UNIVERSIDAD DE GRANADA

Programa de Doctorado: PSICOLOGÍA

TESIS

EFECTOS DE LA COLINA DIETARIA EN MEMORIA Y ATENCIÓN

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Editor: Editorial de la Universidad de Granada
Autor: Hayarelis Coromoto Moreno Gudiño
D.L.: GR 910-2014
ISBN: 978-84-9028-945-7

DEPARTAMENTO DE PSICOLOGÍA EXPERIMENTAL

DEPARTAMENTO DE PSICOBIOLOGÍA

UNIVERSIDAD DE GRANADA

INSTITUTO DE NEUROCIENCIAS "FEDERICO OLÓRIZ"

TESIS DOCTORAL

EFECTOS DE LA COLINA DIETARIA EN MEMORIA Y ATENCIÓN

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Memoria presentada para optar por el título de doctor por la Universidad de Granada

Línea de Investigación: Aprendizaje/Psicobiología del Aprendizaje y la Memoria

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Que el trabajo de investigación titulado: EFECTOS DE LA COLINA DIETARIA EN MEMORIA Y ATENCIÓN ha sido realizado por Dña. Hayarelis Coromoto Moreno Gudiño para optar al grado de Doctor Internacional de Psicología en el Programa de Doctorado de Psicología con mención de Excelencia 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.

Y para que conste donde proceda se firma este certificado en Granada a 19 de Noviembre de 2013.

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Fdo. Milagros Gallo Torre

Fdo. Hayarelis Moreno Gudiño

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Effect Of Dietary Choline On Memory and Attention

FINANCIACION

"*Choline dietary supplementation improves LiCl-induced context aversion retention in adult rats*". Financiado con fondos de PSI2008-03933, PSI2009-07513, PSI2011-23702 (MINECO, España); con fondos de HUM 02763 (Junta de Andalucía. España); y parcialmente con fondos de FEDER.

"*Long-lasting effects of prenatal dietary choline availability on object recognition memory ability in adult rats*". Financiado con fondos de PSI2009-07513, PSI2011-23702, PSI2012-31641 (MINECO, España); con fondos de HUM 02763 (Junta de Andalucía. España) y parcialmente con fondos de FEDER.

"*Chronic dietary choline supplementation modulates attentional change in adult rats*". Financiado con fondos de PSI2009-07513, PSI2012-31641 (MINECO, España); con fondos de HUM 02763 (Junta de Andalucía. España) y parcialmente con fondos de FEDER.

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RESUMEN

RESUMEN

La Colina (Ch), una amina cuaternaria y clasificada como una de las vitaminas del complejo B, es el precursor del neurotransmisor acetilcolina (ACh). Aunque puede ser sintetizada por el hígado, la Ch debe ser consumida a través de la dieta para el mantenimiento de la salud en humanos y animales. Algunas funciones del sistema colinérgico, incluyendo aprendizaje y memoria, pueden ser moduladas por la disponibilidad de la vitamina. Así, el incremento o la disminución del precursor de la ACh puede facilitar o inducir un deterioro en funciones cognitivas dependientes del sistema colinérgico, respectivamente. Diversos estudios han demostrado que la disponibilidad de Ch afecta el desarrollo del sistema nervioso central durante los períodos prenatal y postnatal temprano afectando funciones cognitivas que pueden permanecen durante la vida del individuo. Particularmente, mientras la mayor disponibilidad de esta vitamina mejora ciertas funciones cognitiva, el déficit provoca un deterioro en el desempeño. Estos hallazgos y la pérdida de neuronas colinérgicas observada en pacientes con demencia senil y Alzheimer estimularon investigaciones para evaluar el uso de la vitamina como tratamiento terapéutico. Sin embargo, son escasos los trabajos dirigidos a estudiar el efecto de este nutriente como facilitador cognitivo en sujetos adultos sanos. El objetivo del presente trabajo de investigación es determinar el efecto de la disponibilidad de Ch dietaria en la memoria y atención en ratas adultas.

En el Capítulo 2, se investigó el efecto de la suplementación con Ch en ratas adultas utilizando una tarea de aversión contextual. Ratas de 3-4 meses de edad alimentadas con una dieta suplementada o estándar con Ch durante 7 semanas fueron entrenadas en una tarea de aversión dependiente del contexto. Los resultados mostraron

que los animales suplementados mejoraron la retención del contexto aversivo al implementar dos intervalos de demora (3 y 15 días) después del condicionamiento.

Los experimentos del Capítulo 3, avaluaron el efecto de la disponibilidad de colina dietaria en ratas gestantes (Estudio 1) y adultas (Estudio 2) en un paradigma de reconocimiento de objetos. Con el Estudio 1 se pretendía reproducir el efecto de la manipulación de colina dietaria prenatal en funciones cognitivas observados en investigaciones previas. Sin embargo, en este estudio se utilizó una tarea que no había sido evaluada antes, la tarea de reconocimiento de objetos espontánea (SOR). Debido a que la memoria de reconocimiento es comúnmente deteriorado durante la vejez, tareas como estas son ampliamente utilizadas para investigar déficit cognitivo senil. En este experimento, tres grupo de ratas gestantes fueron alimentadas desde el día E12 hasta E18 con una dieta deficiente (0 g/Kg cloruro de colina), estándar (1.1 g/Kg cloruro de colina) o suplementada (5 g/Kg de colina). Los descendientes fueron criados por madres adoptivas alimentadas con la dieta estándar durante la gestación, y probados en SOR con dos tiempos de retención (24 y 48 h) cuando alcanzaron la edad adulta. Todos los animales de los grupos suplementado y estándar reconocieron el objeto cuando fueron probados en el intervalo de retención corto (24 h). Sin embargo, se observó un efecto mejorador en la memoria de reconocimiento en los sujetos suplementados en la prueba de retención larga (48h), mientras que los animales alimentados con la dieta deficiente fallaron en la prueba de reconocimiento en este intervalo de demora.

En el Estudio 2 (Experimentos 1 y 2) se exploró el efecto de la colina dietaria en ratas adultas de 6-7 meses de edad. Con los experimentos se intentaba verificar si el efecto de suplementación en SOR reflejado durante el período sensible del desarrollo

(Estudio 1) se observa en edades adultas. En el Experimento 1, dos grupos dietarios, suplementado y estándar, fueron probados en el mismo procedimiento conductual (intra-grupo) con dos intervalos de demora (24 y 48 h) utilizado en el Estudio 1. Los resultados no arrojaron diferencia entre los grupos en ninguno de los tiempos de retención. Los animales suplementados y estándar reconocieron el objeto familiar en la retención corta (24 h) y larga (48 h), sin que se encontraran diferencias entre ellos. En el Experimento 2 se utilizó un diseño entre-grupo para avaluar si la repetida exposición al objeto familiar (1 en la fase de adquisición y 2 en las prueba de 24 y 48 h) implicado en el diseño intra-grupo pudiera haber facilitado la representación del objeto familiar y, consecuentemente su reconocimiento. Para probar esto, ratas adultas de 7 meses de edad fueron manipuladas con el mismo tratamiento dietario y experimento conductual utilizado en el Experimento 1 pero aplicando un diseño entre-grupo. Así, un grupo de animales suplementados (n=16) y no suplementados (n=16) fueron probados en un tiempo de retención corto (24 h, 8 suplementado y 8 estándar) o largo (48 h, 8 suplementado y 8 estándar) en la tara SOR. Con este diseño se pretende medir la capacidad de los animales para retener la representación del objeto familiar después de una única exposición (fase adquisición) y poder reconocerlo durante la prueba, 24 ó 48 h posterior a la adquisició. Ambos grupos dietarios, suplementado y estándar, reconocieron el objeto familiar a las 24 h de retención. Sin embargo, sólo el grupo suplementado reconoció el objeto familiar a las 48 de retención. Los resultados sugieren que, en ratas adultas la Ch mejora la retención cuando la demanda de memoria es incrementa.

Una vez demostrado que la suplementación con colina en la edad adulta afecta la memoria en algunas tareas cognitivas, el objetivo de los experimentos realizados en el

Capítulo 4 fue determinar si procesos antencionales dependientes del sistema colinérgico son sensibles a la disponibilidad de Ch. En los experimentos, ratas adultas (7 meses) recibieron una dieta crónica suplementada con colina o estándar. Los paradigmas utilizados es estos estudios miden la normal pérdida de atención a un CS que predice de forma segura su consecuencia, bien sea ésta una ausencia de consecuencias (Inhibición latente) o una consecuencia determinada (Transfer negativo). Esta disminución en atención trae como consecuencia una menor asociabilidad del estímulo y por tanto un retraso en un aprendizaje posterior sobre el CS pre-expuesto. Los resultados en ambos experimentos reflejaron que, mientras los animales estándar mostraron el retraso esperado en aprendizaje durante el condicionamiento posterior, los sujetos suplementados aprendieron fácilmente una segunda asociación. Estas observaciones indican que la suplementación con Ch afecta los procesos responsables de la normal disminución de aprendizaje de estímulos que han sido preexpuestos.

Juntos, los resultados del trabajo de investigación estarían sugiriendo que la suplementación con Ch en edades adultas afecta funciones cognitivas dependientes del sistema colinérgico.

ABSTRACT

ABSTRACT

Choline (Ch), a quaternary amine classified within the vitamin B complex, it is the precursor to the neurotransmitter acetylcholine (ACh). Although Ch can be produced by the liver, it must be consumed through the diet to maintain health in humans and animals. Therefore, some functions of the cholinergic systems, including those related to learning and memory, may be modulated by increased or deficient dietary choline availability either inducing improvements or deficits, respectively. Particularly, previous studies have demonstrated that choline is an essential nutrient during prenatal and early postnatal developmental periods. Thus, the availability of choline during these periods produces some beneficial effects on cholinergic system-dependent learning and memory in rats. However, research on the effect of adult choline supplementation on learning and memory abilities is scarce. The main purpose of the present PhD research was to determine the effect of the dietary Ch availability during prenatal and adulthood periods on memory and attention in rats.

In Chapter 2 was aimed to investigate the effect of the dietary choline in adult rats implementing a task considered of a high level of complexity as it is the paradigm of contextual aversion. Thus, in the present study, 3-4 month-old rats receiving for 7 weeks choline-supplemented diet and control rats receiving a standard diet were trained in a LiCl-induced contextual aversion task. Short and long-term context aversion retention was enhanced by dietary choline supplementation in adult rats.

The experiments of the Chapter 2, assessed the effect of the dietary choline in prenatal (Experiment 1) and adult (Experiments 2 and 3) rats on object recognition memory. The Experiment 1 seeks to replicate the effect of the prenatal choline dietary

manipulation on cognitive processes in adult rats according to the outcomes observed in previous investigations. However, this cited manipulation incorporates a task that had not been evaluated before, such as the memory of recognition of objects (SOR). The deterioration of the recognition memory commonly has been observed in senile subjects, that is why these kinds of tests are widely used to investigate dependent cognitive deficit according to the aging. In this experiment, three groups of pregnant rats were fed from E12 to E18 with choline-deficient (0 g/Kg choline chloride), standard (1.1 g/kg choline chloride) or choline supplemented (5 g/kg choline chloride) diets. The offspring was cross-fostered to rat dams fed a standard diet during pregnancy and tested at the age of 3 months in an object recognition memory task applying retention tests 24 and 48 hours after acquisition. The results indicated a long-lasting beneficial effect of prenatal choline supplementation on object recognition memory at longer retention intervals, which was evident when the rats reached adulthood. On Experiment 2 and 3, the effect of dietary choline on recognition memory (SOR) was evaluated in rats around 7 months of age. These experiments attempt to verify if the effect observed during sensitive periods of the development (Experiment 1) it is observed in adult ages. In Experiment 2, an experimental intra-subjects design was used, so that 16 rats (8 supplemented and 8 controls) were trained in a SOR task with delays of 24 h. and 48 h. The results did not show significant differences between the supplemented rats and the control ones. Both groups recognized the familiar object at 24 h. as well as 48 h. The Experiment 2 was designed to evaluate if the repeated pre-exhibition to the familiar object during both tests of retention (24 h and 48 h) used in the design intra-subjects influences in the memory of recognition of this familiar object. For it, a inter-subjects design was used where 32 adult rats (7 months of age, n = 16 supplemented and n = 16 standard) were given the same dietary treatment and cognitive task applied in

Experiment 1. Each dietary group was subdivided in turn into two groups (n= 8 each one). A first group (8 supplemented and 8 controls) was proven with a retention time of 24 h. whereas the second group was exposed to a retention test of 48 h. No significant differences have found in the performance of the two groups (supplemented and control) during the first retention test. Nevertheless, during the retention of 48 h, only the supplemented group recognized the familiar object. By this way, the result of SOR indicates that the intra-subjects design is an artifact. Being left in evidence an effect "enhancer" of the dietary choline in the long term memory in adult rats.

Once verified that the suplementación with choline in adult ages affects mnemonic functions dependent of the system colinérgico, the aim of the Experiments 1 and 2 (Chapter 4) was to determine if other tasks (e.g. Attention) depending on the colinérgico system are affected by the dietary choline also. In two experiments, adult rats of 8 months age were maintained on a diet enriched with added choline for 12 weeks prior to behavioural testing; control rats remained on the standard diet during this time. In both experiments were decided to examine training procedures that are effective in producing decrements in stimulus associability in normal animals. In Experiment 1 all rats received training in the Hall-Pearce negative transfer paradigm in which prior training with a conditioned stimulus (CS) paired with a small reinforcer retards further learning when the size of the reinforcer is increased. The Experiment 2 investigated the effect of prior non-reinforced exposure of the to-be-CS (latent inhibition). Both experiments produced results suggesting that choline supplementation modifies the processes responsible for learned changes in attention to significant environmental stimuli – specifically that the mechanism responsible for reducing the associability of stimuli in certain circumstances fails to operate normally after choline supplementation.

CAPÍTULO 1

INTRODUCCIÓN

CAPITULO 1: INTRODUCCIÓN

1. La Colina: Estructura y Metabolismo

La colina (Ch) un amonio cuaternario compuesto (2-hidroxietil-trimetil-amonio, Figura 1), clasificado como una de las vitaminas del complejo B, fue reconocida como un nutriente esencial para humanos por el Instituto de Medicina (Academia Nacional de Ciencia, Estados Unidos**,** 1998). La Ch está presente en muchos alimentos, principalmente como molécula libre o fosfátidos en forma de lecitina.

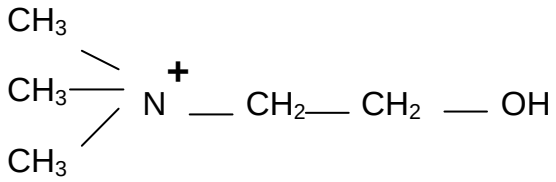

Figura 1. Estructura de la Colina

Endógenamente la Ch puede ser obtenida principalmente a través de la biosíntesis “de novo” a partir de la metilación de la fosfatidiletanolamina (PE). Esta reacción es catalizada por la enzima fosfatidiletanolamina N-metiltransferasa (PEMT) para formar fosfatidilcolina (PC) (Bremer & Greenberg, 1961) y finalmente, acoplada con el catabolismo de la PC por la acción de fosfolipasas, la Ch es liberada. Se ha determinado que esta ruta utilizada por el hígado, representa cerca del 15% del requerimiento de Ch en humanos, el resto es suplido principalmente por los alimentos (Zeisel, 1981). En menores cantidades, la Ch también puede obtenerse a partir de su liberación durante la biosíntesis de fosfatidilserina catalizada por la enzima

fosfatidilserina sintetasa-1 (Vance, 2002). Adicionalmente, el catabolismo de la esfingomielina y ACh también pueden generar Ch. Por su parte, la Ch dietaria la cual está presente en los alimentos principalmente en forma de lecitina, es absorbida por el intestino para ser hidrolizada a lisofosfatidilcolina por la enzima pancreática, fosfolipasa, y de esta forma es capturada a través de transportadores (Li & Vance, 2008) para entrar a circulación portal hasta los órganos. Una vez en el interior celular, ej. hígado (el principal destino de la Ch) y riñón, la vitamina puede ser fosforilada para producir PC, oxidada a betaína a través de una reacción irreversible para formar parte del metabolismo de los mono-carbonos o acetilada para la síntesis del neurotransmisor acetilcolina (ACh) en el cerebro. La Ch, tanto exógena como endógena, en forma de PC representa el 95% del total del pool de Ch en el tejido animal. El 5% restante incluye Ch libre, fosfocolina, glicerolfosfocolina, CDP-colina y ACh (Zeisel & Blusztajn, 1994**,** Zeisel et al., 2003). En el hígado, el 95% de la PC es dirigida a secreciones biliares, de la cual sólo el 40% es retornado a este órgano (Robins, 1975). De esta manera, la Ch y sus metabolitos son balanceados por dos rutas de adquisición de Ch (ingesta de Ch en la dieta y su síntesis por la ruta PEMT) y dos rutas de depleción (oxidación de la Ch y la secreción biliar) (Li & Vance, 2008).

2. Sistema Colinérgico Central en Procesos Cognitivos

Extensivas evidencias confirman que mecanismos colinérgicos modulan diversas funciones cognitivas, los cuales, se pueden reflejar en el desempeño de tareas asociadas a dichos sistemas (Gold, 2003). El sistema colinérgico en el cerebro, se distribuye en una variedad de diferentes núcleos pero, la fuente más importante de ACh se encuentra en el prosencéfalo basal (PB). El PB es una región localizada en la base del cerebro, en posición anterior al hipotálamo y ventral a los ganglios basales. A nivel frontal, limita

con el tubérculo olfatorio y el núcleo accumbens (NAc), mientras que lateralmente lo hace con el complejo amigdaloide y la corteza piriforme (Détári et al., 1999; Semba, 2000). A pesar de ser relativamente escazas en el cerebro, las neuronas colinérgicas del PB inervan la mayor parte de éste agrupándose en núcleos cercanos los cuales comprende el núcleo septal medial (SM) y banda diagonal vertical de Broca (BVB) comúnmente llamada ruta septohipocampal, estos núcleos proyectan al hipocampo; la banda diagonal horizontal de Broca (BHB) se dirige al bulbo olfatorio y finalmente, el núcleo basal magnocelular (NBM), inerva la amígdala y al córtex cerebral (Bigl et al., 1982; Struble et al., 1986; Butcher & Woolf, 2003).

La ACh, neurotransmisor sintetizado por las neuronas colinérgicas, además de ser fundamental en el sistema nervioso periférico, es un neuromodulador que facilita diversas funciones cognitivas incluyendo atención, aprendizaje y memoria y control motor a través de sus proyecciones en el PB. Numerosos estudios en modelos animales usando ratas como sujetos, han permitido el desarrollo de novedosas técnicas que permiten determinar la modulación de regiones colinérgicas en funciones cognitivas específicas. Así el refinamiento de diversos test de comportamiento, toxinas selectivas para lesiones colinérgicas (por ejemplo, la colinotoxina saporina-192 IgG), determinación de liberación de ACh en vivo durante el desempeño de una tarea a través de estudios con microdiálisis, modificaciones genéticas y farmacológicas de la transmisión colinérgica con antagonistas muscarínicos o nicotínicos, han hecho posible conocer en más detalles la implicación del sistema colinérgico de PB en el funcionamiento cognitivo. Algunos estudios sugieren que la naturaleza del efecto en la transmisión colinérgica es dependiente del área del cerebro, de la tarea conductual y del subtipo del receptor afectado. Así, infusiones antagonistas colinérgicos administradas directamente en el hipocampo provocan un deterioro significativo de la memoria en

tareas dependientes de hipocampo como la habituación a ambientes nuevos y evitación inhibitoria (Izquierdo et al., 1992); memoria de trabajo espacial (Felix & Levin, 1997); condicionamiento de miedo contextual (Wallenstein & Vago, 2001). Asimismo, diversos paradigmas de aprendizaje han encontrado un incremento de la ACh hipocampal durante el condicionamiento palpebral (Meyer et al., 1997), condicionamiento operante (Orsetti et al., 1996), entrenamiento de discriminación visual (Yamamuro et al., 1995), en el desempeño de tareas de memoria de trabajo (Fadda et al., 1996), durante la ejecución de memoria espacial (Stancampiano et al., 1999), condicionamiento de miedo al contexto (Nail-Boucherie et al., 2000), en la presentación de estímulos y ambientes nuevos (Inglis & Fibiger, 1995). Igualmente, otros estudios muestran la participación de la ACh en el procesamiento de estímulos. Las investigaciones señalan principalmente al sistema colinérgico cortical en la modulación de la atención. En efecto, lesiones colinérgicas en el cortex prefrontal incrementa la sensibilidad a estímulos distractores en una tarea de atención sostenida (Newman & McGaughy, 2008), mientras que lesiones en el córtex parietal posterior disminuye la atención en una tarea de aprendizaje asociativo (Bucci et al., 1998). Lesiones selectivas en neuronas colinérgicas en el NBM disminuye el incremento normal de atención que ocurre cuando un estímulo cambia sorpresivamente su consecuencia (Chiba et al., 1995), mientras que lesiones en MS /VDB interfiere con el detrimento de atención a claves ambientales cuando su consecuencia se predicen de forma segura (Baxter et al., 1997). Estudios con microdiálisis, reflejan un incremento en la liberación de ACh en el córtex prefrontal durante el desempeño de una tarea de atención visual (Passetti et al., 2000), en el córtex frontal cuando la atención aumenta por la exposición a un ambiente nuevo (Giovannini et al., 2001), en el córtex frontotemporal en una tarea de atención sostenida (Himmelheber et al., 2000, Himmelhebe et al., 2001). Asimismo, Fournier et

al. (2004), demostraron que la liberación de ACh en áreas (somato) sensoriales es específica de la modalidad de estímulo. Así, mientras la estimulación visual incrementa el eflujo de ACh en el córtex visual, la estimulación táctil incrementa la actividad de ACh somatosensorial.

La especificidad de dominios cognitivos en las diferentes regiones colinérgicas del PB, sin embargo, no compiten con la integración de los diversos aspectos psicológicos que pudieran estar implicados en la generación de la mayoría de los fenómenos del comportamiento. La relación entre demanda de atención y aprendizaje y memoria son complejas y se ha sugerido que las funciones cognitivas parecen ser el producto de la interacción dinámica entre los sistemas múltiples en el cerebro. A la par de ser usado como un marcador de activación de diferentes sistemas neurales durante el aprendizaje, una visión más específica es que la liberación de ACh puede ser un neuromodulador importante que regula el equilibrio relativo entre los sistemas neuronales. De aquí que, el sistema colinérgico del PB con la participación simultánea de sus diversos núcleos, pudiera procesar diferentes atributos de una misma experiencia cada uno modulado por un núcleo determinado (Gold, 2003). Además, se ha propuesto que las proyecciones de los sistemas colinérgicos hipocampales y corticales cooperan, en niveles más complejos de procesamiento en la formación de nueva memoria. Así, siendo la atención una de las primeras fase del proceso de aprendizaje y memoria, alteraciones en la atención y en el procesamiento de información podría perturbar la adquisición de nueva información causando un subsecuente deterioro de memoria **(**Furey et al. 2000). Igualmente, la optimización del desempeño basado en el conocimiento previo, podría evidenciar disfunciones en el procesamiento atencional causado por un deterioro en la memoria. De aquí que, debe considerarse que la relación entre procesos atencionales y memoria es bidireccional (Sarter & Bruno, 2004) y, por

ende, un deterioro (o mejora) en el sistema colinérgico incide sobre el proceso de aprendizaje como un todo.

3. La Colina Dietaria

Entre los alimentos con un alto contenido de colina publicado por el Departamento de Agricultura de USA (USDA) se encuentran vegetales (espinaca, papa, coliflor, tomate, banana, naranja, lentejas, almendras, nueces, maní, amaranto), cereales (avena, maíz, cebada, germen de trigo) pero principalmente está en las carnes y alimentos de origen animal (hígado, pescado, pollo, leche, huevos).

La concentración basal de Ch libre en plasma en humanos y animales experimentales se mantiene en alrededor de 8-11 μM (Hirsch et al., 1978; Klein et al., 1991). En estas condiciones fisiológicas, los niveles circulantes de colina en plasma proviene de la síntesis ¨*de novo* ¨ en el hígado y riñón, y de PC en los tejidos de membrana (Wurtman, 1992). Sin embargo, estudios en humanos han determinado que, mientras la ingesta de alimentos ricos en colina incrementa los niveles de la vitamina en plasma (Hirsch et al., 1978) ayunos prolongados lo reducen (Savendahl et al., 1997). Estos estudios estarían indicando que la disponibilidad de Ch en la dieta altera sus concentraciones plamáticas. Asimismo, en otros estudios se ha observado que esta variabilidad de Ch en plasma afecta los niveles de la vitamina en el cerebro y, consecuentemente, modula la síntesis y liberación de ACh en neuronas colinérgicas.

3.1. Ch dietaria: Ch en sangre y cerebro y ACh

La remoción en la dieta de alimentos ricos en colina durante aproximadamente 20 días, disminuye la colina en plasma de 10.6 mM a 8.4 mM (Zeisel et al., 1991) y de 12.1 mM a 6.3 mM (Klein et al., 1998), en humanos y ratas respectivamente. Estos hallazgos sugieren que los niveles de Ch en plasma pueden ser parcialmente pero no

totalmente mantenida endógenamente. Adicionalmente, se ha determinado que los niveles de colina en plasma pueden ser incrementados hasta 50 μM en humanos (Zeisel et al., 1980_a) y en ratas (Zeisel et al., 1980_b) después de una ingesta de alimentos ricos en Ch. Las moléculas de colina libre en circulación pueden atravesar la barrera hematoencefálica bidireccionalmente entre la sangre y el fluido extracelular del cerebro por difusión facilitada a través de una proteína de transporte de baja afinidad (Conford et al., 1978) afectando, de esta manera, la concentración de Ch en el cerebro. Debido a que dicha proteína no está saturada en concentraciones fisiológicas, el aumento de sus concentraciones en plasma, provoca la entrada de Ch al cerebro. Se ha estimado que en ratas la concentración de colina en plasma requerida para que haya un flujo neto de la sangre al cerebro es de 15 μM; por debajo de esta concentración, el flujo neto de colina probablemente es inverso (Klein et al., 1990).

Por su parte, las variaciones de Ch en el cerebro pueden modular la síntesis de la ACh en las neuronas colinérgicas. La biosíntesis de la ACh se realiza en el citoplasma del soma de las neuronas presinápticas por medio de la actividad de la enzima colina acetiltransferasa (ChAT) y, en la hendidura sináptica, el neurotransmisor es degradado en acetato y colina por la enzima colina acetilcolinesterasa (AChE) para su recaptura en la neurona presináptica (Wurtman, 1992). Tanto la ChAT como la AChE son proteínas marcadoras específicas de la actividad fisiológica de las neuronas colinérgicas, y ambas juegan un papel importante en la homeostasis de la ACh neuronal. Sin embargo, la velocidad de síntesis de ACh es controlada por la capacidad de una proteína de alta afinidad para transportar Ch al interior del terminal presináptico (para una revisión ver, Lockman & Allen, 2002**;** Ferguson & Blakely, 2004). Debido a que sólo cerca del 50% de la Ch que proviene de la hidrólisis de la ACh en la hendidura sináptica es recuperada a través del transporte de alta afinidad (Amenta & Tayebati, 2008), es

probable que las neuronas requieran de fuentes adicionales de Ch para la síntesis de ACh. Es conocido que la AChT no está saturada a la concentración del sustrato, la Ch, en el interior del terminal celular (Tucek, 1990). Así, se ha sugerido que la variación de los niveles de Ch afecta la velocidad de producción de ACh. En efecto, la suplementación dietaria con lecitina o Ch produce elevaciones secuenciales en los niveles de Ch en suero, Ch cerebral y, a partir de de este último, un aumento en la síntesis de ACh en regiones con una importante presencia de neuronas colinérgicas (Hirsch et al., 1978; Cohen & Wurtman, 1976; Hirsch & Wurtman, 1978). Al contrario, la restricción de Ch reduce sus niveles en suero y, en consecuencia disminuye la síntesis y liberación de ACh (Zeisel et al., 1991; Savendahl & col., 1997; Nakamura et al., 2001). Otros mecanismos se suman para dar soporte a la probable avidez de las neuronas colinérgicas por la Ch. En caso de que esta vitamina no esté provista de manera suficiente, las neuronas pueden obtener Ch a partir de la hidrólisis de los fosfolípidos de membrana (ej. PC) a través de las enzimas fosfolipasas. Se ha sugerido que este mecanismo denominado como *autocanibalismo* puede ser parte de la degeneración celular observadas en procesos de envejecimiento neuronal y en pacientes de Alzheimer (Blusztajn et al., 1986; Ulus et al., 1989).

3.2. Ch dietaria: Funciones Cognitivas

Diversos estudios han relacionado los niveles de Ch y consecuentemente de ACh, con el desempeño de tareas dependientes del sistema colinérgico. Dichas investigaciones han mostrado una mejora o un deterioro en la ejecución cognitivas cuando hay un incremento o un déficit en la disponibilidad de Ch, respectivamente (discutido en más detalle abajo).

Disfunciones en el sistema colinérgico han sido consistentemente identificados en enfermos con déficit de memoria senil y Alzheimer, (de Toledo- Morrel et al., 1984**;**

Tanila et al., 1997). Estos resultados estimularon una extensiva investigación en la búsqueda de posibles propuestas terapéuticas para mejorar el deterioro de neurotransmisión colinérgica. Entre los tratamientos establecidos se incluyen la intervención con precursores de la ACh, agonistas muscarínicos y nicotínicos, e inhibidores de la AChE o ChE.

Particularmente, la terapia del precursor, incrementando la disponibilidad de Ch o lecitina, fue una de las primeras prácticas para aliviar el detrimento cognitivo observado en sujetos envejecidos o con Alzheimer. En especial, tratamientos durante períodos sensibles del desarrollo en modelos animales han arrojado un indudable efecto de la colina dietaria en el desarrollo del sistema colinérgico del PB y en procesos cognitivos dependientes de dicho sistema en la adultez. El sistema colinérgico de ratas durante los días 11-17 de embarazo (E11-E17) y los primeros 7 días postnatales (PN0-7) coinciden con la neurogénesis colinérgica (Semba & Fibiger, 1988; Brady et al., 1989) y el inicio de inervaciones colinérgicas al hipocampo y neocortex (Koh & Loy, 1989; Gould et al., 1991) respectivamente. Así, la manipulación de Ch dietaria durante estos períodos ha evidenciado que además de neurotransmisor, la ACh puede actuar como un agente morfogénico que regula el desarrollo del sistema colinérgico inmaduro. Particularmente, la variación de la disponibilidad de colina dietaria durante E12-18, altera la génesis, migración, diferenciación y apoptosis de las células progenitoras neuronales en el hipocampo y septo medial (Albright et al., 1999). Asimismo, se ha encontrado que la suplementación con colina durante el período pre y postnatal temprano en ratas provoca un aumento en la densidad de receptores muscarínicos en el hipocampo y córtex frontal (Meck et al., 1989) e incrementa la densidad de espinas dendríticas en las regiones CA1 y girus dentado del hipocampo (Meck et al., 2008). Estos hallazgos resultan de gran importancia toda vez que prominentes pérdidas de

neuronas colinérgicas en PB es una característica bien definida en la enfermedad de Alzheimer (Auld et al., 2002), y en el envejecimiento el cual, está asociado con una pérdida de conectividad sináptica tanto en el hipocampo como en el córtex (Feldman & Dowd, 1975; Markham et al., 2005), junto con una disminución en la neurogénesis (Rao y col., 2006). Por otra parte, numerosas investigaciones encontraron que la suplementación con colina mejoran significativamente la memoria espacial y temporal así como también la atención cuando es implementada durante E12-18 (Meck et al., 1988, 1989; Meck & Williams, 1997a,b,c, 1999; Meck & Williams, 2003) y la memoria espacial cuando la manipulación se lleva a cabo en PN 16-30 (Meck et al., 2008**)**. Igualmente, se ha observado que estos efectos permanecen durante la vida del individuo protegiéndolos contra déficit cognitivos relacionados al envejecimiento (Blusztajn, 1998; Meck & Williams, 2003; Meck et al., 2008; Wong-Goodricha et al., 2008). Al contrario, la deficiencia durante E12-18, provoca una declinación en la atención (Meck & Williams, 1997c) y en procesos cognitivos dependientes del contexto (Lamoureux et al., 2008). En adicionales investigaciones, Meck, Jones, Williams, Pauldine, & Holland (1997; reportado en Meck & Williams, 2003), examinaron el efecto de la disponibilidad prenatal de Ch (suplementado, estándar y deficiente) en la habilidad de los sujetos para seleccionar estímulos en donde es posible evaluar el incremento o detrimento de atención a estímulos que cambian inesperadamente o predicen confiablemente sus consecuencias, respectivamente. Los descendientes deficientes mostraron un deterioro en el incremento normal de atención a un estímulo que ocurre cuando éste introduce un factor sorpresa en su consecuencia. Por su parte, la suplementación prenatal con colina disminuyó el detrimento de atención que se manifiesta cuando claves ambientales prevén de manera segura sus consecuencias. Aún más, la suplementación con colina durante períodos del desarrollo temprano ha demostrado ser un tratamiento efectivo con

propiedades neuroprotectivas en ratas. La suplementación postnatal disminuye la hiperactividad y alteraciones cognitivas en tareas de condicionamiento (Wagner & Hunt, 2006**;** Thomas & Tran, 2012) y memoria espacial provocado por el consumo de alcohol postnatal temprano (Thomas et al., 2007; Ryan et al., 2008). Asimismo, ratas expuestas al alcohol y suplementadas con Ch prenatalmente mostraron un menor déficit de aprendizaje y memoria (Thomas et al., 2010).

Con la intención de mitigar los efectos de la vejez y de las enfermedades degenerativas como el Alzheimer en el déficit de memoria, diversas investigaciones han sido implementadas en humanos y animales al alcanzar la senectud. Desafortunadamente, los resultados fueron inconsistentes. Los primeros estudios observaron que una vez que el déficit cognitivo relacionado al envejecimiento se manifiesta, el precursor no es capaz de restituir tales deterioros (para revisión ver Bartus et al., 1985; Bartus, 2000). Se ha sugerido que, sólo la mayor disponibilidad del precursor no es suficiente para revertir diversos desórdenes funcionales relacionados al detrimento de memoria que se manifiesta en esta edad. Estudios anteriores han reflejado disfunciones neuronales en cerebros viejos como por ejemplo, deterioro en el metabolismo oxidativo y consiguiente alteración en la producción del otro constituyente de la ACh (la acetil CoA) (Sylvia & Rosenthal, 1979), la disminución de la captura de Ch a través del mecanismo de alta afinidad (Gallagher & Pelleymounter, 1988). Así cualquiera de estos factores o, simultáneamente, podrían estar afectando el funcionamiento colinérgico en sujetos geriátricos.

No obstante, los trabajos en adultos no hicieron distinciones entre los diferentes períodos del desarrollo de la vida de un individuo antes de alcanzar la vejez. Los estudios llevados a cabo en ratas incluyeron tanto a los sujetos jóvenes - maduros (3 - 12 meses) como los viejos (13-24 meses de edad) como un único período, la adultez.

En algunos de estos estudios la dieta fue manipulada durante la edad adulta joven-madura y la tarea cognitiva fue aplicada en la vejez, mientras que en otros, ambos procedimientos (dieta y tarea) se realizaron en la senectud. No es extraño suponer que las diferencias metabólicas entre ambos períodos influyan en el efecto de la dieta en funciones cognitivas. Así, son realmente escasas las investigaciones cuando la dieta y la prueba se ejecutan absolutamente a lo largo del período adulto joven-maduro o previo a la ancianidad. Por ejemplo, Teather & Wurtzman (2005) mostraron que 12 semanas de acceso a una dieta rica en colina en ratas de 3 meses atenuaron un déficit de la memoria causado por la exposición a un ambiente empobrecido. Otros estudios que podrían incluirse en este grupo han utilizado animales muy jóvenes, por lo que es discutible si se podría clasificar como adultos. Así, la restricción dietética de la colina durante 12 semanas en ratas de dos meses provocó un deterioro en la retención en una tarea de evitación inhibitoria (Nakamura et al., 2001).

4. Algunas tareas dependientes del sistema colinérgico del PB

Las evidencias de la regulación colinérgica a sistemas múltiples de aprendizaje y memoria resaltan la posibilidad de que su manipulación fisiológica y funcional puede mejorar o deteriorar el desempeño de tareas cognitivas asociadas a dichos sistemas. Una tarea conductual debe reflejar, por un lado, el proceso cognitivo implicado y, por el otro, debe ser sensible a la manipulación fisiológica del sistema neuronal envuelto. Así, las tareas empleadas en el presente trabajo fueron elegidas atendiendo a la posible intervención de estructuras neuronales colinérgicas, ej. El PB, lo cual lo haría sensible a la disponibilidad de Ch y, en consecuencia la modulación de la ACh en funciones cognitivas asociadas a dicha estructura como se presume son los procesos atencionales y de memoria.

4.1. Condicionamiento de Aversión Contextual

El condicionamiento de miedo contextual es un paradigma en donde un estímulo condicionado (EC) emocionalmente neutro (ej. estímulo contextual) es emparejado con un estímulo incondicionado (EI), ej. un malestar (shock eléctrico o nausea) durante la fase de adquisición. Particularmente, cuando el malestar de nausea es provocado por una inyección intraperitoneal (i.p.) de cloruro de litio (LiCl), se establece una asociación entre el contexto (EC) y la sensación de nausea (EI) y, como consecuencia, el estímulo contexto por sí solo o en ausencia de la sensación de nausea, elicita una respuesta condicionada de miedo o aversión, en este caso, la supresión de la ingesta de una solución saborizada en el contexto aversivo durante la fase de prueba. Este procedimiento ha sido previamente usado como evidencia de una asociación Pavloviana contexto-malestar (Simonds & Hall, 1997; Aguado et al., 1998; Simonds et al., 1998; Rodríguez et al., 2000).

Investigaciones previas han mostrado que, la discriminación de claves contextuales para controlar respuestas condicionadas es sensible al hipocampo. Cuando se administra un estímulo aversivo (EI, ej un shock o malestar visceral), la respuesta de miedo no condicionada (RI, freezing o supresión) puede llegar a asociarse con estímulos discretos (EC, ej. tono o luz), interesantemente lo mismo ocurre con el contexto en la cual sucede la experiencia. Los resultados apuntan a la disociación entre el condicionamiento EC-EI y contexto-EI. Numerosas investigaciones sugieren que la asociación contexto-EI es dependiente de hipocampo (Kim & Fanselow, 1992; Phillips & LeDoux, 1992; Maren et al., 1997; Rudy et al., 2002). No obstante, algunos investigadores no reportan estos mismos efectos **(**McNish et al., 1997; Frankland et al., 1998; Good & Honey, 1991; Winocur, 1997). En contraste, otros estudios señalan que variaciones en la tarea de condicionamiento de miedo, tipo de lesiones, medidas de condicionamiento del miedo

(para revisión Phillips & LeDoux, 1994; Holland & Bouton, 1999; Gewirtz et al., 2000) son responsables de dichas discrepancias. En adición, Moses et al. (2007) encontraron que la complejidad del ambiente como EC es un elemento importante a considerar en la implicación del hipocampo. En efecto, las ratas con lesiones en el hipocampo reflejaron una disminución significativa de la asociación contexto-EI en un ambiente complejo en relación a uno simple. Los autores sugieren que el condicionamiento de ambientes complejos en donde se requiere la representación configuracional de diversos estímulos presentes es dependiente del hipocampo mientras que el condicionamiento de ambientes simples como un estímulo discreto no lo es. Estos resultados son consistentes con la función atribuida al hipocampo en el aprendizaje de relaciones entre elementos múltiples (Rudy & Sutherland, 1995; Fanselow, 1999; Moses & Ryan, 2006). De esta manera, es en un entorno complejo en el cual un conjunto de claves contextuales es asociado a la RI, en donde lesiones hipocampales provocan un deterioro en la representación contextual y, por ende, pueden afectar el condicionamiento de miedo contextual.

Aun más específicamente, algunas investigaciones indican que el sistema colinérgico hipocampal juega un rol importante en el condicionamiento del miedo contextual. Estudios con microdiálisis han evidenciado la participación colinérgica en la tarea de miedo contextual con el incremento de la liberación de ACh durante el desempeño (Nail-Boucherie et al., 2000). Infusiones con escopolamina en el hipocampo produce una disminución en la adquisición de la tarea (Anagnostaras et al., 1995; Anagnostaras et al., 1999; Gale et al., 2001). En adición, Wallenstein y Vago (2001) encontraron que la administración directa de escopolamina en el hipocampo provoca un deterioro cuando es aplicada antes de la adquisición y en la fase post-entrenamiento (1 min, 24 y 48 h después del condicionamiento). Justos estos resultados sugieren que

procesos colinérgicos en el hipocampo estarían implicados tanto en la adquisición como en la memoria (o consolidación) del miedo al contexto.

4.2. Memoria de Reconocimiento de Objetos

La memoria de reconocimiento de objetos es un modelo de memoria declarativa (recuerdo de hechos y eventos) en donde se debe discriminar un objeto familiar presentado anteriormente (fase muestra), en relación a un objeto nuevo expuesto durante la prueba (fase prueba), separada por una demora (tiempo de retención). Normalmente los humanos y otros animales (ej. monos y roedores) prefieren mirar (o explorar) el objeto nuevo, indicando que recuerdan el objeto original. Así, se registra el tiempo que pasa el sujeto mirando o explorando el objeto nuevo en relación al familiar. La tarea de memoria de reconocimiento de objetos puede referirse a la memoria de un objeto (Que), la localización de dicho objeto (Donde) y ocurrencia temporal del encuentro del objeto (Cuando) (Clark & Squire, 2010). La memoria implicada en la tarea representa un episodio de la vida de estos individuos, pero la prueba de preferencia de exploración a un objeto nuevo, como consecuencia de la discriminación del objeto familiar, sólo evalúa un aspecto particular de ese episodio; se refiere a la memoria de los atributos físicos de un objeto (Ennaceur, 2010). Una de las tareas para evaluar la memoria de reconocimiento de objetos es la preferencia espontánea a la novedad (SOR, siglas en ingles de spontaneous object recognition). SOR es una adaptación del paradigma de reconocimiento visual, la cual, es aplicada comúnmente en humanos (Fagan, 1970), para ser utilizada en roedores (Ennaceur & Delacour, 1988). La tarea SOR mide la tendencia natural de los roedores a explorar más el objeto nuevo en comparación a uno familiar, de manera que no se requiere de la privación de alimentos para motivar su desempeño y no está asociada con altos niveles de estrés o actividad física (Mumby, 2005).

Particularmente, la memoria de reconocimiento de objetos es considerada un componente crítico de la memoria declarativa en humanos el cual es dependiente del lóbulo temporal medial (LTM) (Squire & Zola, 1996). El LTM, está conformado por la región hipocampal (el hipocampo, el giro dentado y el complejo subicular) y los córtices entorrinal, perirrinal y parahipocampal (postrrinal en ratas) (Squire & Zola-Morgan, 1991; Eichenbaum & Cohen, 2001). De aquí que, un déficit en la memoria de reconocimiento de objetos es comúnmente observada en pacientes con daño cerebral en el LTM (Buffalo et al., 1998; Holdstock, 2005; Lee et al., 2003). En adición, estudios previos han encontrado que la memoria de reconocimiento de objetos es dependiente del tiempo de retención. Si el periodo de tiempo entre el aprendizaje y la recuperación de la información (fase muestra y fase prueba, respectivamente) es prolongado, los pacientes con daño en el LTM manifiestan un déficit en la memoria a largo plazo mientras que la memoria a corto plazo se mantiene intacta (ver revisión de Winter et al., 2010). De esta manera, el modelo animal de la tarea de reconocimiento de objetos, SOR, variando el tiempo de retención ha sido de gran utilidad para estudiar las bases moleculares, neuronales y cognitivas de la memoria y amnesia humana.

Los resultados de diversas investigaciones relacionan principalmente el córtex perirrinal y el hipocampo como las estructuras del LTM implicadas en la memoria de reconocimiento de objetos. Específicamente, el rol modulador de la ACh en dichas estructuras se ha hecho evidente a través del aumento en su liberación en el hipocampo, córtex temporal inferior y perirrinal durante el desempeño en un paradigma de reconocimiento visual implementado en monos (Tang & Aigner, 1996; Tang et al., 1997). Receptores colinérgicos han sido implicados; así, la nicotina sistémica mejora la adquisición, consolidación y restitución de la información en la tarea SOR en ratas (Puma et al., 1999) mientras que, la administración sistémica del antagonista

muscarínico escopolamina provoca un deterioro en la memoria de reconocimiento visual en humanos (Robbins et al., 1997), monos (Aigner & Mishkin, 1986; Aigner et al., 1991), y SOR en ratas (Bartolini et al., 1997). Aun mas, infusiones de escopolamina específicamente en el córtex perirrinal antes de la fase muestra reveló un deterioro significativo en SOR tanto en la retención corta (15-20 min) (Waburton et al., 2003) como retención larga (24 h) (Winter et al., 2006). Sin embargo, la infusión antes de la fase prueba no afectó el desempeño de los sujetos (Winter et al., 2006). Estos resultados sugieren que el neurotransmisor ACh, a través de los receptores muscarínicos del córtex perirrinal, está implicado en la adquisición de información del objeto, mientras que, la independencia del deterioro en relación al tiempo de retención pudiera indicar que esta estructura no está envuelta en el proceso de memoria. Por su parte, un único estudio en ratas llevado a cabo recientemente mostró que, lesiones específicas en neuronas colinérgicas que proyectan del MS al hipocampo producen un deterioro en la memoria de reconocimiento de lugar pero no en SOR con un tiempo de retención entre la fase muestra y fase prueba de 24 h (Cai et al., 2012).

4.3. Inhibición Latente y Transferencia Negativa

La exposición a un estímulo (CS, ej. tono o luz) que predice sus consecuencias (bien ausencia de éstas o una información redundante, ej. un shock eléctrico) de forma confiable, pierde la capacidad para retener la atención. La asociación formada en esta primera fase, CS-No consecuencia o CS-shock eléctrico, provoca una pérdida de atención a la clave predictora (CS) y, por tanto, hay un retardo en un subsiguiente condicionamiento en la segunda fase (Pearce & Hall, 1980). En contraste, cuando en este segundo encuentro las consecuencias que predice el CS son repentinamente alteradas, la atención es recuperada y, la capacidad de asociación restablecida.

La inhibición latente (Lubow, 1989) y la transferencia negativa (Hall & Pearce, 1979) son paradigmas ampliamente utilizados para medir específicamente el detrimento de atención a CS que son predictores seguros de sus consecuencias. De acuerdo a Pearce & Hall (1980), el efecto de la la inhibición latente (IL) y la transferencia negativa (TN) tienen la misma fuente. En ambos, hay una pérdida de asociabilidad causada por la preexposición de un CS seguido por una ausencia o reiterada información en IL y TN, respectivamente.

Estudios previos han demostrado la mediación del sistema colinérgico del PB en la doble disociación de atención (mencionada en sección anterior) que participan durante el procesamiento de estímulos. Particularmente, lesiones en el núcleo central de la amígdala interrumpe el incremento de atención provocado cuando la clave asociada a un CS es cambiada de forma inesperada (Holland & Gallagher, 1993). Los autores sugieren que el procesamiento cortical de atención, regulado a partir del núcleo central a través de sus proyecciones al NBM/SI (región que proporciona la entrada colinérgica más importante a la corteza), interviene en el incremento de asociación. Estudios posteriores sustentan esta hipótesis así, lesiones selectivas colinérgicas con saporina en el NBM reflejaron un deterioro en el incremento de atención al CS cuando su relación a una clave fue modificada (Chiba et al., 1995). Por otro lado, lesiones en el septum medial/banda diagonal vertical de Brocca, las cuales proyectan al hipocampo, dificulta (interfiere en) el detrimento de la atención a claves ambientales irrelevantes o predictores consistentes (Baxter et al., 1997). En conjunto, estos estudios evidencian la implicación de áreas selectivas del sistema colinérgico del PB en las dos rutas complementarias atencionales, incremento y detrimento, que intervienen en el procesamiento de CS.

5. Justificación y Objetivos

Como se discutió anteriormente, es evidente el vacío existente en relación al efecto de la manipulación de colina dietaria en ratas durante períodos de vida comprendida exclusivamente entre 3-12 meses de edad. El conocimiento de la importancia de los nutrientes en procesos cognitivos implica necesariamente saber su efecto en todas las etapas del desarrollo del individuo (Munakata et al., 2004; Wainwright & Colombo, 2006). No obstante, los estudios de las consecuencias de la colina dietaria sobre procesos cognitivos en adultos jóvenes-maduros son casi inexistentes. De aquí que, el objetivo de este trabajo de investigación es determinar si la suplementación crónica con colina en la edad adulta afecta la memoria (Capítulos 2 y 3) y la atención (Capítulo 4).

En el Capítulo 2 se busca determinar si la suplementación con colina en la edad adulta afecta el desempeño de una tarea dependiente del contexto, la cual es modulada por el sistema colinérgico. Para ello, después de conseguir una asociación aversiva a un contexto siguiendo el procedimiento de investigaciones previas (Experimento 1), en el Experimento 2 se examina el efecto de la colina dietaria durante 7 semanas en ratas adultas implementado una tarea considerada con un alto nivel de complejidad como es el paradigma de aversión contextual. Como se explicó anteriormente dado que el sistema colinérgico hipocampal pudiera estar implicado en la consolidación de una experiencia de aversión contextual, el protocolo de esta tarea nos permite evaluar si la colina ejerce un efecto en la retención de la asociación contexto-malestar introduciendo diferentes tiempos de retención entre la condicionamiento y la prueba.

En Capítulo 3 se pretende obtener el efecto de la manipulación de colina dietaria prenatal en la memoria (Experimento 1) y evaluar algún efecto cuando el tratamiento es implementado durante la adultez (Experimentos 2 y 3). Para ello, se

utilizó una tarea que no había sido valorada antes, la memoria de reconocimiento de objetos (SOR). El deterioro de la memoria de reconocimiento es comúnmente observado en sujetos seniles de aquí que, dichas pruebas son ampliamente utilizadas para investigar déficit cognitivo dependiente del envejecimiento. Determinar el efecto de la disponibilidad de colina prenatal y adulta en SOR permitiría disponer de una herramienta confiable para posteriores estudios a lo largo de la vida de un individuo.

Una vez comprobado que la suplementación con colina en edades adultas modula la memoria implicada en tareas dependientes de sistema colinérgico, con los Experimentos del Capítulo 4 se pretende investigar si otras funciones cognitivas, como la atención, relacionada al sistema colinérgico cortical, son afectadas por la colina dietaria. Así, dos tareas fueron implementadas, IL (Experimento 1) y TN (Experimento 2), con la finalidad de medir específicamente el efecto de colina durante la edad adulta en el detrimento de atención a estímulos que predicen confiablemente sus consecuencias.

CAPÍTULO 2

CHOLINE DIETARY SUPPLEMENTATION IMPROVES LICL-INDUCED CONTEXT AVERSION RETENTION IN ADULT RATS

CAPÍTULO 2: CHOLINE DIETARY SUPPLEMENTATION IMPROVES LICL-INDUCED CONTEXT AVERSION RETENTION IN ADULT RATS

Physiology & behavior. 2012, 106(4), 451-456.

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ABSTRACT

Previous studies have demonstrated that choline is an essential nutrient during prenatal and early postnatal developmental periods. Thus, the availability of choline during these periods produces some beneficial effects on hippocampal-dependent learning and memory in rats. However, research on the effect of adult choline supplementation on learning and memory abilities is scarce. In the present study, 3-4 month-old male Wistar rats receiving a 7-week choline- supplemented diet (4.5 fold that of a standard diet) and control rats receiving a standard diet were trained in a LiCl-induced contextual aversion task. Short and long-term context aversion retention was assessed by recording the consumption of a flavored solution in the aversive and safe contexts over two subsequent tests. Statistical analysis showed that the supplemented group exhibited greater intake suppression in the aversive context than in the safe context when two retention tests were applied 3 and 15 days after conditioning. These results suggest that increasing dietary choline availability during adulthood may favor the retention of a context aversion.

Keywords: choline, classical conditioning, aversion, dietary supplement, lithium, memory, rat.

2.1. Introduction

Choline is an essential nutrient in humans [1] since it is required for maintaining cell functions due to its several roles in the synthesis of the phospholipid membrane. Moreover, choline is a major source of methyl donors, and it directly affects acetylcholine (ACh) synthesis [2].

It is well known that levels of circulating choline may be altered by changes in the diet. In particular, dietary choline supplementation induces sequential increases of choline in both serum and brain levels, leading to increased ACh levels in brain areas containing cholinergic neurons [3,4,5]. In contrast, dietary choline restriction decreases choline serum levels leading to reduced brain ACh synthesis and release [6, 7, 8]. Therefore, some functions of the cholinergic systems, including those related to learning and memory, may be modulated by increased or deficient dietary choline availability either inducing improvements or deficits respectively.

Among the extensive evidence showing the involvement of the brain's widespread cholinergic systems on cognitive tasks [9, 10], the hippocampal ACh levels have been related with performance in a variety of learning and memory tasks [11, 12, 13, 14, 15, 16, 17, 18, 19, 20]. Particularly there is specific evidence to suggest that contextual fear conditioning may be linked with the modulation of the hippocampal system by cholinergic neurons, since an increased ACh release has been found during both the acquisition and retention of this type of learning [19]. Additionally, hippocampal lesions have been reported to induce a deficit in the acquisition of contextual fear conditioning [21,22,23,24,25], while it is has also been shown that dorsal hippocampal electrolytic lesions disrupted the acquisition of a context aversion induced by lithium chloride (LiCl) injections [26].

Due to its protracted maturation during early development and the vulnerability of the cholinergic hippocampal system to normal and pathological aging [27,28] most of the research aimed to enhance memory functions by dietary choline supplementation have been centred on prenatal and early postnatal periods (see, for a review [29,30]. From this evidence two main findings have been raised. Firstly, developmental effects of choline supplementation can be seen in adults many months, and in some cases several years after early treatment [31,32]. Secondly, several lines of work have shown that choline supplements given postnatally through weaning can have immediate effects on learning/memory [33,34,35]. The beneficial effects of choline supplementation in these studies are evident in attenuating learning deficits induced by early alcohol exposure. There are also studies that focus on applying the supplemented diet during adulthood in order to assess its effect on aging-related memory deficits at advanced ages. Such experiments have been carried out in both animals and humans but they have yielded divergent and contradictory results (see, for a review [29,36]) .

However, evidence relating to the cognitive effects of dietary choline supplementation in adulthood is scarce. Teather and Wurtman [37] found that dietary supplementation during 3 months with cytidine (5´)-diphosphocholine, a source of cytidine and choline, prevented retention deficits induced by exposure to an impoverished environment in 3-month-old rats trained in a water maze spatial task. Other studies that could be included in this group have used very young animals and so it is arguable whether they could be classified as adults. For instance, dietary choline restriction for 12 weeks in two-month-old rats has been shown to impair retention in an inhibitory avoidance task [6].

The relative difficulty in demonstrating beneficial effects of dietary supplementation in adulthood might not be due to insufficient supplementation, but

could instead be attributed to ceiling effects, given that adult rats show a high level of performance in many learning and memory tasks. Previous studies have showed that choline concentrations between 4,5-5 and 10-fold higher than the concentration of the standard diet are needed to increase ACh synthesis and cognitive performance in rodents [29,32,38,39,40,41,42]. In addition, there are data showing that six weeks of supplementation with the dietary choline should be able to induce an improvement in cognition [37].

Given that choline supplementation clearly has the potential to enhance cognition, it seemed appropriate to search for an effect of increasing dietary choline upon learning in adult rats, using a task which may be less susceptible to the performance-related ceiling effects previously mentioned. The focus of the present study, therefore, is to use a well-established version of a hippocampal-dependent contextual learning task in order to assess the effects of dietary choline supplementation in adult rats. Thus, in the present experiments we investigate if the retention of context aversions could be modulated by 7 weeks of dietary choline supplementation in adult rats.

Contextual conditioning was accomplished by pairing a novel context with visceral malaise induced by an intraperitoneal (i.p) injection of LiCl. Suppressed intake of a flavored solution in the aversive context has been previously used as evidence of a Pavlovian context-illness association [26,43,44,45,46,47,48]. Therefore, in the first experiment a procedure similar to that previously reported by Rodríguez at al. [46] was employed to obtain a hippocampal-dependent contextual aversion. The second experiment was designed to explore if the retention of a contextual aversion may be favored by increased dietary choline availability. The Ethics Committee for Animal

Research of the University of Granada approved all the procedures in accordance with the European Communities Council Directive 86/609/EEC.

2.2. Experiment 1

Rodríguez et al. [46] reported that the animals given an injection of LiCl before being placed in a distinctive experimental context would subsequently suppress intake of a taste solution in the aversive context relative to a group that had received unpaired presentations of these events. It is widely accepted that this finding occurs as a result of the formation of an association between the context and a state of nausea, and can thus be used as a reliable procedure for demonstrating context aversion learning. The aim of the present experiment was to replicate this effect in our laboratory. Thus, a similar behavioural procedure was used except that a non- nutritive flavored solution (vanilla) was used instead of the sucrose solution employed by Rodríguez et al. [46]. In addition, a within-subjects design allowed us to reduce the number of animals needed. On the basis of the results reported by these authors [46], we expected that the animals would show suppressed consumption of the vanilla solution in a context which has been paired with illness, relative to a context which has not been experienced in conjunction with gastric malaise.

2.2.1. Methods

2.2.1.1.Subjects and materials

Sixteen 3-month-old male Wistar rats with a mean weight of 232 g (range: 209-254 g) were used in this study. The subjects were individually housed in a room with a constant temperature kept between 22 and 24 ºC, with a 12:12 h. Light-dark cycle. Food

was available ad libitum throughout the experiment. The water deprivation schedule is described in the behavioural procedure section. The contexts consisted of two cages different from the home cage and located in different rooms. Context A was a 20x20x23 cm box without bedding material placed in a room brightly lit by a white light, while a 32x21x12 cm cage with the floor covered by cat litter and located in a room with cues formed by both a red light and white noise (75 dB) was used as Context B.

2.2.1.2 Behavioural Procedure

All the animals were adapted to a water deprivation schedule with two daily 30 min drinking sessions at 10:00 hr. and 17:00 hr . This deprivation schedule was maintained throughout the rest of the behavioral procedure, except in the last three days of the testing phase in which the flavored solution was available at 12:00 hr. and water at 17:00 hr. In order to counterbalance the aversive context they were randomly assigned to one of two groups (n=8 each). At 12:00 hr on the first conditioning day (day 3) all the subjects received an i.p. injection of the visceral malaise inducing agent LiCl (0.15 M; 2% b.w.). Immediately afterwards, the first group was transferred to Context A and the second group to Context B where they remained for 30 min before being returned to their home cage. On day 4 a similar procedure was followed except that an i.p. injection of NaCl (0.15 M; 2% b.w.) was applied and the first and second groups were exposed to Context B and Context A respectively. This 2-day cycle was repeated twice, so that a total of three conditioning trials were administered within the 6-day conditioning phase. Following conditioning the animals remained in their home cages during two recovery days. On day 10, all the animals had access for 30 min to 20 ml of a flavored solution (Vanilla, 2% in tap water) in their home cage at 12:00 hr. in order to avoid neophobia during the retention tests. On days 11 and 12 the retention tests took place during the 12:00 hr. drinking session. On day 11 half of the animals were given

free access to the vanilla solution for 15 min in Context A; for the other half of the animals, this testing took place in Context B. On the second day of testing (day 12) this arrangement was reversed.

2.2.2. Results

A significance level of $p<0.05$ was adopted in all the statistical analyses. There were no significant differences between the groups in the amount of flavored solution drank during the home cage familiarization session ($F(1,14) = 0.047$, $p > 0.96$). The results of central interest, i.e. those of the retention tests, are displayed in Figure 1. It is clear from Figure 1 that the animals showed a higher suppression of intake of the vanilla solution in the context paired with the LiCl injection (aversive) than in that paired with a NaCl injection (safe), the mean intakes being 4.67 ml and 6.35 ml respectively. A repeated measures ANOVA yielded significant differences in consumption between the aversive and safe contexts ($F(1, 15) = 2.87$, $p < 0.05$). These findings are therefore consistent with previous data [46], showing that context cues had acquired aversive properties as a consequence of the LiCl-context pairings.

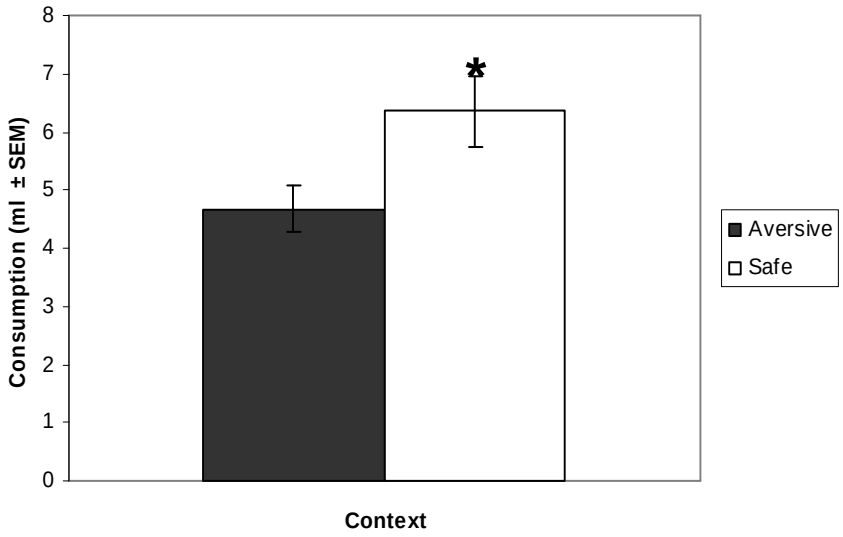

Figure 1. Mean (± SEM) vanilla solution intake in each context (aversive and safe) during the retention test of Experiment

2.3 Experiment 2

Because dietary choline may act as a precursor for ACh, thus being able to improve cholinergic functions [49], it can be proposed that dietary choline supplementation could improve retention of a context aversion . Thus, in Experiment 2 the effect of chronic choline supplementation on memory retention was explored using the protocol established in Experiment 1 for demonstrating a contextual aversion induced by LiCl.

2.3.1. Methods

2.3.1.1. Subjects and diet

A timeline diagram for treatment and training of the two groups of rats used in this experiment (supplemented and standard) is shown in Table 1. Fourteen 3-4 month-old male Wistar rats with a mean weight of 344 g (308-404 g) were randomly assigned to

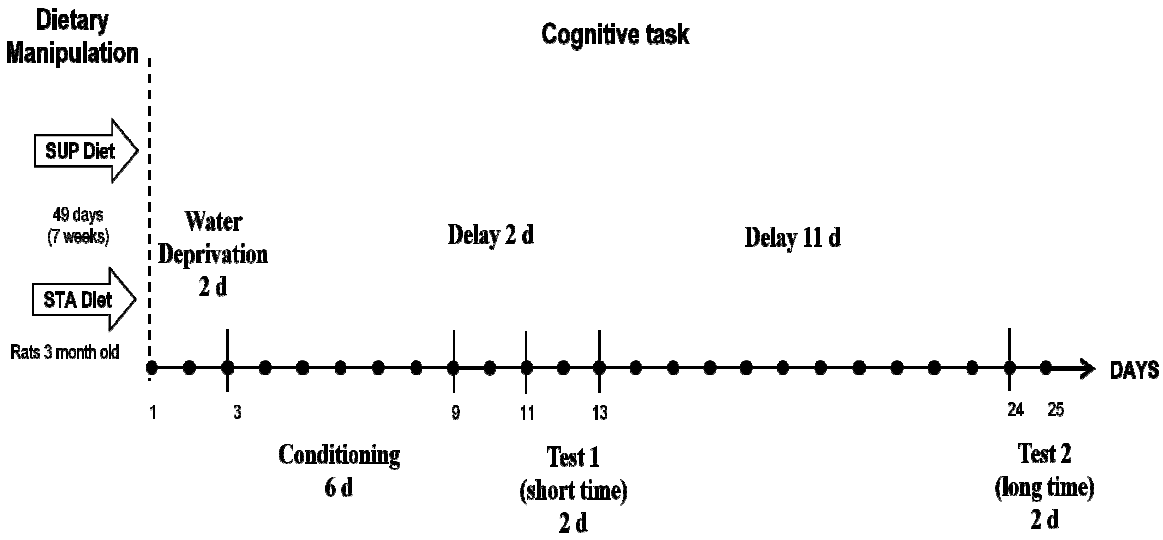

Table 1. Experimental timeline of procedure. Rats 3 month old were divided into tow groups. One group received choline supplemented (SUP) diet, while the other group received standar (STA) diet. After 7 weeks of the diet manipulation, all rats were maintened on standar diet. Two days before to begin the conditionig, rats were adapted to a water deprivation schedule. Then, rats were given 6 days conditioning session. After two days both SUP and STA groups were implemented a short time retention (test 1). Additionaly, after 11 days of dalay the subjects were implemented a long time retention (test 2).

either the Supplemented (n=7) or the Control (n=7) group. They were housed in standard cages (4 per cage) located in a room with a constant temperature. Food and water were available ad libitum during the supplementation period. Water deprivation during the behavioural training was similar to that described in Experiment 1. During the dietary intervention, both groups were fed AIN-76A purified synthetic diet with 7.9 mmol/kg choline chloride (Harlan laboratories Models, SL.). Additionally, the supplemented group was given water containing 25 mM choline chloride [50]. This amount guarantees a concentration about 5 g/Kg of Choline Chloride in the diet, which produces a 4.5-fold increase with respect to the AIN-76A diet (1.1 g/Kg). Following the procedure developed by the Williams and Meck group in pregnant rats [50] the water was sweetened with 50 mM saccharin to neutralize the bitter taste of choline in the water. The drinking water available to the control group also contained saccharin (50

mM) but not added choline [50]. In order to assess the individual intake the rats were singly housed during the first week of the supplementation period and water and food consumption was recorded. Then, the animals were housed in groups during the following 6-weeks of the supplementation period. After 7-weeks of dietary supplementation, all the animals were fed with the standard diet and saccharin-free water was available. Immediately after this, the behavioural procedure for inducing a context aversion was started.

2.3.1.2. Behavioural procedure

A similar behavioural procedure to that described in Experiment 1 was applied, except for the addition of a second retention test which took place 15 days after the end of the conditioning phase. Thus, the interval between ending the diet treatment and assessing memory in the second test was 25 days.

2.3.3. Results

There were no significant differences between the groups in the amount of food and water ingested during the first supplementation week (data not shown). During this first week, the average intake of food was 16 g/day/rat. This provided an average daily choline intake about 0.37 mmol/kg. On the other hand, the average intake of fluid was 18 ml/day/rat. In the supplemented group this resulted in an average daily choline intake of 1.68 mmol/kg [1.31 mmol (water source) plus 0.37 mmol (food source)]. A similar absence of differences in the amount of water drank has been previously reported using a less palatable standard chow [50].

With respect to the behavioral testing, data from 2 rats (1 supplemented and 1 standard) were excluded from our analysis because they were statistical outliers. The

groups did not differ in the consumption of the flavored solution during the familiarization session ($F(1, 10) = 0.363$, $p > 0.5$). A mixed ANOVA 2x2x2 (Group x Context x Retention) including the between-group factor of Group (Supplemented vs. Control) and two within groups factors: Context (aversive vs. Safe) and Retention (test 1 vs. test 2) showed a significant effect of the main factor of Context ($F [1, 10] = 6.139$, $p< 0.05$) and of the interaction Context x Group ($F [1, 10] = 8.26$, $p< 0.05$). There was no effect of the main factors Group ($F [1, 10] = 0.418$, $p>0.5$), Retention ($F [1, 10] = 0.897$, $p> 0.3$) nor the interaction Group x Context x Retention ($F [1, 10] = 0.847$; $p> 0.3$). The interaction Context x Group evidenced a lower mean vanilla solution intake in the aversive (8.99 ± 0.72 ml) than in the safe context (10.72 ± 0.49 ml) by the supplemented group ($t_{(5)}= 3.61$, $p < 0.05$), while the control group exhibited no differences since it drank 9.88 ± 0.59 ml in the aversive context and 9.81 ± 0.72 ml in the safe context ($t_{(5)}= 0.148$, $p > 0.8$). These results suggest an enhancement of contextual learning retention in the supplemented group which is evident using a demanding long-delay test applied 15 days after conditioning (Figure 2).

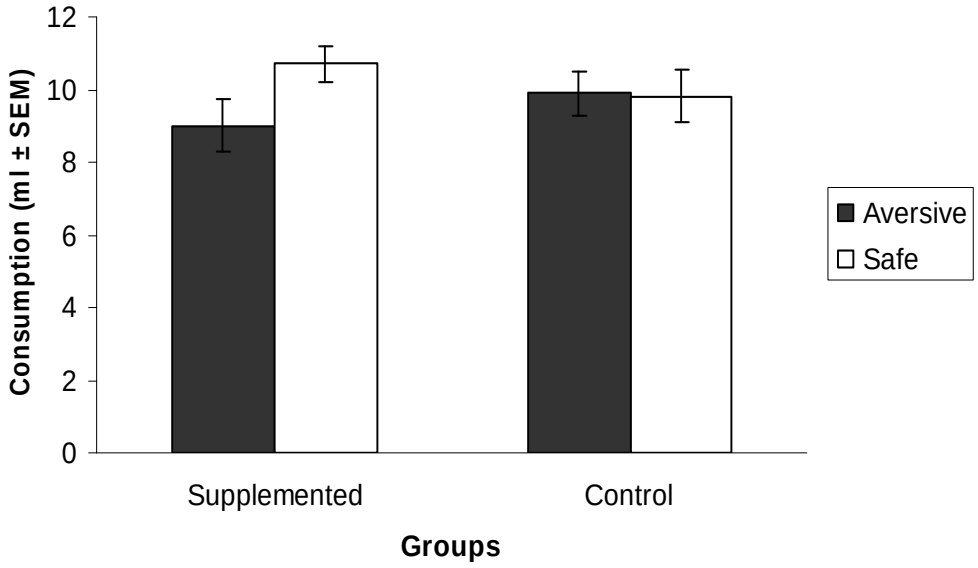

Figure 2. Mean (± SEM) vanilla solution intake in each context (aversive and safe) by choline supplemented and control group in the Experiment 2.

2.4. General Discussion

The main finding of the present study is an enhancement of contextual learning retention by 7 weeks of dietary choline supplementation in adult rats. To our knowledge this is the first report demonstrating an effect of dietary choline on context aversions induced by LiCl. In addition, we have found a beneficial effect on cognition using one of the shortest choline supplementation period used to date in adult rats. The memory-enhancing effect was evident when two retention tests were applied 3 and 15 days after conditioning. In particular, only the group that was fed a choline-supplemented diet exhibited selective intake of suppression in the aversive context, confirming a context aversion. No such effect was evident in the control group. Given the fact that intake of suppression was found in the short-term retention test (3 days after conditioning) both in

Experiment 1 and Experiment 2 (not shown), it can therefore be proposed that choline supplementation had a particularly beneficial effect on long-term retention.

These results are consistent with the role of the cholinergic system in memory [11,12,13,17,18] and the well-established relationship between the hippocampus and context aversions induced by LiCl [26]. Since the task applied in the present study has minimal motor requirements, a relevant contribution of potential ACh increases at the neuromuscular junction is not likely to account for these findings. Instead, we propose a crucial role for brain ACh levels, consistent with previous findings.

The choline supplementation applied was chosen in accordance with previous work [50] and it did not affect food and water intake. Since the first demonstration of increased brain ACh synthesis by dietary choline intake there has been an extensive body of work on the effect on cholinergic neurons activity. Some studies have used choline concentrations within the range of those usually present in the human and animal diet. In this regard, taking into account the variety of foods in the human diet, it has been estimated around 0.5-0.9 g as the mean daily intake. However, due to the occasional consumption of food rich in choline-at a given day it might increase up to 5 g, thus inducing variations up to tenfold with respect to the mean [3]. In accordance, several studies have found that tenfold increase with respect to a standard diet raise plasma choline and ACh synthesis in the rodents' brain [3,39,41]. Nevertheless, the consumption of large doses of choline depresses the food intake whereas the lower doses have no effect on food consumption [3]. Thus, lower choline concentrations between 2.6-5 times higher than the standard diet but effectively increasing choline plasma levels [48] have been often used. The results have shown improvement in the performance of cognitive tasks in rodents whether they were applied perinatally [29,32] or during adulthood [37,38].

The enhanced retention of the conditioned context aversion reported may be attributed to the additional supply of choline in a highly demanding memory task since a long-term retention test was added 15 days after conditioning. Accordingly, an increased requirement of ACh has been demonstrated during the retention test in a contextual fear conditioning protocol. Nail-Boucherie et al. (2000) [19] using microdialysis, demonstrated similar ACh release increases in trained and control groups during acquisition, but both during conditioning and testing. However, the conditioned group exhibited a 4-fold ACh release increase in comparison with the control group during a retention test applied 24 hr. later, thus pointing to a more relevant role for ACh during retention than during acquisition of contextual learning.

The proposal that the supplemented group in the present experiment shows a more prolonged memory of the context-illness association than the control group, as evidenced by a consumption test, due to an additional dietary choline supply is supported by previous reports showing that dietary choline increases the cholinergic metabolism relevant for hippocampal-dependent memory. Besides ACh synthesis, free choline allows the cholinergic neurons to synthesize phosphatidylcholine (PC) which is the most abundant membrane phospholipid [52]. Increases of phospholipidic membrane components such as PC, phosphatidylethanolamine (PE) and phosphatidylserine (PS), as a result of dietary CDP-choline supplementation have also been reported [53,54]. Thus, after a maintained ACh release the incorporation of choline to PC decreases [55] and the mobilization of choline from the PC pool increases in order to support ACh synthesis [56,57,58]. It is conceivable that an increased choline PC pool after dietary choline supplementation might support the long term effect on retention reported in the present study. This is consistent with the fact that increases of ACh release in active cholinergic neurons have been related with improved performance in tasks with high

memory demands [9,59,60,61]. The protective effect of exogenous choline against decreasing ACh levels in hippocampal neurons persists 24 h after the choline brain levels return to normal following the moderate increase induced by acute administration [62]. These findings show that there is some additional source of brain choline which is consistent with the presence of PC choline pool and also that the effect of exogenous choline has a long-term beneficial effect on ACh synthesis.

Thus, given that in our study a chronic supplementation was applied (7 weeks) it can be proposed that the beneficial effects on cholinergic function have extended during the period between the end of supplementation and the retention test (25 days). It cannot be discarded also that the long-term beneficial effects on the retention of a hippocampal-dependent contextual memory in the present study could have been mediated by other mechanisms such as increased adult hippocampal neurogenesis. Previous research has demonstrated that adult choline supplementation induces an increase in hippocampal dentate cell proliferation in 12-month-old rats [32].

Furthermore, the relatively short period of 7 weeks during which the supplementation was applied in this experiment is one of the shortest efficient supplementation periods reported so far. The increase in phospholipidic membrane components after of dietary CDP-choline supplementation required a minimum of 6 weeks to induce significant increases in PC, PE and PS [53,54]. However, Teather and Wurtman [63] demonstrated that choline supplementation for 8 weeks was required in 15-month-old rats to prevent age-induced memory deficits, while 4 weeks or shorter supplementation periods were not effective. In a later study, Theather and Wurtman [37] reported the need for continuous supplementation for 3 months to treat memory impairments in younger adult rats that had been reared during 3 months in an impoverished environment. Taken together, these findings suggest that the minimum

supplementation period required for improving memory functions may be modulated by different variables such as the age and the previous experience of the subjects. The present findings show that a 7 week dietary choline supplementation was enough to obtain a beneficial effect on memory, even if this period was only one week longer than the minimum required for increasing phosphatidylcholine in neuronal membranes. Therefore, we consider that exploring the lower limits of the required time period for memory enhancing intervention is of great value in itself.

In summary, greater choline availability administered throughout the diet during 7 weeks in adult rats enhance retention of a context aversion induced by visceral illness, thus showing that performance in this memory task is modulated by dietary changes in choline levels during adulthood.

Acknowledgments

This research was supported by grants PSI2008-03933, PSI2009-07513, PSI2011-23702 (MICINN. Spain), and grant HUM-02763 (Junta de Andalucía, Spain) partially funded by FEDER. The authors are grateful to Dr. Michelle Symonds for her help relative to some useful technical comments and editing the English text.

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CAPÍTULO 3

EFFECTS OF PRENATAL AND ADULT DIETARY CHOLINE AVAILABILITY ON OBJECT RECOGNITION MEMORY IN RATS

CAPÍTULO 3: EFFECTS OF PRENATAL AND ADULT DIETARY CHOLINE AVAILABILITY ON OBJECT RECOGNITION MEMORY IN RATS

3.1 ESTUDIO 1

EXPERIMENTO 1: LONG-LASTING EFFECTS OF PRENATAL DIETARY CHOLINE AVAILABILITY ON OBJECT RECOGNITION MEMORY ABILITY IN ADULT RATS

Nutritional Neuroscience. 2013.

DOI: 10.1179/1476830513Y.0000000055

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3.2 ESTUDIO 2

EXPERIMENTOS 1 y 2: LA SUPLEMENTACIÓN CRÓNICA CON COLINA MEJORA LA MEMORIA DE RECONOCIMIENTO EN RATAS ADULTAS

Hayarelis C. Moreno, Geoffrey Hall, Milagros Gallo, Isabel de Brugada

ESTUDIO 1: Long-Lasting Effects of Prenatal Dietary Choline Availability on Objects Recognition Memory Ability in Adult Rats

Abstract

Choline is an essential nutrient required for early development. Previous studies have shown that prenatal choline availability influences adult memory abilities depending on the medial temporal lobe integrity. The relevance of prenatal choline availability on object recognition memory was assessed in adult Wistar rats. Three groups of pregnant Wistar rats were fed from E12 to E18 with choline-deficient (0 g/Kg choline chloride), standard (1.1 g/kg choline chloride) or choline supplemented (5 g/kg choline chloride) diets. The offspring was cross-fostered to rat dams fed a standard diet during pregnancy and tested at the age of 3 months in an object recognition memory task applying retention tests 24 and 48 hours after acquisition. Although no significant differences have been found in the performance of the three groups during the first retention test, the supplemented group exhibited improved memory compared to both the standard and the deficient group in the second retention test, 48 h after acquisition. In addition, at the longer retention interval the deficient group did not differ from chance. Taken together, the results support the notion of a long-lasting beneficial effect of prenatal choline supplementation on object recognition memory at longer retention intervals, which is evident when the rats reach adulthood. The results are discussed in terms of their relevance for improving the understanding of the cholinergic involvement in object recognition memory and the implications of the importance of maternal diet for lifelong cognitive abilities.

3.1.1 Introduction

A variety of studies has linked the availability of choline during gestation and perinatal development to brain function and performance of the offspring in cognitive and behavioral tasks (1).

There are a number of underlying choline-sensitive biochemical processes that might influence cognition. In particular, Choline is a precursor of the neurotransmitter acetylcholine (ACh) and the most abundant membrane phosphatide, phosphatidylcholine (PC), both of which are involved in neuronal plasticity related to memory and other cognitive functions. Since the enzymatic reactions involved depend on the local concentration of the substrates and choline circulation through the BBB, the synthesis of these compounds is altered by varying its dietary availability (2).

Moreover, recent research suggests that the nutrients that are part of methyl-group metabolism, such as choline, can significantly influence epigenetics, thus leading to lifelong changes in gene expression (3). The lifelong effect of maternal dietary choline availability on memory seems to be related to the formation and development of the forebrain cholinergic system. Behavioral research in rats varying the availability of dietary choline during critical periods of the forebrain cholinergic system development showed that choline supplementation significantly improved spatial and temporal memory when implemented between embryonic days (E) 12 to 18 protecting it against age-related cognitive deficits (4). In contrast, choline deficiency during E12-18 causes a decline in context-dependent tasks (5). Most of the previous animal studies assessing the effect of early dietary choline availability on adult memory functions have used hippocampal-dependent spatial and contextual tasks. However, to our knowledge there have not been previous attempts to explore a potential effect of maternal dietary choline

supplementation on the performance of offspring in object recognition memory. The spontaneous object recognition (SOR) task applied in rodents is based on the innate tendency to explore novel objects more than familiar.

Evidence obtained in rats has shown the involvement of the cholinergic system in SOR. Systemic nicotine has been shown to enhance performance in the object recognition task (6). Furthermore, systemic administration of the muscarinic antagonist scopolamine induced a deterioration of recognition memory (7). In addition, selective removal by the immunotoxin 192 IgG-saporin of the cholinergic basal forebrain inputs into the perirhinal cortex has the effect of impairing SOR (8). The aim of the present study was to investigate whether exposure to a change in prenatal dietary choline availability influences adult performance in a demanding SOR memory task including 24 and 48 h retention intervals. The effect of maternal dietary choline supplementation and deficiency during E12-E18 was assessed in comparison with the case in which the subjects were fed a standard diet.

3.1.2 Materials and Methods

*Subjects and Diet***.**

Seventeen pregnant Wistar rats fed with the standard AIN 76-A diet containing 1.1 g/kg of choline chloride were maintained at room temperature (22°C-23°C), individually housed in cages (54 x 33 x 18 cm^3) and kept under a 12 h light/dark cycle daily. Food and water were available ad libitum. On the afternoon of E11 the pregnant rats were divided into three groups: supplemented, standard and deficient. Animals belonging to the standard group (n=7) were fed with the standard diet, while those in the supplemented group (n=5) received an AIN 76-A diet with 5g/kg chloride choline and those in the deficient group (n=5) an AIN 76-A diet containing 0 g/kg chloride choline.

These treatments continued until gestational day 18 (E18). At the end of the treatments all the rats were fed the standard diet. During gestational days E12-E18 the daily food intake was recorded.

On the first postnatal day (PN 0), all male pups were cross-fostered to one of seven dams which had received standard diet during pregnancy to avoid potential effects of the diet on the maternal care. Each dam fostered a litter formed by 4-5 male pups belonging to one of the treatment conditions (supplemented, standard and deficient). None of them were obtained from the same original litter. Thus, three new groups (n=10 each) were formed including 3 pups from each treatment group (supplemented, standard and deficient). The group contributing with an extra pup was balanced. Therefore, more than one litter was contributing to each treatment group, meaning that the litter effects were not strictly controlled for. The remaining female pups, not included in the present experiment, were used for other studies performed in the laboratory. The offspring were weaned on PN 21, housed in groups of 4 and fed ad libitum with the standard diet AIN 76-A. A total number of 30 adult male Wistar rats were tested in the behavioral task at the age of 90 days. Figure 1 shows a time-line summary of the dietary and behavioral treatments applied. 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.

Behavioral Procedure.

The behavioral procedure took place in a black opaque open plastic chamber (50cm x 50cm x 40cm). The stimuli used that were two identical copies of two porcelain jars

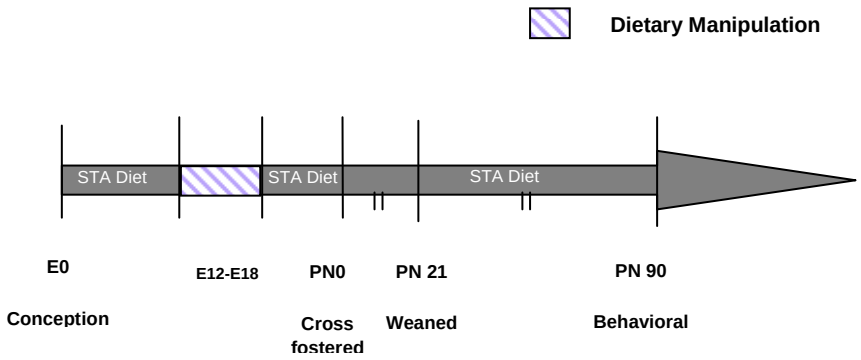

Figure. 1. Schematic timeline of the experimental procedures. Dietary treatment took place during the embryonic period covering E12-E18 gestational days. Three groups received different choline content diets: deficient (0 g/Kg), standard (1.1 g/kg), supplemented (5 g/kg) of choline. (E: gestational day; PN: post-natal day; STA : standard).

differing in shape (rounded vs. elongated) and size (about 10 cm high and 5 cm wide) and a plastic yellow apple (5 cm high). Velcro was attached to each object to be secured to the floor of the testing boxes (9 cm apart), both to ensure correct object position 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 illuminated the testing area reducing room context information.

The procedure consisted of three phases: habituation to the chamber, acquisition/familiarization, and recognition memory tests. In the habituation phase, all the animals were handled for 2 min. They were acclimatized to the room (30 min) and to the empty chamber (5 minutes exploration). Twenty-four hours later during the acquisition session, the rat was allowed to explore the chamber containing two identical objects for a period of 10 minutes. Recognition memory was assessed in two testing sessions taking place after a retention interval of 24 and 48 h. respectively. In the testing sessions, a similar procedure to that previously used during the acquisition session was applied. However, while one of these objects was identical to that presented in the

acquisition session, the other one was an entirely new object. Both the novel object and this position in the chamber were counterbalanced within each group. Each rat was allowed to explore for 5 min. Exploration was defined as contact time with the objects (nose within 2 cm of objects and vibrissae moving). The time that the rat spent exploring the novel and the familiar object during the first 30 s. and the total 5 min. exploration time was recorded. The recorded behavioral data were analyzed by two additional blind experimenters and their results were consistent between them (inter-observer reliability: Pearson r = 0.929) and with those of the main experimenter (blind 1: r = 0.887; blind 2: r = 0.897). For the statistical analyses the exploration ratio (ER) was used as a retention index. As has been previously proposed, ER was calculated as the ratio of exploration time for the novel object in relation to the total exploration time (familiar and novel objects) during the test phase; $t_{novel} / (t_{novel} + t_{familiar})$.

Data analysis.

Two-way mixed ANOVA analyses with “diet” as one of the main between-subjects factors and either “day” as within-subject factor for analyzing food intake during the prenatal dietary treatment or “object and retention” as within-subjects factors for analyzing the performance during the acquisition and the retention tests sessions respectively, were performed using the SPSS statistical package. Bonferroni tests were applied for post hoc analyses. T-tests were used to make within subjects comparisons. A significance level of $p < 0.05$ was adopted in all of the statistical analyses.

3.1.3 Results

There were no body weight differences between the groups formed by either the pregnant rats during the dietary intervention or the pups during the first seven postnatal days.

A mixed 3 x 7 (diet x day) ANOVA analysis of food intake during the prenatal dietary treatment period (E12-E18) yielded only a significant effect of the main factor, day, ($F(6,66) = 13.65$; $p < 0.01$) but not diet ($F(2,11) = 0.940$; $p > 0.42$).Nor was there a significant interaction between diet and day ($F(12,66) = 0.14$; $p > 0.99$). Post hoc analyses by Bonferroni tests showed that the rats consumed more food during E17 ($p < 0.05$) and E18 ($p < 0.01$) compared with the previous days of dietary manipulation.

There was no effect of the prenatal dietary treatment on the exploration time of the two identical objects during the acquisition session (Figure 2). A 3 x 2 (diet x object) mixed ANOVA analysis did not yield any significant effects of the main factors, diet ($F(2,27) = 0.354$; $p > 0.70$) and object ($F(1,27) = 0.713$; $p > 0.40$), or of the interaction diet x object ($F(2,27) = 0.894$; $p > 0.40$).

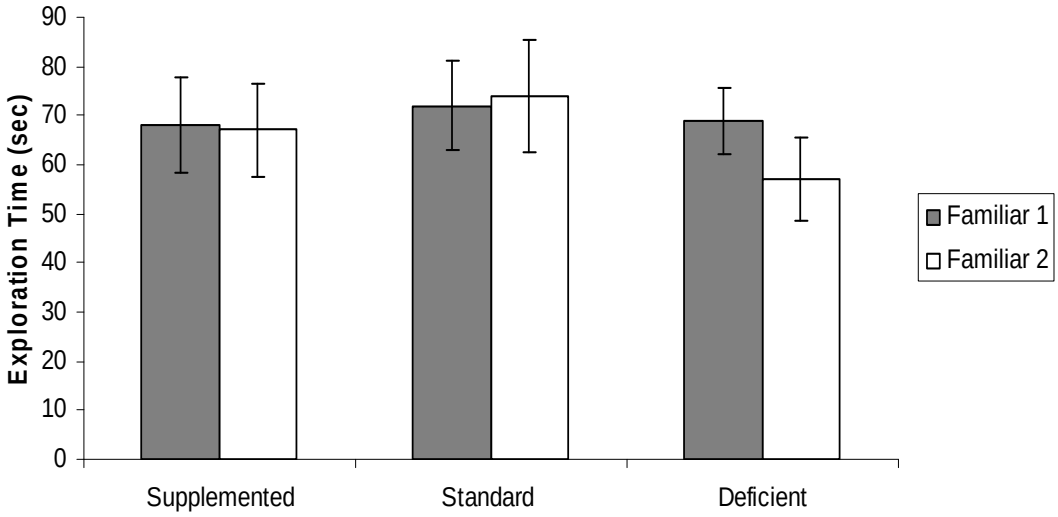

Figure 2. Mean (±SEM) exploration time of each object during the acquisition session by the different groups indicating no effect of the prenatal dietary treatment on the exploration time of the two identical objects.

Given that the total exploration time yielded similar results to those obtained taking into account the first 30 s. period, only the later statistical analyses are reported. Figure 3 shows the mean (±SEM) ERs of the supplemented, standard and deficient groups

during the testing phase at each retention interval (24 and 48 h.). A mixed ANOVA analysis of 3 x 2 (diet as between-group factor x retention as within-subject factor) in ER showed a significant effect of the main factor of diet ($F(2,27) = 11.27$; $p<0.01$) and of the interaction between diet and retention ($F(2,27) = 5.48$; $p <0.05$). The effect of retention was not significant ($F (1,27) = 2.46$; $p> 0.12$). Further analyses by a one-way ANOVA revealed no significant differences between the groups at the 24 h delay ($F(2,29) = 1.92$; $p<0.16$) while the groups showed different ERs at the 48 h delay ($F(2,29) = 14.71$; $p<0.01$). Bonferroni post hoc tests revealed that the supplemented group exhibited a significantly higher ER (ER = 0.83) than those of the standard (ER = 0.61; $p <0.01$) and the deficient (ER = 0.49; $p <0.01$) groups. There were no significant differences between the standard and the deficient group ($p > 0.14$). Comparisons of the ERs at 24 h versus 48 h delays by paired t- tests indicated that the deficient group exhibited a significant decrease at the longer retention interval ($t(9) = 2.689$; $p<0.05$). No significant differences were found either in the supplemented ($t(9) = -1.55$; $p>0.15$) or the standard groups ($t(9) = 0.211$; $p<0.83$).

Additionally, the ER values of each group were compared with the "chance level" (0.5) using one sample t test vs. 0.5. At a 24 h retention interval the supplemented, standard, and deficient groups showed ER values significantly higher than the chance level ($t_{(9)} = 6.22$, $p <0.01$; $t_{(9)}= 2.3$, $p <0.05$ and $t_{(9)} = 5.06$, $p <0.01$, respectively). However, at 48 h only the deficient group failed to show a significant difference relative to the chance level ($t_{(9)}= 0.199$, $p> 0.846$). Also, only the deficient group was affected when the retention time increased from 24 to 48 h ($t_{(9)}= 2.69$, $p <0.05$), with mean ERs of 0.76 and 0.49 respectively.

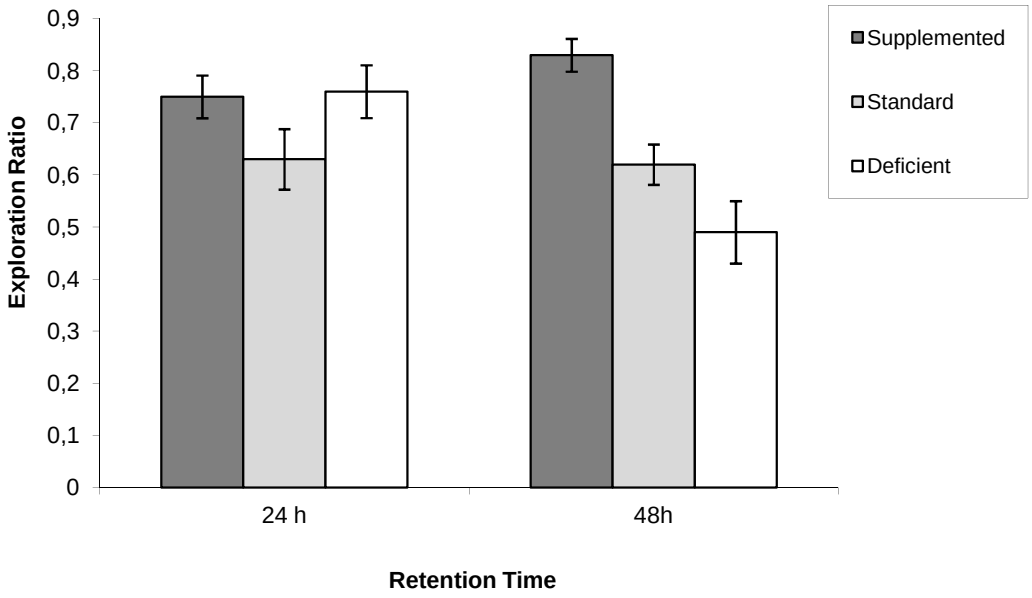

Figure 3. Mean (±SEM) exploration ratio (ER) of the different groups during each of the retention tests taking place 24 and 48 hours after the acquisition session. The dotted line shows the "chance level". A ER value of 0.5 shows no differences between the exploration time of the novel, and the familiar objects, while values close to 1 show longer exploration of the novel object, thus evidencing memory of the object previously explored during the acquisition session.

A further mixed 3 (diet) x 2 (retention interval) ANOVA analysis performed on the total exploration time showed longer exploration time at the 24 h delay (77.57 s) than at the 48 h delay (61.73 s); $F(1,27) = 7.47$ and $p < 0.01$. Neither the main factor of diet ($F(2,27) = 1.57$; $p = 0.23$) or the interaction diet x retention interval were significant ($F(2,27) = 0.05$; $p = 0.95$). The mean total exploration times of the supplemented, standard and deficient groups were 86,20 s, 76,20 s y 70,3 s at the 24 h retention interval and 72 s, 57,9 s y 55,3 s at the 48 h retention interval, respectively.

3.1.4 Discussion

The present results constitute the first demonstration that maternal dietary choline availability plays a critical role in the memory processes involved in spontaneous object recognition tasks during adulthood in rats.

Two main findings have been reported in this work. First, adult rats receiving choline supplementation from E12 to E18 exhibited increased retention ability. Second, a choline deficient diet during this gestational period impaired adult memory. The memory enhancement in the supplemented group was evident upon increasing the memory demands at a 48 h retention interval but not at a shorter 24 h interval. While at 24 h the three groups (deficient, standard and supplemented) exhibited memory of the familiar object with no significant differences among them, a 48 h retention interval revealed that the rats belonging to the supplemented group showed a higher retention index than the standard group. In spite of this, both groups performed over the chance level, thus demonstrating memory of the familiar object. However, the ER of the deficient group did not differ significantly in relation to the chance levels at the longer 48 h retention interval. Therefore, the longer 48 h retention interval in the present experiment increased the demand for memory allowing us to reveal the effect of prenatal choline on SOR memory.

However, given the fact that we applied two consecutive retention tests using the same familiar object, an alternative explanation cannot be ruled out. It is feasible that the general improvement in the choline supplemented subjects and impairment in the deficient subjects at 48 h is more related to relative resistance/susceptibility to proactive interference. Nevertheless, whatever the mechanism involved, our results lend support to the notion that performance in memory tasks can be modified by changing dietary choline availability during adulthood.

It is conceivable that the maternal dietary intervention had affected adult SOR memory by influencing the development of the forebrain cholinergic system. The dietary treatment implemented was carried out during a critical gestational period (E12-18), during which the forebrain cholinergic neurons undergo major developmental

changes (9). Thus, it has been demonstrated that neonatal (PN 0-7) forebrain cholinergic lesions impair neither acquisition nor memory using short retention intervals in a similar task (10). This is consistent with an interpretation of our results in terms of a potential effect on the development of the cholinergic system, since retention at the shorter 24 h interval was not affected by dietary choline availability during prenatal development. Rather,the effect was only evident at a long 48 h retention interval.

The behavioral data reported in the present study do not allow us to draw any firm conclusions with respect to the brain areas and mechanisms involved. Whist there is some controversy about the brain areas involved in SOR memory, there is a growing number of studies that point to the involvement of the perirhinal cortex (PER) as a key area (8). The long-lasting decrement in PER activity associated with object familiarity has been related to NMDA receptors-dependent long term depression (LTD) which in turn is mediated by AMPA receptors (11). Therefore, given that PER and the glutamatergic receptors AMPA and NMDA seem to be critically involved in consolidation of object recognition memory, it could be proposed that the early diet intervention had altered a cholinergic modulatory role on glutamatergic neurotransmission. It is well known that both choline and cholinergic agonists modulate non-cholinergic neurotransmission, including that mediated by NMDA receptors in cortical areas, such as the auditory cortex (12). Critically, cholinergic-glutamatergic interactions have proven to be important during early cortical morphogenesis. Thus, lesions of the basal forebrain cholinergic system in neonatal mice (PN 0) cause a decrease in AMPA and NMDA receptors in the neocortex during the PN 14 (13). It is therefore possible that an abnormal development of the cholinergic system, induced by prenatal choline deficiency in the present study, had disrupted the cholinergic-

glutamatergic interaction and consequently had caused a deficit in the retention of the familiar object.

With respect to the potential increase in adult retention abilities found in the supplemented group, a possible explanation in terms of adaptive metabolic changes triggered by prenatal choline availability can not be ruled out. Thus, it is known that prenatal choline-deficient rats develop an efficient mechanism to synthesize ACh from circulating choline, which serves to increase the gene expression of the high-affinity protein transporter (14). However, there is a decrease of the choline content in the membranes (PC) and an inability to sustain the release of acetylcholine evoked by depolarization, which could be responsible of the retention deficits. In contrast, previous work in hippocampal slices has found that prenatally supplemented rats synthesize a high proportion of ACh from the membrane-bound choline pool in the form of PC (15). Thus, it is possible that the effects at the increased retention intervals found in the present study could be related to this. Our results might therefore be interpreted in terms of the fact that the availability of choline during sensitive periods can generate phenotypes with different metabolic pathways of choline that affect cognitive performance.

In addition, the fact that the prenatal dietary intervention produced permanent effects on the animals' performance in a SOR task that were evident in adult rats could be attributed to non-cholinergic epigenetic mechanisms inducing long-lasting effects. Choline, as a precursor of the membrane phospholipid phosphatidylcholine favours hippocampal neurogenesis (4). It is also known that choline acts as a methyl donor influencing DNA methylation, thus inducing life-long changes in health status (3).

Our results showing that variation in choline availability during this specific developmental period (E12-18) altered performance in a SOR task are consistent with those previously reported using spatial and temporal memory tasks (1-2). Therefore, our findings add to the bulk of knowledge about the influences of specific nutrients on emotional and cognitive processes, and they also shed light on the issue of determining nutritional requirements during sensitive periods of development. In this regard, some significant contributions have also been achieved by manipulating dietary lipid macronutrients such as polyunsaturated fatty acids and gangliosides. Similarly, an effect on emotional and cognitive functions has been observed by varying the availability of the micronutrients, such as iron and zinc in the early phases of life (16).

Furthermore, our results might be relevant for developing a better understanding of the role of maternal nutrition during human development. In particular, there are similarities between the physiological responses of women and rats to depletion of hepatic choline reserve. Such depletion is often caused during gestation and nursing by the large amounts of choline transmitted to the fetus throughout the amniotic liquid and maternal milk (17). Therefore, it might be advisable to increase choline supplementation during pre- and perinatal periods. Interestingly, the formal recognition of choline as an essential nutrient for humans did not occur until 1998 (18). Even most recently choline has been incorporated into the database including the nutritional composition of common foods in the human diet (19). There is increasing evidence to show reduced choline intake in women (20) with respect to the recommended doses during pregnancy (450 mg/day) and lactation (550 mg/day). In addition, variations in choline content of infant formula with respect to the maternal milk choline concentration (about 131 mg/L of choline equivalents) have been reported (21). In fact, there are significant variations in the dietary choline requirement of women during perinatal periods that can be

explained by very common genetic polymorphisms (3). Thus, genetic differences should be taken into account in order to understand the effects of choline supplementation in humans and to make practical recommendations.

Finally, an additional value of the present findings for potential applications to treat human memory disorders is the use of a SOR task. This task has potential as an appropriate procedure for preclinical studies of human memory due to the similarity of procedures in human and rodent versions of recognition memory tasks (22) as well as its dependence on MTL brain areas (23). Previous work has demonstrated that this delay-dependent deficit is critical in the diagnosis of amnesia (24). Given the results of the present study with rodents, we cannot rule out the possibility that choline deficient diets might similarly be impairing memory abilities in humans. Although a human study did not find any correlation between the choline status in maternal serum and offspring IQ at the age of 5 years (25), it did not include choline supplementation, and the behavioral tasks applied were different to those of the present study. In addition, since plasma choline derives from dietary choline, endogenous synthesis and liberation from its reservoir within the membrane's phospholipids, the plasma choline concentrations might not be an appropriate index to assess the intake of choline. Thus, further research is required to determine the influence of pre- and perinatal choline availability on infant and adult cognitive abilities.

Acknowledgments

The authors are grateful to Dr. Michelle Symonds for English editing. This research was supported by grants PSI2009-07513 (MICINN. Sapin), PSI2012-31641 (MINECO.

Spain), PSI2011-23702 (MINECO. Spain), and grant HUM-02763 (Junta de Andalucía, Spain) partially funded by FEDER.

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ESTUDIO 2: La Suplementación Crónica con Colina Mejora la Memoria de Reconocimiento de Objetos en Ratas Adultas

3.2.1 Introducción

Dos han sido los períodos que han centrado el interés de los investigadores para evaluar el efecto del precursor de la acetilcolina, la colina (Ch), en procesos cognitivos: el desarrollo temprano y la vejez. La Ch es una vitamina que se encuentra en muchos alimentos principalmente en forma de lecitina o fosfatidilcolina (USDA, Database for the Choline Content of Common Foods, 2004). Debido al deterioro colinérgico observado durante la vejez y en enfermedades degenerativas (Terry & Buccafusco, 2003), el objetivo principal del uso de esta vitamina ha sido evitar, relentecer e incluso revertir el detrimento cognitivo senil o provocado por dolencias como el Alzheimer. Administrando Ch a través de la dieta, algunos trabajos han creado expectativas para aliviar dichos padecimientos. Así, los estudios del desarrollo con modelos animales han demostrado que la disponibilidad de Ch prenatal o postnatal temprano, coincidente con el desarrollo del sistema colinérgico, incide en funciones cognitivas, los cuales perduran durante toda la vida del individuo (Meck & Williams, 2003; McCann et al., 2006). Por su parte, la suplementación con Ch en animales y humanos como tratamiento contra el déficit cognitivo senil han sido poco alentadora. Los resultados obtenidos no fueron convincentes y, muchos de ellos contradictorios (Bartus, 2000).

Pese al alto número de investigaciones, poco es conocido acerca del efecto de la Ch cuando la manipulación dietaria y las tareas cognitivas son implementadas entre la edad adulta-joven y adulta-madura en individuos sanos. Aun los escasos estudios en animales han insistido en evaluar la disponibilidad de Ch como un tratamiento para aliviar déficit cognitivo provocado por factores ambientales (Teacher and Wurtman, 2005) o determinar el efecto cuando el nutriente está ausente en la dieta (Nakamura et al., 2001). De aquí que, la evaluación de la Ch exclusivamente como un mejorador cognitivo en sujetos adultos sanos ha permanecido usualmente olvidado.

En el Capítulo II (Experimento 2) del presente trabajo de investigación, se evaluó el efecto de la suplementación con colina durante 7 semanas en ratas adultas-jóvenes (3 meses de edad) sobre la retención de una aversión asociada al contexto, una tarea compleja dependiente del hipocampo. Los resultados mostraron un mejor desempeño en los sujetos suplementados en comparación a los estándares. Con el propósito de confirmar la sensibilidad del cerebro adulto sano a la mayor disponibilidad de Ch, con el Estudio 2 se pretende determinar el efecto de la suplementación con colina en la memoria en ratas adultas-maduras. Asimismo, se busca establecer diferencias en el efecto de la Ch cuando la suplementación es implementada en distintos períodos en la vida del individuo. El Estudio 1 mostró claramente que ratas enriquecidas durante la gestación mejoran la memoria utilizando un paradigma SOR. Así, en el Estudio 2 ratas entre 6-7 meses de edad fueron suplementadas con Ch durante 10 semanas y probadas en la tarea SOR empleando el mismo diseño experimental utilizado en las ratas prenatales.

3.2.2 Experimento 1

3.2.2.1. Materiales y Métodos

Sujetos y dieta

Dieciséis ratas Wistar machos entre 6-7 meses de edad con un peso promedio de 545 g (470-618 g) fueron mantenidos a temperatura ambiente (22-23 °C). Las ratas fueron alimentadas con una dieta estándar *ad libitum* antes de comenzar la fase experimental. Los sujetos, asignados aleatoriamente a un grupo estándar (n= 8) o suplementado (n= 8), recibieron la dieta AIN-76A estándar con un contenido de 1.1 g/Kg ó 5 g/Kg de cloruro de colina respectivamente, durante 10 semanas. Durante la manipulación dietaria, alimento y agua fueron suministrados *ad libitum*. Al finalizar este

período, todos los animales fueron alimentados con la dieta AIN 76-A estándar, hasta que los ensayos conductuales fueron completados.

Procedimiento Conductual

El procedimiento conductual incluyendo los objetos y el diseño experimental intra-sujetos fueron los mismos a los utilizados en el Estudio 1.

3.2.2.2. Resultados

Un análisis de ANOVA mixto de 2 x 2 (dieta x objeto) no mostró ninguna diferencia significativa en el tiempo de exploración a los dos objetos idénticos en el factor dieta ($F(1,14) = 0.13$; $p = 0.73$), objeto ($F(1,14) = 0.81$; $p = 0.38$, ni de la interacción (dieta x objeto, $F(1,14) = 0.20$ y $p > 0.66$) durante la sesión de adquisición. La Figura 1, la cual muestra los valores promedios de ER en los dos tiempos de retención, evidencian que ambos grupos, suplementado y estándar, reconocen el objeto familiar a las 24 y 48 h. El análisis mixto ANOVA con dieta como factor entre-sujeto y retención como factor intra-sujeto confirmaron esta hipótesis. Así, no hubo diferencia de la dieta ($F(1, 14) = 0.51$; $p = 0.49$), la retención ($F(1,14) = 0.47$; $p = 0.51$) ni de la interacción dieta x retención ($F(1,14) = 0.04$; $p = 0.85$). Adicionalmente, todos los grupos reflejaron diferencias significativas en relación al "nivel chance" ($t_s > 0.05$) en ambas retenciones.

3.2.3. Experimento 2

Los resultados del Experimento 1 podrían sugerir que, a diferencia de la manipulación prenatal, la suplementación con colina en la edad adulta no afecta la memoria SOR. Sin

embargo, se planteó la posibilidad de que el procedimiento implicado en el diseño intra-sujeto en esta tarea influyera en la adquisición de la representación del objeto familiar. Es

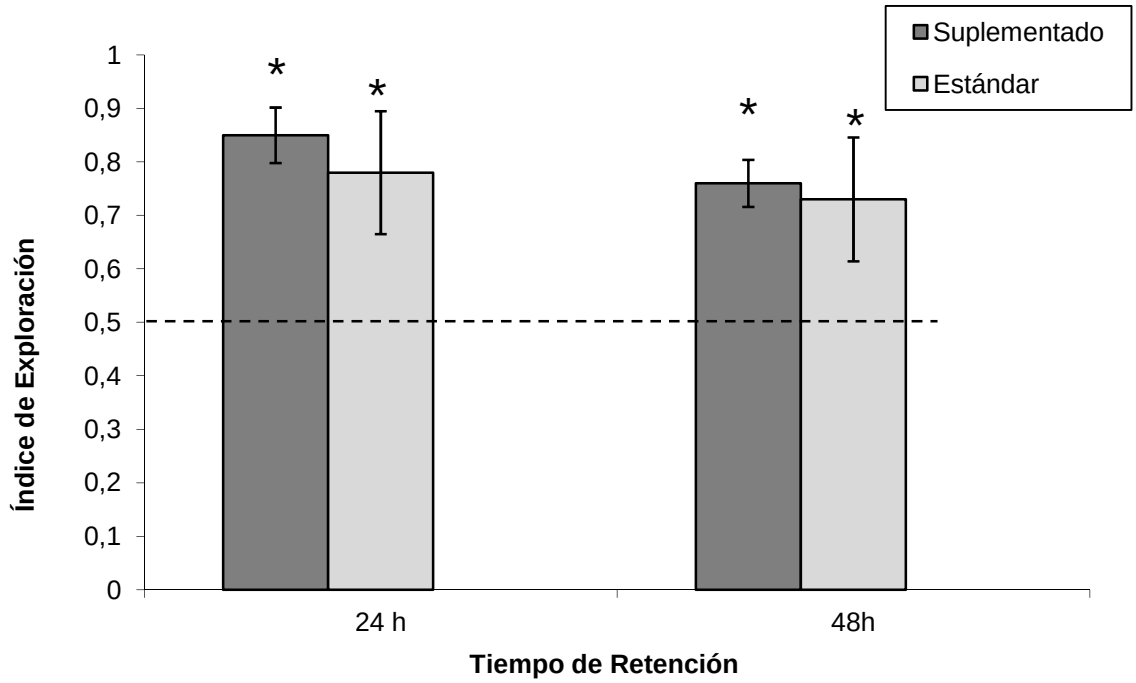

Figura 1. Medias (±SEM) de los índices de exploración (ER) de los diferentes grupos durante cada prueba de retención (24 y 48 h después de la fase de adquisición). La línea punteada muestra El "nivel chance". Un valor de ER igual a 0.5 refleja el mismo tiempo de exploración para el objeto familiar y nuevo, Un valor cercano a 1 muestra un mayor tiempo de exploración al objeto nuevo.

viable que la reiterada exposición al objeto familiar (fase de adquisición y dos pruebas de retención, 24 y 48 h. Figura 2) haya facilitado dicha adquisición lo cual se reflejaría en el reconocimiento del objeto familiar en ambos tiempos de retención. Para probar esta hipótesis, en el Experimento 2 el mismo tratamiento dietario fue llevado a cabo, pero con un diseño entre-sujeto en el procedimiento conductual. De esta manera, los sujetos habían estado expuestos sólo una vez al objeto familiar para el momento de su desempeño durante la sesión de prueba (Figura 2).

3.2.3.1. Materiales y Métodos

Sujetos y dieta

El presente experimento se llevó a cabo en los laboratorios de Psicología Experimental de la Universidad de York, UK. El tratamiento dietario, idéntico al

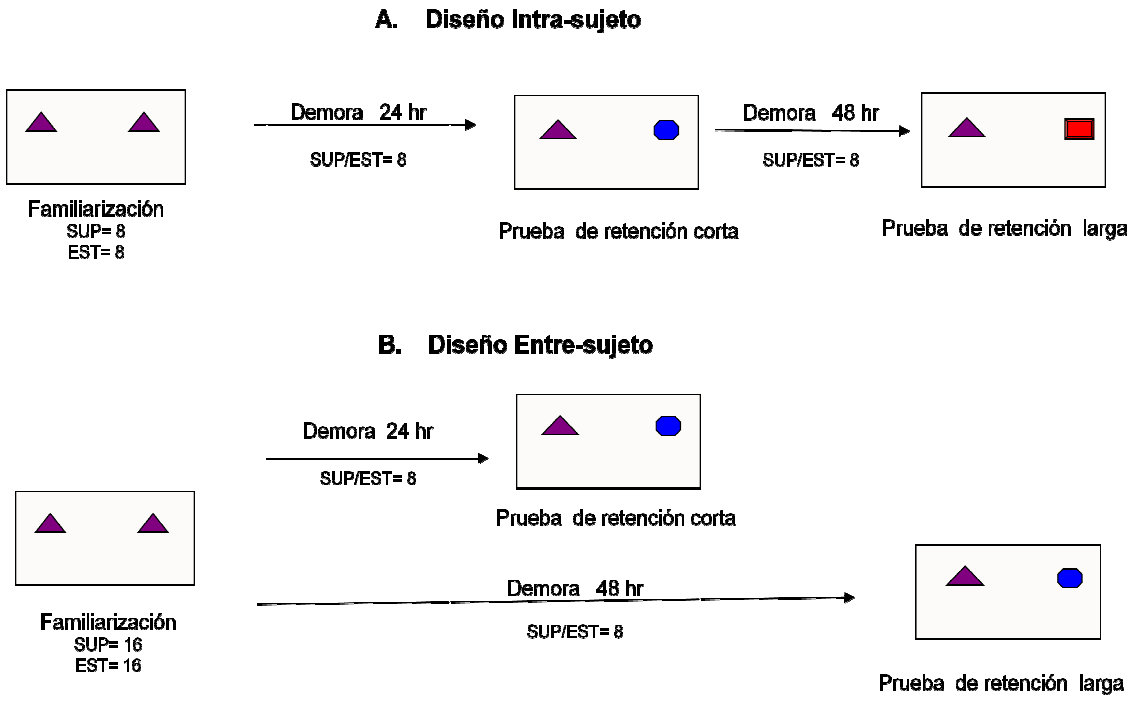

Figure 2. La Figura A y B representan el diseño intra-sujeto y entre-sujeto en una tarea SOR, respectivamente. SUP= ratas con dieta suplementada, EST= ratas con dieta estándar.

empleado en el Experimento 1, se implementó en treinta y dos ratas Lister machos provistos por Laboratorios Harlan UK. Así, los sujetos de aproximadamente 7 meses de edad con un peso promedio de 639 g (550-760 g), fueron asignados aleatoriamente a un grupo suplementado (n= 16) y otro estándar (n= 16).

Procedimiento Conductual

El mismo procedimiento conductual descrito en el Experimento 1 fue aplicado, excepto que en el presente ensayo se utilizó un diseño entre-sujeto. Así, cada grupo dietario, suplementado (n= 16) y estándar (n=16), fue subdividido a la mitad (n= 8) y distribuidos de forma aleatoria a un grupo de retención corta (24 h.) y el otro a un grupo de retención larga (48 h.). Después de 24 h ó 48 h de la fase de adquisición, los grupos de retención corta y larga respectivamente, fueron expuestos por segunda vez al objeto familiar y por primera vez al objeto nuevo (Figura 2).

3.2.3.2. Resultados

Dos datos de ratas suplementadas, una del grupo de 24 h (out layer) y otra del grupo de 48 h de retención (no exploró los objetos), fueron excluidos. Ninguno de los grupos (24 y 48 h de retención) mostró diferencias significativas en el tiempo de exploración de los dos objetos idénticos en la fase de adquisición. Así, el análisis de ANOVA mixto de 2 x 2 (dieta x objeto) no arrojó ningún efecto significativo en los factores principales dieta ($F(1,28) = 0.45$, $p = 0.51$; objeto ($F(1,28) = 0.67$, $p = 0.422$, ni de la interacción dieta x objeto ($F(1, 28) = 0.07$ $p = 0.8$.

La figura 3 muestra las medias (±SEM) de los valores ERs de los animales suplementados y estándares durante la fase de prueba a las 24 ó 48 h de retención. Un ANOVA de 2 x 2 (dieta x retención) arrojó un efecto significativo de la dieta ($F (1, 26) = 5.17$; $p < 0.05$), la retención ($F (1, 26) = 9.02$; $p <0.01$) y, de la interacción dieta x retención ($F (1, 26) = 4, 3$; $p < 0.05$). Un análisis de una vía en los ERs en los dos tiempos de retención no reveló diferencias significativas entre los grupos dietarios a las 24 h ($F (1, 13) = 0.014$; $p = 0.91$). En contraste, las medias de la ER entre los animales suplementados (ER = 0.71) y estándar (ER = 0.51) difieren significativamente ($F (1, 13) = 14.49$; $p < 0.05$) cuando el tiempo de retención es mayor (48 h).

Los valores de ERs de cada grupo fueron comparados con el "nivel chance" (0.5) usando la prueba t para una muestra. A las 24 h de retención, ambos grupos dietarios mostraron valores ER_s significativamente mayores en relación al "nivel chance", t (6) = 4.71, p <0.05 y t (7) = 4.58, p <0.05 para los sujetos suplementado y estándar, respectivamente. Sin embargo, a las 48 h de retención únicamente los animales suplementados reflejaron una diferencia significativa con el "nivel chance" (t (6) = 5.44; p < 0.05), mientras que los valores de ER_s del grupo estándar no difieren del "nivel chance" (t (7) = 0.24; p = 0.81).

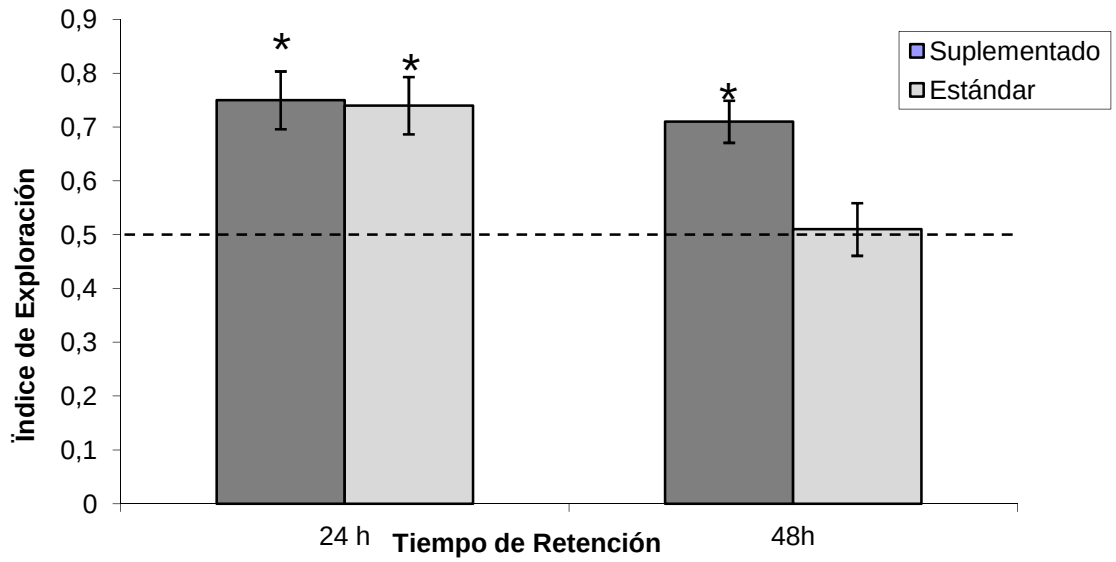

Figure 3. Medias (±SEM) de los índices de exploración (ER) de los animales suplementados y estándar durante las pruebas de retención (24 y 48 h después de la fase de adquisición).

3.2.4 Discusión General

Los resultados del Estudio 2 permitieron demostrar que la suplementación con colina durante la edad adulta-madura afecta el desempeño de los sujetos cuando la demanda de memoria es incrementada en una tarea SOR.

Mientras que el diseño experimental intra-sujeto (Experimento 1) no reflejó ningún efecto, el diseño entre-sujeto implementado en el Experimento 2 reveló que la mayor disponibilidad de colina en la dieta mejora la memoria cuando hay un incremento en el intervalo de demora entre la fase de adquisición y la prueba. En efecto, ninguna diferencia significativa en el reconocimiento del objeto familiar a las 24 h y 48 h de retención fue encontrada entre los grupos suplementado y estándar en el Experimento 1. En contraste, en el Experimento 2, ambos grupos dietarios pasaron más tiempo explorando el objeto nuevo en la retención corta (24 h), sin embargo, sólo los animales suplementados reconocieron el objeto familiar después de 48 h de la adquisición. Diferencias en el procedimiento entre los diseños experimentales utilizados en este estudio pudieran estar afectando la ejecución de la tarea SOR. Así, en el diseño intra-sujeto los animales tuvieron tres exposiciones consecutivas al objeto familiar (una en la fase de adquisición y posteriormente, dos durante las pruebas de retención, 24 y 48 h). Es posible que este procedimiento haya provocado una mayor habituación, un proceso definido como la disminución de una respuesta (ej. exploración) evocada por la repetida exposición a estímulos nuevos sin ninguna consecuencia relevante (Leussis & Bolivar, 2006). Así, una mayor habituación al objeto encontrado previamente pudiera facilitar su representación y, en consecuencia un mejor reconocimiento. Por su parte, el diseño entre-sujeto utilizado en el Experimento 2 permite evaluar la capacidad de los animales para reconocer el objeto familiar después de un único encuentro (fase de adquisición). En este procedimiento, los animales deben retener la representación del objeto familiar durante un intervalo de demora hasta la fase prueba. Los resultados indicaron que la suplementación con Ch mejora la memoria, lo cual se hizo evidente cuando el tiempo de retención fue incrementado de 24 a 48 h.

Por otra parte, los resultados de los estudios cuando la disponibilidad de la vitamina es incrementada en el adulto (Experimento 1, Estudio 2) difieren de los encontrados en la suplementación prenatal (Experimento, Estudio 1). Mientras que los sujetos suplementados prenatal y adulto reconocieron el objeto familiar a las 24 h de retención, sólo los animales prenatales reflejaron una mejor retención 48 h después de la adquisición en SOR. Si, tal como se discutió anteriormente, las exposiciones repetidas al estímulo implicado en el diseño intra-sujeto (utilizado en estos dos experimentos) facilita la retención del objeto, entonces es la habituación el proceso cognitivo que propicia el incremento de memoria observada en este paradigma utilizando este procedimiento. Estudios previos sugieren una modulación colinérgica en el proceso de habituación. Thie et al. (1998), en sus investigaciones de microdiálisis con ratas, encontraron una relación entre el incremento de ACh extracelular hipocampocampal durante la exploración de un ambiente nuevo. Interesantemente, la re-exposición al mismo ambiente disminuyó la conducta exploratoria pero mantuvo altos los niveles de ACh. Es posible presumir que la habituación es sensible a la variabilidad de Ch prenatal durante E12-E18, período de neurogénesis colinérgico en ratas (Semba y Fibiger, 1988; Brady y col., 1989). Estos resultados estarían sugiriendo que la colina dietaria afecta la memoria facilitada por el proceso de habituación sólo en periodos tempranos del desarrollo. No obstante, estudios adicionales son necesarios para evaluar el efecto de la Ch durante la suplementación temprana y adulta.

Algunos mecanismos pudieran fundamentar a favor de la modulación del sistema colinérgico en procesos cognitivos en sujetos adultos. En la actualidad es ampliamente aceptado que la neurogénesis no es exclusiva de organismos inmaduros sino que, el cerebro adulto es capaz de generar y desarrollar nuevas neuronas funcionales (Abrous et. al, 2011). Interesantemente, se ha demostrado que el sistema colinérgico regula la

neurogénesis en el hipocampo adulto (Cooper-Kuhn et al., 2004) modulando consecuentemente la plasticidad cognitiva de cerebros maduros (Mohapel et al., 2005; Van Kampen & Eckman, 2010). Por otro lado, recientes estudios indican que procesos epigenéticos son altamente dinámicos en el cerebro adulto (ver revisión McGowan et al., 2008). Debido a que la programación epigenética responde a factores ambientales, ej. la dieta (Gottlieb, 2000), el suministro de Ch podría alterar epignéticamente la expresión de genes dando lugar a fenotipos conductuales. De aquí que, la dieta en general y, particularmente la Ch como un nutriente portador de grupos metilos implicados en cambios epigenéticos (Niculescu, 2012), podrían contribuir a la plasticidad fenotípica no sólo durante períodos tempranos del desarrollo, sino también, durante toda la vida del individuo.

Juntos, los Estudios 1 y 2 demostraron que la variabilidad de Ch en la dieta afecta funciones cognitivas tanto en edades tempranas como en adultas. Comparando los grupos de Ch temprana y adulta, los resultados reflejaron diferencias en el efecto de la colina dietaria según la edad de suplementación en la tarea SOR. Si la modulación de la Ch difiere en sujetos inmaduros y maduros implica que, la edad es un factor crítico en establecer la precisa función de este nutriente en una tarea determinada. Así, investigaciones adicionales tomando en cuenta esta variable son requeridos.

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CAPÍTULO 4

CHRONIC DIETARY CHOLINE SUPPLEMENTATION MODULATES ATTENTIONAL CHANGE IN ADULT RATS

CAPÍTULO 4: CHRONIC DIETARY CHOLINE SUPPLEMENTATION MODULATES ATTENTIONAL CHANGE IN ADULT RATS

Behavioural Brain Research. 2013, 243: 278–285

Hayarelis Moreno, Isabel de Brugada, Geoffrey Hall

ABSTRACT

In two experiments adult rats were maintained on a diet enriched with added choline for 12 weeks prior to behavioral testing; control rats remained on the standard diet during this time. In Experiment 1 all rats received training in the Hall-Pearce negative transfer paradigm in which prior training with a conditioned stimulus (CS) paired with a small reinforcer retards further learning when the size of the reinforcer is increased. This effect, which has been attributed to a loss of associability by the CS, was obtained in control subjects but not in those given the supplement. Experiment 2 investigated the effect of prior nonreinforced exposure of the to-be-CS (latent inhibition). Such exposure retarded subsequent learning in control subjects, but latent inhibition was not obtained in those given the supplement. We conclude that the mechanism that reduces the attention paid to a stimulus that accurately predicts its consequences does not operate effectively after choline supplementation. These results are consistent with a role for the cholinergic system of the basal forebrain in modulation of attention.

Key words: choline; attention; conditioning; latent inhibition; basal forebrain; rats

4.1. Introduction

Choline, is a quaternary amine classified within the vitamin B complex, present in many foods. It is regarded as an essential nutrient [1, 2]. It is necessary for the normal functioning of all cells due to the role that it plays in the synthesis of phospholipid components of the membrane; it is also a precursor of the neurotransmitter acetylcholine (ACh) [3]. The availability of this precursor can determine the speed of production and liberation of the neurotransmitter and will be influenced by diet. Choline transport at the blood-brain barrier depends on plasma concentration; in basal conditions this is between 8-11 μM of free choline in humans and experimental animals, but this can increase to about 40 μM in humans and up to 50 μM in rats [4, 5] after the ingestion of choline-rich food. Dietary supplementation will thus increase levels of cerebral choline, and promote the synthesis and emission of ACh in the brain[6, 4, 7]. Conversely, choline restriction reduces serum concentration, and diminishes the production of ACh in cholinergic neurons [8, 9, 10].

Studies in which levels of dietary choline have been manipulated have shown an effect on cognitive functioning in experimental animals given tasks taken to depend on the cholinergic system of the forebrain. For the most part these studies have focused on the role played by choline availability very early in development, usually perinatally (see [11], and [12], for reviews; also [13]). Evidence on the effects of choline in older subjects is sparse and contradictory (see [14], for a review), although there is some evidence for effects in rats that might be classified as adolescent or young adult at the time of the dietary manipulation. Thus, rats given supplementary choline for 21 weeks from the age of 5 weeks have been found to show improved performance on a temporal discrimination task [15], and rats given a choline-deficient diet for 12 weeks from the

age of 2 months showed a memory deficit in a task of passive avoidance [10]. There are few studies available examining cognitive functioning after dietary manipulation exclusively in adulthood. However, Teather and Wurtzman (2005) [16] have shown that 12 weeks of access to a high-choline diet for 3-month old rats attenuated a memory deficit caused by exposure to an impoverished environment, and Moreno, Gil, Carias, Gallo, and de Brugada (2012) [17] found better retention of a context aversion in rats of the same age given 7 weeks of supplement. These findings were enough to encourage us to investigate the effects of supplementation on fully adult subjects.

We chose to investigate the effects of dietary choline on behavioral tasks designed to assess an aspect of attention. The ability of manipulations of choline levels to influence cognitive functioning may be assumed to operate by way of an effect in the basal forebrain cholinergic system, the main source of cholinergic input to the cortex and the limbic system. Lesions of this system have been found to generate a wide range of effects, but, according to Sarter and Bruno (1997) [18], they are largely consistent with the hypothesis that cholinergic input to the cortex mediates the subject's ability to select stimuli for processing (see also [19]). This form of attention has been intensively studied by Holland and Gallagher and their collaborators, using a set of behavioral paradigms that allows specification of the detailed mechanisms involved (see [20], for a review). One paradigm of particular interest assesses the ability of rats to resume attending to a stimulus with which they have grown familiar, when the consequences of that stimulus are changed. In this procedure (devised by Wilson, Boumphrey, & Pearce, 1992, [21]) rats are trained initially with a target stimulus (a light) reliably followed by another (a tone). When, in the test phase, the light is used to signal the immediate availability of food, conditioned responding develops slowly. This is taken to indicate

that, during the first phase, the light, being a reliable predictor of its consequences, loses the power to govern attention (suffers a decline in its *associability*,[22]); subsequent conditioning is thus retarded. This retardation can be eliminated, however, if, between the two phases, the rats experience some trials on which light-tone trials are intermixed with light alone trials. The surprising change in the consequence of the light restores its lost associability, allowing learning to occur normally on the test.

Holland and Gallagher (1995) [23] investigated the effects of lesions of the central nucleus of the amygdala on the task just described. They found that after such lesions the interpolated "surprise" trials were without effect, so that learning in the test phase remained slow. They concluded that the lesions had disrupted the mechanism responsible for restoring lost associability. Their interpretation was that the central nucleus regulates the surprise-induced increase in associability by way of its interaction with the basal forebrain cholinergic system. Direct support for this interpretation came from a study by Chiba, Bucci, Holland, and Gallagher (1995) [24] who gave rats given central infusions of a form of saporin that produces selective lesions of cholinergic neurons. When tested in the procedure of Wilson et al. (1992) [21], subjects with saporin-induced lesions in the caudal region of the forebrain (the region that provides the primary cholinergic input to the cortex) behaved like rats with lesions of the amygdala, in that they failed to show the normal, surprise-induced restoration of associability.

In a subsequent study, Baxter, Holland, and Gallagher (1997) [25] reported a parallel investigation of the effects of saporin-induced lesions of the rostral region of the basal forebrain, a region that projects primarily to the hippocampus. Rats given this treatment learned readily in the test phase of the Wilson et al. (1992) [21] procedure,

and did so whether they had experienced the surprise trials or not. This result suggests that in these animals the normal loss of associability produce by the first phase of training had failed to occur. Han, Holland, and Gallagher (1995) [26] have observed a similar effect in rats given neurotoxic lesions of the hippocampus itself. These and related results have been taken to support the general conclusion that increase and decreases in associability are mediated by distinct and separate brain mechanisms (see, e.g., [20], for a review).

Prompted by these observations Meck, Jones, Williams, Pauldine, and Holland (1997; reported in [11]) examined the effects of variation in dietary choline on performance in the Wilson et al. (1992) [21] procedure. Choline was manipulated prenatally (i.e., via the diet of pregnant dams, whose offspring were the experimental subjects). There were three groups: one in which the mothers were maintained on a standard diet, one in which they were given a diet with supplementary choline for 7 days during the second half of gestation, and one in which they were given a choline-deficient diet during this period. When tested in adulthood the offspring of mothers in the choline-deficient condition, like rats with lesions of the amygdala and rats treated with saporin in the caudal region of the basal forebrain, showed slow learning in the test phase even after the surprise trials – in these animals the mechanism responsible for reducing the associability of a consistent predictor appeared to work normally, but that responsible for restoring lost associability did not. Performance on this task was also influenced by supplementation of choline. Subjects in this condition learned readily in the test phase, and did so whether they had experienced the surprise trials or not; that is, like rats with lesions of the rostral region of the basal forebrain or with hippocampal lesions, these subjects appeared to be resistant to the loss of associability normal

induced by the first phase of training. Thus, choline deficiency disrupts the processes necessary for an increase in associability when this has fallen to a low level, but choline supplementation prevents loss of associability on the first place.

Accordingly, in our initial studies of the effect of choline supplementation in adult subjects, we decided to examine training procedures that are effective in producing decrements in stimulus associability in normal animals. The first of these, used in Experiment 1, and sometimes known as Hall-Pearce negative transfer [27], has something in common with the well-established latent inhibition effect [28] – the retardation of conditioning produce by prior nonreinforced exposure to the to-be-conditioned stimulus. However, it shares with the procedure of Wilson et al. (1992) [21] that in the initial phase of training, the target stimulus is followed by a consistent consequence. According to Pearce and Hall (1980) [22] subsequent poor learning about this stimulus results from a loss of stimulus associability during the first phase of training. The second procedure (used in Experiment 2) was latent inhibition itself. This effect may be multiply determined (see [29], for a review) but an important component is the loss of associability generated by the preexposure treatment [30].

4.2. Experiment 1

In this experiment we compared rats that had been maintained throughout their lives on a standard laboratory diet with rats given a diet containing supplementary choline for 12 weeks from the age of 8 months. The rats were tested using the conditioned suppression procedure. The design of the experiment is summarized in Table 1. The rats received an initial phase of training in which the conditioned stimulus (CS; a tone for half the subjects, a light for the rest) was followed by a relatively weak

footshock, the intensity of the shock being chosen to generate a moderate level of suppression of the baseline response (food-reinforced lever pressing). The second stage assessed the acquisition of further suppression with a shock of increased intensity, all the rats now experiencing the tone as the CS. The control subjects can be expected to

Table 1: Experimental designs

Experiment 1		
Group	***Phase 1***	***Phase 2***
SUP- T	$T \rightarrow US_{Weak}$	$T \rightarrow US_{Strong}$
SUP- L	$L \rightarrow US_{Weak}$	
CON-T	$T \rightarrow US_{Weak}$	
CON-L	$L \rightarrow US_{Weak}$	
Experiment 2		
Group	***Preexposure***	***Conditioning***
SUP	*T or L*	$T \rightarrow US_{Shock}$
CON		*and*
		$L \rightarrow US_{Shock}$

Note: SUP = supplemented diet; CON = standard diet; T and L = tone and light CSs; US_{weak} = electric footshock of 0.25 mA; US_{strong} = electric footschock of 0.5 mA; US_{schock} = electric footshock of 0.25 mA.

show the negative-transfer effect of Hall and Pearce (1979) [27], with subjects pretrained with the tone learning more slowly than those pretrained with the light. According to Pearce and Hall (1980) [22] this effect occurs because the initial phase of training produces a reduction in the associability of the CS used in that stage. The

question of interest was whether rats given the choline supplement would show this effect. If supplementation disrupts the mechanism responsible for reducing associability, we might expect the negative-transfer effect to be absent in these subjects.

4.2.1. Method

4.2.1.1. Subjects and diet.

The subjects were 32 male Lister hooded rats supplied by Harlan Laboratories UK. After arriving in the York laboratory at the age of 3 months, they were housed in pairs in an environmentally controlled colony room, with a 12 hr light/dark cycle. Experimental sessions occurred during the lit periods of the cycle. Before the start of the present experiment the rats were maintained with ad libitum access to Certified Rodent Diet 5002 (supplied by LabDiet; PMI Nutrition International). This is the standard diet used in our laboratory; it contains 2 g/kg of choline chloride. The rats were initially used in a study of flavor preference conditioning, but were naïve with respect to the procedures of the present experiment, which commenced when they were aged 8 months (when the rats had a mean weight of 608 g; range: 470 – 710 g).

Previous work on perinatal [11] and adult [31, 16, 17] supplementation has demonstrated effects with choline concentrations between 2.6 and 5 times higher than that of the standard diet. We made use, therefore, of a supplemented version of the rodent diet of the American Institute of Nutrition (AIN) which produces a 4.5-fold increase of choline with respect to the AIN-76A standard diet. (The standard AIN 76-A diet supplies 1.1 g/kg of choline; the supplemented diet supplies 5 g/kg.) Sixteen rats were assigned to the AIN 76-A standard diet and the remainder were given the supplemented formulation. For the next 12 weeks of dietary manipulation the

appropriate food was provided ad libitum. At the end of this period, all were transferred to a restricted feeding regime with the AIN 76-A standard diet, and were maintained at 85% of their free-feeding body weights until behavioral testing was complete.

4.2.1.2. Apparatus.

Eight Skinner boxes (Med Associate Inc, St. Albans, VT) were used. These measured 30 cm x 24 cm x 21 cm and were housed in sound-attenuating chests. The ceiling and two longest sides of the chamber were made of clear plastic, and the front and back walls of stainless steel. A houselight, set high on the rear wall, provided dim illumination. Each box was equipped with a response lever on the front wall. Situated to the right of the lever was an aperture (5 cm x 5 cm), that gave access to a food cup to which 45-mg Noyes food pellets could be delivered. The floor of the chamber consisted of stainless steel rods to which a scrambled shock could be delivered from a Coulbourn Instruments (Allentown, PA) shock source. Two different stimuli were used as CSs. Set above of the lever was a 100-mA 28-V lamp, which provided the light stimulus. The second CS was 2.5 KHz tone, at 80 dB, generated by a speaker adjacent to the houselight. Both stimuli had a duration of 60 s.

4.2.1.3. Procedure.

Training consisted of daily 40-min sessions. The baseline response of lever pressing was established over the first five sessions. In the first session, food pellets were delivered on a variable-time (VT) 30-s schedule while lever press responses were continuously reinforced by delivery of a food pellet; in the second, each lever press again yielded a single food pellet again but in addition a delivered VT 60-s schedule

was in effect. Reinforcement was delivered according to a variable interval (VI) 30-s schedule in Session 3 and a VI 60-s schedule in Sessions 4 and 5. The VI 60-s schedule remained in force throughout the rest of the experiment.

For the first phase of conditioned suppression training, half the rats in each of the main groups (supplemented, SUP, and control, CON) were assigned to conditioning with the light (L) as the CS and half to the tone (T), making the four groups of 8 (SUP-T, SUP-L, CON-T, CON-L) of Table 1. There were five sessions of Phase-1 training, each containing 4 trials, consisting of presentation of the CS followed immediately by a 0.5-s, 0.25-mA footshock. Stimulus presentations occurred 5, 15, 25 and 35 min after the start of the session. All subjects were treated identically during the five sessions of Phase 2. The four groups received 2 trials per session in which the tone was followed by a shock of 0.5 mA for 0.5 s. Presentations of the tone occurred 5 and 25 min after start of the session. Responding was recorded during the CS and during the 60-s period (the preCS period) that preceded each trial. Suppression ratios were calculated for each session after pooling all CS and preCS scores for a given animal. These ratios took the form $a/(a+b)$, where a represents the rate of response in the CS and b the rate in the preCS.

4.2.1.4. Results and Discussion

The rats readily acquired the baseline response during the pretraining sessions. Unexpectedly, an effect of diet was evident at this stage, with rats in the supplemented condition responding much more frequently than the control subjects. On the last day of pretraining rats in the SUP groups had a mean rate of response of 25.65 responses per

min; those in the CON groups had a rate of 10.76 responses per min. These scores differed significantly, $F(1, 29) = 15.51$, $p < .01$ (here and throughout a significance level of $p < .05$ has been adopted).

This difference in baseline responding was maintained throughout conditioned suppression training. The left panel Figure 1 shows group mean responses per preCS period over the five sessions of Phase 1. For all groups, response rate tended to increase, but a higher rate was consistently shown by the SUP than the CON groups. An analysis of variance (ANOVA) with session, diet (CON or SUP), and type of CS (T or L) as the variables, showed there to be significant effects of session, $F(4, 108) = 9.38$, $p < .01$, and of diet, $F(1, 27) = 14.97$, $p < .01$. There was no significant effect of CS-type, and none of the interactions among variables achieved significance; largest $F(1, 27) = 2.30$, for the Group x Diet interaction.

Figure 2 shows the development of conditioned suppression during Phase 1. It is evident that all four groups acquired a moderate level of suppression by the final session and it appears that the light was somewhat more effective as a CS than was the tone, in that means for the L groups were consistently lower than those for the T groups. The overall levels of suppression were less in the SUP than in the CON groups, but a direct comparison of the suppression ratios of these groups would not be legitimate given that they are derived from substantially different baseline response rates. Accordingly we conducted separate analyses for the CON and SUP groups, with CS-type and session as the variables. One subject in the CON-T group lost baseline responding during Phase 1, making it impossible to compute a suppression ratio; this subject was excluded from further analysis, resulting in n = 7 for the CON-T group. For both the CON and the SUP groups, there was a significant effect of session: $F(4, 56) = 8.69$, $p < .01$, for the SUP

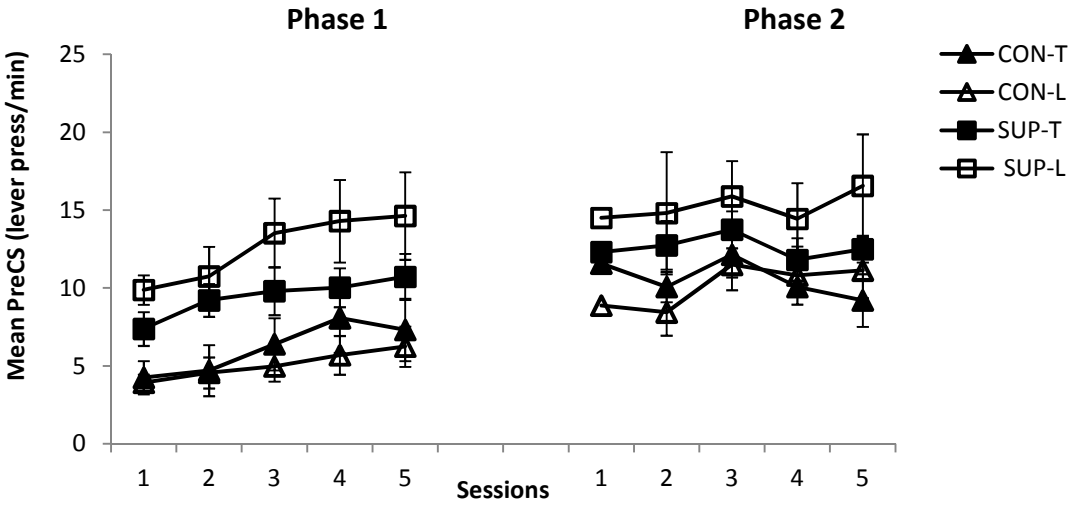

Figure 1. Experiment 1: Mean (±SEM) baseline (PreCS) response rates recorded during Phases 1 and 2. SUP = supplemented; CON = control; T = tone; L = light.

groups, and $F(4, 52) = 5.32$, $p < 0.01$ for the CON groups. The difference between tone and light turned out to be nonsignificant; there was no significant effect of CS type in either dietary condition (Fs < 1.2), and in neither was the interaction of the variables significant (Fs < 1.5).

The acquisition of suppression in Phase 2 (in which all animal received the tone followed by the stronger shock) is shown in Figure 3; the baseline preCS scores, from which the ratios were derived, are shown in the right panel of Figure 1. An error on the part of the experimenter meant that data were lost on Session 1 for four of the subjects in groups SUP-L and CON-L (although the rats experienced the events as scheduled). Accordingly the mean scores shown in the figure for the first session for groups SUP-L

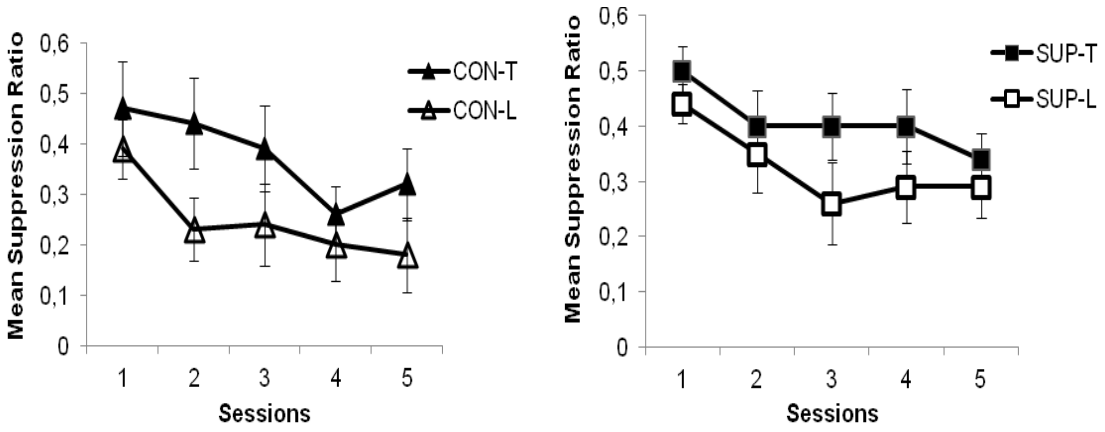

Figure 2. Experiment 1: Mean (±SEM) suppression ratios during Phase 1 for supplemented (SUP) and control (CON) groups; T = tone; L = light.

and CON-L are derived from the remaining four subjects in each of these groups. A full set of data was available for the remaining four sessions, and statistical analysis was confined to the data from these sessions. Introduction of the stronger shock resulted in a reduction in the baseline response rate, but the rate of the SUP groups remained higher than that of the CON groups (Figure 1). An ANOVA conducted on the data shown in the figure with CS-type, dietary condition and session as the variables revealed only a significant main effect of diet, $F(1,27) = 5.01$, $p < .05$; for other main effects and interactions, $F < 1$. Given this difference, analyses were again conducted separately on the suppression ratios of the CON and the SUP groups.

As Figure 3 (left panel) shows, the CON group that had received Phase-1 training with the tone acquired suppression rather poorly compared with the CON group pretrained with the light, thus replicating the negative transfer effect of Hall and Pearce [27]. An ANOVA with group (Phase 1 with T or with L) and session as the variables

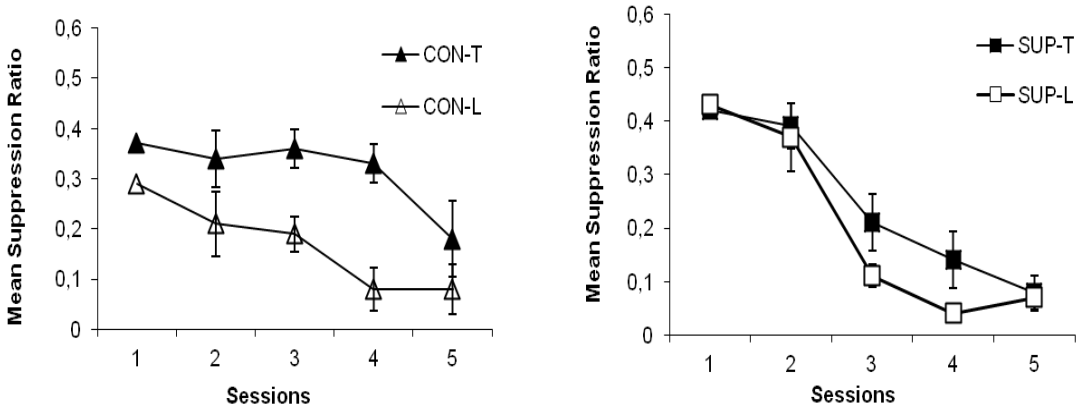

Figure 3. Experiment 1: Group mean (±SEM) suppression ratios during Phase 2 for choline supplemented (SUP) and control (CON) groups. (T = tone; L = light.)

revealed significant main effect both of group, $F(1, 13) = 9.18$, $p < .05$, and of session $F(3, 39) = 5.30$, $p < .01$; the interaction between the variables was not significant, $F(3, 39) = 1.1$. The SUP groups by contrast (Figure 3, right panel) both learned readily in Phase 2 and at much the same rate. For these groups the equivalent ANOVA revealed only a significant main effect of session, $F(3, 42) = 42.8$, $p < .01$; neither the effect of group, $F(1, 14) = 1.8$, nor the Group x Session interaction, $F(3, 42) = 1.5$ was significant.

The results of this study demonstrate that chronic dietary supplementation with choline can produce behavioral effects even in fully adult rats. This is shown both in the elevated rate of food-reinforced lever pressing shown by rats given the supplement and also by that fact that these rats failed to show retarded acquisition of conditioned suppression after pretraining with the CS. The first effect is novel and needs to be confirmed by further work; the second is consistent with the results reported by Meck and Williams (2003) [11], suggesting that choline supplementation disrupts the process

by which the associability of a consistent predictor is normally reduced. This proposal was examined further in Experiment 2, which also allowed a further examination of the effects of supplementation on food-reinforced leverpress responding.

4.3. Experiment 2

According to Pearce and Hall (1980 [22]; see also[30]) the negative transfer effect of Experiment 1 and the latent inhibition effect have the same source. In both, it is suggested, experience of a stimulus followed by a consistent consequence (the absence any event, in the case of latent inhibition) leads to a loss of associability, so that subsequent conditioning is retarded. On the basis of the results of Experiment 1, therefore, we might expect that choline supplementation would abolish or attenuate the latent inhibition effect.

The design of the experiment is shown in Table 1. As before, there were two main groups of subjects, those given choline supplementation in adulthood (SUP), and those maintained on a standard diet (CON). During the first phase of training all received nonreinforced presentations of a stimulus (a tone for half of each group; a light for the rest) that was to be used as a CS in conditioned suppression training in the test phase. During the test all subjects received reinforced trials both with the tone and with the light. Latent inhibition should be evident as slower acquisition to the stimulus preexposed in the first phase; the question of interest was whether such an effect would be obtained in the SUP group.

4.3.1. Method

The experiment was conducted at the University of Granada. The subjects were 16 male Wistar rats supplied by Harlan Laboratories. After arriving in the Granada laboratory at the age of 3 months, they were housed four to a cage in an environmentally controlled room under a 12-hr light/dark cycle, with ad libitum access to the standard diet, AIN 76-A. At age 8 months (mean weight 519 g; range: 422-614 g) eight subjects were assigned to the SUP condition and for 12 weeks were given access to the supplemented formula of the AIN 76-A diet; the CON group remained on the standard diet. At the end of period of dietary manipulation all were given the standard diet but feeding was restricted to reduce the animals to 80% of their free-feeding weights, prior to behavioral testing. The apparatus consisted of four Med Associates operant chambers. These measured 32 x 25 x 34 cm, and the speaker supplying the auditory stimulus was located on the front wall above the stimulus light; otherwise they were identical to those described for Experiment 1.

Pretraining established a baseline of food-reinforced lever-pressing on a VI 60-s schedule, and this schedule was maintained throughout the experiment. The preexposure phase consisted of five 40-min sessions with a 60-s stimulus being presented four times, 5, 15, 25, and 35 min after the start of the session. For half the animals in each group this was the light, and for half it was the tone. The conditioning phase consisted of a single session of four trials in which presentation of the CS was followed by a 0.5-s, 0.25-mA footshock. On two of the trials the CS was the tone and on two it was the light, the trial sequence was counterbalanced, being presented in the sequence TTLL for half the subjects in each group and in the sequence LLTT for the remainder. For each individual subject, the scores for both trials of a given type were

pooled to compute a suppression ratio for that session. Details not specified here were the same as those described for Experiment 1.

4.3.1.1. Results and Discussion

Apparatus failure meant that data were lost for two subjects in the CON group (one preexposed to the light. the other to the tone), reducing the group size to six.

The leverpress responding established by pretraining was maintained at a high rate throughout preexposure and conditioning. In contrast to Experiment 1, however, there was no substantial difference in rates between the SUP and CON groups. Thus on the last day of the preexposure phase the mean response rates recorded during the preCS periods were 29.3 responses per min for the CON group and 30.6 responses per min for the SUP group; these rates did not differ reliably ($F < 1$). The mean baseline response rates pooled over all preCS periods for the conditioning phase were 16.77 responses per min for the CON group and 17.34 responses per min for the SUP group; again, these did not differ reliably ($F < 1$). The marked difference between this and the previous experiment in the effect of diet on baseline responding raises the possibility that the different strains used in this investigation (Lister rats in Experiment 1, Wistar rats in Experiment 2) are differently sensitive to the effects of diets. However this may be, the lack of a difference between the SUP and CON groups in this experiments has a positive feature in that it is possible to make a direct comparison of the suppression ratios of the two groups that is not complicated by differences in baseline levels of responding.

Presentations of the stimuli (the tone and light) during the preexposure phase generated some unconditioned suppression of responding, that persisted throughout the

phase. Thus on the last session of preexposure the mean suppression for subjects exposed to the tone was .39 in the SUP group and .44 in the CON group; suppression scores for the light were .33 for the SUP group and .37 for the CON group. An ANOVA with stimulus (tone or light) and dietary group as the variables showed there to be no significant effect of diet, $F(1, 10) = 3.70$, $p > .05$, but there was a significant effect of stimulus, $F(1, 10) = 9.48$, $p < .05$. The interaction was not significant ($F < 1$.)

The results of the conditioning test session, group mean scores for suppression to the preexposed and the novel CS, are presented in Figure 4. It is evident that, for the CON group, suppression was acquired readily to the nonpreexposed stimulus but not to the preexposed stimulus; that is, the standard latent inhibition effect was obtained. The

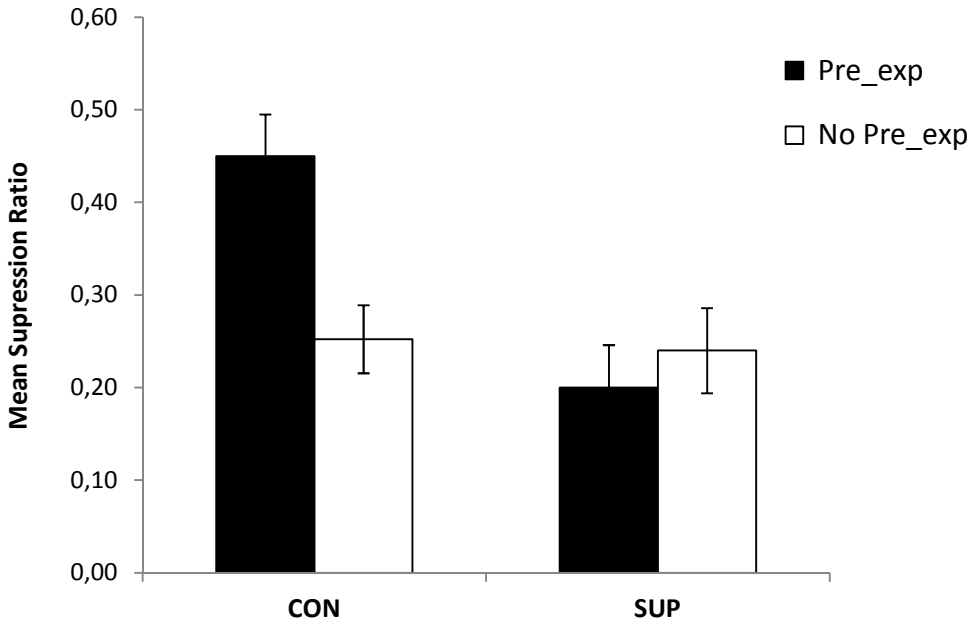

Figure 4. Experiment 2: Mean (±SEM) suppression ratios on the conditioning session shown to a CS that had been presented in the preexposure phase (Pre_Exp) and to a novel CS (No Pre_exp). Subjects in the SUP group had received choline supplementation; control (CON) subjects had not.

SUP group, by contrast failed to show latent inhibition, suppressing to both the preexposed and the nonpreexposed CS, at a level similar to that shown by CON subjects to the nonpreexposed CS. Statistical analysis confirmed this description of the results. We conducted an ANOVA with dietary condition and stimulus novelty (preexposed or not) as the variables; and given the difference between tone and light observed during preexposure we also included stimulus-type (light or tone) as a variable. This yielded a significant main effect of diet, $F(1, 10) = 8.17$, $p < 0.05$, and of the interaction between diet and stimulus novelty, $F(1, 10) = 11.53$, $p < .01$. No other effects or interactions were significant; largest, $F(1,10) = 4.74$. Analysis of the interaction confirmed that the score for the preexposed condition differed from that for the nonpreexposed condition in the CON group, $F(1, 5) = 13.95$, $p < .05$, but not in the SUP group ($F < 1$). Further, the CON and SUP groups differed for the preexposed condition, $F(1, 13) = 15.67$, $p < 0.01$, but not for the nonpreexposed condition ($F < 1$).

Although these results are consistent with the proposal that the CON group shows latent inhibition (i.e., retarded acquisition to the preexposed CS) and the SUP group does not, other possibilities should be mentioned. First, (for those conditions that showed it) suppression was acquired very rapidly; and it was not evident at all in the preexposed CON condition, whose test performance was closely similar to the level shown at the end of preexposure. One interpretation of this pattern of results is that the test scores reflect not conditioned suppression, but an enhancement of the unconditioned suppression evoked by the stimuli as a consequence of the introduction of shocks. The performance of the preexposed CON condition would thus reflect the fact that the unconditioned response to the CS had habituated during preexposure; in this case the occurrence of suppression in the preexposed SUP condition would indicate

not an absence of latent inhibition but a failure to habituate the unconditioned response to the preexposed stimulus in the first phase of training. Although this possibility would be of interest in itself, the results of the preexposure phase argue against it. In that phase unconditioned suppression was observed, but it was seen to the same extent in both groups, suggesting that there was no difference between them in the degree of habituation they showed.

A second possible alternative interpretation arises from our use of a within-subject testing procedure. With this procedure, for subjects to show latent inhibition to just one of the test stimuli, it is obviously necessary that they be able to discriminate between the stimuli. Similar levels of suppression to the two stimuli, as shown by the SUP group, might thus indicate an inability to discriminate between them rather than the absence of latent inhibition to the preexposed stimulus. Evidence against this interpretation comes from a comparison of the levels of suppression shown by the two groups in Figure 4. If the SUP subjects do suffer from latent inhibition but fail to discriminate the preexposed from the nonpreexposed stimulus, then we would expect that learning about both these stimuli would be retarded. Thus the performance of the SUP subjects to both stimuli should be similar to that shown by the CON subjects to the preexposed stimulus. But in fact their performance matched that of the CON subjects to the nonpreexposed stimulus; that is they learned readily to both cues, consistent with the suggestion that latent inhibition influenced acquisition to neither of them.

4.4. General Discussion

The experiments reported here demonstrate that chronic exposure to a choline-supplemented diet can alter the behavior of fully adult rats. In both experiments the

subjects were 8 months old at the start of the dietary manipulation (and 12 weeks older than that at the start of behavioral testing). Both experiments produced results suggesting that choline supplementation modifies the processes responsible for learned changes in attention to significant environmental stimuli -- specifically that the mechanism responsible for reducing the associability of stimuli in certain circumstances fails to operate normally after choline supplementation. Our results thus confirm that an attentional effect previously observed after perinatal supplementation can be obtained in adults, and do so using behavioral assays that complement that used in previous research.

Previous work (e.g., [20]) has suggested that there are separable cholinergic mechanisms in the basal forebrain for producing learned changes in associability, with the rostral region being responsible for loss of associability and the caudal region being responsible for restoration of lost associability. In these circumstances there are no grounds for predicting what the effects of a general increase in ACh levels, such as will be produced by choline supplementation, are likely to be. The results reported by Meck and Williams (2003) [11], however, showed that supplementation prevented the loss of associability normally seen in the appetitive training paradigm of Wilson et al. (1992) [21]. In this paradigm, control rats that have had training in which a light is reliably followed by a tone learn poorly in a subsequent test in which the light is followed food, a result interpreted as indicating that experience of the tone as a reliable predictor on a consequence produces a loss of associability. Our Experiment 1 used a related procedure [27] in which initial training with the target CS reliably signaling a weak shock was followed by a test stage in which the shock intensity was increased. Control subjects showed retarded learning in the test stage, interpreted as being the consequence

of loss of associability in the first phase. Subjects given the dietary supplement learned readily in the test stage, suggesting that the initial loss of associability had failed to occur.

Support for this interpretation came from Experiment 2 in which conditioning was assessed after prior nonreinforced presentations of the event to be used as the CS. According to Pearce and Hall (1980) [22] the retarded learning produced by such preexposure (latent inhibition) is also a consequence (in part; see [30]) of a reduction in the associability of the preexposed stimulus. Latent inhibition was obtained in our control subjects, but not in the subjects given choline supplementation, supporting the view that the mechanism responsible for reducing associability is dysfunctional in the latter.

Before pursuing the implications of these findings it would be worthwhile to establish that the effects obtained are specific to attentional learning and are not a consequence of some more general learning deficit. The ready acquisition of food-reinforced lever press responding by the SUP group of Experiment 1 may seem to suggest quite the opposite, but these results need to be treated with caution. A high rate of response may reflect an effect on performance rather than on the acquisition of the relevant association. As for central neurons, ACh synthesis and liberation in motor neurons will depend on choline availability and some studies have shown that small doses of choline will improve neuromuscular transmission [32] and increase liberation of ACh at the neuromuscular junction [33]. The heightened activity shown by the SUP group of Experiment 1 could thus be a peripheral effect. A further reason for caution is that the effect was obtained only with the rats used in Experiment 1 and not with those used in Experiment 2. This result is interesting in itself and highlights the possibility the

effects of dietary choline levels might interact with genotypic differences; it does, however, preclude us from concluding that choline supplementation enhances lever press acquisition generally.

When it comes to classical conditioning, there is no reason to think that choline availability modifies simple acquisition. Thus, for example, Lamoureaux, Meck, and Williams (2008) [34] found no effect of prenatal supplementation on the acquisition and extinction of a noise->food association (although the sensitivity of the rats to contextual factors was modified). In our aversive conditioning procedure the overall level of suppression established by phase-1 training in Experiment 1 was somewhat less in SUP than in CON subjects, but the difference in baseline response rates makes a direct comparison of the suppression ratios of the two groups difficult to interpret. In Experiment 2, however, where baseline rates were comparable, there was no difference between the SUP and CON groups in acquisition of suppression to the nonpreexposed stimulus, supporting the conclusion that the effect of supplementation was confined to the learning process responsible for retarded learning about the preexposed stimulus in the CON group (i.e., for the latent inhibition effect).

It remains to explain why long-term choline supplementation should impair the ability to reduce attention. The results reported here parallel those reported for the effects of lesions of the cholinergic system of the basal forebrain [25] prompting the speculation that this form of supplementation produces compensatory changes in that system making it less able to operate effectively. Although there is empirical support for this possibility (e.g., the demonstration by Li et al., 2003 [35], for knockout mice lacking acetyl cholinesterase, of a down-regulation of muscarinic receptors) it will need further research to confirm its applicability to the present case.

An inability to reduce attention to stimuli that do not deserve or require it has often been taken as a hallmark of schizophrenia, and, indeed, the latent inhibition phenomenon has been advocated as a model system for study of the mechanism that is dysfunctional in schizophrenia (e.g., [36]). Accordingly, our present findings lend support to the developing hypothesis that a disturbance of the normal functioning of cholinergic mechanisms plays a role in schizophrenia (see, e.g., [37, 38]). Relevant observations include the fact that neuropathological investigation has demonstrated a decrease in muscarinic receptors in the prefrontal cortex of patients with schizophrenia (see, e.g., [39, 40]) and the early observation [41] that chronic exposure to high levels of extracelleuar ACh can produce an increase in some of symptoms associated with schizophrenia.

It is true that much previous research in this area has focused on the role of mesolimbic dopaminergic mechanisms; for example the observation that treating rats with amphetamine disrupts normal latent inhibition has been taken to reflect an effect on the dopaminergic system of the nucleus accumbens (NAcc) (see, e.g., [42], for a review). But the dopaminergic and cholinergic systems are intimately linked; the NAcc projects to the basal forebrain and the activity of the corticopetal cholinergic system of the basal forebrain has been found to vary according to the level of activity in the NAcc [43]. Such observations have led to the hypothesis that the symptoms of schizophrenia derive from dysfunctions in a chain of mechanisms that ultimately influence a cholinergic cortical process that selects or discards certain stimuli for attentional processing [44]. Our results prompt the suggestion that choline supplementation may provide a useful model system in which this hypothesis could be tested further.

Finally we should comment on the fact that cholinergic mechanisms have often been linked not so much with attention as with memory, primarily as a consequence of the suggestion (e.g., [45]) that a dysfunction of the cholinergic neurons of the basal forebrain is responsible for the memory deficits of aging. This characterization has been disputed (see, e.g., Voytko, 1996 [46], who specifically assesses the alternative view that the basal forebrain controls attention rather than memory). The issue is not easy to resolve, partly, we suggest, because terms like "memory" and "attention" are relatively ill-defined. Each covers a covers a range of psychological processes and aspects of each will be involved in generating most behavioral phenomena. Thus, although we have used the term attention in summarizing our findings, our behavioral analysis has been concerned with a specific psychological process – that responsible for reducing the associability of stimulus followed by consistent consequences – a process that involves learning, and thus a contribution from some aspect of memory. It remains to investigate if such changes in associability can be shown to be occurring in other experimental paradigms that have been said to show cholinergic involvement in attention and memory.

Acknowledgments

This work was supported by grants from Ministerio de Ciencia e Innovación PSI2009-07513, Ministerio de Economía y Competitividad PSI2012-31641 and HUM-02763 (Junta de Andalucía, Spain) partially funded by FEDER. We thank Scott Franklin, Juan Carlos Rodriguez, and Fernando Garzón for experimental assistance.

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DISCUSION GENERAL

DICUSIÓN GENERAL

El objetivo de este trabajo de investigación fue determinar si la suplementación con colina durante la edad adulta afecta el desempeño de tareas cognitivas dependientes del sistema colinérgico.

Debido al deterioro del sistema colinérgico en funciones cognitivas, hasta ahora los estudios de la Ch dietaria se han centrado principalmente en evaluar su efecto como un nutriente terapéutico en el tratamiento y prevención de la pérdida de memoria senil y en enfermedades degenerativas como el Alzheimer. Sin embargo, son escasas las investigaciones dirigidas a estudiar su efecto de facilitador cognitivo en sujetos adultos sanos. Con ese objetivo se suplementaron ratas adultas entre 3-7 meses de edad durante periodos de 7 a 12 semanas y posteriormente se comparó su ejecución en diferentes tareas cognitivas dependientes del sistema colinérgico con sujetos controles no suplementados. Gracias al desarrollo de diversas técnicas (lesiones, microdiálisis, producción de agonistas o antagonista colinérgicos) ha sido posible evaluar la modulación del sistema colinérgico en funciones cognitivas específicas. Así, las tareas utilizadas en este trabajo fueron seleccionadas atendiendo a su sensibilidad al neurotransmisor y, a la disponibilidad de Ch.

En el Capítulo 2 exploramos si la suplementación crónica con colina durante la edad adulta afecta la retención de una aversión al contexto provocada por su emparejamiento a un malestar gástrico. Los resultados mostraron que el grupo suplementado logró una mejor retención de la aversión al contexto condicionado en relación al grupo alimentado con la dieta estándar. El beneficio de la Ch dietaria en este primer estudio debe ser destacada considerando que el paradigma de aversión

contextual es una tarea compleja, la cual requiere configurar la representación de diversas claves presentes de dicho ambiente. Asimismo, el tiempo de suplementación implementado en esta investigación (7 semanas), está entre los más cortos utilizados previamente con éxito para observar una mejora cognitiva en ratas adultas. (Teather & Wurtman, 2003; Teather & Wurtman, 2005)

En el Capítulo 3 se llevaron a cabo dos Estudios. En el Estudio 1 se pretendió evaluar si la variación de Ch en la dieta (suplementada, estándar o deficiente) en ratas gestantes afecta la memoria de los descendientes cuando alcanzan la edad adulta utilizando una tarea de SOR. En el Estudio 2 se evaluó el efecto de la suplementación en el desempeño de esta misma tarea cuando la vitamina es administrada en la edad adulta. Los resultados mostraron que la variación en la disponibilidad de Ch durante ambos periodos afecta la memoria en un paradigma de SOR. Estos hallazgos constituyen la primera demostración de que la variabilidad de colina juega un papel crítico en el desempeño en la tarea SOR en ratas. Así, en el estudio de manipulación prenatal, mientras todos los grupos (suplementado, estándar y deficiente) reconocieron el objeto familiar en la prueba de retención corta (24 h), sólo los animales suplementados reflejaron un mejor reconocimiento 48 h después de la fase de adquisición. En contraste, el grupo deficiente falló en reconocer el objeto familiar. Los resultados son consistentes con investigaciones previas en donde se ha encontrado que mientras la suplementación con colina durante períodos perinatales mejora funciones cognitivas dependientes del sistema colinérgico, una dieta deficiente provoca un déficit (para revisión Mekc & Williams, 2003).

El estudio con suplementación adulta (Estudio 2, exp. 1) reveló que ambos grupos, suplementado y estándar, recordaron el objeto familiar tanto a las 24 h. como a

las 48 h. de retención sin que se observara ninguna diferencia entre ellos. Los resultados podrían estar sugiriendo que, a diferencia de la suplementación prenatal, la Ch dietaria en la edad adulta no afecta el desempeño en la memoria en la tarea SOR. Sin embargo, se propuso la posibilidad de que la reiterada exposición al objeto familiar implicado en un diseño intrasujeto (una durante la fase de adquisición y dos más en las pruebas de retención, 24 h y 48 h) facilita la representación del objeto familiar y, consecuentemente mejora su reconocimiento. Para probar esto, en el Experimento 2 (Estudio 2) se utilizó el mismo tratamiento dietario, pero con un diseño entre-grupo. De esta manera, un grupo de cada condición dietarias (suplementado y estándar) fue probado 24 h y otro 48 h. después de la fase de adquisición. A las 24 h., tanto los sujetos suplementados como los estándares reconocieron el objeto familiar. Sin embargo, sólo los animales suplementados probados en el período de retención más largo (48 h) reconocieron el objeto familiar, mientras que los animales estándares no lo recordaron. Estos resultados sugieren que la suplementación de colina dietaria durante la edad adulta mejora la retención en una tarea SOR.

Comparando los Estudios de suplementación prenatal (Estudio 1) con los adultos (Estudio 2, exp. 1,), ambos con diseño intra-sujeto, se puede observar que mientras los animales suplementados prenatales presentaron, en comparación a los estándar, un mejor reconocimiento del objeto familiar tras una demora larga (48 h), los animales suplementados adultos no manifestaron tal ventaja.

Al menos dos implicaciones pueden extraerse de los resultados obtenidos en los Estudios del Capítulo 3. Por una parte, la diferencia reflejada en SOR entre los grupos

suplementado prenatal y adulto utilizando un diseño intra-sujeto (Estudio1 y Estudio 2, exp. 1), podría sugerir que la habituación es sensible a la mayor disponibilidad de Ch en edades inmaduras. Este razonamiento se basa en hallazgos previos en donde se ha demostrado que el sistema colinérgico juega un rol en la modulación de la habituación (Thie et al., 1998). Es posible, que la habituación sea más sensible a la mayor disponibilidad del nutriente durante el desarrollo temprano. Específicamente durante el período de neurogénesis del sistema colinérgico en ratas durante E12-E18 (Semba & Fibiger, 1988), produciendo alteraciones permanentes en el funcionamiento de dicho sistema y facilitando el desempeño de SOR en el adulto. Estudios comparativos, sin embargo, variando la edad de suplementación y las tareas cognitivas son necesarios.

Por otra parte, cuando se utiliza un procedimiento entre-grupo (Estudio 2, exp. 2), la retención de la información después de una única exposición al objeto familiar, le otorga una mayor complejidad a la tarea. Esto último debido al incremento en la demanda de memoria cuando la demora entre la fase de familiarización y la fase prueba, aumenta de 24 a 48 horas (Hammond et al., 2004). Esta misma razón es viable para sustentar los resultados observados con los animales manipulados nutricionalmente en edades adultas y probados en una tarea de aversión contextual (Capítulo 2). La mejor retención del contexto aversivo mostrado por el grupo suplementado en comparación al estándar cuando dos pruebas de retención (3 y 15 días después del condicionamiento) fueron aplicadas, confirma la facilitación de la Ch adulta en tareas con una alta demanda de memoria.

Sea el mecanismo implicado y/o la edad de suplementación utilizada, juntos, los datos obtenidos en los experimentos del Capítulo 3 sugieren la implicación del sistema colinérgico en la memoria cuando se utiliza la tarea SOR. Siendo el déficit de memoria

de reconocimiento uno de los primeros síntomas de pérdida de memoria senil o demencia (Buffalo et al., 1998; Lee et., al., 2003), los resultados encontrados en este experimento cobran gran relevancia al exhibir una sensibilidad a la disponibilidad del precursor del neurotransmisor ACh, la Ch, en la dieta. Así, futuros estudios preclínicos y clínicos en diferentes períodos de la vida de los individuos podrían se avizorados.

Finalmente, el objetivo de los estudios del Capítulo 4 fue evaluar el efecto de la suplementación crónica con colina en la edad adulta utilizando la atención como función cognitiva. Es ampliamente aceptado que el sistema el colinérgico, principalmente los inputs de áreas corticales, está implicado en el proceso de atención (Klinkenberg et al., 2010). Transfer Negativo (NT; Hall & Pearce, 1979) en el Experimento 1 e Inhibición Latente (LI; Lubow, 1989) en el Experimento 2, fueron los paradigmas utilizados en el estudio. Estos paradigmas miden la normal pérdida de atención a un CS que predice de forma segura su consecuencia, bien sea esta una ausencia de consecuencias (Inhibición latente) o una consecuencia determinada (Transfer negativo). Esta disminución en atención trae como consecuencia una menor asociabilidad del estímulo y por tanto un retraso en un aprendizaje posterior sobre el CS pre-expuesto. Los resultados en ambos experimentos reflejaron que, mientras los animales estándar mostraron el retraso esperado en aprendizaje durante el condicionamiento posterior, los sujetos suplementados aprendieron fácilmente una segunda asociación, sugiriendo que la Ch provocó una disminución en el retraso de aprendizaje normalmente observado. Esto estaría indicando que el mecanismo que reduce la atención prestada a estímulos que predicen consistentemente sus consecuencias no opera efectivamente después de una suplementación crónica con Ch

en ratas adultas. Asimismo, los resultados confirman la modulación del sistema colinérgico en la atención.

La ausencia de Inhibición latente es un déficit cognitivo común que presentan los pacientes con esquizofrenia. De hecho, el fenómeno de LI se ha propuesto como un modelo animal para estudiar los mecanismos implicados en esta enfermedad. Algunas investigaciones previas están en concordancia con nuestras observaciones en este trabajo. Así, se ha encontrado que pacientes esquizofrénicos presentan una disminución de receptores muscarínicos en la corteza prefrontal (Crook et al., 2001; Raedler et al., 2003). Adicionalmente, en un estudio preliminar, Bowers et al. (1964) encontraron que un incremento de ACh extracelular puede provocar la manifestación en algunos de los síntomas asociados con la esquizofrenia. Estos hallazgos parecen sugerir que algunos mecanismos colinérgicos desempeñan un papel en la esquizofrenia. Estos resultados resaltan la necesidad de llevar a cabo investigaciones con modelos animales de esquizofrenia en donde se controlen variables de dosis y tiempo de suplementación.

CONCLUSIONES

CONCLUSIONES

1. La colina dietaria modula funciones cognitivas dependientes del sistema colinérgico en edades adultas. Los resultados de la presente investigación mostraron que la suplementación con colina durante periodos adultos (jóvenes-maduros) afecta el desempeño en tareas atencionales y aquellas con una alta demanda de memoria.
2. La suplementación crónica con colina mejora la retención en una tarea de condicionamiento contextual. Los resultados apoyan la intervención del sistema colinérgico en la memoria y, específicamente en tareas dependientes de contexto, las cuales han sido relacionadas con el hipocampo.
3. La variación de colina en la dieta afecta a la memoria de reconocimiento de objetos (utilizando un paradigma SOR) cuando la manipulación se lleva a cabo tanto durante períodos prenatal como en la etapa adulta.
4. La disponibilidad de colina durante el período prenatal afecta la memoria en SOR en la edad adulta. A las 24 h de retención ambos grupos, suplementado y deficiente, reconocieron el objeto familiar. Tras un intervalo de retención largo (48 h), los animales suplementados reflejaron una mejor retención del objeto familiar en comparación al estándar y deficiente, mientras que los sujetos deficientes no reconocieron el objeto familiar.
5. El mejor desempeño en SOR de los sujetos suplementados prenatalmente a las 48 h de la fase de adquisición (después de tres exposiciones consecutivas al objeto familiar) sugiere que la colina puede estar modulando el proceso de habituación cuando la dieta es manipulada durante periodos temprano del desarrollo.

6. La administración de una dieta crónica con colina en ratas adultas no afecta al reconocimiento del objeto familiar en la tarea SOR cuando se usa un diseño intra-grupo. Todos los animales, suplementados y no suplementados, reconocieron el objeto familiar durante las pruebas de retención corta y larga. Esto pudiera estar indicando que, la suplementación con colina en adultos no afecta el proceso de habituación.
7. La mayor disponibilidad de colina en edades adultas mejora la retención del objeto familiar en intervalos de memoria largos. Utilizando un diseño entre-grupo, los resultados reflejaron que, mientras los sujetos suplementados y no suplementados lograron retener la representación del objeto familiar por 24 h, solo los animales suplementados reconocieron el objeto familiar 48 h después de la adquisición.
8. La exposición crónica a una dieta rica en colina durante la etapa adulta modula los procesos atencionales. Los estudios mostraron que la suplementación con colina disminuye la reducción de atención que normalmente ocurre cuando un estímulo predice de forma segura sus consecuencias. Los resultados sustentan la hipótesis de que el sistema colinérgico modula procesos atencionales.

CONCLUSIONS

1. Dietary choline during adulthood modulates cognitive functions dependent on the cholinergic system. The results reported in the present thesis show that choline supplementation applied to adult (young-mature) rats modifies their performance in tasks requiring attention and high memory demands.

2. Chronic dietary choline supplementation improves retention using a context conditioning task. The results support a role of the cholinergic system in memory which is evident in context -dependent tasks widely associated with the hippocampal function.

3. Both prenatal and adult changes in dietary choline availability enhance object recognition memory using a spontaneous object recognition (SOR) task.

4. Prenatal choline availability induces long-lasting effects on object recognition memory that can be seen during adulthood. While both the supplemented and deficient group remembered the familiar object 24 h after acquisition, only the supplemented group exhibited retention at a longer 48 h retention intervals.

5. A better performance in SOR at the longer retention period of the group receiving prenatal choline supplementation suggests that choline availability at early developmental stages might modulate the habituation process.

6. Chronic choline supplementation in adult rats does not affect recognition of the familiar object in a SOR task if a within-subject design is applied. All the groups, whether supplemented or not, remembered the familiar object both at short and long retention intervals. This might indicate that the habituation process is not affected by choline supplementation during adulthood.

7. However, the use of a between-group design indicated that adult dietary choline availability enhances memory of the familiar object when long retention intervals are used. While both the supplemented and non-supplemented groups exhibited retention of the familiar object representation during 24 h, only the suplemented group recognized the familiar object 48 h after acquisition.

8. Chronic intake of a choline-rich diet during adulthood modulates attentional processess. The present results indicated a decrease of the attention normally induced by a stimulus that successfully predicts its consequences. Thus, these data support a modulatory role of the cholinergic function in attention.

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