugar de convergencia de conexiones recíprocas con múltiples áreas corticales y subcorticales le coloca en una situación privilegiada para jugar un papel crucial en aquellos recuerdos que implican representaciones multimodales. Aunque hay que tener en cuenta que los diversos efectos que el contexto puede tener sobre diversos tipos de memoria abren múltiples posibilidades sobre la participación del

Hipocampo (Holland & Bouton, 1999) su papel en la memoria espacial está ampliamente apoyado por la evidencia experimental. En este sentido, una cuestión de intenso debate ha sido si las representaciones espaciales son el resultado de una función específicamente espacial del hipocampo (O'Keefe, 1993) o bien son el resultado de la posibilidad de establecer relaciones complejas multimodales no únicamente espaciales entre estímulos (Eichenbaum, 1996).

Por su parte, el aprendizaje aversivo gustativo o la habituación de neofobia gustativa permiten adquirir memorias gustativas aversivas y apetitivas tradicionalmente integradas entre los diversos tipos de memorias "no declarativas" que no requieren la integridad del sistema hipocampal. La memoria de reconocimiento gustativa depende de un circuito neural que involucra áreas cerebrales situadas en diversos niveles de integración desde el tronco cerebral, diencéfalo y corteza cerebral (Bermudez-Rattoni, 2004). En el caso del aprendizaje aversivo gustativo el circuito incluye el NTS, PBN, Amígdala y Corteza Insular (CI). Además, la posibilidad de introducir largas dilaciones entre los estímulos a asociar durante el proceso de adquisición sugiere la participación de sistemas de memoria capaces de mantener el engrama gustativo durante el intervalo, que parecen depender de proyecciones ipsilaterales entre PBN y CI (Gallo, Roldan, & Bures, 1992).

La organización de los sistemas de memoria en circuitos neurales disociables es coherente con los resultados obtenidos sobre el papel de PKMζ en el mantenimiento de la memoria a largo plazo. Así, la administración bilateral de ZIP en el Hipocampo Dorsal elimina los recuerdos consolidados adquiridos en tareas de aprendizaje espacial (Pastalkova, et al., 2006; Serrano, et al., 2008), pero también interfiere con otros tipos de memoria dependientes del contexto, como por ejemplo el miedo al contexto (Serrano, et al., 2008). Otros tipos de memoria en los que PKMζ parece jugar un papel relevante son los inducidos en tareas de reconocimiento de la localización de objetos (Hardt, Migues, Hastings, Wong, & Nader, 2010) y condicionamiento palpebral de huella (Madronal, Gruart, Sacktor, & Delgado-Garcia, 2010). Por el contrario, son las infusiones bilaterales de ZIP en la CI las que eliminan aversiones gustativas consolidadas (Shema, Hazvi, Sacktor, & Dudai, 2009; Shema, et al., 2007). No se ha explorado el efecto de la infusión de ZIP

en la Amígdala sobre los procesos de memoria gustativa durante la adquisición y consolidación de aversiones condicionadas. Sin embargo, la evidencia experimental indica la relevancia de la actividad de PKMζ en la Amígdala para el mantenimiento del miedo condicionado a un tono (Kwapis, Jarome, Lonergan, & Helmstetter, 2009; Serrano, et al., 2008).

La existencia de circuitos neurales disociables para distintos tipos de memoria no excluye la interacción entre ellos. Se acepta que habitualmente en las situaciones de aprendizaje múltiples sistemas están procesando la información en paralelo de forma simultánea. Sin embargo, cada sistema permite crear diferentes representaciones y a establecer recuerdos de diversa naturaleza. Dependiendo de las exigencias de la tarea, los diversos sistemas pueden actuar de forma cooperativa o competitiva. Por un lado, existen algunas formas de aprendizaje que no pueden conseguirse sin la participación de más de un sistema. Este es el caso de ciertos fenómenos complejos de aprendizaje en tareas de memoria gustativa, como bloqueo (Gallo & Candido, 1995a, 1995b) o la dependencia del contexto temporal de la inhibición latente (Manrique, et al., 2004; Molero, et al., 2005; Moron, Manrique, et al., 2002). En ambos casos, tanto la integridad del circuito responsable del aprendizaje aversivo gustativo como la del Hipocampo dorsal son necesarias para la adquisición de recuerdos más ajustados a las condiciones de contingencia experimentadas en diversas situaciones. Por otro lado, las interacciones competitivas se ponen de manifiesto cuando la eliminación de un circuito, mediante lesiones permanentes o reversibles, no sólo impide la adquisición del tipo de aprendizaje con el que está relacionado, sino que, además, facilita la adquisición de otro tipo de aprendizaje. Este es el caso de la inactivación bilateral del Hipocampo Dorsal mediante infusiones de muscimol que facilitan la adquisición de aversiones gustativas (Stone, Grimes, & Katz, 2005). Resultados similares se han obtenido en ratas envejecidas naïve con lesiones permanentes de esta zona que muestran dependencia temporal de la aversión gustativa condicionada a pesar de no exhibir la dependencia temporal de la inhibición latente que requiere la integridad del Hipocampo. Aún más el efecto aparece empleando un procedimiento conductual incapaz de producirlo en animales intactos (Manrique, Gamiz, Moron, Ballesteros, & Gallo, 2009).

Dada la naturaleza plástica de los circuitos neurales implicados en la adquisición y mantenimiento de la memoria no es de extrañar que sufran reorganización y ajustes durante el desarrollo ontogenético (Manrique, Gamiz, et al., 2009; Manrique, Moron, Ballesteros, Guerrero, & Gallo, 2007) hasta el final de la vida. En este sentido, el estudio de la memoria gustativa durante el envejecimiento representa una oportunidad privilegiada para estudiar los efectos de la experiencia y recuerdos acumulados a lo largo de toda una vida. Resultados previos han puesto de manifiesto que el efecto de las memorias gustativas previas sobre la respuesta neofóbica ante los sabores se modifica con la edad, siendo mayor en ratas envejecidas que en adultas (Moron & Gallo, 2007). Asimismo, las ratas envejecidas muestran una mayor capacidad que las adultas para adquirir memorias gustativas aversivas pudiendo desarrollar aversiones condicionadas con intervalos de más duración entre el sabor y el malestar gastrointestinal (Misanin, Collins, et al., 2002). Por último, la modulación hipocampal de la memoria gustativa sufre grandes cambios a edades avanzadas. Mientras que no se observa la dependencia temporal de la inhibición latente en ratas intactas, la lesión bilateral del Hipocampo dorsal induce una facilitación de la dependencia temporal de la aversión gustativa condicionada (Manrique, Gámiz, Moron, Ballesteros, & Gallo, 2009). Ello pone de manifiesto el decaimiento asociado a la edad de determinadas funciones hipocampales responsables del primer fenómeno mientras que parece mantener una interacción competitiva que al ser eliminada por la lesión facilita el segundo fenómeno.

En conjunto, se pone de manifiesto que la naturaleza de las experiencias de aprendizaje previas así como variaciones en los procedimientos experimentales empleados en las tareas de memoria adquieren una mayor relevancia a edades avanzadas. Ello puede estar relacionado con la gran variabilidad existente en la ejecución de animales envejecidos en tareas de memoria, lo cual produce informes contradictorios como es el caso de la memoria de reconocimiento de objetos. Aunque la mayoría de los resultados apuntan a deficiencias en el mantenimiento de los recuerdos durante periodos de 24 horas o superiores no existe acuerdo unánime y los datos son aún más contradictorios durante el periodo de consolidación anterior.

A partir de lo expuesto se hace evidente que un estudio completo de los mecanismos neurales responsables de la memoria gustativa requiere la combinación de aproximaciones desde el nivel de análisis molecular hasta el de sistemas que permitan comprender la naturaleza de las interacciones entre el circuito neural básico del aprendizaje aversivo gustativo y el sistema hipocampal, así como su reorganización a lo largo de la vida estableciendo comparaciones con la ejecución en otras tareas de memoria.

Por ello, el objetivo de esta tesis doctoral es aplicar tanto el nivel de análisis molecular en ratas adultas (Parte I) como el nivel de análisis de sistemas combinado con una aproximación de desarrollo en ratas de edad avanzada (Parte II) al estudio de los mecanismos cerebrales responsables del aprendizaje gustativo empleando fenómenos de aprendizaje complejo y estableciendo comparaciones con tareas de evitación activa (Parte I) y de reconocimiento de objetos (Parte II).

La Parte I se plantea a partir de una revisión teórica exhaustiva de estudios previos que han investigado los efectos de la inactivación de PKMζ mediante la infusión del péptido inhibidor ZIP en diversas áreas cerebrales sobre diferentes tipos de memoria (Capítulo 1). A partir de los conocimientos revisados en el Capítulo 2 se proponen dos objetivos en relación con la actividad de PKMζ en la Amígdala y su papel en las memorias inducidas mediante la tarea de aprendizaje aversivo gustativo y de evitación activa en ratas adultas.

En primer lugar, dado que la Amígdala basolateral forma parte del circuito implicado en la adquisición aversiones gustativas y puesto que no existen datos sobre el efecto de la inactivación de PKMζ en dicha zona, se plantea la posibilidad de que participe en los periodos de adquisición y/o consolidación previos al establecimiento de la memoria permanente dependiente de la CI. Para investigar esta cuestión se explorará el efecto de la infusión bilateral de ZIP en la Amígdala basolateral sobre la adquisición de aversiones gustativas empleando diversos

parámetros temporales (pre-adquisición, durante el intervalo entre el sabor y el malestar, y con diversos intervalos postadquisición desde inmediato hasta 24 horas).

En segundo lugar, puesto que se ha informado de la relevancia de la actividad de la PKMζ amigdalina en tareas de evitación pasiva, se investigará el efecto de la administración bilateral de ZIP en la Amígdala basolateral aplicada 24 horas después de la adquisición sobre el recuerdo consolidado de una respuesta de evitación activa aprendida. Asimismo, se investigará la posible existencia de memoria preservada mediante el reentrenamiento posterior.

La Parte II se centra en el estudio de las capacidades de memoria de ratas envejecidas haciendo especial hincapié en la relevancia de los efectos acumulativos de la experiencia previa (Capítulo 3) y de variaciones en los procedimientos experimentales (Capítulo 4) sobre la organización de los circuitos neurales responsables a la hora de interpretar los resultados.

El Capítulo 3 contiene una revisión crítica de los efectos del envejecimiento sobre la memoria gustativa que conduce a una interpretación de las facilitaciones y deficiencias asociadas a la edad en términos de alteraciones de la función hipocampal y, como consecuencia, de su interacción con el circuito básico de memoria gustativa. Las series experimentales están dirigidas a comprobar dos hipótesis. En primer lugar, con el fin de investigar si el entrenamiento en tareas de aprendizaje discretas puede reactivar la función hipocampal a edades avanzadas, se compara la ejecución de ratas envejecidas sometidas a tareas de aprendizaje previas y ratas envejecidas naïve en una tarea que en adultas evidencia la modulación de la inhibición latente por parte del contexto temporal, fenómeno que decae con el envejecimiento. En segundo lugar, dado que la lesión permanente del Hipocampo dorsal en ratas envejecidas induce la emergencia de la modulación temporal de la aversión condicionada se pone a prueba la posibilidad de inducir dicho fenómeno mediante la infusión bilateral de tetrodotoxina (TTX) para inactivar la zona bien con diversos parámetros temporales (adquisición versus recuperación), tanto en animales envejecidos naïve como en preentrenados.

En el capítulo 4 se explora exhaustivamente la contribución de la escasa información y la potencial variabilidad de las características de los objetos empleados en la tarea de reconocimiento de objetos a la controversia establecida sobre la naturaleza de los déficits asociados al envejecimiento en este tipo de memoria. Dado que existe asimismo controversia sobre el papel del Hipocampo y áreas corticales relacionadas en la adquisición y mantenimiento de la memoria de reconocimiento de objetos en ratas adultas, la exploración rigurosa de las capacidades en animales de edad avanzada se propone como especialmente relevante para explorar los efectos de la edad sobre la función hipocampal. Para ello, en un primer experimento se evalúa el recuerdo de ratas envejecidas durante diferentes intervalos de retención hasta las 24 horas, empleando objetos de la vida diaria convencionalmente utilizados por investigadores previos. En función de los resultados obtenidos en el segundo experimento se compara el recuerdo durante el periodo de consolidación en función de la naturaleza de los objetos empleados. Disociamos entre los efectos de ambigüedad y complejidad de los objetos. Utilizando figuras con una alta ambigüedad pero simples y con una alta complejidad pero poco parecidos entre ellos.

PARTE 1:

CAPÍTULO I: PKMZ Y EL MANTENIMIENTO DE LA MEMORIA A LARGO PLAZO

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RESUMEN

En el último siglo se han realizado importantes avances en el conocimiento de los mecanismos moleculares responsables de las primeras fases de adquisición de los recuerdos. Sin embargo, existe un gran desconocimiento sobre los mecanismos implicados en el mantenimiento de la memoria. En los últimos cinco años se ha propuesto la actividad automantenida de PKMζ como sustrato del mantenimiento de la memoria consolidada.

El capítulo revisa los resultados de los trabajos publicados en los últimos cuatro años en los que se induce la inactivación de PKMζ mediante administración i.c. del péptido inhibidor ZIP en el Hipocampo, Amígdala y Corteza Insular. En conjunto, los datos indican un efecto amnésico horas, días e incluso meses después de la adquisición en una variedad de tareas (memoria espacial, miedo condicionado, respuestas condicionadas de evitación activa y pasiva, aversiones gustativas condicionadas) en función del área cerebral inyectada. Por otra parte, la administración inmediata o en los siguientes minutos a la adquisición puede interferir con el procesamiento de situaciones novedosas o estresantes, abriendo la posibilidad de nuevas funciones de PKMζ relacionadas con la memoria a corto plazo.

La participación de PKMζ en el mantenimiento de una gran variedad de recuerdos a largo plazo, que dependen de circuitos neurales disociables, abre nuevas posibilidades para avanzar en el conocimiento de la compleja organización anatómico-funcional que sustenta la memoria. En conjunto, los resultados obtenidos son coherentes con los planteamientos vigentes en la actualidad, ya que las estructuras cerebrales abordadas que han podido relacionarse con el efecto amnésico han sido relacionadas previamente con la adquisición de los diferentes tipos de memoria estudiadas. Sin embargo, la interpretación de los resultados y de su posible relevancia para la aplicación clínica debe ser extremadamente cautelosa.

INTRODUCCIÓN

Cómo se crean los recuerdos y cómo se mantienen durante largos periodo de tiempo son cuestiones a las que la humanidad está tratando de responder desde la antigüedad. Aunque las civilizaciones egipcia, griega y romana sugirieron respuestas más o menos acertadas, los planteamientos actuales se han beneficiado de los logros obtenidos en el campo de las Neurociencias durante el último siglo. Los retos actuales a la hora de desentrañar la naturaleza de los recuerdos pueden agruparse en torno a dos aspectos: su contenido y su duración.

Con respecto al primer aspecto, una vez superados los debates entre localizacionistas y holistas, hoy en día está ampliamente aceptado que existen diversos tipos de memoria disociables que dependen de una compleja organización de circuitos neurales situados en distintos niveles de organización cerebral. Desde el descubrimiento de la existencia de capacidades de memoria preservadas en pacientes amnésicos con daño del lóbulo temporal medial (Milner, 1965), han predominado las propuestas teóricas dicotómicas que distinguen entre memoria declarativa/explicita versus memorias no declarativas/implícitas (Squire, 2009). La memoria declarativa, que incluye memoria semántica (conocimiento general) y episódica (autobiográfica) depende del lóbulo temporal medial y de sus proyecciones corticales, especialmente con la corteza prefrontal. Este tipo de memoria se describe como el recuerdo consciente al que llamamos *memoria* en seres humanos y muestra características, tales como la dependencia del contexto, rapidez de adquisición, flexibilidad y complejidad representacional, que permiten su estudio en modelos animales. En roedores las tareas más empleadas para estudiar memoria declarativa incluyen el reconocimiento de objetos novedosos con ensayo único y la navegación en laberintos siempre y cuando se base en representaciones complejas y flexibles del contexto, siendo la búsqueda de una plataforma oculta en el laberinto acuático la más empleada. Por su parte, las memorias no declarativas se definen por defecto como todas aquellas que no cumplen las condiciones mencionadas para la memoria declarativa, lo que en términos concretos suele suponer todas las que permanecen preservadas

después del daño hipocampal. El término memoria no declarativa incluye, por tanto, una diversidad de recuerdos, no necesariamente conscientes, adquiridos gracias a las capacidades plásticas de los sistemas sensoriales, motores y emocionales, que se ponen de manifiesto, de manera más automática, cuando las situaciones lo requieren. En roedores se emplean tareas de aprendizaje asociativo (miedo condicionado, evitación activa y pasiva, condicionamiento aversivo gustativo, condicionamiento palpebral, etc,..) y no-asociativo (habituación, sensibilización) para investigar estos tipos de memorias. Los resultados de intervenciones cerebrales han mostrado que se trata de recuerdos que dependen de una diversidad de circuitos neurales disociables y que exhiben características diferentes entre sí, lo que está poniendo en tela de juicio el valor de las propuestas radicalmente dicotómicas. Ello, sumado a los datos que demuestran la interacción entre sistemas (Poldrack & Packard, 2003) ha conducido a propuestas alternativas (McDonald, Devan, & Hong, 2004; Mizumori, Yeshenko, Gill, & Davis, 2004) que enfatizan la necesidad de abordar un nivel de integración superior a los planteamientos relativamente simplistas actuales para la comprensión de los sistemas de memoria.

Con respecto al segundo aspecto, desde que los trabajos de Müller y Pilzecker en 1900 y las propuestas de Donald Hebb (1949) dieron lugar a la teoría de la consolidación (McGaugh, 2000), se acepta que, en función de su duración, un recuerdo puede encontrarse en diversas fases de estabilización. Sin entrar en el debate sobre si estas fases se organizan de manera secuencial o en paralelo, puede distinguirse entre memoria inmediata, que se relaciona con el concepto actual de memoria de trabajo, memoria a corto plazo, con una duración de minutos a horas y memoria a largo plazo que puede durar días, semanas, meses o incluso una vida entera. La teoría de la consolidación, que implica el paso del recuerdo permanente de una fase lábil a una estable no afectada por tratamientos tales como estimulación electroconvulsiva o inhibición de la síntesis de proteínas, por ejemplo, permite explicar la amnesia retrógrada en pacientes con daño del sistema temporal que afecta a recuerdos no consolidados. Esta teoría está siendo enriquecida en la actualidad por datos que indican la maleabilidad de los recuerdos incluso una vez establecidos. En este sentido se incluyen procesos de reconsolidación por los cuales los recuerdos previamente consolidados se

desestabilizan parcialmente y se actualizan, integrando nueva información cuando son recuperados (Dudai, 2006; Rodriguez-Ortiz & Bermudez-Rattoni, 2007; Sara, 2000).

Grandes avances se han realizado en las últimas décadas en la identificación de los mecanismos celulares y moleculares responsables de la memoria gracias a fructíferas líneas de investigación en invertebrados, como la de Erick Kandel en la Aplysia por la que recibió el Premio Nobel en 2000 (Kandel, 2001; Kandel & Squire, 2000) y gracias al descubrimiento por parte de Bliss y Lomo (1973) de la potenciación a largo plazo (LTP) en rodajas hipocampales como modelo de plasticidad sináptica (Cooke & Bliss, 2006; Lomo, 2003), hallazgo al que han seguido otros fenómenos plásticos tales como depresión a largo plazo y metaplasticidad. De acuerdo con la teoría de la huella dual de Donald Hebb, que describe una primera fase lábil basada en la actividad neural reverberante, la actividad neuronal mantiene la memoria a corto plazo gracias a cascadas de transducción mediadas por quinasas. Dichas señales cuando son transportadas al núcleo activan procesos de regulación génica que conducen a la síntesis de proteínas y a los cambios plásticos responsables de la alteración duradera en la eficacia sináptica que se propone como subyacente a la memoria a largo plazo. Aunque es objeto de debate, existen datos que apoyan la existencia de mecanismos moleculares compartidos para los procesos de consolidación y reconsolidación (Rodriguez-Ortiz & Bermudez-Rattoni, 2007).

Dado el gran progreso en los conocimientos sobre los acontecimientos celulares e intracelulares implicados en los cambios responsables de la adquisición y primeras fases de consolidación de los recuerdos, los retos actuales están centrados en dilucidar los mecanismos moleculares implicados en los procesos que median la persistencia, extinción y reconsolidación de los recuerdos. Así, en el año 2006, se ha propuesto un mecanismo molecular responsable del mantenimiento de los recuerdos consolidados. Todd Sacktor y su equipo defienden la importancia funcional de una isoforma atípica de PKC con actividad automantenida (Sacktor, et al., 1993). Esta proteína, llamada PKMζ (proteína quinasa M zeta), al carecer de dominio regulador mantiene su actividad de manera persistente. Resultados aplicando inyecciones intrahipocampales del péptido inhibidor de la proteína

quinasa Mzeta (ZIP) han demostrado que PKMζ es necesaria para el mantenimiento, pero no para la inducción, de LTP (Ling, et al., 2002; Pastalkova, et al., 2006; Serrano, et al., 2005). Todo parece indicar que PKMζ actúa, principalmente, potenciando la transmisión sináptica mediada por los receptores AMPA, modificación indispensables para el mantenimiento de LTP (Ling, et al., 2002). Otros hallazgos recientes indican que el mantenimiento de LTP por PKMζ se debe a la regulación del flujo de GluR2 en los receptores AMPA postsinápticos, ya que se ha demostrado que el bloqueo de PKMζ correlaciona con el descenso de GluR2 postsináptico (Migues, et al., 2010).

El hecho de que la inyección bilateral en el hipocampo dorsal del péptido inhibitorio seudosubtratoζ (ZIP), que actúa como dominio autorregulador e inactiva a PKMζ (Hernandez, et al., 2003; Ling, Benardo, & Sacktor, 2006; Ling, et al., 2002; Pastalkova, et al., 2006; Sacktor, 2008; Serrano, et al., 2005), elimine el recuerdo adquirido 24 horas antes en una tarea de evitación espacial abrió la posibilidad de que la persistente actividad automantenida de la proteína quinasa Mzeta (PKMζ) pueda representar el fundamento molecular de la memoria a largo plazo (Pastalkova, et al., 2006).

Sin embargo, la exploración del papel de PKMζ en el mantenimiento de los recuerdos exige una amplia investigación en el nivel de análisis de sistemas. Efectivamente, tal y como señalan voces reconocidas en el tema (Cahill, McGaugh, & Weinberger, 2001), sólo una aproximación que enfrente la complejidad de las interacciones entre las áreas y circuitos cerebrales implicados en los distintos tipos de memoria puede dar sentido a los resultados obtenidos en el nivel celular y molecular. Independientemente de los circuitos cerebrales específicos implicados en la adquisición de cada tipo de memoria, los recuerdos dependen de diversas áreas cerebrales en función de la fase en que se encuentran durante el proceso de consolidación. Determinadas áreas cerebrales, tales como el Hipocampo y la Amígdala, parecen jugar un papel importante en los procesos de consolidación de la memoria declarativa y no declarativa respectivamente. Como consecuencia de dichos procesos los recuerdos pueden estabilizarse en áreas diferentes de aquellas en que se formaron, lo que se ha denominado migración del engrama.

A continuación se revisan los datos disponibles hasta la actualidad con respecto al efecto de la administración intracerebral de ZIP en diversas áreas cerebrales sobre el mantenimiento de diversos tipos de memoria (Tabla I.1).

Tabla I.1. Resumen esquemático de los efectos amnésicos de la inyección i.c. de ZIP en roedores en función del área cerebral y del tipo de tarea comportamental.

TAREA / ZIP	EVITACIÓN ACTIVA DE LUGAR	LABERINTO RADIAL	LABERINTO ACUÁTICO	MIEDO COND.	EVITACIÓN PASIVA	COND. AVERSIVO GUSTATIVO
HIPOCAMPO	??	?	?	=		=
AMÍGDALA				?	?	
CORTEZA INSULAR						??

TAREA / ZIP	RECUERDO TRAUMÁTICO (laberinto elevado, respuesta de sobresalto y petrificación contextual)	COND. PALPEBRAL DE DEMORA	RECONOCIMIENTO DE OBJETOS (OBJETOS / LUGAR)	FAMILIARIZACIÓN AL CONTEXTO
HIPOCAMPO	?	?	= | ??	!
AMÍGDALA	=			
CORTEZA INSULAR	?			

?? = Eliminación total del recuerdo

? = Deterioro parcial del recuerdo o de una característica específica

! = Mejora en la tarea

= = Ningún efecto registrado

EFECTO DE LA INHIBICIÓN DE LA ACTIVIDAD DE PKMZ EN LOS CIRCUITOS CEREBRALES RESPONSABLES DE LA MEMORIA.

Efecto de la inyección bilateral de ZIP en el Hipocampo Dorsal

De manera coherente con el papel propuesto para el Hipocampo en el sistema de memoria declarativa, así como su función en los procesos de consolidación, es, con diferencia, la estructura que más estudios sobre la relación entre la actividad de PKMζ y memoria ha generado. Todos ellos han aplicado intervenciones similares en roedores inyectando bilateralmente ZIP (10 nmol/1µl de salino) en el Hipocampo Dorsal (HCd).

En 2006 se publicó en Science el primer estudio que vínculo la inhibición de PKMζ con el deterioro de la memoria espacial en una tarea de evitación compleja en ratas (Pastalkova, et al., 2006). Los autores eliminaron en el mismo estudio LTP inducida in vivo por estimulación de alta frecuencia y memoria espacial en ratas. Dado que la intervención se retrasó 22 horas, se trataba de modificaciones plásticas consolidadas. Los resultados permiten excluir un efecto inespecífico sobre la actividad neural, ya que no altera la transmisión sino ha sido previamente potenciada. La eliminación del recuerdo adquirido en la tarea de aprendizaje es consistente con la relevancia del Hipocampo en el tipo de memoria que involucra representaciones complejas del entorno. La tarea exigía que el animal aprendiera a evitar una ligera descarga en las patas asociada con un sector fijo en relación con el contexto de la habitación en una arena que giraba constantemente. Ello inducía una respuesta de evitación activa por parte del sujeto que debía depender de las señales contextuales. Se trata de un efecto selectivo sobre la memoria a largo plazo que no se produce al inyectar otros inhibidores de PKC, como la stauroporina, que interfieren selectivamente con la inducción de LTP pero no con su mantenimiento. Por otro lado, parece producir selectivamente la eliminación del recuerdo

consolidado sin dañar el mecanismo de consolidación, ya que la capacidad de adquirir nuevos recuerdos no resultó afectaba si las mismas ratas eran entrenadas de nuevo.

El mismo grupo ha informado de resultados similares empleando otras tareas de aprendizaje y memoria espacial en el laberinto radial y la piscina de Morris (Serrano, et al., 2008). En ambos casos los datos han permitido disociar los efectos de la inactivación de PKMζ en distintos tipos de memoria, confirmando un deterioro selectivo de la memoria de referencia a largo plazo sin que resulte afectada la memoria de trabajo. Así, en el laberinto radial de 8 brazos que contiene un pellet de alimento en cuatro de ellos, lo que se interrumpe es el recuerdo de cuales son los brazos con comida, pero no la memoria de los brazos que ya se han visitado en cada ensayo. Del mismo modo otros tipos de memoria no declarativa, tal como los aspectos procedimentales de la tarea no resultan afectados.

El papel de PKMζ en el hipocampo dorsal también ha sido evaluado utilizando otras tareas de memoria dependientes del hipocampo, tales como reconocimiento de de la localización de los objetos (Hardt, et al., 2010), condicionamiento palpebral de huella (Madronal, et al., 2010) y miedo al contexto (Serrano, et al., 2008). En el primer caso la administración intrahipocampal de ZIP 1 y 6 días tras la adquisición eliminó el recuerdo de la posición del objeto, sin afectar al recuerdo del objeto (Hardt, et al., 2010). En el segundo caso, el grupo que recibió la inyección de ZIP 22 horas después del entrenamiento, en comparación con el grupo inyectado con la versión inactiva scrambled-ZIP, mostró un significativo descenso tanto de las respuestas condicionadas de parpadeo como de los potenciales postsinápticos excitatorios en CA3 y CA1 durante el intervalo entre el tono (EC) y la estimulación por pulso eléctrico de las colaterales de Schaffer (EI). Por el contrario, el miedo al contexto adquirido al establecer una asociación entre descarga en la patas y un sonido dentro de un contexto determinado, que se pone de manifiesto cuando el animal permanece *petrificado,* no resulta afectado por la inhibición de PKMζ en el HCd.

Por último, dado que diversas teorías han relacionado la función hipocampal con la detección y respuesta ante la novedad, Moncada y Viola (2008), han explorado el papel de la actividad de la PKMζ hipocampal en la familiaridad

espacial. Partiendo del hecho de que los niveles de PKMζ hipocampal descendían tras aplicar un protocolo de familiarización a un contexto de campo abierto, los autores utilizaron la infusión de ZIP en el HCd para hacer descender los niveles de PKMζ de manera artificial y evaluar su efecto sobre la conducta exploratoria. Así comprobaron que la inhibición de este péptido inducía familiaridad espacial, y por lo tanto una menor exploración del contexto, a diferencia del grupo inyectado con scrambled-ZIP. Sin embargo, en este caso la administración de ZIP se aplicaba sólo 1 hora después de la exposición al contexto, lo que permite vislumbrar la participación de PKMζ en otros procesos además del mantenimiento de la memoria del contexto a largo plazo.

Efecto de la inyección bilateral de ZIP en la Amígdala Basolateral

Con respecto a la adquisición y mantenimiento de recuerdos emocionales, especialmente asociados a situaciones aversivas y al miedo, la Amígdala parece jugar un papel crucial. La Amígdala basolateral (AMbl), posiblemente por representar la vía de salida del complejo amigdalino y por sus amplias proyecciones sobre los circuitos de memoria, ha sido el objetivo de los experimentos dirigidos a explorar el efecto de la inactivación de PKMζ mediante inyecciones bilaterales de ZIP.

Los primeros datos utilizando una tarea de miedo condicionado (Serrano et al., 2008) muestran que la inyección de ZIP en la AMbl, 24 horas después de que se forme la asociación entre contexto/tono y descarga, atenúan la respuesta condicionada de petrificación tanto ante el contexto como ante el tono, sin que interfiera con la respuesta incondicionada de miedo producida la descarga. Se trata de la misma intervención que no produjo efecto sobre el miedo aprendido cuando se aplicó en el HCd.

En un estudio del grupo de Helmstetter se obtuvieron conclusiones similares (Kwapis, et al., 2009). La inhibición de PKMζ en la AMbl, pero no en el

HCd, deteriora la memoria de miedo al contexto y a un tono condicionado dos días antes.

Asimismo, la inyección bilateral de ZIP en la AMbl 24 horas después del entrenamiento en una tarea de aprendizaje de evitación pasiva, en la que se asocia una descarga con la entrada en un compartimento oscuro, deteriora el recuerdo en una prueba posterior. En nuestro laboratorio hemos obtenido resultados similares empleando una tarea de evitación activa. En ambos casos se trata de una atenuación en vez de la eliminación del recuerdo.

Efecto de la inyección bilateral de ZIP en la Corteza Insular

La corteza insular (CI), especialmente la corteza gustativa insular, ha sido objeto de interés en relación con el papel de PKMζ en el mantenimiento de la memoria gustativa. El grupo de Yadin Dudai fue el primero en estudiar el papel de PKMζ en el mantenimiento de aversiones adquiridas a sabores cuya ingestión fue seguida de malestar gastrointestinal en ratas (Shema, et al., 2007). Este tipo de asociación produce en un sólo ensayo intensas aversiones condicionadas difíciles de extinguir que dependen de un circuito neural independiente del hipocampo. Los resultados de Shema et al., publicados en Science en 2007, resultaron especialmente sorprendentes ya que la inhibición de PKMζ mediante la inyección bilateral de ZIP en la CI, 3, 7 y hasta 25 días después del entrenamiento borraba por completo la aversión, sin dejar rastro alguno que pudiera detectarse con pruebas de recuperación espontánea o reactivación del recuerdo. La intervención parece eliminar selectivamente el recuerdo aversivo sin impedir la adquisición de nuevas aversiones. Los resultados han sido replicados posteriormente (Shema, et al., 2009) ampliando el periodo de consolidación previo a la inyección a 3 meses, ya que algunos autores han indicado que el proceso de consolidación en ratas puede durar un mes en ocasiones (Anagnostaras, Maren, & Fanselow, 1999; Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999). Aún más, la inyección de ZIP en la CI eliminó aversiones condicionadas previamente a dos sabores distintos e incluso

cuando se incrementa el número de ensayos de condicionamiento hasta tres (Shema, et al., 2009; Shema, et al., 2007).

De acuerdo con los resultados obtenidos con tareas hipocampales, parece tratarse de un efecto específico sobre la memoria a largo plazo, ya que el recuerdo no resultaba alterado si la inyección de ZIP se aplicaba dos horas antes de la presentación del sabor, durante el intervalo entre el sabor y el malestar, o una hora tras la asociación sabor-malestar. Estos datos implican que el bloqueo de PKMζ en la CI no afecta a la memoria a corto plazo del sabor y posiblemente tampoco a la codificación del malestar. Parece únicamente afectar al mantenimiento del recuerdo consolidado (Shema, et al., 2009; Shema, et al., 2007).

La huella itinerante de un recuerdo traumático

La relevancia de la actividad de PKMζ en la CI para el mantenimiento de recuerdos aversivos es coherente con otros resultados obtenidos en el trabajo de Cohen et al. (2010). En su estudio utilizaron un modelo animal del trastorno por estrés postraumático, en el que las ratas eran expuestas al olor de la cama de un depredador, lo que inducía una incremento posterior de las respuestas de ansiedad, detectable en la ejecución de un laberinto elevado, el reflejo de sobresalto y petrificación contextual. Comprobaron que si se inyectaba ZIP en la CI 10 días tras la exposición estresante la respuesta de ansiedad descendía a niveles basales, a diferencia del grupo que recibió la versión inactiva de ZIP o bien ZIP en el HCd, el ventrículo lateral (VL) y AMbl. Sin embargo cuando la microinfusión de ZIP se realizaba inmediatamente después del estímulo ansiógeno únicamente se eliminaba el recuerdo estresante si era inyectado en el VL o en el HCd, pero no en la CI ni en AMbl. Los autores interpretan que los recuerdos traumáticos están localizados en diferentes áreas cerebrales en función del grado de consolidación, lo que es coherente con los conocimientos existentes. Los resultados parecen indicar

que los procesos de consolidación dependerían del sistema hipocampal mientras que a largo plazo los recuerdos serían almacenados en la CI.

Por otra parte, el trabajo aporta información acerca del posible efecto reforzante de la situación estresante sobre recuerdos aversivos anteriores. Así, el recuerdo de una aversión gustativa adquirida 48 horas antes del inicio de la exposición estresante no resultó afectado por la inyección de ZIP en la CI, a pesar de interferir con el recuerdo traumático. La disparidad con los resultados previamente mencionados del grupo de Dudai en ausencia de estimulación traumática (Shema, et al., 2009; Shema, et al., 2007) pone de manifiesto la complejidad de la interacciones entre diversos tipos de memoria.

DISCUSIÓN

Los hallazgos revisados en torno a la participación de PKMζ en el mantenimiento de una gran variedad de recuerdos a largo plazo abren nuevas e interesantes posibilidades para avanzar en el conocimiento de la compleja organización anatómico-funcional que sustenta la memoria.

Buena parte de los resultados obtenidos son coherentes con los planteamientos vigentes en el área en la actualidad. Así, la actividad de la enzima en diversas estructuras cerebrales resulta selectivamente implicada en los diferentes tipos de memoria con los que han sido relacionadas previamente.

Efectivamente, la inactivación de PKMζ en el HCd interfiere con la memoria a largo plazo del contexto necesaria para la navegación espacial (Pastalkova, et al., 2006) y el reconocimiento de la localización de los objetos (Hardt, et al., 2010),así como con el condicionamiento palpebral de demora (Madronal, et al., 2010). A pesar de que el miedo al contexto, cuya adquisición ha resultado sensible a lesiones hipocampales en trabajos previos, no parezca resultar afectado por la inyección de ZIP en el HCd (Serrano, et al., 2008), ello no permite excluir la participación hipocampal, debido a que el Hipocampo ventral no ha sido explorado. Asimismo, los resultados obtenidos con la inactivación de PKMζ en la AMbl confirman su participación en los recuerdos emocionales adquiridos en tareas de miedo condicionado y evitación. Por último, el hecho de que la inyección bilateral de ZIP en la CI elimine las aversiones gustativas condicionadas aporta un contundente apoyo a planteamientos ya existentes, en función de datos anteriores, que defendían un papel crucial de la zona en la memoria gustativa a largo plazo.

En conjunto los datos apoyan versiones actuales de la teoría de la consolidación por la que los cambios moleculares que conducen a la estabilización de la memoria se producen en circuitos neuronales diversos, pudiendo añadirse otras áreas, tales como la Amígdala , Acumbens, Estriado Ventral, neocorteza e Hipocampo (Bermudez-Rattoni, 2010). En este sentido, los resultados revisados indican que la

actividad de PKMζ juega un importante papel en el mantenimiento a largo plazo de recuerdos específicos, en todas las áreas estudiadas hasta la fecha.

Sin embargo, otra parte de los resultados obtenidos hasta el momento plantean la necesidad de considerar el papel de PKMζ más allá de interpretaciones simplistas.

Por un lado, diversos resultados parecen indicar que su inactivación puede interferir con una variedad de procesos mnésicos, además del mantenimiento de la memoria a largo plazo. Es el caso de los procesos de consolidación de recuerdos traumáticos (Cohen, et al., 2010) o la detección de la novedad (Moncada & Viola, 2008), en el Hipocampo, en los que se emplean inyecciones de ZIP con cortos periodos de dilación. Por otra parte, el grado de interrupción en el caso de las memorias a largo plazo parece variar en función de la zona o del tipo de memoria. Ello pone de manifiesto la posibilidad de la existencia de una variedad de mecanismos con participación diferencial de PKMζ y la necesidad de continuar investigando con una variedad de protocolos conductuales alterando los parámetros temporales.

En definitiva, la posibilidad de eliminar recuerdos no deseados ha abierto enormes expectativas en la comunidad. Sin embargo, aunque prometedores, los resultados obtenidos hasta el momento necesitan ser replicados, extendidos e integrados con el amplio cuerpo de conocimientos existente acerca de los mecanismos responsables del aprendizaje y la memoria. La manipulación de la actividad de PKMζ sólo debe ser considerada en la actualidad como una útil herramienta experimental en este sentido.

CONCLUSIONES

1. La actividad automantenida de PKMζ se ha revelado como necesaria para el mantenimiento a largo plazo de una variedad de tipos de memorias tanto declarativas como no declarativas.

2. Los efectos de la inactivación de PKMζ sobre recuerdos consolidados específicos de diversa naturaleza dependen del área cerebral intervenida selectivamente.

3. Se abre la posibilidad de que PKMζ participe en otras funciones mnésicas relacionadas con las primeras fases del proceso de consolidación.

4. Los resultados son congruentes con la existencia de múltiples sistemas de memoria disociables interaccionando a lo largo del tiempo durante los procesos de adquisición, consolidación y mantenimiento de los recuerdos.

5. Aunque la posibilidad de borrar recuerdos no deseados ya consolidados resulta prometedora, la investigación se encuentra en una fase inicial muy alejada de planteamientos relacionados con aplicaciones clínicas potenciales.

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CAPÍTULO II: INTRA-AMYGDALAR ZIP INJECTIONS IMPAIR THE MEMORY OF ACTIVE AVOIDANCE LEARNED RESPONSES AND CONDITIONED TASTE AVERSION ACQUISITION IN RATS

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Gámiz, F. and Gallo, M.

ABSTRACT

We have investigated the effect of PKMζ inhibition in the basolateral amygdala (BLA) upon the retention of a non-spatial learned active avoidance response and conditioned taste-aversion (CTA) acquisition in rats. ZIP (10nmol/µl) injected into the BLA 24 hours after training impaired retention of a learned avoidance-jumping response assessed 7 days later when compared with control groups injected with scrambled-ZIP. Nevertheless, a retraining session applied 24 hours later indicated no differences between the groups. Additionally, a similar ZIP injection into the BLA during the CS-US interval attenuated CTA acquisition. These findings support the BLA PKMζ role in various forms of memory.

INTRODUCTION

Previous findings using the myristoylated ζ-pseudosubtrate inhibitory peptide (ZIP) have demonstrated the essential role of the atypical protein kinase C (PKC), known as protein kinase Mzeta (PKMζ), for maintaining long-term potentiation (LTP) (Hernandez, et al., 2003; Ling, et al., 2006; Ling, et al., 2002; Sacktor, 2008, 2011; Sacktor, et al., 1993; Serrano, et al., 2005) and different types of memory (Cohen, et al., 2010; Hardt, et al., 2010; Madronal, et al., 2010; Pastalkova, et al., 2006; Serrano, et al., 2008; von Kraus, Sacktor, & Francis, 2010), including conditioned taste-aversion (CTA) (Shema, et al., 2011; Shema, et al., 2009; Shema, et al., 2007).

The role of the basolateral amygdala PKMζ in memory maintenance remains a subject open to some debate. Bilateral ZIP injections into the basolateral amygdala (BLA) induced retrograde amnesia for learned contextual and auditory fear responses such as freezing and passive avoidance responses (Kwapis, et al., 2009; Migues, et al., 2010; Serrano, et al., 2008). Most of the reported results indicate preserved fear expression (Serrano, et al., 2008) and the ability to acquire and maintain new fear memories (Kwapis, et al., 2009; Migues, et al., 2010). Some recent results, on the other hand, have suggested that ZIP injected into the BLA may disrupt the expression of fear memory instead of erasing memory. In fact Parsons and Davis (2011) found that ZIP had no effect on fear-potentiated startle if assessed a long time after the injection unless a previous test had been applied shortly afterwards. Since similar results have been found after amygdala lesions using two-way active avoidance learning tasks (Choi, Cain, & LeDoux, 2010), investigations into the possible role of PKMζ in the long-term maintenance of discrete learned avoidance responses are required.

Previous results have also suggested the relevance of the BLA in CTA acquisition (Barot, Kyono, Clark, & Bernstein, 2008; Bermudez-Rattoni, Ramirez-Lugo, Gutierrez, & Miranda, 2004; Bures, Bermudez-Rattoni, & Yamamoto, 1998; Gallo, et al., 1992; Koh & Bernstein, 2003; Nishijo, Uwano, Tamura, & Ono, 1998; Yasoshima & Yamamoto, 1997). Thus it has been proposed that CTA learning requires an increased functional connectivity between the BLA and IC (Grossman,

Fontanini, Wieskopf, & Katz, 2008), which may involve enhanced LTP (Bermudez-Rattoni, 2004). This role may be mediated by PKC activity since PKC inhibitors applied in the BLA during the acquisition session impeded the formation of CTA (Yasoshima & Yamamoto, 1997). This is consistent with the deleterious effect of chelerythrine, a PKC inhibitor that blocks PKMζ activity, on the CTA acquisition processes taking place in the parabrachial area (Sacchetti & Bielavska, 1998). Whilst the absence of CTA savings after post-training ZIP injection in the IC may exclude a role for PKMζ activity in the BLA in maintaining learned taste aversions, other possible roles for PKMζ activity in the BLA during CTA acquisition and early consolidation have not yet been explored.

To understand more fully the role of amygdala PKMζ in memory we have investigated the effect of ZIP microinjections into the BLA upon both CTA acquisition and active avoidance learning retention. In the first experiment BLA bilateral ZIP microinjections were applied either during the CS-US interval or 30 min after training. Additionally the effect of ZIP infusion before drinking and 24 hours after training was assessed. In a second experiment we explored the effect of bilateral ZIP injections into the BLA on the retention of a learned active avoidance response without navigational requirements, consisting of a vertical jump on hearing a tone in order to avoid a shock (Candido, Gonzalez, & de Brugada, 2004; Candido, Maldonado, & Vila, 1988, 1991; Manrique, Molero, Candido, & Gallo, 2005).

MATERIALS AND METHOD

Seventy adult male Wistar rats (280-390gr) were individually housed in an isolated room at 21°C and a 12:12 h. light–dark cycle. Food and water was available *ad libitum* except during the CTA protocol. To implant chronic guiding cannulae 22 g (0.7mm o.d.; 0.39-0.43mm i.d.) in the BLA (AP: -2,5. LM: ±4,8. D-V: -8 from Bregma) (Paxinos & Watson, 1998) each rat was deeply anesthetized with acepromacine (1-2 mg/kg) and a ketamine and clobutol mixture (IMALGENE) (150 mg/kg) before undergoing stereotaxic surgery**.** The 10 mm-long cannulae were inserted bilaterally 4 mm below the skull and fixed with acrylate. In order to perform the BLA injections, the 30g injection needles (0.3 mm o.d.; 0.13-0.16 mm i.d.) connected to 10 µl Hamilton microsyringes were inserted 14.5 mm deep into the 10 mm-long guiding cannulae to administrate 1µl of ZIP or scrambled ZIP per hemisphere at a rate of 0.5 µl per min using an injection pump (Harvard, USA). Two minutes later the injection needle was slowly removed. The procedures were approved by the University of Granada Ethics Committee for Animal Research and were in accordance with the European Communities Council Directive 86/609/EEC.

In the CTA experiment rats were randomly assigned to 4 groups: CS-ZIP-US (n = 21), CS-Scram-US (n =16), CS-US-ZIP (n = 20) and CS-US-Scram (n = 13). Following a 10-day recovery period the rats were adapted for 4 days to a water restriction schedule with two daily drinking sessions at 10 am (15 min) and 5 pm (30 min). Conditioning took place during the morning session of day 5, in which all the rats received an i.p. injection of LiCl (0.15M; 1% b.w.) as the US, 30 minutes after drinking a 0.1% saccharin solution (CS). ZIP (10nmol/µl) or scrambled ZIP were injected bilaterally into the BLA either 15 min after the end of the drinking period (CS-Scram-US and CS-ZIP-US), or 30 min after LiCl injection (CS-US-scram and CS-US-ZIP) (Fig.II 1a, 1b). A one-bottle test was applied 48 hours later. Forty six of these animals were reused with a different taste solution (NaCl 1%) in order to assess the effect of ZIP infusions either before drinking or 24 hours after CTA (Fig.II 1c,1d).

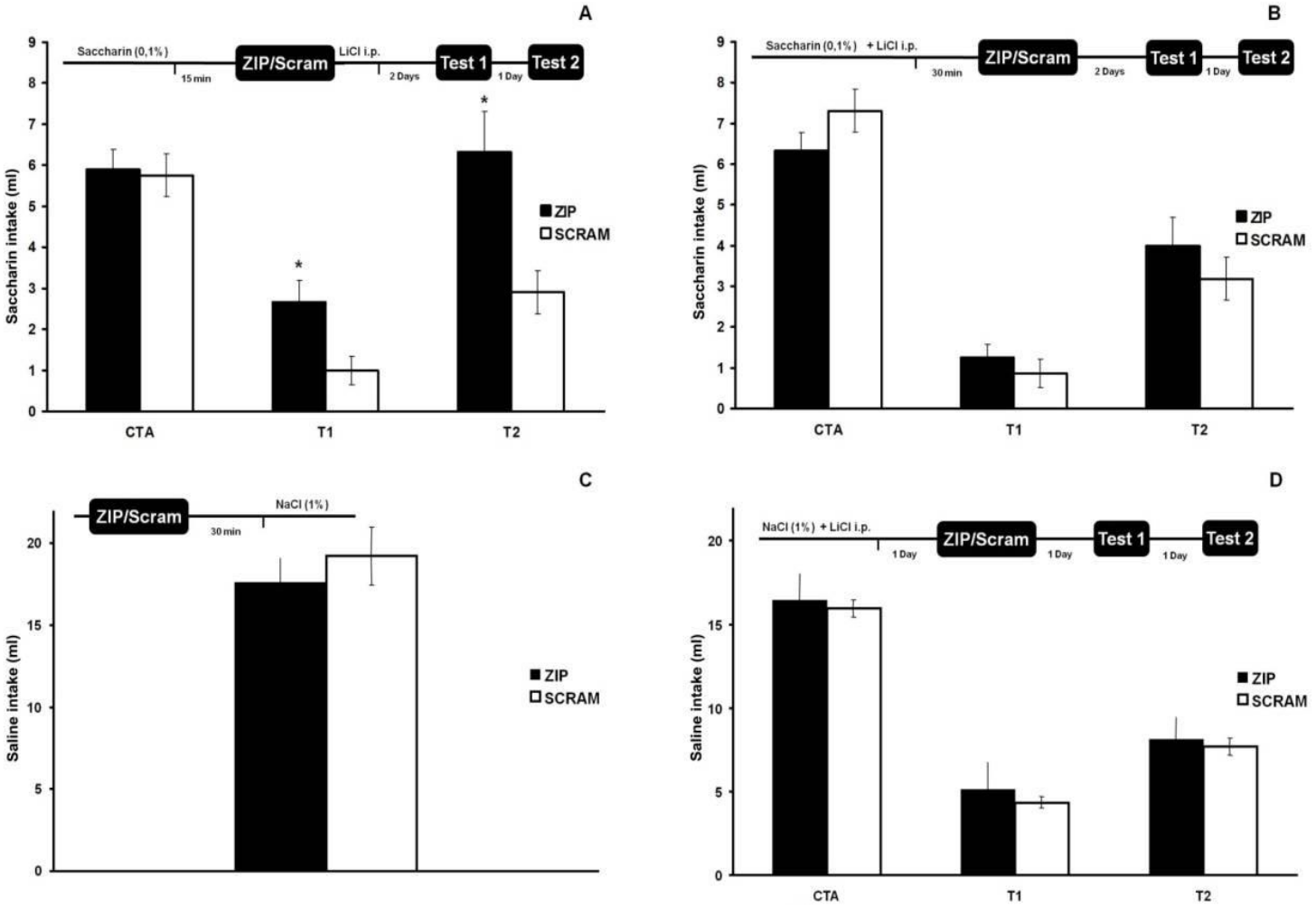

Figure II 1. Mean (± SEM) taste-solution intake by the groups receiving ZIP/Scram infusions in the BLA at different stages of the behavioural procedure*. a) Saccharin solution intake during acquisition and testing in the CS-ZIP/Scram-US groups which were injected during CS-US interval. b) Saccharin-solution intake during acquisition and testing in the CS-US-ZIP/Scram groups which were injected 30 minutes after conditioning. c) Saline-solution intake in groups receiving ZIP (n=11) or Scram (n=11) infusions 30 minutes before drinking. d) Saline-solution intake during acquisition and testing in the groups receiving ZIP (n=12) or Scram (n=12) infusions 24 hours after conditioning. ZIP injection during the CS-US interval impaired conditioned taste aversion acquisition.*

The rest of the animals were subjected to a signaled active avoidance jumping experiment two weeks later. They were assigned to two groups counterbalancing both morning or afternoon sessions and the previous treatment with SCR or ZIP: ZIP (n = 13) and Scram (n = 10). The learning procedure followed was similar to that described in detail elsewhere (Candido, et al., 2004; Candido, et al., 1988, 1991; Manrique, et al., 2005). In brief, the animals learnt to jump on hearing an 80-dB SPL/1000Hz warning tone (CS) in order to avoid a 0.8 mA footshock (US) delivered by a LETICA LI 2700 shock-source module. Avoidance and escape latencies were measured by a LETICA LE 130/100 digital chronometer, accurate to 0.1 sec. The temporal sequence of events was controlled by the LI 2700 module connected to a computer. Each daily session consisted of a maximum of 75 trials. An avoidance response was taken to be one which occurred within 5 sec of the onset of the warning signal. Training lasted until the rat reached the acquisition criterion of 5 consecutive avoidance responses (CARs), which is considered to be a medium difficulty task (Manrique, et al., 2005). In most cases (65.3%) the rats reached the learning criterion during the first session. The rest of them reached in the second session. No animals had to be eliminated since none of them met the exclusion criterium, i.e., failing to escape the shock for 3 consecutive trials.

Twenty-four hours after training 1µl per hemisphere of ZIP or scrambled ZIP (10nmol/µl) were injected into the BLA. Seven days later a testing session consisting of 25 trials with no shocks was applied. The number of avoidance-jumping responses was recorded. To assess the rats' ability to relearn, a retraining session identical to the one previously conducted was applied 24 hours after testing.

At the end of the behavioural procedure the rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with saline and formalin solutions. Their brains were removed and processed for histological verification of the injection needle trace location. Figure II.2 shows the placement of the injection cannula tips.

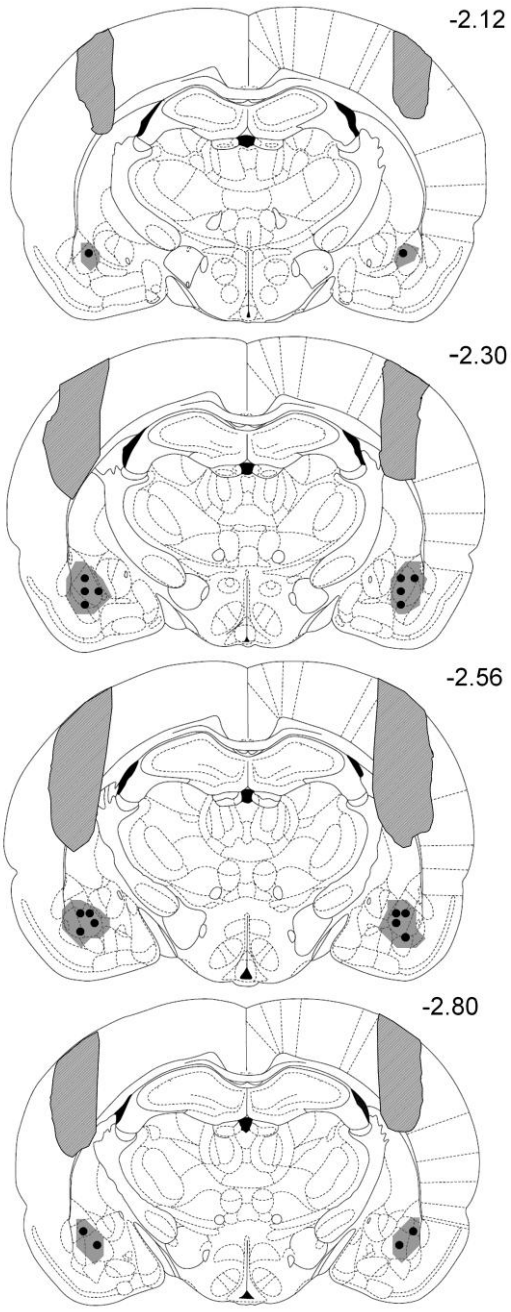

Figure II. 2. Placement of the injection cannula tips in the BLA. *Infusion sites in a subset of the rats are plotted on schematics of the brain at the indicated distance in millimetres posterior to the bregma. The hatched area represents the largest footprint of the guide cannula. All the cannula tips were located within the gray region in the BLA.*

RESULTS

In the CTA experiments, the groups differ neither in the water intake during the baseline nor in taste-solution intake during conditioning. All the conditioned groups exhibited learned aversion (Fig. II.1a,1b,1d), being significant the interaction 2X3 (Group X Day) only when CS-ZIP-US and CS-Scram-US were compared [$F(2,70) = 4.00$; $p<0.01$]. One-way ANOVA analyses of saccharin-solution intake during the two one-bottle tests indicated significant group effects both in test 1 [$F(1,35) = 6,24$; $p<0.01$] and test 2 [$F(1,35) = 7,60$; $p<0.01$]. (Fig. II.1a). Even though the saccharin intake of both groups fell during test 1 only the CS-ZIP-US group showed no differences between conditioning and test 2 ($p>0.5$), thus returning to the saccharin conditioning level after only one extinction test. No effect of ZIP infusion on taste-solution consumption was evident (Fig. II.1c).

As depicted in Figure II.3a, in the active avoidance task there were no significant differences between ZIP and Scram control groups in the number of trials required to reach the learning criterion during the acquisition phase [$F(1,21)= 0.22$; $p>0.6$]. Nevertheless, an ANOVA analysis of the number of avoidance responses during the test applied one week after i.c. injections revealed that the ZIP group performed fewer avoidance-jumping responses than the Scram group [$F(1,21) = 8.24$; $p<0.01$], thus showing that their retention had been impaired (Fig. II.3b). Nevertheless, as is shown in Figure II.3c, both groups required a similar number of trials to reach the learning criterion during the relearning session [$F(1,21) = 0.4$; $p>0.5$]. Moreover, a mixed ANOVA 3X2 (Group x Day) of the number of trials required for reaching the learning criterion during the first and second training sessions indicated a significant day effect [$F (1,21) = 24.8$; $p<0.001$], but not group effect [$F(1,21) = 0.54$; $p>0.46$] or interaction group x day [$F(1,21) = 0.005$; $p>0.94$]. Both groups required fewer trials during relearning, thus indicating savings.

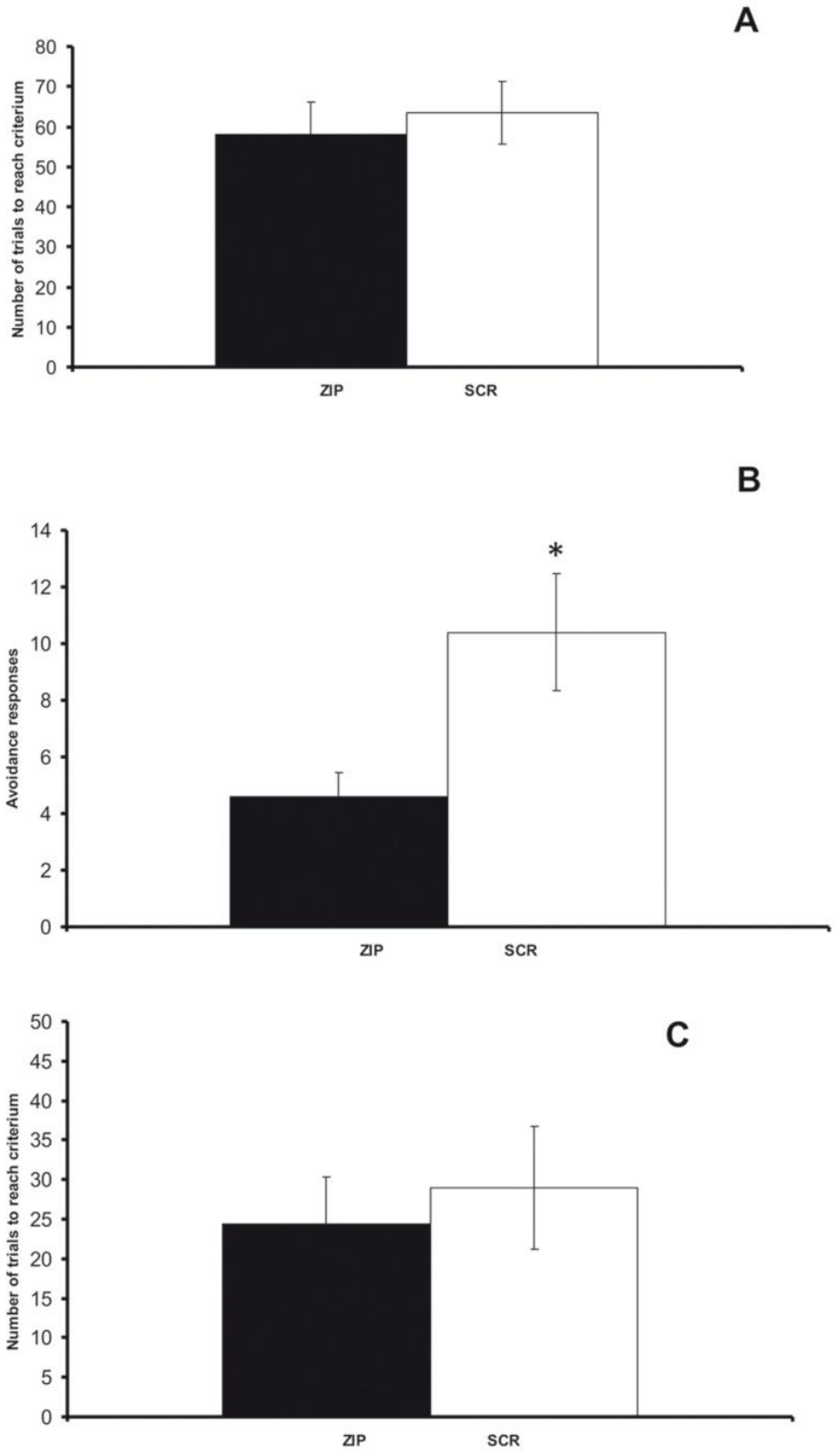

***Figure II.3**. a) Mean (± SEM) number of trials to reach the avoidance jumping learning criterion required by ZIP and SCRAM groups during the acquisition phase before receiving i.c. injections. b) Mean (± SEM) number of learned avoidance-jumping responses during the test session applied one week after i.c. injections 24 hours posttraining. Post-training ZIP injection impaired memory maintenance. c) Mean (± SEM) number of trials to reach the avoidance-jumping learning criterion required by the ZIP and SCRAM groups during the reacquisition phase applied 24 hours after the previous testing session.*

DISCUSSION

Two findings deriving from our present experiments merit discussion. The main one is that the inhibition of PKMζ activity by ZIP injections into the BLA twenty four hours after training impaired the retention of a learned non-spatial active avoidance response in rats. It should be stressed that the avoidance tasks applied in other studies have usually included navigational and contextual requirements. This is the first study to our knowledge reporting the relevance of PKMζ activity in the BLA to the maintenance of a discrete, non-navigational active avoidance memory.

It seemed to be a long-lasting rather than a temporary effect because the animals were tested seven days after ZIP injection. Permanent unspecific sensory, motor or motivational deficits induced by the ZIP injection can be ruled out since the rats were able to relearn the task 24 hours after the testing session. These results add to previous data showing the impairment of retention but not new acquisition in spatial active avoidance tasks (Pastalkova, et al., 2006; Serrano, et al., 2008), CTA (Shema, et al., 2009; Shema, et al., 2007) and fear conditioning (Kwapis, et al., 2009; Migues, et al., 2010; Serrano, et al., 2008). Also, the results allow us to discard explanations based on retrieval or expression deficits (Parsons & Davis, 2011), because impairment was still evident 7 days after ZIP infusion without any previous retrieval session. It should be stressed that these authors used a lower dose than that used previously. This might have contributed to the return of memory under certain conditions, since the effect of ZIP has been reported to be dose-dependent (Shema, et al., 2009).

Otherwise, the fact that both the groups injected with ZIP and those with scrambled-ZIP required a similar number of trials to reach the criterion during relearning could be open to different interpretations. Firstly, it might be due to the incomplete erasure of the learned response in question; this would be consistent with previous results showing the attenuation of contextual conditioned freezing (Kwapis, et al., 2009; Serrano, et al., 2008). Nevertheless, if this were true,

differences in savings between the ZIP and Scram groups should still be expected. Thus, the most feasible interpretation relies on the possibility that the post-ZIP savings during relearning may be due to extra-BLA memory processes. In fact the rats needed an average of 60 trials to reach the learning criterion, which may be considered as extensive training that may have involved additional brain areas. This learning criterion has also been applied in other studies (Gale, Bingley, Emmett, & Collier, 2004; Maren, 1998; Ponnusamy, Poulos, & Fanselow, 2007).

One additional finding concerns the attenuation of CTA acquisition induced by PKMζ inactivation in the BLA during the CS-US interval. However no effect of post-acquisition PKMζ blockade was found. ZIP infusions either 30 minutes or 24 hours later did not interfere with learned aversions. This is consistent with the results obtained by Yasoshima and Yamamoto (1997) using other PKC inhibitors. Nevertheless, these authors also reported CTA disruption by post-training injections applied 30 min after CS-US pairing, but no effect either 4 hours after CS-US pairing or 30 min before the retention test (Yasoshima & Yamamoto, 1997). This discrepancy might be attributed to a more general effect on cell metabolism by non-specific PKC inhibitors. Similarly, the impairment of CTA acquisition was lower in our experiments, since all the groups exhibited learned saccharin aversions. In fact, the use of one-bottle tests, which are able to discriminate between aversions of different magnitudes (Batsell & Best, 1993; Reilly & Bornovalova, 2005), allowed us to detect differences among the groups.

The fact that the BLA is selectively involved in CTA acquisition is also supported both by permanent (for reviews see Gallo, Marquez, Ballesteros, & Maldonado, 1999; Reilly & Bornovalova, 2005) and reversible lesion studies (Gallo, et al., 1992; Roldan & Bures, 1994), being CTA attenuation the most common finding. Among the different explanations proposed to account with CTA attenuation by BLA interventions those related with disruption of the perceived taste novelty (Reilly & Bornovalova, 2005; St Andre & Reilly, 2007) do not seem feasible in the present experiment since ZIP injections were applied after drinking the taste solution. Moreover, the results indicated no differences in the amount of novel taste solution drunk after either ZIP or Scram injections (Fig. II.1c). A likely explanation for the reported attenuation may well be related to a possible role for

PKMζ in processing the attributes of the taste memory trace, such as, familiarity (Moncada & Viola, 2008), salience, etc. It is quite conceivable that this memory process might involve the amygdala and other brain areas and so any interference with taste memory during the acquisition session could lead to an attenuation of taste aversion. A role of BLA PKMζ on early CTA consolidation is not supported by our results. Post-training ZIP infusions up to 24 hours did not interfere with acquisition. Further research would be needed to explore a potential role of BLA PKMζ in long-term CTA memory maintenance as well as other amygdala nuclei involvement.

Whatever the specific role of PKMζ in the BLA on CTA learning might be, our findings add support for a modulatory function relevant to the acquisition of new memories. Overall, our results suggest that PKMζ activity in the BLA is required to retain a learned avoidance-jumping response and to facilitate CTA formation to some extent, thus supporting the idea that persistent PKMζ activity plays a wide role in memories that depend on different brain areas.

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PARTE 2

CAPÍTULO III: TASTE LEARNING AND MEMORY: A WINDOW TO THE STUDY OF BRAIN AGING

Invited paper for publication in Frontiers in Neuroscience

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ABSTRACT

Taste learning exhibits advantages for research on memory brain systems and its reorganization along the life. A review of the effects of aging on taste memory abilities offers a complex picture showing preserved, impaired and enhanced functions. Some of the age-related changes in taste memory seem to be related with temporal processing. Longer taste-illness delays can be introduced for acquisition of conditioned taste aversions and the modulation of taste learning by the temporal context is absent in naïve aged rats. Evidence is presented suggesting that the effect of aging in hippocampal-dependent taste memory modulatory functions can be reactivated by previous learning experiences. Since temporary hipocampal inactivation may represent a better model of the aged hippocampus than permanent damage, results are reported indicating the need of taking into account interactions between the previous experiences and functional brain changes when applying taste learning and memory tasks at advanced ages.

INTRODUCTION

A developmental approach may greatly benefit our understanding of the brain memory and learning systems. The brain plasticity responsible of learning and memory evolves throughout the life in interaction with developmental plastic mechanisms which should not be overcome. This is especially complex when studying the effect of aging. At advanced age the learning and memory abilities have been shaped by several decades of previous learning experiences and face new adaptive challenges due to the modifications of the biological conditions. One of the greatest problems to study learning and memory during aging is to dissociate the effects of aging and those of pathological conditions. This is especially difficult because aging is associated with a greater susceptibility to a variety of pathological conditions, including neurodegenerative diseases affecting the brain and memory systems. It is well known that in humans early symptoms of Alzheimer's disease can be confounded with normal memory changes associated to aging. In addition to brain diseases, a collection of age-related diseases may interfere with learning and memory abilities. In fact, the higher incidence of pathological conditions has led to a view emphasising a common ground between aging and age-related diseases. Thus, the global normal aging approach emphasises a process driven by the lifetime accumulation of a wide variety of molecular-damaging events (Kirkwood, 2010). In this line, several data support that aging might be associated with cognitive decline, even in absence of pathological disorders. However, such deficits do not seem to be unavoidable since there are great individual differences. Moreover, when they appear, they are not consistent with a global memory decay effect but they affect selectively some memory functions while others remain relatively unimpaired. Age-related impairment is thought to be more evident in functions categorized as declarative or explicit memory depending on the medial temporal lobe and related cortical areas while non-declarative or implicit memory remains stable or it is even enhanced (Rieckmann & Backman, 2009). Furthermore, a meta-analysis of the data

reported by functional neuroimaging human studies comparing old and young adults indicates different cortical activity patterns driven by memory encoding and retrieval tasks even when the performance is equal (Spreng, Wojtowicz, & Grady, 2010). This means that aging induces reorganization of the brain memory systems.

The global aging theory explain individual differences in the aging process by the influence that external factors, such as stress, nutrition or exercise, impinge on the mechanisms repairing the cellular machinery. Thus, higher susceptibility to the damaging external agents would be the explanation of the selective decay of some memory systems. According to this view, the reported reorganization of the brain memory systems in old subjects is interpreted as compensatory changes to the selective decay of specific areas.

The interpretation of functional reorganization in terms of compensation stands on the fact that the brain does not loose its ability for neural plasticity even at advanced ages. Surprisingly, little attention has been paid to the effect of the accumulation of previous learning and memory experiences throughout a long life. Given the plasticity of the brain memory systems, it can be envisaged that changes of the brain systems connectivity have been the obvious outcome of previous learning in order to enhance adaptation to the environmental conditions. It can be envisaged that the relationship between higher brain areas involved in declarative memory and the variety of brain systems responsible of non-declarative memory might have been modified along the life. Thus, we suggest that the peculiar memory performance of healthy subject at advanced ages might reflect the accumulation of the normal impact of previous leaning experiences on the brain memory systems organization instead of compensation for age-related losses. Although adaptation to potential damage of the systems might play a role, it seems that such explanation cannot fully account for the variety of changes, including not only decay but also enhancement of specific memory functions.

We have previously proposed taste learning and memory as a privileged behavioural model for studying the interaction between hippocampal and non-hippocampal memory systems both in adult (Gallo, Ballesteros, Molero, & Morón, 1999) and aged rodents (Manrique, et al., 2007). The impact of aging on different taste memory domains is complex, including impaired, preserved and enhanced

functions. The fact that taste memory relies on brain circuits located at different levels from the lower brain stem to the higher cortical and hippocampal areas (Figure III.1) prompts an explanation based on different susceptibility of specific brain areas to the impact of aging. However, scarce attention has been paid to the effect of previous events and learning experiences on the emergence of the peculiar features in taste memory abilities during aging, both in human and animal research. A systematic approach to explore the changes induced by normal aging in taste learning and memory should take into account potential modifications induced by life events at several steps. First, aging can alter sensory processing, thus modifying the salience of the stimuli to be used in the learning procedures. Second, aging can affect the associative and memory mechanisms themselves, either favouring or interfering with acquisition and/or retention of learned responses. Third, aging can be associated with a reorganization of the taste memory circuits, thus modifying the role of the brain areas involved. This review will focus on these issues emphasising the role of previous learning experiences on shaping the taste memory abilities.

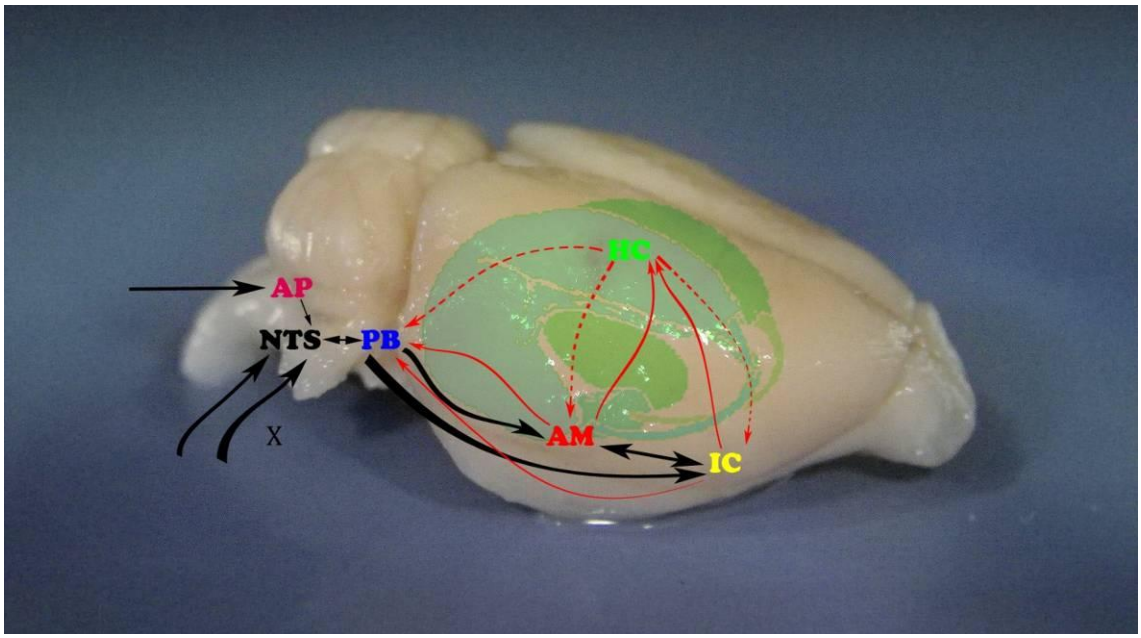


Figure III.1. Schematic representation of the basic neural circuit of the taste aversion and potential modulation by the hippocampal system*. Arrows do not represent the variety of anatomical pathways but proposed interactions. AP (area postrema), AM (amygdala), IC (insular cortex), HC (hippocampus), NTS (nucleus of the tractus solitarius), PB (parabrachial nucleus).*

TASTE SENSITIVITY AND AGING

Anatomical observations in humans do not support reliable decreased taste sensitivity with normal aging. Although great inter-individual differences in the number of fungiform papillae have been reported, little or no decline in the number of taste buds was evident until very advanced ages, older than 90 year-old (Arvidson, 1979; Miller, 1988). Consistently, the peripheral taste system seems to be also well preserved in old monkeys and rats (Mistretta & Baum, 1984).

Psychophysical studies reporting increased taste detection thresholds with aging indicate that it is not a global effect, since it was limited to specific tastants and exhibited great individual differences. Similar results showing large variations have been obtained in electrogustometric (EGM) studies reporting increased taste detection thresholds above 60 years of age (Murphy, Quinonez, & Nordin, 1995; Nakazato, Endo, Yoshimura, & Tomita, 2002).

The reason of such large individual differences may well be attributed to the not well known confounding effect of pathopysiological conditions affecting peripheral taste processing. Boucher et al. (2006) have proposed a critical role for dental deafferentation when investigating decay of taste sensitivity along the life. They included five age groups (20-29 yr; 30-39 yr.; 40-49 yr.; 50-69 yr. and 60-80 yr). The results showed that in all age groups, including 60-80 yr group, the dental status was the best predictor of increased threshold instead of age. Thus, irrespective of age, subjects lacking more than 7 teeth exhibited higher thresholds than subjects with less than 7 deafferented teeth. A topographical relationship between dental deafferentation and taste deficits found in the older group further support the relevance of dental impairment for explaining taste sensitivity impairment previously attributed to aging. Also, impairment and distortion of gustatory function reported in elderly may have been produced by certain medical conditions, pharmacologic interventions, radiation, and exposure to toxic chemicals (Schiffman, 2009).

Consistently, neurophysiological responses to various tastes, such as KCl, sucrose, quinine-hydrochloride, HCl, monosodium glutamate and glutamic acid do not change with age (Osada, et al., 2003). In spite of the fact that a decreased olfactory sensitivity has been associated with aging, old rats have been reported to discriminate between odours (Brushfield, Luu, Callahan, & Gilbert, 2008) and flavours conventionally used in flavour preference tasks, such as grape and cherry (Renteria, Silbaugh, Tolentino, & Gilbert, 2008). Given the standard concentrations used in conventional taste learning protocols no taste sensitivity deficit has been reported in aged rodents that could significantly affect the outcome.

TASTE NEOPHOBIA AND HABITUATION DURING AGING

Animal research data have yielded controversial results regarding the impact of aging on taste neophobia. Although some studies have found enhanced neophobic responses (Collier, Greene, Felten, Stevens, & Collier, 2004); no effect of ageing has been reported in the amount drunk of a novel taste when using a grape juice solution (Gallagher & Burwell, 1989; Koh, Wheeler, & Gallagher, 2009) or 0.1% sodium saccharin (Moron & Gallo, 2007), 1% sodium chloride (Manrique, Moron, et al., 2009), 0.5% sodium chloride and 3% cider vinegar solutions (Moron, Ballesteros, Candido, & Gallo, 2002). Since the full demonstration of the neophobic response to a new taste requires taking into account not only the reduction in taste consumption in comparison with a previous water intake baseline but also later increases upon subsequent taste presentations, the results of the ageing effect on taste neophobia might be confounded with that on the attenuation of neophobia. In fact, a lower rate of neophobia attenuation has been reported in old rats (Pelleymounter & Cullen, 1993) although no age-related differences have been found using a low NaCl concentration (Manrique, Moron, et al., 2009).

While increased taste neophobia has been proposed to contribute to anorexia in elderly humans, no effect of age on the neophobic response to food has

been reported except for a decrease in those elderly subjects with poor olfaction (Pelchat, 2000).

A difficulty to compare data reported in animal and human research may rest in the different previous exposure to taste. Earlier studies showed the relevance of previous aversive taste learning on neophobia to later encountered taste solution in adult rats (Best & Batson, 1977; Domjan, 1975; Franchina & Dyer, 1985; Kristal, Steuer, Nishita, & Peters, 1980). Regarding aging studies, in contrast to the richness of taste experiences throughout the human life, rather naïve animals are used in many experiments devoted to taste memory. Therefore, studying the effect of previous taste experiences on the aged rat willingness to accept novel tastes may contribute to understand controversial results. In this line, previous exposure and habituation of the neophobic response to a sodium saccharin solution disrupted subsequent neophobia to a NaCl solution both in young and old adult rats. However, inducing a previous aversion by pairing saccharin and lithium chloride injections induced a larger increase on later neophobic response to the salty solution in aged rats than in young adult rats (Moron & Gallo, 2007). The amount of solution drunk by the older group was lower than that of the younger group during both the first and the second exposure, although the latter reached the water baseline level indicating attenuation of neophobia. Given that the strength of the previous aversive experience was equated across the age groups, the results seem to be due to a greater impact of aversive memories at advanced ages.

Thus, taste neophobia at advanced ages depends on the number and the nature of previous learned responses to other tastes, thus leading to individual differences. The fact that previous aversive experiences have had higher impact as long as ageing proceeds, it is consistent with reports of increased food neophobia in elderly people, since it is conceivable that aversive taste experiences had been accumulated. Therefore the outcome at an advanced age will depend on the balance between the appetitive, safe and aversive taste memories stored throughout the life.

Different explanations could account for the enhanced effect of aversive taste learning on the neophobic responses to other tastes, being the most suitable a

superior learning ability to develop stronger taste aversions at advanced ages. Whatever the explanation, it can be seen as an adaptation of the memory systems to benefit from previous noxious experiences to reject potentially lethal substances threatening the life especially at advanced ages.

ASSOCIATIVE TASTE LEARNING AND MEMORY IN AGED RATS

The impact of aging in associative taste learning abilities seems to be different on appetitive and aversive tasks in rats.

On one hand, consistent with the reported age-related deficits in odour-reward learning, it has also been found a decreased ability to form flavour-reward associations at advanced ages in rats (Renteria, et al., 2008). In this study, the authors reported no age-related differences in the stimuli reward value, since aged rats readily discriminate between different sucrose concentrations and they distinguished a flavoured solution containing sucrose from the unsweetened one, thus increasing consumption during the training phase. However, the older 24-month-old group, unlike the 7-month-old group, did not exhibit a preference during the testing phase for the flavour previously paired with sucrose. The authors discarded odour discrimination impairments since the stimuli used (cherry and grape) have proven to be discriminated at advanced ages (Brushfield, et al., 2008). Therefore, the results point to an impairment of associative appetitive flavour learning induced by aging. Nevertheless, it should be stressed that 43% of aged rats performed similarly to the younger group (Renteria, et al., 2008), thus confirming individual differences in the impact of the aging process in learning abilities.

On the other hand, taste aversion learning seems to be facilitated at advanced ages. As it has been reviewed elsewhere (Manrique, et al., 2007), stronger aversions are evident in old rats in comparison with young-adult rats

during the first extinction test, provided that floor effects are avoided. One of the most intriguing (puzzling, striking) features of taste aversion facilitation in aged rats is the possibility of introducing longer intervals between the taste and the LiCl injections than in younger adult rats. Using a relatively low dose of LiCl (1% b.w.) and a 24 hours two-bottle test, saccharin aversions have been found in aged rats but not in young-adult rats with taste-LiCl intervals ranging from 180 (Misanin & Hinderliter, 1989) to 360 minutes (Misanin, Collins, et al., 2002). This ability to associate a taste with an illness over long intervals develops gradually as rats get older. Thus, rats older than 18 months exhibit taste aversion at the 180-min interval, while only 24- and 30-month old rats acquire learned taste aversions at 360-min delays (Misanin, Collins, et al., 2002).

Different explanations for the age-related facilitation of long-trace taste illness associations have been proposed. Previous results suggest that they cannot be attributed to age differences in taste sensitivity or increased efficacy of LiCl injection (Misanin & Hinderliter, 1994). Other explanations based on deficits of learned irrelevance (Misanin & Hinderliter, 1995), age differences in the use of interval context cues (Hinderliter & Misanin, 1995b), context-illness associations (Hinderliter & Misanin, 1995a), relative taste novelty (Hinderliter & Misanin, 1993) or memory for specific taste attributes (Misanin, Hoefel, Riedy, & Hinderliter, 1997) have been also ruled out. Misanin et al. (2002) have proposed a longer availability of the taste memory trace in aged rats, because increasing the illness intensity extends the interval over which trace conditioning is evident in old but not in young-adult rats. In order to explain how a memory trace can be available to old-age rats at a time when it is not longer available to young adult rats, the authors have proposed the slowing down of a metabolic pacemaker. The hypothesized pacemaker is compared with a countdown timer that regulates trace decay after taste processing. The timer would stop at a given duration. Thus, aging can slow the pace at which the clock counts down, thus extending the memory trace decay delay. The effect of aging in this metabolic pacemaker would be independent of that on other circadian clocks or brief interval timers (Misanin, Collins, et al., 2002; Misanin, Goodhart, Anderson, & Hinderliter, 2002). Support for that metabolic pacemaker has been obtained from studies with adult rats showing correlations between decreased metabolic rate and the ability to establish long-

trace taste aversions. First, at low body temperatures rats displayed learned taste aversions with delays up to 225 minutes, which was attributed to a cold-induced slowing of the biochemical clock (Misanin, Anderson, et al., 2002). Second, chronic water deprivation that increased metabolic rate reduced the interval that can be introduced in a taste aversion learning protocol (Anderson, Hinderliter, & Misanin, 2006). Explanation based on an altered sense of time may be related with other reports in animals (Walton, 2010) and humans (Fitzgibbons & Gordon-Salant, 2004; Gooch, Wiener, Wencil, & Coslett, 2010) pointing to age-related differences in temporal processing using other tasks.

A similar explanation could account for the higher resistance to extinction of learned taste aversions in old than in young-adult animals even if no significant differences in acquisition are detected. Thus, Ingram and Peacock (1980) reported that aged rats showed delayed extinction of a LiCl-induced saccharin aversion monitored over a period of 32 days. Similarly, resistance to the extinction of a saccharin aversion induced by a low dose of LiCl has been reported in aged rats (Moron & Gallo, 2007). Impaired retention at advanced ages has also been reported using other learning tasks, such as fear conditioning(Kaczorowski, Davis, & Moyer, 2011), passive avoidance or learned helpleness (Martinez & Rigter, 1983), among others (Bevilaqua, et al., 2008). Given the proposals considering the relevance of a time-induced context differentiation process during extinction, it is conceivable that an altered sense of time could contribute to slower extinction during aging. An alternative explanation of the slower extinction rate found in older subjects can be related with the greater robustness of the aversion. Nevertheless, even though the age-related superiority in taste aversion learning might rest on the associative mechanisms acting during the acquisition session, enhanced taste memory abilities cannot be excluded given the long intervals supported at advanced ages. Thus, whatever the explanation, a neural reorganization of the taste memory systems favouring the acquisition and retention of taste aversion seems to be evident.

TASTE LEARNING FACILITATION AN THE AGED HIPPOCAMPUS

Basic taste memory is widely considered as a type of implicit or non-declarative memory. Thus, it has long been thought as hippocampal-independent. Consistently permanent lesion studies have proven the hippocampus not to be necessary for acquisition of safe or aversive taste memories using conventional protocols. However, both dorsal and ventral hipocampal neurotoxic lesions have been reported to impair selectively taste aversion learning when 3 h. intervals were introduced between taste and illness (Koh, et al., 2009). As we have previously noted, a comparison between the effects of permanent hippocampal lesions in young adult rats and those of aging in conditioned taste aversion does not support an explanation of aging based exclusively on the decline of the hipocampal function (Manrique, et al., 2007). Aged but not hippocampal-damaged rats showed an enhancement of taste aversion learning in a conventional one-trial protocol using a standard delay (Moron, Ballesteros, et al., 2002). Moreover, hippocampal grafts did not reverse this age-induced enhancement (Moron, Ballesteros, Valouskova, & Gallo, 2001). Additionally, the effects of hipocampal lesions (Koh, et al., 2009) and aging (Misanin, Collins, et al., 2002) on long- delay conditioned taste aversion seem to be opposite, since old rats, but not young-adult hipocampal rats, acquire strong aversions when long intervals are introduced between taste and illness. Therefore, the experimental evidence from permanent lesion studies does not support an explanation of the aging-related enhancement of taste learning based on hippocampal damage.

However a contribution of an altered functioning of the aged hippocampus to the potentiation of taste learning in old rats cannot be excluded. If this were the case, acute hipocampal inactivation in adult behaving animals could be a better model to study the potential hipocampal involvement in the age-induced facilitation of taste aversion learning. Congruently, temporary inactivation of the dorsal hippocampus by muscimol infusions during acquisition has been shown to enhance learned aversions in a procedure that involved no delay, two different taste solutions and two conditioning trials (Stone, et al., 2005). The authors point out to the potential relevance of avoiding ceiling effects due to the use of the

relative complex two-tastants protocol used. The same study found also an increased latent inhibition effect, i.e., increased effect of previous safe taste presentation on later conditioning attenuation (Stone, et al., 2005).

Both enhancement of safe and aversive taste memory after hipocampal reversible inactivation may be interpreted as the result of releasing some interference on the basic taste memory circuits. It has been proposed that the interaction between multiple memory systems working in parallel might induce competitive interference between them (Schoenbaum & Stalnaker, 2005). Thus, the hipocampal processing, that may allow taste learning to exhibit complex phenomena depending on the previous experience, would be normally interfering with the acquisition of basic taste learning. It is conceivable that the aging process would be related with a change in the hippocampal function that could have altered the interaction between hippocampus and the taste memory systems without disrupting it. Thus, the Stone et al. (2005) results could account for the superiority shown by aged rats in basic taste learning. To our knowledge there are no data on the possibility that temporary hipocampal inactivation could increase the duration of the taste-illness intervals that can be introduced in conditioned taste aversion protocols. Since the disruptive effect of permanent hipocampal lesions on long-delay taste aversions have been attributed to a potential role of the area in processing the temporal attributes of the learning situation, age-related alterations of the hipocampal function cannot be discarded as potential explanation of the enhanced long-delay taste learning at advanced ages. Temporal processing deficits may also be at the basis of other peculiar features of older subjects' performance in taste learning tasks modulated by the temporal context.

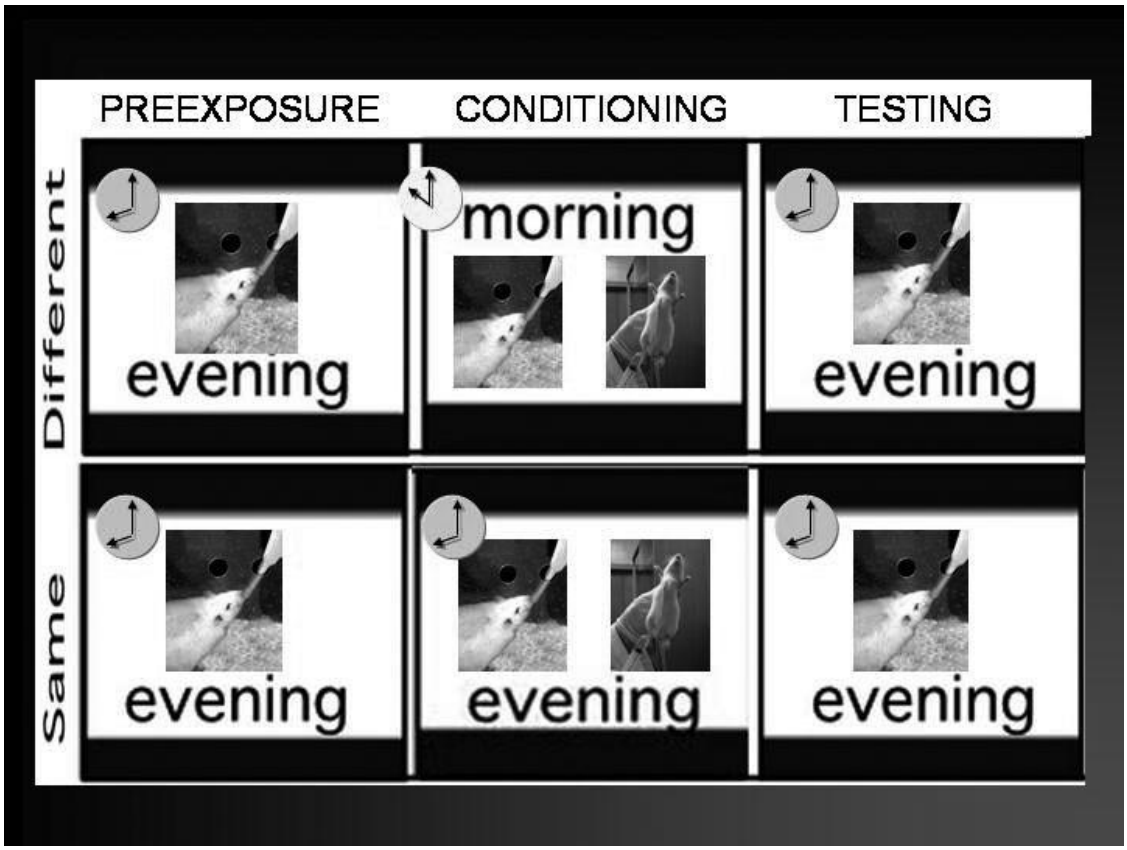
DOES THE HIPPOCAMPUS PLAY A ROLE IN TASTE MEMORY?

Current views of taste learning go beyond considering it exclusively as a type of implicit learning. Thus, Bermudez-Rattoni has proposed both CTA and taste exposure tasks as models for studying taste recognition memory (Bermudez-Rattoni, 2004). Though taste recognition memory may depend on associative mechanisms, it also exhibits features of episodic memory, a type of declarative memory. Thus a single trial is enough to encode a taste learning episode linked with information concerning context and time, that can be related, recalled and applied flexibly and independently of the way in which was originally learned. This can be seen in a variety of complex learning effects that are evident using taste aversion tasks (Gallo, Ballesteros, et al., 1999). Consistently, both aversive and safe taste memories are bound to specific time and space cues, so that a change of context during subsequent learning episodes with the familiar taste solution will modify the learning outcome. An example is the latent inhibition phenomenon in which a safe memory induced by a previous exposure to a taste solution will attenuate later taste aversion learning. It has been shown that latent inhibition of CTA is context-dependent. Thus, a context change between taste preexposure and conditioning interferes with the latent inhibition effect (Manrique, et al., 2004). Alternatively aversive taste memories induced by pairing a taste solution with visceral discomfort seem to be also linked to context cues. They have been proven to be also context-dependent, since a context change between conditioning and testing interferes with learned taste aversions retrieval (Moron, Manrique, et al., 2002).

Evidence from adult rat studies has shown that temporal cues may act as a context, in a similar way to spatial cues, in order to modulate taste memory. Depending on the behavioural procedure, the same change of the time of day interferes either with latent inhibition (Manrique, et al., 2004) or with the retrieval of a learned taste aversion (Moron, Manrique, et al., 2002). The latent inhibition protocol used included two preexposures to a 1% NaCl solution followed by a single saline-LiCl pairing and several one-bottle extinction tests. In addition to the control non-preexposed groups, the critical groups to be compared are those

receiving the taste-illness pairings either at the same (SAME) or at a different (DIFF) time of day than preexposure and testing (Table III.1). Subtle changes in the extent of previous habituation to drink water twice a day lead to opposite behavioural results In a short-habituation (2 days) protocol, DIFF groups exhibit weaker aversions than SAME groups, thus indicating the context dependency of the learned aversion (Moron, Manrique, et al., 2002). However, if the protocol included longer habituation (5 days) DIFF groups show stronger taste aversions than SAME groups (Manrique, et al., 2004). This result is consistent with the context-dependency of latent inhibition.

Table III.1. Experimental procedure for assessing the effect of a time-of-day shift on taste learning*. In the DIFFERENT conditions acquisition took place at a different time of day than preexposure and testing. Groups SAME received all the experimental sessions at the same time of day.*



The opposite pattern of results yielded by short and long duration of the previous habituation to the temporal context points out to different memory processes involved. It has been suggested that the short-habituation protocol may lead to the formation of a compound-conditioned representation of the stimulus (taste-time of day) associated with illness (Manrique, Moron, et al., 2009). Thus, a weaker aversion is evident when the animals are tested at a different time of day (DIFF group) than at the same time of day (SAME group). However, it is unlikely that in the long-habituation protocol the time of day combines with taste to form a compound representation to be associated with illness. In fact, the results were opposite as weaker aversions in the SAME than in the DIFF groups were evident. This indicated that the taste memory was represented separately from the time information.

This ability to segregate elements of an episode into separate internal representations contributes to the features of episodic memory and it has been related with the hipocampal function (Eichenbaum, 2000; Kubik & Fenton, 2005; Schoenbaum & Stalnaker, 2005). According to this view, hippocampal damage may compromise the ability to encode, maintain or use segregated representation of experience. However, since episodic memory is bound to the context the interpretation of lesion studies is sometimes difficult given the well known hippocampal role in processing spatial information. Therefore, the use of temporal context cues combined with a learning task such as taste learning which does not require spatial processing offers a powerful preparation for investigating the specific participation of the hippocampus and related cortical circuits in episodic memory.

Previous data have shown that neurotoxic lesions of the dorsal hippocampus selectively disrupted the effect of a time-of-day change in the long habituation procedure but they have no effect on the short-habituation protocol (Molero, et al., 2005). Whilst there were no differences between the strength of the aversions exhibited by SAME and DIFF hipocampal groups using a long-habituation protocol, weaker aversions were seen in DIFF than in SAME groups in the short-habituation protocol (Figure III.2). Thus, the hippocampus seems not to be required for the ability to form compound-conditioned stimulus

representations that may be responsible of the temporal context dependency of the aversion. However, the hippocampus may play a critical role for flexibly using segregated representations of previous episodes, a memory function that may be compromised during aging.

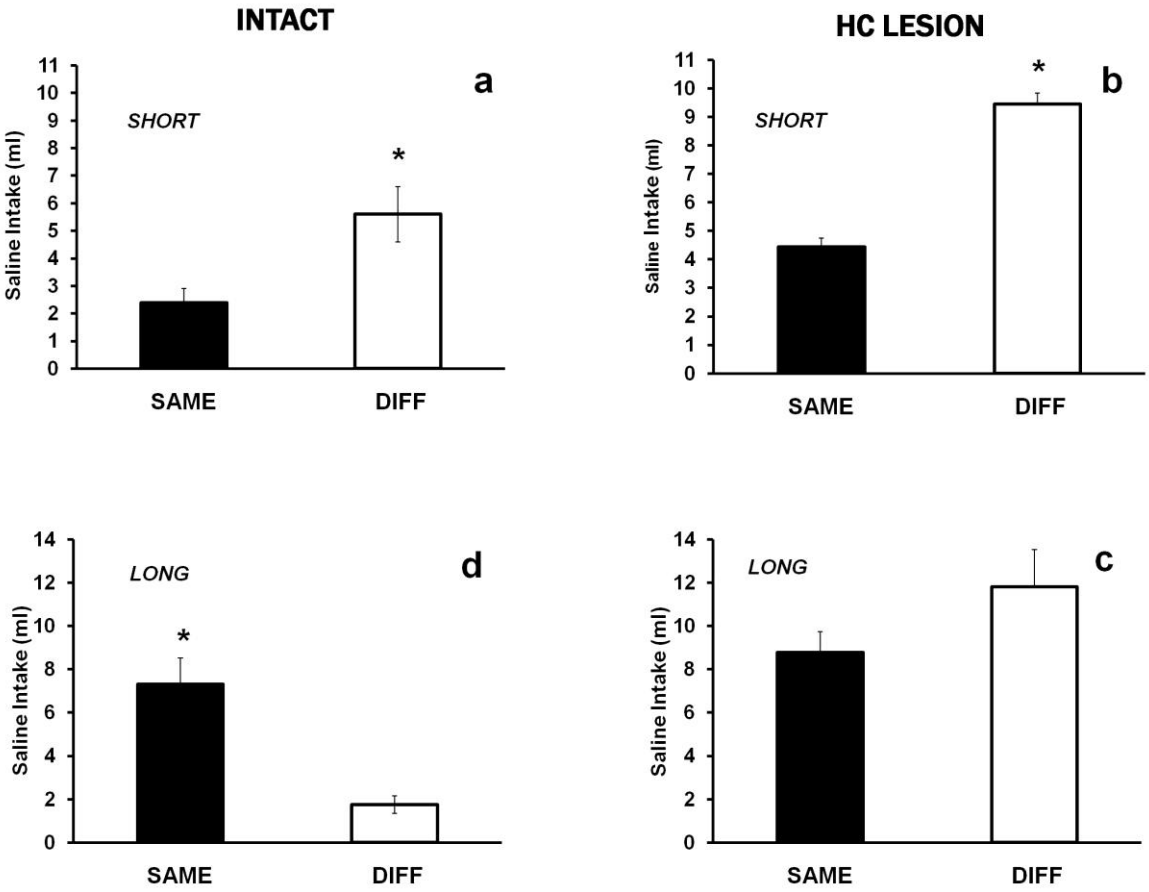


Figure III.2. Summary of hippocampal damage effect on the time-of-day shift effects in taste learning*. Mean (± SEM) saline intake by the groups SAME and DIFF with intact (a, c) or hippocampal (HC) lesion (b, d) using different behavioural procedures for assessing the temporal context modulation of taste learning. Groups a) and b) were subjected to a short-habituation (2 days) protocol and c) and d) were subjected to a long-habituation (5 days) protocol.*

THE HIPPOCAMPAL INVOLVEMENT IN TEMPORAL CONTEXT-DEPENDENT TASTE MEMORY DURING AGING AND THE EFFECT OF PREVIOUS LEARNING EXPERIENCES

The same behavioural preparation has proven to be useful to investigate the memory abilities of aged rats. Consistent with a selective age-related decay of the declarative memory systems, intact aged rats have been reported to exhibit deficits in the long-habituation protocol similar to those induced by hippocampal lesions in young-adult rats (Manrique, Moron, et al., 2009). There were no differences between the aversions exhibited by SAME and DIFF groups when a time-of-day shift was applied during conditioning (Figure III.3a), showing that latent inhibition did not exhibit time dependency. This finding does not seem to be explained by a disruptive effect of ageing on either latent inhibition (Moron, Ballesteros, et al., 2002) or the ability to use the time of day as a context. In fact, an additional experiment indicated that the time of day modulated taste learning in hippocampal aged rats (Manrique, Moron, et al., 2009). A more feasible interpretation has been related with a compromised episodic memory ability in aged rats due to an impairment of the segregation function depending on the hippocampus.

However we have also found that such impairment it is not an ineludibly consequence of normal aging since it can be avoided by previous training. Previous training in our experiment included several tasks: a) exposure to a first novel taste solution and subsequent attenuation of taste neophobia, b) a latent inhibition protocol using a second novel taste solution and c) a novel object recognition task. Surprisingly the temporal context dependency of latent inhibition was reinstated in 28 month-old rats with previous learning experience (Figure III.3b). Whilst the naïve SAME and DIFF aged groups did not differ in the magnitude of the learned taste aversions, previously trained aged rats exhibited a pattern of differences similar to that seen in adults. The DIFF group exhibited stronger aversions than the SAME group. This pattern of results can be attributed to the disruption of latent inhibition by the time-of-day change between preexposure and conditioning.

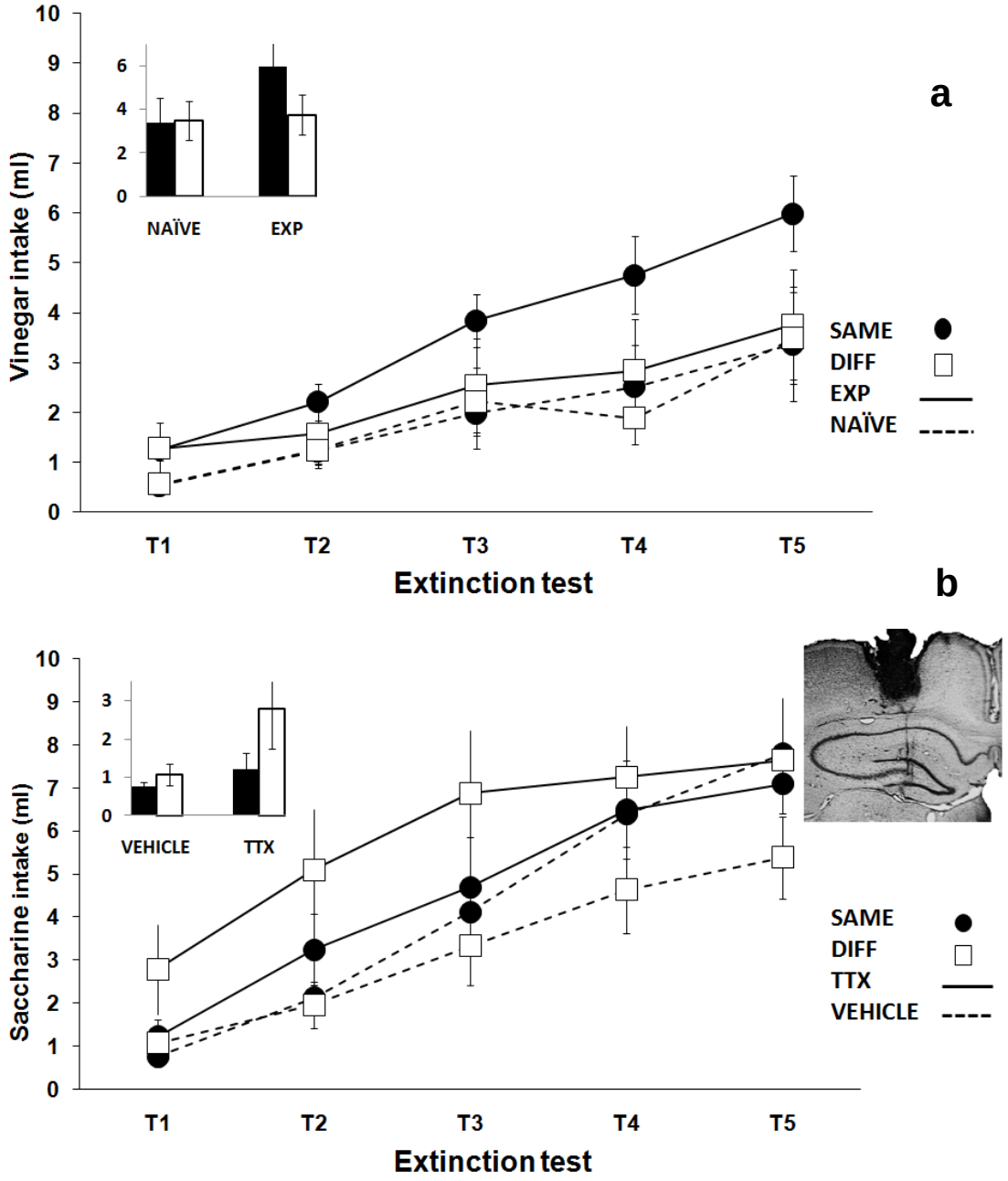


Figure III.3. Mean (± SEM) taste solution intake by the groups SAME and DIFF using a behavioural procedure of temporal context-dependent latent inhibition of conditioned taste aversion using a long-habituation protocol*. a) Groups previously trained (EXP) versus groups without previous experience (NAÏVE). b) Previous trained groups receiving tetrodotoxin (TTX) injection in the dorsal hippocampus versus those receiving vehicle injections (VEHICLE).*

The reinstatement of this adult pattern, that it is known to require an intact hippocampus (Molero, et al., 2005), in previously trained aged rats suggests preserved functions of the aged hippocampus. In our experiments these functions

were reactivated only by discrete learning experience involving the above mentioned taste and visual learning tasks. However, a similar two-month-long exposure to unspecific environmental enrichment did not prove to be effective. Since either longer exposure or increased complexity of the enriched environment conditions may be needed, further research is required on this issue.

Moreover, a surprising finding reported by Manrique et al. (2009) was that dorsal hipocampal neurotoxic lesions facilitated the time modulation of aversive memories. The time-dependency of taste aversion which was evident in adult rats using the short-habituation protocol appeared in lesioned aged rats subjected to the long-habituation procedure. Since this effect does not require an intact hippocampus in adult rats (Molero, et al., 2005), these results throw relevant implications. First, they confirm previous data showing that an aged hippocampus cannot be considered as a damaged hippocampus. Second, they support a competitive interaction between hippocampus and the basic CTA memory circuit. Thus, as it has been proposed (Schoenbaum & Stalnaker, 2005; Stone, et al., 2005), output from hippocampus in adults would normally interfere with basic taste aversion learning because it would contribute to the segregation of learning experiences into distinct episodes. It is conceivable that the aged hippocampus of naive old rats would exhibit decay of the function relevant for episodic memory while maintaining the competitive action on the basic CTA circuit. Thus, permanent hipocampal damage would facilitate non-hippocampal learning effects, such as the temporal context dependency of learned taste aversion.

Given that temporary hipocampal inactivation may represent a better model of the aged hippocampus, we have applied TTX infusions in the dorsal hippocampus of aged rats for exploring potential reorganization of the taste memory circuits by previous learning experience. The general finding was that temporary hippocampal inactivation during conditioning, but not during testing, induced a similar effect to that described in permanent lesioned aged rats. However, the previous learning experience seems to have a critical role in determining the effects of temporary hippocampal interventions during aging. While permanent neurotoxic lesions of the dorsal hippocampus in naïve aged rats enhanced the non-hippocampal modulation of taste aversion by the time of day

(Manrique, Moron, et al., 2009), temporary inactivation by TTX during conditioning induced a similar effect only in experienced old rats, but not in naïve rats. The effect cannot be attributed to changes in the attenuation of neophobia during conditioning since hippocampal inactivation during exposure to a 3% cider vinegar solution had no effect either in the neophobic response or its habituation. It is, therefore, conceivable that reversible temporary inactivation may release functions modulated by the aged hippocampus previously reactivated by learning experience. However a permanent damage would be required for the reorganization of neural circuits in naïve animals. Therefore, the study of the interaction between the hippocampus and other taste memory systems at advanced ages should take into account to the nature of the learning experiences throughout the life.

FINAL REMARKS

Ageing offers a privileged opportunity to investigate neural systems plasticity. Brain systems reorganization is an unavoidable consequence of the accumulated experiences as well as sensory, motor and other body changes throughout a long life since neural plasticity is present along the entire life cycle. Such aging-induced reorganization should be especially evident in the learning and memory systems given their specialized adaptive function to deal with environmental changes. Thus, applying a developmental approach culminating at advance ages may be especially useful for illuminating the present views on the interaction among multiple and parallel memory circuits.

The complexity and variety of life experiences in human studies prevent conclusions on the specific nature of the relevant previous learning experiences and the mechanisms involved in the functional reorganization of brain memory systems, thus leading to interpretation problems. In contrast with human studies, aging research in animals is typically performed in naïve subjects. When previous learning performance is taken into account is limited to the water maze conventionally applied as screening to dissociate impaired and unimpaired learning abilities in aged rodents. However, scarce attention has been paid to the effect of previous experience itself, except for those studies applying environmental enrichment. Our results suggest a greater impact of discrete learning experiences than environmental enrichment on memory systems reorganization. Therefore, aging research can benefit of developing behavioural animal models using specific learning tasks, such those leading to safe or aversive taste memories.

Taste learning exhibits advantages for research on memory brain circuits and its reorganization along the life. First, taste learning task do not impose sensory or motor requirements that are compromised during normal aging.

Second, taste learning and memory depends on dissociable neural circuits depending on the behavioural procedure applied. Basic taste memories such as learned taste aversions rest on a well known neural circuit that do not require other cognitive abilities usually difficult to dissociate in other learning paradigm often applied to aged subjects. Our data support the hippocampal involvement in some taste learning phenomena such as blocking, and the time-of-day dependency of latent inhibition. Finally, aging reshapes taste learning abilities in different domains inducing both facilitation and impairment.

The fact that previous discrete learning experiences in aged rats determine the outcome of hipocampal inactivation in taste learning show up a complex interaction between parallel memory systems which can not be obviated for understanding both brain learning and memory systems and aging.

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CAPÍTULO IV: SPONTANEOUS OBJECT RECOGNITION MEMORY IN AGED RATS: RELEVANCE OF COMPLEXITY VERSUS AMBIGUITY.

Manuscript submmitted

Gámiz, F. and Gallo, M.

ABSTRACT

Previous work on the effect of aging on the performance of spontaneous object recognition (SOR) memory tasks in rats has yielded controversial results. Although there is agreement regarding the presence of age-related impairments at retention intervals of 24 hours, studies using shorter delays have reported conflicting results. In a series of experiments we have assessed the potential relevance of the type of object used in determining the performance of aged Wistar in SOR tasks at different retention intervals. In the first experiment using standard objects that differed in material, size, shape and color naïve male 24-month-old rats exhibited retention impairment compared with adult rats at 24 h but not 10 sec, 60 sec or 1 h. delays between acquisition and testing. At 1 h retention intervals no differences between adult and old rats were found in a high-ambiguity SOR task using pyramids that differ only in one plane (Exp. 2) but aged rats exhibited inability to recognize the novel object when clearly different complex forms were applied (Exp. 3). Additionally no relationship was evident between aged-related impairments in SOR and a spatial version of the water maze tasks. These findings support a critical role of complexity but not ambiguity when elemental objects are used in the recognition memory deficits associated with aging, thus stressing the relevance of careful control of the objects used in SOR aging studies. Also, the results provide insight on the potential brain areas involved in the age-related memory deficits.

INTRODUCTION

Age-related memory decline has received a great deal of attention in rodents. Selective impairment in memory tasks involving spatial information such as the Morris water maze (Morris, 1984) is well documented and it has been associated to a greater vulnerability of the hippocampal system and related cortical areas to the effect of aging. (Gallagher & Nicolle, 1993; Sharma, Rakoczy, & Brown-Borg, 2010; van der Staay, 2002). In fact this task is often applied as a screening tool for identifying age-related cognitive impairment (Ainge, et al., 2006; Burke, Wallace, Nematollahi, Uprety, & Barnes, 2010).

However, there is no agreement about the extent and nature of the age-related impairment in recognition memory. The spontaneous object recognition (SOR) task applied in rodents is based on the innate tendency to explore novel objects more than familiar (eg Ennaceur & Delacour, 1988). Although there is some controversy about the brain areas involved in object recognition memory (Broadbent, Squire, & Clark, 2004; Clark, Zola, & Squire, 2000; Mishkin, 1978; Saunders, Murray, & Mishkin, 1984; Zola-Morgan, Squire, & Mishkin, 1982; Zola, et al., 2000), there is a growing number of studies that point to the perirhinal cortex (PER) as the key structure (Baxter & Murray, 2001; Burke, et al., 2010; Ennaceur & Aggleton, 1997; Ranganath, et al., 2004; Wiig & Burwell, 1998; Winters & Bussey, 2005a, 2005b; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). There are several advantages of using SOR for exploring memory abilities at advanced ages. The task does not require either a rule to be learned nor does it rely on positive or negative reinforcers (eg Ennaceur & Delacour, 1988). Also the task offers the possibility of introducing several variations that might explain contradictory findings.

The main controversy on the effects of aging in SOR memory lies in the relevance of the retention interval. Although there is agreement regarding the presence of retention deficits in recognition tests applied 24 h after (Bartolini, Casamenti, & Pepeu, 1996; Burke, et al., 2010; de Lima, et al., 2005; Leite, Wilhelm, Jesse, Brandao, & Nogueira, 2011; Pieta Dias, et al., 2007), the evidence is controversial using shorter delays. While some authors found no age-related

impairment in SOR memory at 1 min delay (Lukaszewska & Radulska, 1994), 2 min delay (Burke, et al., 2010), 3 min delay (Bergado, Almaguer, Rojas, Capdevila, & Frey, 2011), 5 min (Cavoy & Delacour, 1993) and 1.5 hour (de Lima, et al., 2005; Leite, et al., 2011; Pieta Dias, et al., 2007), there are studies reporting deficits in aged rats at 2 h (Burke, et al., 2010), 1 h (Da Silva Costa-Aze, Dauphin, & Boulouard, 2011; Pitsikas, Rigamonti, Cella, Sakellaridis, & Muller, 2005; Scali, Casamenti, Pazzagli, Bartolini, & Pepeu, 1994; Scali, Giovannini, Bartolini, et al., 1997; Vannucchi, Scali, Kopf, Pepeu, & Casamenti, 1997) and even at 60 or 30 sec (Bartolini, et al., 1996; Willig, et al., 1987).

One of the relevant variations that might explain the controversial findings could be related with the stimuli used in SOR memory tasks applied to aged rats. In general, there is a great variety of objects used and they are not always well defined. They could be classified as standard objects, solid geometric forms and complex objects built with Lego blocks and toys. The standard objects used in the SOR task applied to aged rats have been elemental everyday and junk objects ranging from cups, brackets, transparent bottles filled with sand, "spray nozzle for a garden hose","a plastic hand soap dispenser" to multicoloured plastic children's toys (Aggleton, Blindt, & Candy, 1989; Da Silva Costa-Aze, et al., 2011; Hauser, Tolentino, Pirogovsky, Weston, & Gilbert, 2009; Liu, Smith, Appleton, Darlington, & Bilkey, 2004; Lukaszewska & Radulska, 1994). Solid geometric forms have included cubes, pyramids and cylinders (Bartolini, et al., 1996; Pitsikas, et al., 2005; Scali, et al., 1994; Vannucchi, et al., 1997; Willig, et al., 1987). Complex objects made of Duplo Lego bricks, sometimes combine Lego toys (de Lima, et al., 2008; de Lima, et al., 2005; Leite, et al., 2011; Pieta Dias, et al., 2007).

Most of the aging studies have attempted the pair of objects used to greatly differ in several features such as the material, shape, color and size (Bergado, et al., 2011; Burke, et al., 2010; Cavoy & Delacour, 1993). However it is difficult to evaluate the degree of dissimilarity as well as the difficulty level of the discrimination. In fact, lesion studies in adult rats have pointed to the relevant distinction between feature ambiguity and difficulty of discrimination when assessing object recognition deficits induced by PER damage (Bartko, Winters, Cowell, Saksida, & Bussey, 2007a, 2007b; Buckley & Gaffan, 1998; Eacott, Machin,

& Gaffan, 2001; Norman & Eacott, 2004). PER lesions have been shown to result in a disproportionate impairment when feature ambiguity was increased by including a higher number of overlapping features but not when the difficulty of discrimination was increased by enlarging the stimulus set (Norman & Eacott, 2004). Since it has been proposed that the impaired performance of aged rats in SOR tasks might be related with a deficit of disambiguation, a primary function of PER (Burke, et al., 2010) evaluating the contribution of factors such as the object ambiguity and difficulty of discrimination to SOR impairment at advanced age seems to be especially relevant.

Finally, an additional difficulty for comparison and interpretation of the results obtained in SOR experiments with aged rats is the fact that the raw data are not always provided and different indexes are estimated. Among them the indexes most commonly applied are: 1) Discrimination Ratio (DR), i.e., which is the difference in exploration time of the novel and the familiar object divided by the total time spent exploring the objects (Ennaceur & Delacour, 1988); 2) Recognition Index (RI), obtained by dividing the exploration time of the novel object by the total time of exploration (Costa, et al., 2008; Leite, et al., 2011); 3) Novel-Familiar Index (NFI), i.e., the exploration time of the novel object minus the familiar one (Ennaceur & Delacour, 1988). The first two indexes have been more often applied (Bartolini, et al., 1996; Burke, et al., 2010; Da Silva Costa-Aze, et al., 2011; de Lima, et al., 2008; de Lima, et al., 2005; Leite, et al., 2011; Pieta Dias, et al., 2007; Pitsikas, et al., 2005; Vannucchi, et al., 1997) while the use of NFI has been less frequent (Cavoy & Delacour, 1993; Scali, et al., 1994; Scali, Giovannini, Prosperi, Bartolini, & Pepeu, 1997). Even though these indexes tend to yield similar results, to our knowledge there are no studies aimed to assess differential sensitivity and potential advantages or disadvantages of each of them.

In order to explore these issues a series of experiments was performed in rats. The first experiment was aimed to assess SOR memory deficits in naïve aged rats at different retention intervals using standard objects commonly used in previous studies. In a second part the role of ambiguity (Experiment 2) and difficulty of discrimination (Experiment 3) in age-related SOR memory were investigated after a 1 hour retention interval by using elemental solid forms

differing in one plane and complex but clearly different objects built of multiple components, respectively. Additionally, special attention was paid to match the object exploration time during the familiarization phase of adult and aged groups and the results obtained by estimating DR, RI and NFI indexes were compared.

METHOD

Subjects

A total number of 54 adult (6-7 month-old) and 52 aged (24-month-old) male Wistar rats were used. In the first SOR experiment, using standard objects, 36 adult and 34 aged rats were assigned to each of four groups according to the retention interval applied: Adult 10 sec (n= 8), Aged 10 sec (n=7), Adult 60 sec (n= 10); Aged 60 sec (n=7), Adult 1 hour (n=8), Aged 1 hour (n=10), Adult 24 hours (n=10) and Aged 24 hours (n=10). In the second and third experiments adult and aged rats (n= 9 per group) received training in the hidden platform water maze task one week before applying the SOR task either with elemental/ambiguous or complex/different objects respectively. Half the animals were used for elemental-object SOR and the other half for complex- object SOR.

All the animals were housed individually and maintained on a 12-hr light–dark cycle at a constant temperature of 22 °C. The behavioral procedures were performed during the light cycle (from 10:00 am). Food and water were available *ad libitum* during the experiments. Old rats were subjected to caloric restriction from the age of 20 months. The procedures were approved by the University of Granada Ethics Committee for Animal Research and were in accordance with the European Communities Council Directive 86/609/EEC.

Standard objects SOR procedure

The behavioral procedure took place in a black opaque open plastic chamber (40cm x 40cm x 40cm). Figure IV.1a shows the stimuli used that were two identical copies of a plastic apple and porcelain jar (approximately 12 cm high and 8 cm wide). Velcro was attached to each object in order to be secured to the floor of the testing boxes (9 cm apart), both to ensure correct positioning of the object and to prevent the rats from displacing the objects during testing. A video camera mounted above the chamber allowed us to record the sessions. Overhead lighting illuminates the testing area reducing room context information.

The procedure required three phases: habituation to the chamber, acquisition/familiarization and recognition memory test. In the habituation phase all the animals received handling (2 min) and were acclimated to the room (30 min) and to the empty chamber (2 minutes exploration). This procedure was repeated daily for 5 consecutive days. Twenty four hours later, during the acquisition session the rat was allowed to explore the chamber containing two identical objects during 10 minutes. Recognition memory was assessed in a testing session taking place after a retention interval of 10 sec, 60 sec, 1 h or 24 h depending on the group assignment. In the testing session, a similar procedure to that used in the acquisition session was applied but while one of the objects was identical to that presented in the acquisition session, the other one was an entirely new object. Both the novel object and the position in the chamber were counterbalanced within each group. Each rat was allowed to explore until it accumulated 30 seconds of contact time with the objects (nose within 2 cm of objects and vibrissae moving) or for a maximum of 5 min. The time that the rat spent exploring the novel and the familiar object, as well and the total exploration time, were recorded. Subsequently the following indexes were estimated. Discrimination ratio (DR) was taken as the difference in the exploration time of the novel and the familiar object divided by the total time spent exploring the objects in the test phase (DR=N-F/N+F) (Ennaceur & Delacour, 1988) . Recognition Index (RI) consists in dividing the novel-object exploration time by the total exploration time (N/N+F) during the testing trial (Costa, et al., 2008; Leite, et al., 2011). Finally,

a Novel-Familiar Index (NFI) (Ennaceur & Delacour, 1988) was estimated by subtracting the exploration time of novel object minus that of the familiar object N-F).

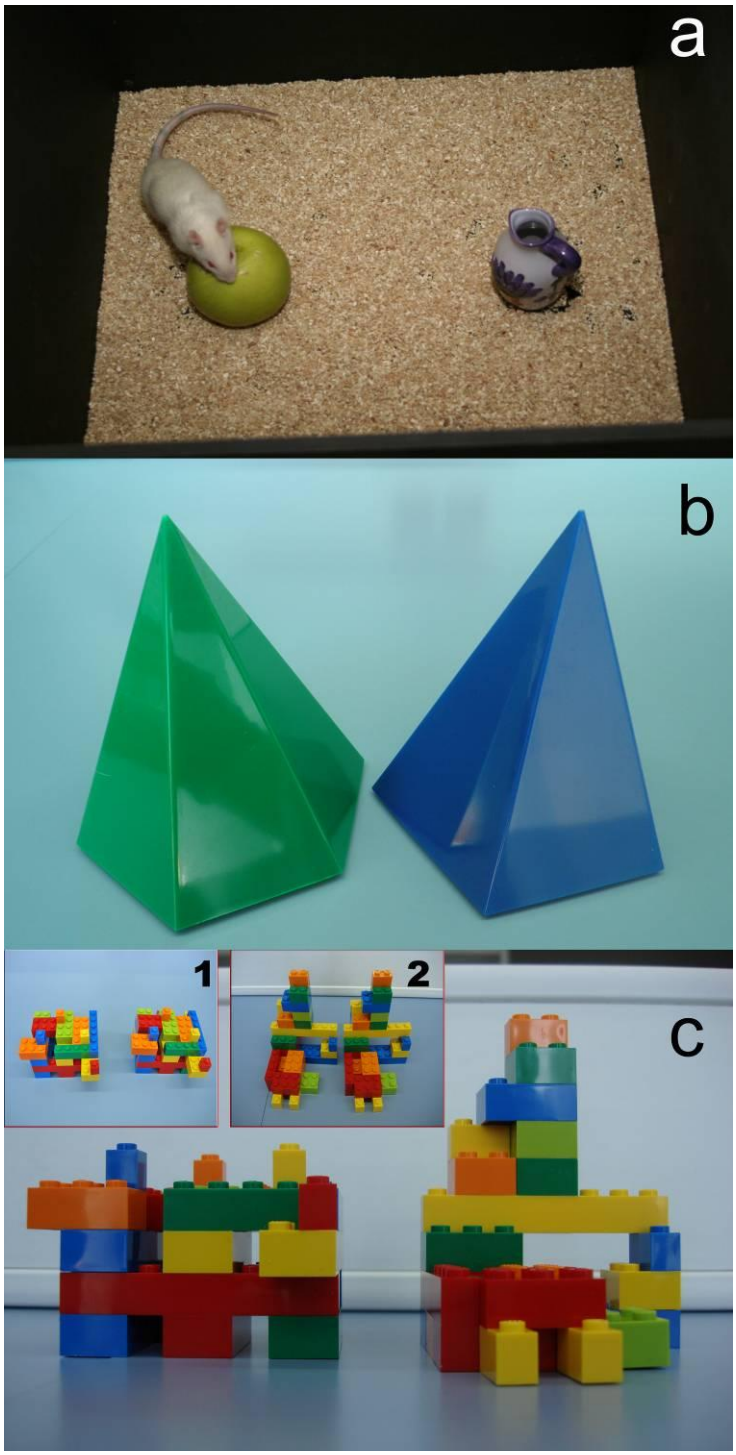

Figure IV.1. Photographs of the objects' pairs used in the SOR experiments*. a) Standard objects consisting of a plastic apple versus a porcelain jar (Exp. 1). b) Elemental/Ambiguous objects consisting of a regular pentagon based pyramid versus a regular hexagon based pyramid (Exp. 2). c) Complex/Different objects built with Duplo Lego bricks of five different colors(Exp. 3) .*

Elemental/ambiguous versus complex/different objects SOR procedure

The behavioral procedure followed in the second (elemental/ambiguous objects) and third (complex/different objects) experiments was similar to the above described except for the following differences. First, the object exploration times of the younger rats during the acquisition phase were matched to those of the older rats, being the maximum 10 min. Thus, each animal in the young yoked group was allowed to explore exactly the same time than one animal in the aged group. Second, the retention interval lasted 1 h. Such a retention interval was chosen because it was the longest retention interval tested in the first experiment not affected by aging. Finally, the critical difference among the experiments lies in the stimuli used. In the second experiment (elemental/ambiguous) a pair of elemental geometric shapes with several overlapping features was chosen (Fig. IV.1b). The solid forms used were a dark blue (Pantone 280) regular pentagon based pyramid versus a dark green (Pantone 349) regular hexagon based pyramid (12 cm high and 8 cm wide). Therefore, the object to be discriminated exhibited a high level of feature ambiguity (they differed only in the number of planes) but low complexity (they are elemental solid forms). In the third experiment (complex/different objects) a pair of complex tridimensional forms were built with Duplo Lego bricks of various colors (Lego® Basic Bricks-Standart ref: 5529) containing each figure 30 pieces. Although being complex, the global shape was intended to differ clearly (Fig. IV.1c). Thus, object 1 measured 12 cm wide and 7 cm high while object 2 measured 15 cm wide and 12 cm high.

Morris water maze

In order to assess age-related impairment in spatial memory and potential interactions with the performance in SOR memory in the second and third experiments the rats were tested on the hidden platform and the visually cued

versions of the water maze task (Morris, 1984). The pool consisted of a 180 cm diameter and 30 cm deep circular plastic tank with a removable 11 cm diameter circular platform. The temperature of the water was maintained at 24–26°C. Water was stained with non-toxic black ink in order to make the platform invisible during the spatial version and for the computerized system to detect the albino rat. The water level was 22 cm and the platform was placed 1 cm below the water surface, except during the cued visual trials of the first block where the platform was visible. The platform was placed approximately 35 cm from the pool border in the center. Training consisted of one daily block. Each training block consisted of 4 learning trials separated by a 5 min inter-trial interval. Each subject was released once from each of the four compass points during a block of four trials. The order varied randomly. The animal was allowed to swim freely for 60 sec or until it climbed onto the platform. If it did not find the platform within 60 sec, it was placed there by the experimenter and remained there for 15 s. All the rats received a block of the visually cued task and 6 training blocks in the spatial task. Immediately after the last block of trials they performed a probe trial without platform, followed by a retention test 24 hours later. A video system and software associated (Noldus EthoVision video tracking software) was used to record escape latency, path length and speed.

RESULTS

Standard objects SOR memory task

There were no differences in object exploration time among the groups during the acquisition phase [$F(1,68) = 2.44$; $p>0.12$]. Main (±SEM) exploration time of the novel and familiar object by the different groups during the testing session performed at different retention intervals are shown in Figure IV.2a. While there were no differences between groups at the shorter delays (10 sec, 60 sec and 1 hour), the effect of aging was evident at 24 hours delay. A 4x2x2 (Retention interval x Age x Novelty) ANOVA analysis indicated a significant effect of the main factor novelty [$F(1,62) = 42.93$; $p<0.01$]. Although no other effect or interactions were significant further analyses were performed in each of the retention interval level. Independent 2x2 (Age x Novelty) ANOVA analyses yielded significant effects of novelty at 10 sec, 60 sec and 1 hour intervals but not age nor interaction effects. However, a 2X2 (Age x Novelty) ANOVA analysis at 24 h retention interval showed a significant effect of the main factor novelty [$F(1,18) = 8.36$; $p<0.01$] and the interaction age x novelty [$F(1,18) = 11.13$; $p<0.01$] . The adult group spent longer time exploring the novel object than the familiar one [$F(1,9) = 16.9$; $p<0.01$ but there were no differences in the exploration time of the novel versus the familiar object in the aged group [$F(1,9) = 0.11$; $p>0.74$]. Moreover, the aged group exhibited lower exploration time of the novel object [$F(1,18) = 11.13$; $p<0.01$] and longer exploration time of the familiar object [$F(1,18) = 11.13$; $p<0.01$] than the adult group.

Consistently, the aged and adult groups differed only at the 24 hour retention interval applying the most commonly SOR indexes: discrimination ratio [$F(1,18) = 11.21$; $p<0.01$] (Fig. IV.2b), recognition index [$F(1,18) = 10.95$; $p<0.01$] (Fig. IV.2c) and novel-familiar index [$F(1,18) = 11.13$; $p<0.01$] (Fig. IV.2d).

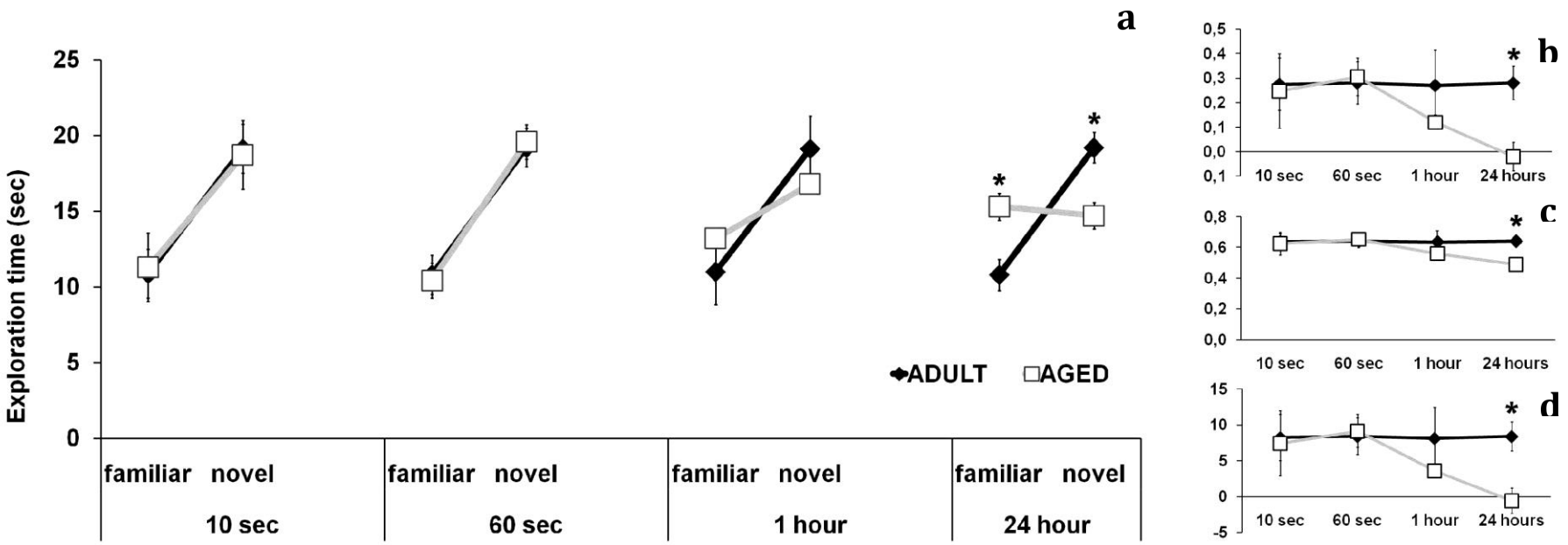

Figure IV. 2. Spontaneous object recognition (SOR) task performance at different retention intervals during the test phase using standard objects. *a) Mean (± SEM) exploration time of adult (filled squares) and aged (open squares). b) Mean (±SEM) Discrimination Ratio scores (DR= N-F/N+F) of the adult and aged group. c) Mean (± SEM) Recognition Index scores (RI=N/N+F). d) Mean (± SEM) scores using the Novel-Familiar Index (NFI). F: familiar object; N: novel object.*

Elemental/Ambiguous versus Complex/Different objects SOR memory task

As expected there were no differences in the total object exploration time between the aged and the adult yoked group during the acquisition phase both in the Exp. 2 [$F(1,16) = 0.007$; $p>0.93$] and 3 [$F(1,16) = 0.015$; $p>0.90$]. However a 2x2 (Age x Object) ANOVA analysis of the total time required to reach the same object exploration level, considering that aged animal were allowed to explore for a maximum of 10 min, yielded significant effects of the main factors and the interaction age x object [$F(1,32) = 5.01$; $p<0.05$]. Analyses of the interaction showed a more active object exploration by the adult group both in the Exp. 2 [$F(1,16) = 58.26$; $p<0.01$] and Exp 3 [$F(1,16) = 158.66$; $p<0.01$]. The adult groups reached the object exploration time exhibited during the entire trial (600 sec) by aged rats in a lower mean time (303 and 190 sec respectively). Moreover, adult animals showed a more active pattern of exploration when exposed to complex/different than elemental/ambiguous objects. A one-way ANOVA of the total exploration time spent by adult groups during the acquisition phase indicated a significant effect of the object type [$F(1,16) = 5.01$; $p<0.05$].

Regarding the testing session performed at 1 hour retention interval a 2x2x2 (Age x Object x Novelty) showed a significant interaction of the three factors [$F(1,32) = 5.34$; $p<0.05$].

A 2x2 (Age x Novelty) ANOVA analysis of the object exploration times in the Exp. 2 (elemental/ambiguous objects) revealed significant main effects of novelty [$F(1,16) = 26.53$; $p<0.01$] and age [$F(1,16) = 13.65$; $p<0.01$] but not the interaction novelty x age. Both adult and aged groups explored longer time the novel object than the familiar one (Fig. IV.3a), thus indicating similar discrimination and recognition memory when using elemental solid forms even if they were ambiguous. The analyses of the SOR indexes (DI, RI NFI) did not evidence any significant difference between the groups (Fig. IV.3b, 3c, 3d, respectively).

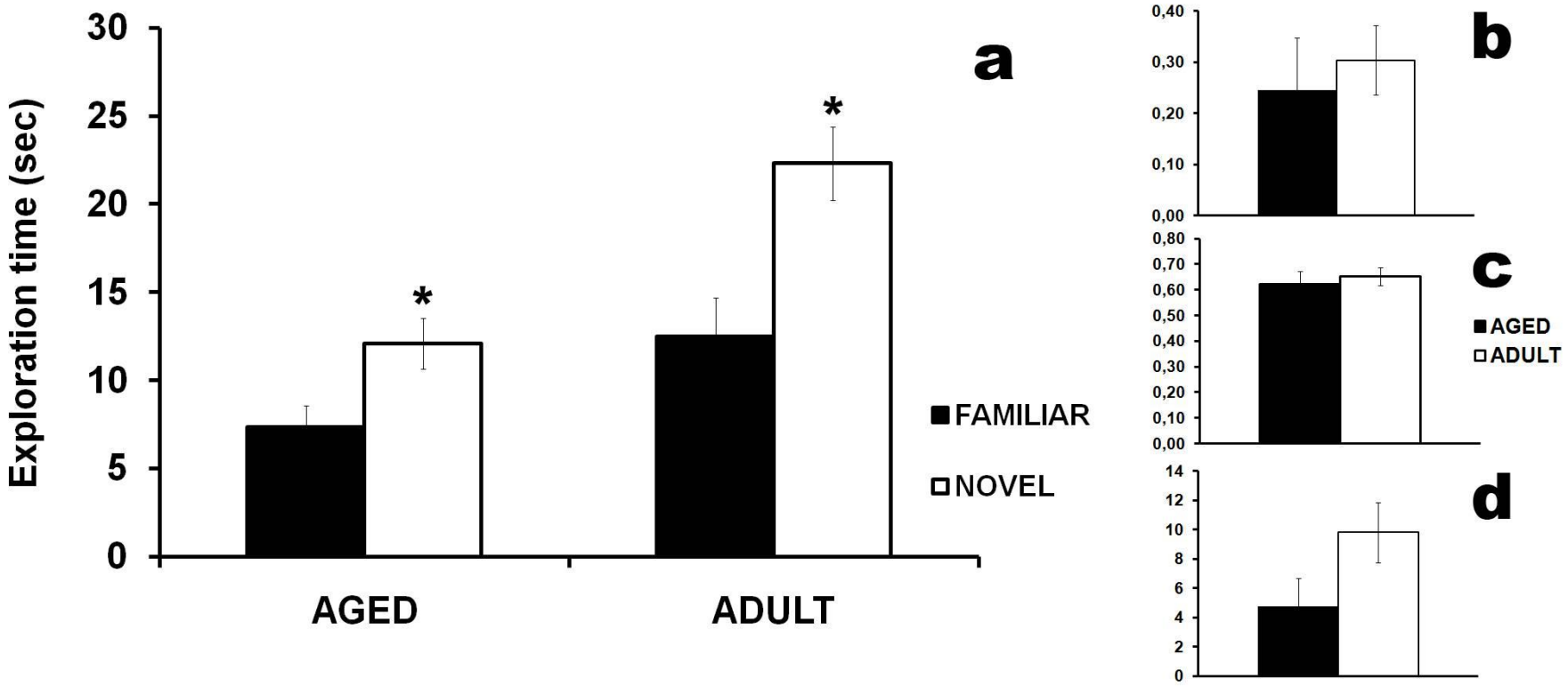

Figure IV. 3. Adult versus aged groups' performance in the SOR task using elemental/ambiguous objects at 1 hour retention interval*. a) Mean (±SEM) exploration time of familiar (black) and the novel (white) object during the test phase. b) Mean (± SEM) DR scores. c) Mean (± SEM) RI scores. d) Mean (± SEM) NFI scores.*

However, a similar 2x2 (Age x Novelty) ANOVA analysis of the object exploration times in the Exp. 3 (complex/different objects) revealed main significant effects of age and the interaction age x novelty [$F(1,16) = 13.07$; $p<0.01$] (Fig. IV.4a). The analysis of the interaction showed that the adult group explored longer the novel object [$F(1,8) = 16.86$; $p<0.01$] than the familiar one, while the aged group did not show differences between the exploration of the novel and familiar object [$F(1,8) = 1.11$; $p>0.32$]. Also the exploration time of the novel object was higher in the young than in the aged group [$F(1,16) = 16.89$; $p<0.01$] but there were no differences between the groups in the time spent exploring the familiar object.

With respect to the analyses based on the estimated indexes, they were consistent with the analyses of the raw data. Thus, none of the SOR indexes (DI, RI NFI) evidenced significant differences between the groups in the Exp. 2 (Fig. IV.3b, 3c, 3d, respectively). However, all the indexes indicated differences between the groups in the Exp. 3, i.e., DI [$F(1,16) = 7.37$; $p<0.01$] (Fig. IV.4b), RI [$F(1,16) = 7.61$; $p<0.01$] (Fig. IV.4c) and NFI [$F(1,16) = 13.07$; $p<0.01$] (Fig. IV.4d). Additionally, a 2x2 (Age x Object) ANOVA including the results obtained in Exps. 2 and 3 for each index yielded a significant age x object interaction only in NFI [$F(1,32) = 5.34$; $p<0.05$]. The analysis of the interaction confirmed the effect of age in the complex/different SOR task but not in the elemental/ambiguous. However, the rest of the indexes did not detect the interaction, being significant only the effect of age and obviating the relevance of the object.

In all, the comparison based on the type of object used suggest that aged-related impairment at one hour delay is only evident when using complex/different objects and that among the indexes estimated NFI has proven to be the most sensitive to the interaction age x object type.

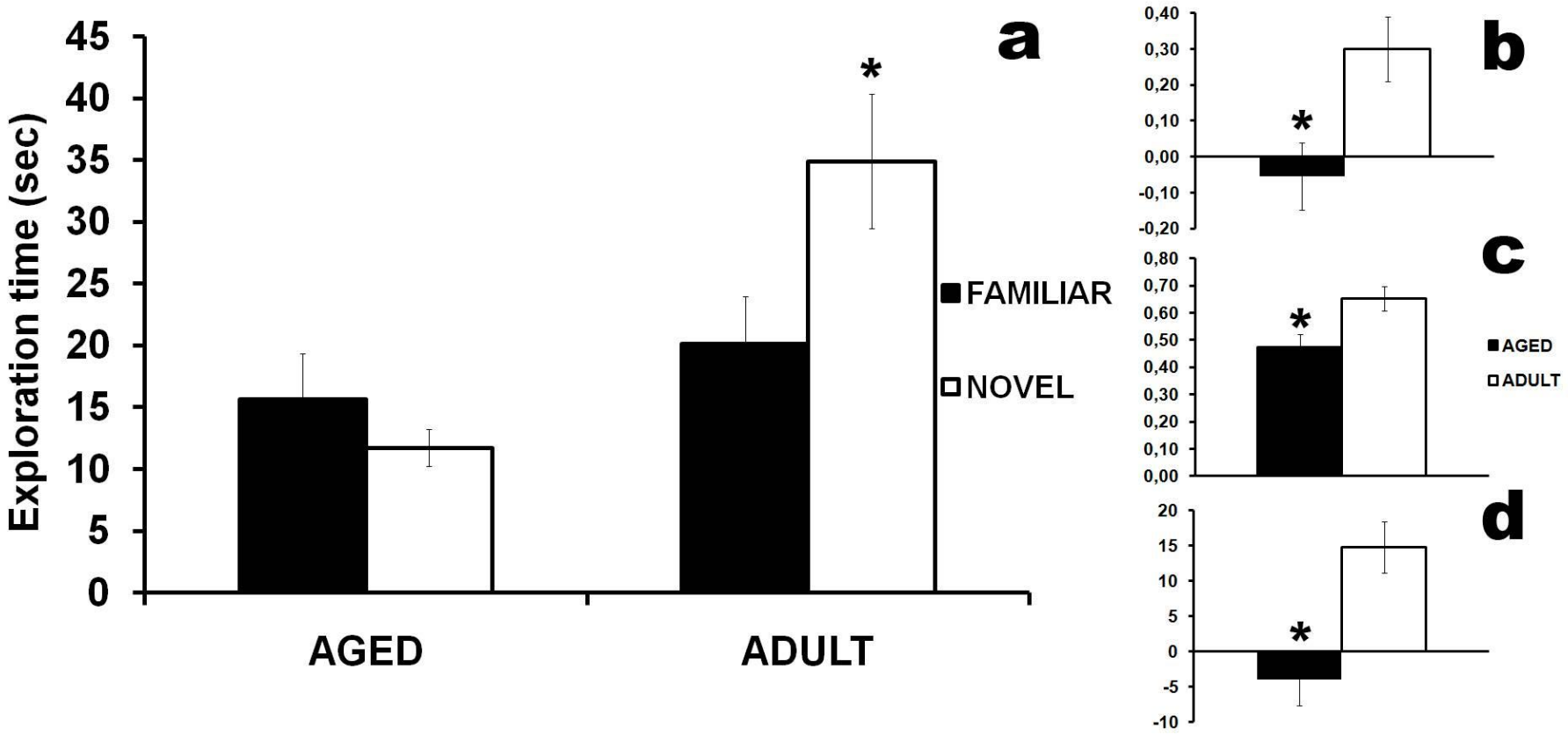

Figure IV.4. Adult versus aged groups' performance in the SOR task using complex/different objects at 1 hour delay*. a) Mean (±SEM) exploration time of familiar (black) and the novel (white) object during the test phase. b) Mean (± SEM) DR scores. c) Mean (± SEM) RI scores. d) Mean (± SEM) NFI scores.*

Morris water maze

In order to compare the performance of the groups belonging to the Exp. 2 and Exp. 3 in the water maze tasks mixed ANOVA including object as an additional factor were performed.

During the visually cued version of the water maze task 3 aged and 1 young rat were unable to reach the visible platform. Thus, they were excluded from further analyses. A 2x2x4 (Age x Object x Trial) ANOVA analysis of the latency to reach the platform showed a main significant effect of age [$F(1,32) = 4.52$; $p<0.05$], the old group being the slowest and of trial [$F(3,96)= 6.29$; $p<0.01$], with reduced latencies after the first trial. The latency differences can be attributed to a lower speed in the aged group than in the adult group in trials 3 and 4 [$F(1,32) = 6.69$ $p<0.05$] and [$F(1,32) = 4.57$; $p<0.05$] respectively, since no significant differences in path length between the groups were found. Thus, age-related visual deficits enabling to locate the platform could be ruled out.

In the spatial version of the water maze a 2x2x6 (Age x Object x Block) ANOVA analysis of the latencies along the blocks of trial showed a significant main effect of age [$F(1,32) = 4,52$; $p<0.05$] and of block [$F(5,160) = 8.91$; $p<0.01$]. Aged rats showed higher latencies and the learning curve was evident in all the groups. In this phase there were no significant speed differences. Although no significant effects were found applying a similar analysis to the latencies to reach the platform during the immediate probe, the data obtained in the probe trial applied 24 hours later evidenced a significant main effect of age [$F(1,32) = 4.16$; $p<0.05$]. Aged rats spent a longer time to reach the target area. In this case the impairment cannot be due to differences in swimming speed, as they did not differ. Figure IV.5 shows the individual performance of aged and adult groups in the spatial memory test. After subdividing the aged group in “unimpaired” (less than 2 SD above the mean of the young animals) and “impaired” (the rest of the animals), the performance of impaired and unimpaired aged rats on the SOR tasks did not differ. Additional linear regression analyses indicated no significant relationship between the performance on the water maze probe and the object recognition using DR index

[F(1,34) = 0.51; p>0.47]. Nevertheless the spatially impaired aged rats were distributed randomly in SOR experiments in order to avoid any possible bias.

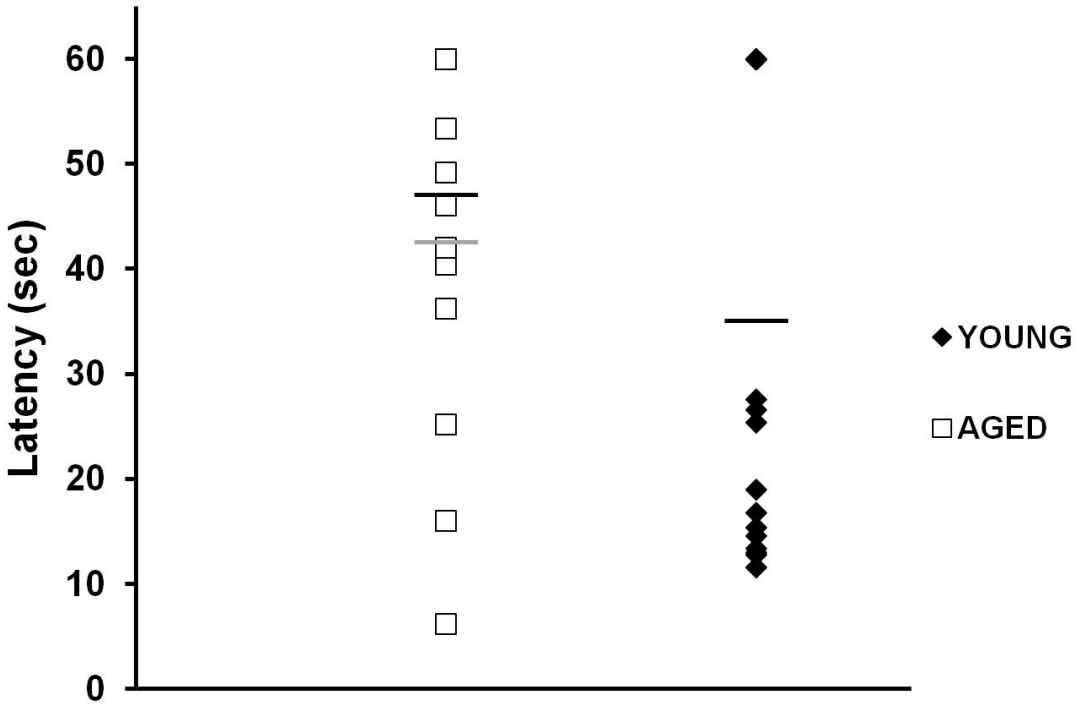

Figure IV.5. Individual performance of adult (filled diamonds) and aged (open squares) rats in the 24 h delayed test of the spatial version of the water maze task*. The horizontal black lines indicate the mean for each age group. In the aged rats, the values below the gray horizontal line represent rats that performed within 2 SD of the adult group mean. Only 6 aged rats met this criterion, whereas the other 12 rats had scores that were at least 2 SD above the adult rat mean.*

DISCUSSION

The present results provide evidence supporting the relevance of establishing dissociations between the processing of complexity and ambiguity in aging studies using the SOR tasks.

The main finding of Exp. 1 is that aged rats, but not adult rats, showed an inability to recognize and discriminate between novel and familiar standard objects 24 hours after acquisition. However, the performance of aged and adult rats did not differ at shorter retention intervals from seconds to 1 hour. These results are consistent with most of the previous reports (Burke, et al., 2010; de Lima, et al., 2005; Leite, et al., 2011; Pieta Dias, et al., 2007). In contrast, some aging studies have found group differences between aged and young rats at this range of retention intervals (Bartolini, et al., 1996; Pitsikas, et al., 2005; Scali, et al., 1994; Vannucchi, et al., 1997).

When considering potential variables responsible of this discrepancy several issues should be taken into account. First, age-related differences in overall exploratory activity can reduce the object exploration time during acquisition, thus leading to different levels of familiarity in adult and aged groups. In fact, the aged rats in Exp. 1 exhibited less exploratory activity than adult rats. However, a reduced exploratory activity in aged rats is not always reported. Even if few papers have reported comparisons between the object exploration time during the familiarization phase of young and aged rats, the results are controversial. On one hand, Cavoy and Delacour (1993), de Lima et al. (2005) and Hauser and colleagues (2009) found no effect of age in the exploratory activity. On the other hand, Bergado and colleagues (2011) and Liu's team (2004) reported that aged rats explored the objects significantly less than adult rats during the familiarization phase. The fact that old rats not always exhibit a reduced exploratory activity is evident in some studies including several experiments where differences between adult and aged groups appear only in one of them (Burke, et al., 2010). Although a reduced object exploration time by the aged groups during acquisition did not seem to be critical in the present experiment given the similar performance of aged

and adult groups at retention intervals up to 1 hour, it could be relevant in other studies. It is conceivable that a greater familiarity in the adult groups will affect the memory of the objects during subsequent test phases, thus leading to confusing results when comparisons are established with older groups. Thus, ensuring similar object exploration time during the acquisition session by different age groups can be a useful strategy in SOR aging studies. Such a point has been taken into account in the Exp. 2 and 3 by using yoked adult groups with object exploration times matched to that of the older groups.

Second, the effect of previous experiences might modify the reaction of aged animals to novel stimuli. Results using taste recognition memory tasks have shown that previous aversive experiences with other taste solutions significantly enhances the response to novel taste solutions in aged rats (Moron & Gallo, 2007). This is consistent with a bulk of data on the beneficial effects of environmental enrichment or discrete learning experiences on memory and other behavioral abilities at advanced ages (Lazarov, Mattson, Peterson, Pimplikar, & van Praag, 2010; Segovia, del Arco, & Mora, 2009). In fact doubts have risen by Paolisso et al. (2000) and var der Staay (2002) among other authors on the validity of using rats which have spent their entire lives in isolation and deficient stimulation as a suitable model of normal aging, since this is developmental stage necessarily marked by previous learning and experience. Although many experiments on aging have used naïve rats, it is frequent in SOR studies to perform previous assessment of aged-related cognitive impairments typically by training the animals in hippocampal-dependent spatial memory tasks. This strategy has had the advantage of allowing comparisons between memory abilities relaying on different brain areas then favoring the knowledge on the aging process. However, the differences among SOR studies regarding the previous learning experience of the aged subjects can be a source of discrepancies. In the Exp. 1 naïve subjects have been used in order to avoid confounding effects. Moreover, the performance on each of the retention intervals tested was assessed in independent groups so that to avoid repeated test sessions. However, the absence of differences between aged and adult groups at the range of retention intervals between 10 sec and 1 hour does not support a potential deterioration by previous impoverished environment along the life. Thereafter, in the rest of the present experiments both visual cued

and spatial versions of the water maze task were applied before the SOR memory task in order to gain insight on the relationship about different types of memory.

A third issue that might be critical for explaining controversial effects of aging in SOR memory tasks concerns the nature of the objects used. In the Exp. 1 we used standard objects present in everyday life that differed in material, shape, size and colour. Aging studies using SOR tasks have applied a great variety of standard objects (Aggleton, et al., 1989; Da Silva Costa-Aze, et al., 2011; Hauser, et al., 2009; Liu, et al., 2004; Lukaszewska & Radulska, 1994), being common the use of clearly different elemental stimuli (Bergado, et al., 2011; Burke, et al., 2010; Cavoy & Delacour, 1993). However, they are often poorly described and not easily found all over the world, rendering difficult to replicate the experiments. Detailed description of the objects used including size, material and color is also required in experiments using solid geometric forms (Bartolini, et al., 1996; Pitsikas, et al., 2005; Scali, et al., 1994; Vannucchi, et al., 1997; Willig, et al., 1987) and complex objects made of Duplo Lego components (de Lima, et al., 2008; de Lima, et al., 2005; Leite, et al., 2011; Pieta Dias, et al., 2007). Since subtle modifications of the object's features in SOR tasks has proven to be relevant for involving different brain areas such as PER cortex (Norman and Eacott, 2004) and it has been suggested that SOR memory impairments in aged rats could be related with PER dysfunction (Burke et al., 2010), a precise description including photographs of the standard objects, reproducible geometric forms and complex stimuli used in the present study are included.

The findings of the Exp. 2 and 3 in the present study confirm the relevance of the behavioral procedure applied regarding the objects to be discriminated at a 1 h retention interval. The type of object used in the SOR task can be crucial for involving discrimination and memory processes that are selectively impaired by aging. The design of Experiments 2 and 3 was aimed to dissociate the effect of object ambiguity and complexity on the performance of aged rats. Old rats did not exhibit difficulties to discriminate and recognize familiar simple geometric forms (pyramids) 1 hour after the acquisition, even if the novel object was a very similar geometric form differing only in one plane. However, they were unable to recognize a familiar complex object made of Lego building bricks at the same

retention interval when presented simultaneously with a very different object but of the same complexity level.

The lower performance of aged rats than adult rats in the SOR task with complex but clearly different objects does not seem to be due to visual deficits problems since these animals performed the cued version of the water maze as well as the high-ambiguity SOR task at the same level than adult rats. It is also not feasible that unspecific impairments in aged rats affecting performance of difficult tasks could explain the results. Although assessing the level of difficulty is not easy, discriminating between the pentagon and the hexagon based pyramids used in Exp. 2 can be considered a very difficult task since they only differed in one plane being both of them similarly dark coloured. It seems that the relevant point rest on the features of highly complex objects. Aged rats can have problems to integrate many features that may even harm in judgments of object recognition with distinctly different but highly complex.

The fact that the deficits found in aged rats are similar to those detected in animals with lesions in the PER cortex (Bartko, Cowell, Winters, Bussey, & Saksida, 2010; Bartko, et al., 2007a, 2007b; Buckley & Gaffan, 1998; Bussey, Saksida, & Murray, 2002; Cowell, Bussey, & Saksida, 2010; Eacott, et al., 2001; Murray & Richmond, 2001) might point to age-related changes affecting the PER cortex functions relevant for the processing of the configural aspects of complex objects in visual tasks. Bartko et al. (2010; 2007b) manipulating the degree of perceptual similarity in complex objects built with Lego forms found that PER cortex lesions impaired recognition even using zero-delays in conditions of high similarity. However, it is difficult to know if their findings were due to the complexity or to ambiguity because these authors always used complex Lego objects. Although brain damage cannot be accepted as a model of normal aging, it cannot be discarded that the age-related deficits in the SOR task would be associated with the potential functional impairment of PER cortex as it has been proposed (Burke et al., 2010). The fact that the PER cortex receives a direct projection from the ventral tegmental area (Furtak, Wei, Agster, & Burwell, 2007) has supported associations between a potential age-associated decline in PER and the mesolimbic dopaminergic system activity (Furtak, et al., 2007). This proposal would explain

the deficits found in SOR tasks with complex and ambiguous objects according to the perceptual-mnemonic feature conjunctive (PMFC) model (Bussey & Saksida, 2002; Bussey, et al., 2002; Bussey, Saksida, & Murray, 2003). However, the present results allow us to dissociate perceptual-mnemonic processes for ambiguous and complex objects differentially vulnerable to aging. Furthermore, the fact that we have not found any relationship between the performance in spatial water maze and SOR tasks provides evidence for a dissociation between the well documented hippocampus role in spatial memory (e.g., Gallagher & Nicolle, 1993; Sharma, et al., 2010) and independent brain areas involved in object recognition memory, as pointed by Burke et al. (2010).

Finally, several retention indexes, in addition to the raw data, have been estimated in each of the reported experiments in order to explore potential advantages or disadvantages of using them in aging research. In the present experiments the type of index applied did not make a difference, except for the fact that the significant interaction between age and the type of object was evident only when comparing the NFI in the Exp. 2 and 3. DI and RI did not detect such interaction, being significant only the effect of the factor age and obviating the relevance of the object. However, when analysing each independent experiment the three indexes used (DI, RI NFI) yielded similar results to that of raw data statistical analyses. Although the use of different indexes did not alter the final outcome in the present SOR experiments they can be useful tools in particular conditions in SOR aging studies. In particular DR and RI ratios are especially appropriate when the total exploration time is low as it is often the case in aging research, even though they can not be applied with extremely low exploration times. Therefore, the results support the need of reporting information about raw data in order to permit comparisons among studies, even if an additional index is estimated due to special requirements.

In summary, it is our point of view that a careful control of procedural variables will help to draw conclusions on the effect of normal aging in object recognition memory. This study have explored the relevance of the retention interval being able to dissociated impaired and unimpaired object recognition memory abilities at 1 h retention interval depending on the complexity and

similarity of the objects used. Therefore, paying a greater attention to the nature of the objects to be discriminated will be of great help not only for increasing reproducibility and allowing comparisons among experiments but also to identify the selective decay of perceptual-mnemonic processes and the brain structures involved.

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DISCUSIÓN GENERAL

De acuerdo con el objetivo de esta tesis doctoral, la investigación desarrollada se ha dirigido a investigar los mecanismos neurales del aprendizaje y la memoria gustativa en ratas siguiendo una aproximación múltiple en la que se combina el nivel de análisis conductual (Capítulo 4) con el molecular (Capítulos 1 y 2) y el de sistemas (Capítulo 3) y se establecen comparaciones con otros tipos de memorias. El nivel de análisis de sistemas se ha beneficiado de la aplicación de una perspectiva de desarrollo empleando animales envejecidos.

Los resultados obtenidos en la Parte I han mostrado que la inhibición de PKMζ en la Amygdala basolateral mediante la infusión bilateral de ZIP (10nmol/µl) 24 horas después del entrenamiento en una tarea de evitación activa mediante salto produce un efecto amnésico que no impide la readquisición de la respuesta condicionada si se aplica reentrenamiento. Se trata de un efecto permanente ya que el recuerdo fue evaluado siete días después de la infusión. Ello descarta explicaciones basadas en dificultades de recuperación o expresión del recuerdo, tal como se ha propuesto por Parsons y Davis (2011). El hecho de que los animales fueran capaces de volver a aprender y recordar excluye efectos inespecíficos debidos a deficiencias sensoriales, motoras o motivacionales. Estos resultados son congruentes con los obtenidos empleando diversos índices de miedo condicionado (Kwapis, et al., 2009; Migues, et al., 2010; Serrano, et al., 2008). La principal aportación con respecto a hallazgos previos que mostraban efectos amnésicos con los mismos parámetros temporales en una tarea de evitación pasiva (Pastalkova, et al., 2006; Serrano, et al., 2008), viene dada por la exclusión de componentes espaciales en el procedimiento empleado. El hecho de que los animales que habían recibido inyecciones i.c. de ZIP requirieran el mismo número de ensayos durante la segunda adquisición que los animales que recibieron inyecciones control de scrambled ZIP indicó memoria preservada, a

diferencia de los informado por otros investigadores (Shema, et al., 2009; Shema, et al., 2007).

A diferencia del papel crucial en el mantenimiento a largo plazo de la respuesta de evitación activa aprendida, los resultados presentados indican que la actividad de PKMζ en la Amígdala basolateral juega un papel modulador facilitando la adquisición de aprendizaje aversivo gustativo durante el intervalo entre el sabor y el malestar gastrointestinal, sin que su inactivación mediante infusiones de ZIP antes de la exposición al sabor ni durante los procesos tempranos de consolidación durante las 24 horas siguientes al emparejamiento tenga efecto alguno. Estos datos representan un hallazgo de gran relevancia ya que ponen de manifiesto funciones adicionales de la actividad de PKMζ a las previamente descritas que enfatizaban su papel en el mantenimiento de memorias consolidadas exclusivamente. Por el contrario estos datos indican la posibilidad de que los procesos de adquisición de aversiones gustativas aprendidas se beneficien de la facilitación de procesos de memoria actuando durante la larga dilación entre estímulos que este tipo de tarea permite introducir.

En conjunto, los resultados contenidos en los Capítulos 1 y 2 apoyan un papel crucial de PKMζ en diversas formas de memoria que dependen de circuitos neurales diferentes. Los resultados obtenidos son coherentes con los planteamientos vigentes en el área en la actualidad. Así, la actividad de esta enzima en diversas estructuras cerebrales resulta selectivamente implicada en una amplia gama de tipos de memoria.

La propuesta de que la actividad automantenida de PKMζ sea el sustrato del mantenimiento de la memoria consolidada representa un hito en la investigación sobre los mecanismos moleculares de la memoria. Aunque existe una larga tradición de investigación que ha identificado múltiples mecanismos y procesos moleculares implicados en la adquisición y primeras fases de consolidación de diversos tipos de memoria (Baddeley, et al., 2000; Cahill, et al., 2001; McGaugh, 2007), no ha sido hasta hace cinco años (Pastalkova, et al., 2006) cuando se ha vislumbrado un mecanismo molecular del mantenimiento a largo plazo. Los resultados presentados en esta tesis forman parte de un esfuerzo llevado a cabo por la comunidad científica para confirmar la generalidad y alcance

de dicho mecanismo. Se trata, por tanto, de un campo que muestra gran actividad y con importantes repercusiones clínicas en el futuro. En este sentido, se han obtenido datos en modelos animales que sugieren su posible aplicación al borrado de recuerdos post-traumáticos (Cohen, et al., 2010), siendo ésta sólo una de las aplicaciones que se atisban.

Por otro lado, la Parte II de esta tesis aplica un nivel de análisis de sistemas con el fin de explorar conexiones del circuito cerebral responsable del aprendizaje aversivo gustativo con circuitos generales de memoria así como establecer comparaciones con otro tipo de memorias de reconocimiento. En el Capítulo 3 se lleva a cabo una revisión de los cambios que se producen durante el envejecimiento en el aprendizaje aversivo. A su vez se emplea un paradigma de aprendizaje complejo aversivo gustativo, en concreto la dependencia temporal de la inhibición latente, para investigar la implicación de otras estructuras cerebrales en el circuito básico conocido.

Los resultados de este capítulo muestran como el envejecimiento ofrece una privilegiada oportunidad de investigar la plasticidad de los circuitos cerebrales responsables del aprendizaje y la memoria. Los cambios que se producen a lo largo de toda la vida, ya sean producto del aprendizaje, experiencia, debidos al deterioro o fallo de algunos sistemas y/o a la adaptación en pos de compensar dichos déficits, ofrecen una ocasión ideal para estudiar los procesos de plasticidad cerebral (revisiones en Burke & Barnes, 2006; Erickson & Barnes, 2003; Kelly, et al., 2006). También hacen del envejecimiento un periodo especialmente complicado de estudiar por la inmensa variabilidad entre los sujetos. El aprendizaje aversivo gustativo muestra una serie de importantes ventajas a la hora de estudiar el envejecimiento como es que se pueda forman en un único ensayo, que tenga bajos requisitos sensoriales y motores y que la tarea básica no se ve perjudicada por el envejecimiento (Manrique, Moron, et al., 2009; Manrique, et al., 2007). A su vez, el circuito cerebral básico de este tipo de aprendizaje se conoce bastante bien (Bermudez-Rattoni, Nunez-Jaramillo, & Balderas, 2005; Bures, et al., 1998; Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994) pero dicha tarea básica permite su manipulación con el fin de hacer partícipes de otros procesos y

sistemas neurales superiores. Datos previos han demostrado la implicación del hipocampo en fenómenos complejos del aprendizaje gustativo como son el bloqueo (Gallo & Candido, 1995a; Moron, et al., 2001) y la dependencia temporal de la inhibición latente (Manrique, Gamiz, et al., 2009; Manrique, et al., 2004; Manrique, Moron, et al., 2009; Molero, et al., 2005). A pesar de estas diferencias individuales existe un amplio acuerdo de que el sistema hipocampal es uno de los primeros circuitos en mostrar problemas durante el envejecimiento (Lister & Barnes, 2009; Ward, Stoelzel, & Markus, 1999). Los datos presentados en el Capítulo 3 ahondan en este tema y muestran que la lesión neurotóxica del hipocampo dorsal que afecta al procesamiento del contexto temporal en ratas adultas, altera el efecto del cambio temporal en el fenómeno de inhibición latente durante el envejecimiento, sin ser equivalente el efecto del daño hipocampal al presumible deterioro de la función hipocampal encontrado en estas ratas envejecidas. Así mismo, en función de los datos presentados, es posible proponer complejas interacciones competitivas entre el Hipocampo y áreas del circuito básico aversivo gustativo, ya que la inactivación del Hipocampo dorsal durante la fase de condicionamiento del CTA en ratas envejecidas muestra efectos del cambio de contexto que habían desaparecido a edades avanzadas. Otro de los resultados destacados del Capítulo 3 es el efecto beneficioso de las experiencias de aprendizaje previas sobre la posterior ejecución en una tarea de inhibición latente del aprendizaje aversivo con cambio contextual en ratas envejecidas. Ratas envejecidas naïve que previamente habían demostrado ser insensibles al cambio de contexto durante un procedimiento de inhibición latente de la aversión condicionada, después de ser sometidas a diferentes tareas de memoria de reconocimiento gustativa y visual recuperaron la capacidad de asociar el contexto temporal con las señales gustativas, y mostraron capacidades similares a las ratas adultas (Manrique, et al., 2004). Los resultados vienen a sumarse a datos previos que muestran los efectos beneficiosos de diferentes experiencias vitales sobre el envejecimiento. Uno de los efectos más estudiados a nivel experimental es el de la exposición a un ambiente enriquecido. El ambiente enriquecido es un modelo experimental en que los animales son mantenidos en sus jaulas en unas condiciones especiales que favorecen las interacciones sociales y la estimulación sensorial y motora (Rosenzweig & Bennett, 1996; Segovia, et al., 2009; van Praag,

Kempermann, & Gage, 2000), lo que provoca importantes mejoras en aprendizaje y memoria y retrasa en muchos casos los efectos del envejecimiento o enfermedad (Mattson & Magnus, 2006; Nithianantharajah & Hannan, 2006; van Praag, et al., 2000). La principal diferencia con la intervención aplicada en esta tesis radica en la naturaleza discreta de la experiencia a que han sido sometidos los animales. Sin embargo, ambos tipos de estudios ponen de manifiesto la importancia de la historia previa a la hora de estudiar los efectos del envejecimiento en tareas de aprendizaje y memoria. Así el hecho de que los animales de experimentación sean naïve o reutilizados y el tipo de experiencias por la que han pasado puede ser un factor fundamental a la hora caracterizar, tanto conductualmente como a nivel cerebral, el envejecimiento. Sin embargo este hecho suele recibir poca atención y los investigadores tienden a utilizar ratas naïve como norma. Aunque esta circunstancia puede ser una buena manera de controlar la influencia de las experiencias previas, lo cierto es que ratas envejecidas que han pasado toda su vida aisladas en ambientes con una estimulación claramente deficiente difícilmente puede ser buenos modelos ecológicos del envejecimiento normal humano, como ya han apuntado otros autores (Paolisso, et al., 2000; van der Staay, 2002).

Por último el Capítulo 4 se dedica a estudiar el efecto del envejecimiento en memoria de reconocimiento visual empleando una tarea de reconocimiento de objetos espontánea. Entre los principales hallazgos de este trabajo puede destacarse en primer lugar el haber explorado la curva de olvido de las ratas envejecidas. Los resultados demuestran ausencia de deterioro en las ratas envejecidas (24 meses de edad) para reconocer el objeto nuevo con demoras desde 10 segundos hasta una hora. Sin embargo las deficiencias se pusieron de manifiesto al introducir intervalos de retención de 24 horas. Estos resultados son coherentes con los obtenidos por otros autores (de Lima, et al., 2005; Leite, et al., 2011; Pieta Dias, et al., 2007), aunque existe cierta controversia sobre los déficits que se producen durante el envejecimiento en este tipo de tarea. Como se discute en el Capítulo 4, posiblemente son las diferencias procedimentales las que puedan marcar la diferencia entre preservación y deterioro. En este capítulo también se aportan datos que demuestran la importancia del tipo de objeto empleado en la ejecución de la tarea de reconocimiento de objetos. Aunque existe algunos estudios

que evalúan el efecto de la complejidad y ambigüedad de los objetos en ratas adultas (Bartko, et al., 2010; Bartko, et al., 2007b; Norman & Eacott, 2004), hasta ahora no se había explorado el papel de las características del objeto en ratas envejecidas. El procedimiento aplicado en esta tesis permite disociar entre el procesamiento de objetos simples pero ambiguos y objetos de alta complejidad pero claramente diferentes. Las ratas envejecidas solo mostraron una clara incapacidad para reconocer los objetos de alta complejidad, así demostrando que más que la ambigüedad o similitud entre los objetos, es la discriminación entre estímulos de alta complejidad, es decir los procesos de integración de numerosas características, la que se ve perjudicada en la vejez. A pesar de que, como se ha apuntado anteriormente, el sistema hipocampal es una de las principales estructuras que muestran déficits durante el envejecimiento, existe una amplia controversia sobre qué estructuras cerebrales son las responsables del reconocimiento de objetos. De hecho parte de los autores que han manipulado el grado de complejidad de los objetos han encontrado claros déficits en los animales con lesión en la corteza perirhinal (Bartko, et al., 2010; Bartko, et al., 2007b; Bussey, et al., 2002; Norman & Eacott, 2004). Estos datos sugieren que durante el envejecimiento también puede verse comprometida la actividad de la corteza perirhinal, afectando a los procesos de integración y reconocimiento visual, aunque no se puede descartar que el deterioro en otros circuitos cerebrales también influya en el desempeño en este tipo de tarea.

El estudio de las complejas interacciones entre los sistemas neurales implicados en la memoria de reconocimiento, incluyendo hipocampo, corteza perirhinal, corteza insular y amígdala, puede enriquecerse de la investigación sobre los cambios plásticos que acompañan al envejecimiento. A su vez, el estudio del envejecimiento puede verse beneficiado por una aproximación múltiple desde el nivel molecular hasta el de sistemas que tenga en cuenta las complejas interrelaciones entre circuitos neurales y el efecto de experiencias discretas de aprendizaje como de las complejas vivencias que se suceden en el transcurso de toda una vida.

CONCLUSIONES

1. La actividad de la proteína quinasa atípica PKMζ en la Amígdala basolateral 24 horas después de la adquisición es necesaria para el mantenimiento de la memoria a largo plazo inducida mediante una tarea de evitación activa de salto que no requiere procesamiento espacial. Ello confirma el papel crítico de PKMζ en el mantenimiento de una diversidad de memorias, independientemente del circuito neural implicado.

2. La actividad de la proteína quinasa atípica PKMζ en la Amígdala facilita la adquisición de aversiones gustativas condicionadas durante el intervalo entre el sabor y el malestar gastrointestinal, pero no parece jugar ningún papel durante el procesamiento del estímulo gustativo ni durante las primeras fases de consolidación. Esto apoya la existencia de mecanismos moleculares compartidos en distintas fases de los procesos de adquisición y mantenimiento de la memoria gustativa.

3. Las ratas de edades avanzadas no muestran modulación de la memoria gustativa por parte del contexto temporal. No se observan efectos del cambio de la hora del día durante el condicionamiento sobre la inhibición latente del aprendizaje gustativo aversivo.

4. Las experiencias de aprendizaje discretas pueden reactivar las funciones hipocampales relevantes para la modulación de la memoria gustativa inducida por la hora del día que resultan deterioradas por el envejecimiento, incluso cuando se apliquen a edades avanzadas.

5. La inactivación reversible de la actividad del Hipocampo dorsal mediante infusiones bilaterales de TTX durante el condicionamiento, pero no durante las pruebas de retención, invierte el efecto del cambio de hora del día en ratas envejecidas previamente entrenadas poniendo de manifiesto la dependencia del contexto temporal de la aversión gustativa condicionada. Ello sugiere la existencia de interacciones competitivas entre el sistema hipocampal y el circuito responsable de la memoria gustativa a edades avanzadas.

6. Durante el envejecimiento la curva del olvido se adelanta con respecto al adulto en la tarea de reconocimiento de objetos espontánea empleando estímulos elementales claramente diferenciados que implican discriminaciones con bajos niveles de complejidad y ambigüedad. Aunque las ratas envejecidas muestran un buen recuerdo con demoras desde pocos segundos hasta una hora, son incapaces de distinguir un objeto cotidiano familiar de uno novedoso a las 24 horas.

7. El envejecimiento deteriora selectivamente la memoria visual con intervalos de retención de 1 hora en una tarea de memoria de reconocimiento cuando se utilizan objetos de una alta complejidad, pero no es afectada si se utilizan objetos simples aunque tengan una alta similitud entre ellos.

CONCLUSIONS

1. The activity of the atypical protein kinase PKMζ in the basolateral Amygdala 24 h. after acquisition is required for maintaining long-term memory acquired in a jumping active avoidance tasks without spatial requirements.

2. The activity of the atypical protein kinase PKMζ in the basolateral Amygdala facilitates the acquisition of learned taste aversions during the taste-illness interval but it does not seem to play any role either for taste processing or the early consolidation phases. These results support shared molecular mechanisms by various processes involved in acquisition and retention of taste memories.

3. Taste memory in aged rats is not modulated by the temporal context. There are not effects of a time-of-day shift during conditioning on the latent inhibition phenomenon in taste aversion learning.

4. Discrete learning experiences can reactivate hippocampal functions impaired by aging that are involved in the modulation of taste memories by the time of day, even though they were applied at advanced ages.

5. Reversible inactivation of dorsal Hippocampus by bilateral TTX infusions during conditioning, but not during retrieval tests, reverse the effect of a time-of-day shift in aged rats previously trained, thus indicating the emergence of the temporal dependency of conditioned taste aversions. These results suggest

preserved competitive interaction between the aged Hippocampus and taste memory neural circuit.

6. During aging the forgetting curve comes forward in the spontaneous object recognition task with respect to adults if elemental stimuli that are clearly different and require low level of complexity and ambiguity discriminations are used. Although aged rats show a good memory with delays of a few seconds to an hour, they are unable to perform satisfactorily the recognition at 24 hours using everyday objects.

7. The aging process selectively impairs visual object recognition memory at 1 hour retention intervals when using highly complex objects, but no effect is found with simple but high-similarity objects.

PERSPECTIVAS

Los resultados incluidos en la presente tesis abren nuevas posibilidades para la investigación futura que pueden resumirse en los siguientes puntos:

1. Continuar explorando la generalidad del mecanismo de mantenimiento de la memoria mediado por la actividad de PKMζ en memorias visuales y gustativas con diversos grados de complejidad y a lo largo del curso temporal de los procesos de consolidación y mantenimiento implicados.

2. Examinar los efectos de la inactivación reversible de PKMζ en el circuito responsable de la memoria gustativa mediante la combinación de infusiones i.c. de ZIP en Corteza Insular y Amygdala con técnicas de identificación de la actividad en el resto de las áreas.

3. Investigar la evolución dependiente de la edad del mecanismo mnésico mediado por PKMζ.

4. Identificar patrones peculiares de organización de los circuitos responsables de la memoria durante el envejecimiento, así como los mecanismos involucrados en la reactivación de funciones hipocampales por parte de la experiencia de aprendizaje discreta.

5. Evaluar características críticas de las tareas de memoria empleadas en roedores para la identificación del efecto de envejecimiento que permitan avanzar en el desarrollo de baterías de pruebas conductuales de aplicación sencilla y sistemática que faciliten la comparación entre los resultados obtenidos por diversos laboratorios.

6. Investigar los circuitos cerebrales compartidos de diferentes memorias de reconocimiento tanto a nivel molecular como sistémico, con el objetivo de conocer y comprender mejor los complejos mecanismos neurales responsables del aprendizaje y la memoria.

PERSPECTIVES

The results reported in the present thesis open new perspectives in the research field being the following representative issues:

1. To continue the assessment of the generality of the memory mechanisms requiring PKMζ activity in visual and taste memories involving various complexity levels along the consolidation and maintenance time course.

2. To examine the effect of the reversible PKMζ inactivation in the neural circuit involved in taste memory by combining i.c. infusions of ZIP in Insular Cortex and Amygdala and techniques allowing the identification of neuronal activity in the rest of the areas.

3. To investigate the age-dependent evolution of the memory mechanism requiring PKMζ.

4. To identify age-related peculiar patterns of the memory neural circuits' organization and the mechanisms involved in the hippocampal functions reactivation induced by discrete learning experiences.

5. To assess critical features of the memory tasks applied to rodents in order to develop easy and systematic tests batteries for facilitating comparisons between results obtained by different laboratories.

6. To investigate s shared brain circuits of different types of recognition memory applying several levels of analysis from the molecular to the systems levels. In order to gain a better knowledge and understanding of the complex neural mechanisms responsible for learning and memory.

PUBLICACIONES RELACIONADAS

Manrique, T., Gámiz, F., Morón, I., Ballesteros, M.A., Gallo, M. 2009. Peculiar modulation of taste aversion learning by the time of day in developing rats. *Dev. Psychobiol* **51**(2) 147- 157.

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ÍNDICE DE ABREVIATURAS

AM: Amígdala.

AMbl: Amígdala Basolateral.

AP: Area Postrema.

b.w.: Body weight.

BLA: Basolateral Amygdala.

CARs: Consecutive avoidance responses.

CI: Corteza Insular .

CS: Conditioned stimulus.

CTA: Condicionamiento aversivo gustativo (Conditioned Taste Aversion).

DIFF: Grupo experimental sometido a un cambio de hora del día entre la preexposición y el condicionamiento de retención en un procedimiento de aprendizaje aversivo.

DR: Discrimination Ratio (razón de discriminación: N-F/N+F).

EC: Estímulo Condicionado.

EGM: Electrogustometría.

EI: Estímulo Incondicionado.

F: Exploración del objeto familiar.

g: Gauge (calibre).

gr: Gramos.

HC: Hipocampo.

HCd: Hipocampo dorsal.

i.c.: Intra-cerebral (inyección).

i.d.: Inner Diameter.

i.p.: Intra-peritoneal (inyección).

IC: Insular Cortex.

kg: Kilogram.

LiCl: Cloruro de Litio (Lithium Cloride)

LTP: Potenciación al Largo Plazo (Long Term Potentiation).

µl: Microliters

M: Molar

mg: Miligram.

N: Exploración del objeto nuevo.

NaCl: Cloruro sódico (sal).

NFI: Novel-Familiar Index (índice nuevo-familiar: N-F).

nmol: Nanomolar.

NTS: Núcleo del Tracto Solitario.

o.d.: Outer Diameter.

PB: Parabraquial nucleus.

PBN: Núcleo ParaBraquial.

PER: Perirhinal Cortex.

PKC: Proteína Quinasa C.

PKMζ: Proteína Quinasa Mζ.

PMFC: Perceptual-mnemonic feature conjunctive model.

RI: Recognition Index (índice de reconocimiento: N/N+F).

SAME: Grupo experimental sometido a preexposición, condicionamiento y prueba de retención a la misma hora del día en un procedimiento de aprendizaje aversivo gustativo.

SCR: Scrambled ZIP.

SOR: Spontaneous object recognition.

TTX: Tetrodotoxina.

US: Unconditioned stimulus.

VL: Ventrículo Lateral.

ZIP: Péptido sintético Inhibidor de PKMζ.

ÍNDICE DE FIGURAS Y TABLAS

CAPÍTULO 3

CAPÍTULO 4