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CIRCUITOS CEREBRALES Y MEMORIA DE RECONOCIMIENTO EN ROEDORES: EFECTO DE EXPOSICIÓN A SABORES

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Circuitos cerebrales y memoria de reconocimiento en roedores: Efecto de exposicion a sabores

RESUMEN

El planteamiento de la presente tesis está basado en una aproximación multidisciplinar al estudio de los efectos de la exposición al sabor aplicando tanto un análisis comportamental como un análisis del Sistema Nervioso en el nivel celular y el nivel de circuito. La exposición repetida a un sabor sin consecuencias negativas puede producir cambios en la actividad neuronal, así como cambios comportamentales, derivados de la transición del sabor de novedoso a familiar, y su consiguiente categorización como sabor seguro. En el estudio de preferencias condicionadas al sabor, estos cambios en familiaridad pueden generar diferentes fenómenos de aprendizaje. Un caso es inhibición latente en la que el grado de preferencia condicionada a un sabor puede ser retrasado cuando el reforzador es emparejado con un sabor familiar (preexpuesto) en comparación con un sabor novedoso (no preexpuesto). Por ello, el objetivo de esta tesis se centra en la exploración de los efectos comportamentales inducidos por la exposición a sabores en el estudio de preferencias condicionadas, así como la implicación de áreas cerebrales relevantes para la memoria de reconocimiento.

En la serie experimental perteneciente al **Capítulo 3** se exploró la disociación de los dos tipos de aprendizaje "sabor-nutriente" y "sabor-sabor" que sustentan las preferencias condicionadas al sabor, mediante la preexposición al sabor, la manipulación del estado motivacional durante la prueba, y mediante el empleo como reforzadores de fructosa y maltodextrina. Ambos reforzadores fueron capaces de generar una preferencia cuya adquisición fue retrasada por la exposición previa al sabor (inhibición latente) en animales privados de comida durante la prueba. Sin

embargo, en animales saciados durante la prueba, la exposición previa al sabor no indujo inhibición latente independientemente del reforzador empleado. Estos resultados sugieren que el uso de fructosa y maltodextrina como reforzadores no permite una disociación clara entre aprendizaje "sabor-nutriente" y "sabor-sabor". Por el contrario, la manipulación del estado emocional durante la prueba tiene un marcado efecto sobre la aparición del fenómeno de inhibición latente, observándose éste únicamente en animales privados de comida durante la prueba.

En la serie experimental perteneciente al **Capítulo 4** se examinó el efecto de la preexposición a sabores con diverso valor hedónico sobre la adquisición de preferencias condicionadas inducida por sacarosa en animales privados de comida durante la prueba. El uso de un sabor neutro como estímulo condicionado reveló la presencia del fenómeno de inhibición latente en animales preexpuestos. Por el contrario, la preexposición a un sabor no-preferido no sólo no produjo inhibición latente, sino que tuvo lugar el efecto contrario, una facilitación de la respuesta condicionada. Estos resultados revelan que el uso de sabores con distinto valor hedónico puede generar resultados contrapuestos en procedimientos experimentales orientados a generar preferencias condicionadas.

En el **Capítulo 5** se estudiaron los cambios que induce la exposición a un sabor sobre la actividad neural desencadenada en el tálamo gustativo como estación central de procesamiento del sistema gustativo, utilizando la determinación inmunohistoquímica de la proteína c-Fos como índice de actividad neural. La exposición a un sabor provocó una respuesta neofóbica inicial que fue atenuada en subsecuentes presentaciones en las que el sabor no iba acompañado de consecuencias negativas. La familiaridad del sabor produjo cambios en la actividad

talámica, encontrándose el mayor número de células c-Fos positivas cuando el sabor era más familiar. Así, la actividad del tálamo gustativo parece estar relacionada con el proceso de habituación de la neofobia, mostrando éste la mayor activación durante la exposición a un sabor que se ha convertido en seguro gracias a un proceso de familiarización.

En el **Capítulo 6** se exploró el efecto de la lesión neurotóxica de la corteza perirrinal inducida por inyección intracerebral de NMDA sobre la memoria de reconocimiento visual y la memoria gustativa segura en el mismo grupo de animales. La lesión de la corteza perirrinal alteró el reconocimiento de objetos tras un intervalo de retención de 24h e indujo un retraso significativo en el proceso de familiarización de un sabor, aunque no lo impidió, y tampoco afectó a la habituación final de la neofobia gustativa. Estos resultados sugieren un importante papel de la corteza perirrinal en el proceso de familiarización durante la transición de lo novedoso a lo familiar.

En conjunto, los resultados de esta tesis muestran que la exposición repetida a sabores puede producir diversos efectos comportamentales relacionados con cambios en la familiaridad y que afectan a procesos de aprendizaje asociativo. Además, estos cambios en familiaridad pueden estar relacionados con la actividad neuronal de áreas cerebrales implicadas en memoria de reconocimiento como el tálamo gustativo y la corteza perirrinal. Por último, los efectos comportamentales inducidos por la exposición a sabores pueden depender de variables como el valor hedónico del sabor, y pueden generar diferentes fenómenos de aprendizaje, como inhibición latente o facilitación, fenómenos que pueden ser claves en el estudio de los mecanismos relacionados con el aprendizaje asociati

ÌNDICE

CAPÍTULO 1

Introducción

1. Memoria de reconocimiento y preferencia condicionada por el sabor.

La memoria de reconocimiento es la habilidad para identificar como familiar un estímulo o situación experimentados previamente. Esta habilidad puede reflejarse en informes verbales o en modificaciones del comportamiento, lo que posibilita su estudio en modelos animales. Los conceptos de novedad y familiaridad juegan un papel relevante en las teorías del aprendizaje y la cognición en relación con los procesos atencionales. Los cambios en la atención dirigida a un estímulo o situación familiar versus la atención dirigida a uno novedoso dependen de su relación bien con consecuencias, ya sean aversivas o reforzantes, bien con la ausencia de consecuencias relevantes para el organismo.

La *memoria de reconocimiento* puede explorarse empleando cualquier modalidad sensorial. Sin embargo, la modalidad más estudiada en modelos animales ha sido la visual, empleando la presentación de objetos y registrando las respuestas de exploración de los mismos. El tiempo de exploración del objeto es mayor cuando éste es novedoso y no ha ido seguido de consecuencias relevantes para el organismo. Así, las tareas de memoria de reconocimiento de objetos, desarrolladas inicialmente en primates (Mishkin & Delacour, 1975) y ampliamente utilizadas en roedores posteriormente (Ennaceur & Delacour, 1988), son eminentemente visuales, a pesar de que modificaciones de la tarea puedan involucrar otras modalidades sensoriales, tales como la somatosensorial o la olfativa (Albasser, Poirier, & Aggleton, 2010; Albasser et al., 2011). Aunque existen interpretaciones alternativas, a raíz de las características generales de este tipo de memoria en seres humanos y primates la *memoria de reconocimiento de objetos* ha sido clasificada como memoria episódica,

un tipo de memoria declarativa que permite recordar una vivencia o hecho concreto ocurrido previamente y que requiere la participación del lóbulo temporal medial (Winters, Saksida, & Bussey, 2008).

Por el contrario, la *memoria de reconocimiento gustativa* se ha explorado tradicionalmente en roedores como un tipo de memoria no declarativa independiente del hipocampo y áreas temporales asociadas. El procedimiento experimental empleado para estudiar la memoria gustativa requiere generalmente la presentación de una solución gustativa. La novedad del sabor provoca una respuesta innata de cautela denominada neofobia gustativa (Domjan & Gillan, 1976), que induce un consumo inicial reducido con el resultado de evitar potenciales efectos tóxicos. Si no se producen consecuencias negativas tras el consumo, la ingesta se incrementa en sucesivas presentaciones, a medida que es reconocido como familiar y seguro. Este proceso, denominado *atenuación de la neofobia* (Domjan & Gillan, 1976), se ha propuesto como modelo de memoria de reconocimiento gustativa (Bermudez-Rattoni, 2004).

El reconocimiento de alimentos a través del análisis del sabor y de las propiedades sensoriales de la comida se produce mediante un sistema funcional de alto valor adaptativo orientado a la identificación correcta de alimentos beneficiosos y nocivos. Los roedores (así como el ser humano) muestran una predisposición genética a preferir sustancias dulces y saladas, y a rechazar aquéllas que son ácidas y amargas (Birch, 1999). Sin embargo, estas preferencias gustativas se refinan través de la experiencia. Así pues, como hemos mencionado anteriormente, la mera exposición reduce la respuesta neofóbica innata, incrementando la aceptación y preferencia por un sabor tras exposiciones repetidas (Hill, 1978). No obstante, la

preferencia a un sabor también puede ser modificada a través de procesos asociativos de aprendizaje (Rozin & Zellner, 1985). Así, cuando el consumo del sabor es seguido de malestar visceral, se modifica el valor hedónico de dicho sabor convirtiéndose en aversivo, siendo rechazado en ulteriores ocasiones. Si el sabor se asocia con consecuencias positivas, como en el caso de nutrientes o de estímulos gustativos agradables al paladar, se incrementa el consumo desarrollándose una preferencia gustativa.

En el caso de las *preferencias gustativas*, el procedimiento estándar más usado en roedores para su estudio experimental es el paradigma de preferencia condicionada al sabor (PCS, Myers & Sclafani, 2006; Rozin & Zellner, 1985). El procedimiento básico, no el único, para inducir preferencias condicionadas por el sabor consiste en la presentación de un sabor novedoso o estímulo condicionado (EC) emparejado con un segundo sabor que produce de forma incondicionada una preferencia (estímulo incondicionado, EI). El condicionamiento se pone de manifiesto en una prueba de elección posterior observándose una preferencia por el sabor emparejado con el EI (EC+) sobre otro sabor no emparejado (EC-) (Drucker, Ackroff, & Sclafani, 1994), así como por el EC+ sobre agua (Harris, Gorissen, Bailey, & Westbrook, 2000).

Se ha establecido que las ratas pueden aprender asociaciones basadas en la palatabilidad o valor hedónico del EI (Holman, 1975), lo que daría lugar a un tipo de aprendizaje denominado “sabor-sabor”, así como asociaciones relacionadas con las consecuencias de su ingestión, como sus efectos calóricos o nutritivos (Harris, Shand, Carroll, & Westbrook, 2004; Sclafani & Ackroff, 1994), un tipo de aprendizaje denominado “sabor-nutriente”. De esta forma, en el aprendizaje “sabor-nutriente”

se producen asociaciones de tipo predictivo y sensibles a cambios en contingencia, mientras que en el aprendizaje "sabor-sabor" se produce un proceso de transferencia de las propiedades hedónicas al sabor condicionado afectado por la relación de contigüidad entre el sabor y el reforzador, y no por la relación de contingencia, conformando un tipo de aprendizaje no predictivo (Drucker et al., 1994).

Diferentes disociaciones experimentales apuntan a la independencia de ambos tipos de mecanismos de aprendizaje (Myers & Sclafani, 2006). El estado motivacional durante el test parece ser de especial relevancia para determinar cuál de los dos tipos de aprendizaje es responsable de una preferencia observada. Así, la preferencia basada en aprendizaje "sabor-nutriente" depende del estado motivacional durante la prueba, manifestándose en animales privados de comida , mientras el aprendizaje "sabor-sabor" parece operar cuando el animal no se encuentra privado (Harris et al., 2000). Es importante señalar que el estado motivacional parece no afectar a la adquisición de ambos tipos de aprendizaje durante el condicionamiento, sino sólo a su expresión durante la prueba (Fedorchak & Bolles, 1987; Yiin, Ackroff, & Sclafani, 2005a, 2005b).

2. Efectos de la exposición al sabor en preferencia condicionada al sabor.

La exposición repetida a sabores puede producir cambios comportamentales que pueden estar relacionados con la actividad neuronal de áreas cerebrales implicadas en memoria de reconocimiento. La ausencia de consecuencias negativas tras la presentación de un sabor produce una *habituación* de la respuesta neofóbica

inicial. El sabor antes novedoso se convierte en familiar y seguro, lo que se traduce en un aumento del consumo. En el estudio de preferencias condicionadas al sabor, estos cambios en familiaridad pueden generar diferentes fenómenos de aprendizaje como la *inhibición latente* o la *facilitación*, donde el grado de preferencia condicionada a un sabor puede ser alterado dependiendo de que el reforzador sea emparejado con un sabor familiar (preexpuesto) o con un sabor novedoso (no preexpuesto). Por ello, explorar éstos fenómenos comportamentales puede ser clave en el estudio de los mecanismos relacionados con el aprendizaje asociativo.

2.1. Inhibición latente.

Se denomina *inhibición latente* a un proceso en el que la exposición previa sin consecuencias a un estímulo da lugar al retraso en la adquisición de una asociación posterior en la que dicho estímulo predice la aparición de una consecuencia (Lubow & Moore, 1959). Se trata de un robusto fenómeno de aprendizaje demostrado en una variedad de procedimientos de aprendizaje, incluyendo condicionamiento aversivo gustativo (Lubow & Weiner, 2010). Sin embargo, el aprendizaje de preferencias gustativas representa un caso excepcional en el que la exposición previa al EC puede desencadenar consecuencias diversas.

Los estudios De la Casa, Marquez, & Lubow, (2009) con animales hambrientos y de Delamater (2011) con animales sedientos parecen mostrar que mientras que el efecto de IL se encuentra claramente utilizando animales hambrientos, en animales sedientos no se produce dicho efecto (no al menos en un test inicial, observándose sólo tras exposiciones adicionales a la sacarosa). Ello se

confirma con los resultados obtenidos por Garcia-Burgos, Gonzalez, & Hall (2013), donde el efecto de IL se obtiene de forma consistente en animales hambrientos durante la prueba, con independencia de su estado motivacional durante la preexposición y el condicionamiento. Por el contrario, en animales no-hambrientos durante la prueba se encuentra una ausencia clara del efecto de IL. Se ha propuesto que cuando los animales se prueban hambrientos el aprendizaje sabor-nutriente controla la ejecución, mostrando el fenómeno de inhibición latente. Sin embargo, cuando los animales están sedientos en la prueba es el aprendizaje sabor-sabor el que controla la ejecución; éste no es sensible al valor predictivo del estímulo, formándose la asociación sabor-sabor por contigüidad temporal durante la fase de condicionamiento, sin efecto de la preexposición. Por tanto, los estudios sobre IL parecen reforzar la idea de que sólo el aprendizaje sabor-nutriente es sensible a los cambios de contingencia EC-EI.

2.2. Facilitación.

Por otra parte, se ha demostrado que, en determinadas circunstancias, la preexposición al EC puede producir el efecto opuesto a la inhibición latente, esto es, facilitar la respuesta condicionada tras la asociación con el EI. La facilitación inducida por la preexposición a un estímulo ha sido ampliamente encontrada en procedimientos de respuesta condicionada al miedo, donde la preexposición al contexto produce un incremento en las respuestas de miedo comparado con la no-preexposición (Asok, Schreiber, Jablonski, Rosen, & Stanton, 2013; Pisano, Ferreras, Krapacher, Paglini, & Arias, 2012; Robinson-Drummer & Stanton, 2016).

Aunque este efecto ha sido raramente encontrado en procedimientos con preexposición al sabor, algunos investigadores han encontrado un efecto de facilitación de la preexposición al EC en procedimientos de condicionamiento aversivo gustativo. Sin embargo, su expresión parece depender de variables como la intensidad del EI, la cantidad de preexposiciones y la complejidad del EC. De este modo, la facilitación del condicionamiento es inducida por los efectos beneficiosos de la preexposición al EC, la cual permite un procesamiento más efectivo de un estímulo complejo tanto en ratas adultas (Bennett, Tremain, & Mackintosh, 1996), como especialmente en crías de rata (Chotro & Alonso, 2001; Gaztañaga, Aranda-Fernandez, Diaz-Cenzano, & Chotro, 2015), en las que la inmadurez del sistema sensorial puede ser responsable de un déficit en el procesamiento del EC. El hecho de que este efecto no haya sido encontrado previamente en PCS subraya la importancia de los resultados obtenidos en los experimentos expuestos en los capítulos 3 y 4 del presente trabajo.

2.3. Habituación de la neofobia gustativa.

La mera presentación repetida de un sabor cuya ingestión no va seguida de consecuencias negativas permite estudiar un tipo de aprendizaje no asociativo denominado habituación. En el caso de la modalidad gustativa dicho proceso se pone de manifiesto al reducirse la respuesta neofóbica inicial ante un sabor desconocido, por lo que se denomina *habituación de la neofobia gustativa*. Esta reducción de la respuesta neofóbica se traduce en un aumento del consumo del sabor a medida que convierte en un sabor familiar. Se ha demostrado que la mera

exposición a un sabor sin consecuencias negativas puede conllevar además un incremento en su palatabilidad (Garcia, Hankins, Robinson, & Vogt, 1972) y, por tanto, un cambio en su valor hedónico (Lin, Amodeo, Arthurs, & Reilly, 2012). Sin embargo, dado que la presentación del sabor implica la ingestión de un fluido en animales privados de agua, no puede descartarse la formación de una asociación entre el sabor y el valor reforzante de reducir la sed (Lin, Amodeo et al., 2012).

La habituación de la neofobia gustativa se pone de manifiesto empleando una variedad de estímulos gustativos, incluyendo sabores básicos (dulce, salado, amargo, ácido, umami) y sabores compuestos obtenidos por la dilución de saborizantes disponibles en el mercado (almendra, vainilla, etc.,..). La respuesta neofóbica se mide no sólo por una reducción significativa del consumo del sabor desconocido con respecto a una línea base de consumo de agua, sino por el incremento posterior a medida que el sabor se convierte en familiar. Hay que tener en cuenta que existen diferentes preferencias previas dependiendo de la palatabilidad del sabor y ello modifica la cantidad ingerida en la primera presentación. Aunque dicha respuesta es constatable en todos los casos, cuando se trata de sabores palatables, tales como una solución salada o dulce, únicamente es evidente por el incremento del consumo en repetidas presentaciones, ya que la cantidad ingerida en la primera presentación puede no diferir de la cantidad de agua consumida durante la línea base (Moron & Gallo, 2007; Reilly & Bornovalova, 2005). Frecuentemente, la habituación de la neofobia gustativa se produce muy rápidamente y es evidente en la segunda presentación del sabor, aunque, en ocasiones, puede requerir un mayor número de exposiciones. Por ello, es aconsejable continuar las presentaciones hasta que se haya alcanzado la asíntota lo

que puede demostrarse cuando no existen diferencias en el consumo entre dos sesiones consecutivas (Gomez-Chacon, Morillas, & Gallo, 2015).

La habituación de la neofobia gustativa representa la preparación comportamental más sencilla para estudiar los efectos de la exposición previa sobre los mecanismos cerebrales implicados en los procesos de aprendizaje y memoria gustativa. Ofrece, por tanto, de una oportunidad privilegiada para explorar los circuitos neurales responsables de una serie de fenómenos que dependen de la exposición al sabor.

3. Circuitos cerebrales responsables de los efectos de la exposición al sabor.

3.1. Neuroanatomía del sistema gustativo.

En los mamíferos, incluyendo los roedores y el ser humano, los receptores gustativos se encuentran en la cavidad oral, distribuidos en la lengua, parte posterior de la boca y faringe. Se disponen en grupos formando botones gustativos alrededor de las papilas gustativas. Las aferencias gustativas que proceden de estos receptores son transmitidas al Sistema Nervioso Central a través de los pares craneales Facial (VII), especialmente la corda timpánica, Glosofaríngeo (IX), y Vago (X) hasta alcanzar la parte rostral del Núcleo del Tracto Solitario (NTS) (Yamamoto, 2006). A su vez, en la rata, éste proyecta ipsilateralmente hacia el Núcleo Parabraquial (PBN) medial (Reilly, 1999). Ya en estos primeros relevos troncoencefálicos el sistema parece llevar a cabo el procesamiento disociado de la cualidad gustativa y sus características hedónicas, capaz de dirigir la conducta de ingestión, como demuestra el hecho de que ratas descerebradas muestren

respuestas de aceptación y rechazo ante soluciones de distintos sabores administradas oralmente (Grill & Norgren, 1978). La información gustativa se distribuye principalmente a través de dos rutas o vías hacia la corteza gustativa, situada en la corteza insular entre otras áreas cerebrales: una vía tálamo-cortical y otra ventral (Nakashima et al., 2000; Norgren, 1976; Reilly, 1998; Tokita, Inoue, & Boughter, 2010). La vía tálamo-cortical incluye proyecciones bilaterales hacia el área parvocelular del núcleo ventroposteromedial del tálamo (VPMpc), también denominada como *tálamo gustativo*, el cual mantiene conexiones con la zona gustativa de la corteza insular (Nakashima et al., 2000; Reilly, 1998). La vía ventral incluye proyecciones principalmente monosinápticas, y en la mayoría de los casos recíprocas, hacia la corteza gustativa, y amígdala. Además una gran variedad de áreas cerebrales implicadas en aprendizaje y memoria gustativa, así como en el control de la conducta alimentaria, reciben aferencias gustativas de forma directa o indirecta, como es el caso del núcleo de la base de la estría terminal (BNST), hipotálamo lateral, núcleo accumbens, corteza perirrinal y corteza prefrontal medial (Nunez-Jaramillo, Ramirez-Lugo, Herrera-Morales, & Miranda, 2010; Yamamoto, 2006). En conjunto, el sistema realiza un complejo procesamiento integrando la información acerca de la cualidad del estímulo con las consecuencias viscerales de su ingestión, gracias a la convergencia de aferencias gustativas y viscerales en todos los niveles desde el primer relevo troncoencefálico en el NST hasta la corteza gustativa insular a través de las interacciones con la amígdala.

Los datos sobre proyecciones aferentes y eferentes de los núcleos de la amígdala provienen principalmente de estudios con trazadores anterógrados y retrógrados inyectados en diversas regiones corticales y subcorticales. Estos

estudios revelan que cada núcleo amigdalino recibe entradas de múltiples regiones del cerebro, y de la misma manera sus proyecciones eferentes son también generalizadas, incluyendo tanto regiones corticales como subcorticales (revisado en McDonald, 1998; Pitkanen, Savander, & LeDoux, 1997; Sah, Faber, Lopez De Armentia, & Power, 2003).

3.2. Organización anatómica y funcional del tálamo gustativo.

Los núcleos talámicos se han clasificado en función de su organización sináptica y conectividad en relevos de primer orden y relevos de orden superior. Los núcleos que establecen relevos de primer orden envían la información sensorial recibida de relevos anteriores o de la periferia hacia la corteza. Este es el caso del núcleo geniculado lateral y el núcleo ventroposteromedial, relevos de la información visual, somatosensorial y gustativa. Por su parte, los núcleos de relevo de orden superior transmiten información entre áreas corticales. Entre ellos se encuentra el núcleo dorsomedial.

El área parvocelular del núcleo ventroposteromedial del tálamo, también conocido como tálamo gustativo, es una pequeña banda de células que se extiende hacia la zona medial desde el núcleo ventroposteromedial del tálamo (VPM). VPMpc contiene neuronas mecanoreceptoras en su parte medial, neuronas que responden a estímulos gustativos en su parte rostromedial y neuronas que responden a diversos estímulos relacionados con el sabor en su parte medial y caudal (Nomura & Ogawa, 1985). Ello le convierte en una de las estructuras más importantes para el

procesamiento de la información gustativa, tanto sensorial como hedónica (Lundy Jr, Norgren, & George, 2004; Sewards, 2004).

En roedores, VPMpc recibe fibras aferentes procedentes de PBN y envía conexiones hacia la corteza insular, constituyendo así un relevo ascendente de la información gustativa y orosensorial (Lundy Jr et al., 2004; Tokita et al., 2010). Además, estudios con trazadores anterógrados y retrógrados arrojan evidencia de conexiones recíprocas entre los núcleos lateral y central de la amígdala con el tálamo gustativo (Nakashima et al., 2000; Ottersen & Ben-Ari, 1979; Turner & Herkenham, 1991; Yasui, Itoh, & Mizuno, 1984), lo que demuestra la interconexión entre la vías ventral y talamo-cortical de procesamiento de información gustativa. En este sentido, se ha propuesto que estas conexiones transportan información sensorial gustativa, información hedónica apetitiva (Sewards, 2004), así como información hedónica aversiva (Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994).

3.3. Sistema gustativo y memoria de reconocimiento.

Se ha sugerido que la corteza insular participa en las reacciones a la novedad y la saliencia de los estímulos gustativos, así como en la memoria del sabor (Gallo, Roldan, & Bures, 1992), pudiendo modular de este modo la adquisición y retención de aversiones gustativas condicionadas (Dunn & Everitt, 1988). Efectivamente, lesiones farmacológicas permanentes y transitorias han demostrado que la corteza insular tiene un papel relevante en la formación de la memoria de reconocimiento gustativa, afectando al establecimiento del condicionamiento aversivo gustativo (Gutierrez, Tellez, & Bermudez-Rattoni, 2003; Lin, Roman, St Andre, & Reilly, 2009;

Stehberg, Moraga-Amaro, & Simon, 2011; Yamamoto, Fujimoto, Shimura, & Sakai, 1995), así como impidiendo la habituación de la neofobia gustativa (Figueroa-Guzman, Kuo, & Reilly, 2006). Estos resultados sugieren que una corteza insular intacta es esencial tanto para la persistencia de la huella de memoria como para su recuperación.

Congruentemente, la presentación de un sabor novedoso produce un incremento en la expresión de la proteína C-Fos en la zona en comparación con el efecto de la presentación de un sabor familiar (Koh, Wilkins, & Bernstein, 2003; Pisano et al., 2012). De la misma forma, el condicionamiento aversivo gustativo de un sabor novedoso produce un aumento de la expresión de C-Fos con respecto al condicionamiento que emplea un sabor familiar (Koh & Bernstein, 2005).

Por otra parte, la amígdala parece desempeñar también un papel relevante en la memoria de reconocimiento gustativa. Por un lado, tanto lesiones electrolíticas como excitotóxicas del núcleo basolateral de la amígdala (BLA) reducen la respuesta neofóbica ante sabores desconocidos (Kesner, Berman, & Tardif, 1992; Kolakowska, Larue-Achagiotis, & Le Magnen, 1984; Lin et al., 2009; Nachman & Ashe, 1974; Reilly & Bornovalova, 2005). Por otro lado, el bloqueo de receptores NMDA en dicho núcleo mediante la infusión de MK-801 (antagonista no competitivo de los receptores NMDA) impide la atenuación de la respuesta neofóbica (Figueroa-Guzman & Reilly, 2008), imposibilitando así la formación de memorias gustativas seguras. Sea cual sea la fase afectada, en cualquier caso la integridad de BLA parece necesaria para la memoria de reconocimiento gustativo. Además, estudios mediante el marcaje de la proteína C-Fos muestran un aumento significativo de la actividad neuronal ante la presentación de un estímulo gustativo novedoso en el núcleo

central y en el núcleo basolateral de la amígdala (Koh et al., 2003; Montag-Sallaz, Welzl, Kuhl, Montag, & Schachner, 1999; Pisano et al., 2012; Wilkins & Bernstein, 2006).

Finalmente, se ha descrito que lesiones electrolíticas y excitotóxicas de VPMpc inducen una disminución en la respuesta neofóbica ante un estímulo gustativo (Loullis, Wayner, & Jolicoeur, 1978), lo que sugiere una posible función talámica en los procesos implicados en la memoria de reconocimiento segura. Además, aunque existen discrepancias acerca de los efectos de lesiones talámicas en la adquisición de la memoria gustativa aversiva (Grigson, Lyuboslavsky, & Tanase, 2000; Scalera, Grigson, & Norgren, 1997), existen informes que indican una marcada atenuación del aprendizaje gustativo aversivo en ratas lesionadas (Lasiter, 1985; Yamamoto et al., 1995). Por su parte, los escasos estudios que emplean marcaje de la proteína c-Fos muestran diferencias en la actividad de VPMpc ante la presentación de un sabor frente a la presentación de agua (Mungarndee, Lundy, & Norgren, 2008). Esta activación se incrementa al presentar una solución novedosa (sacarina, 0,5% wt/vol) frente a la presentación de la misma solución una vez reconocida como familiar después de seis exposiciones (Lin, Roman, Arthurs, & Reilly, 2012).

De especial relevancia para la memoria de reconocimiento parecen ser las conexiones entre la amígdala y el tálamo gustativo, así como las conexiones recíprocas entre los núcleos de la amígdala y la corteza insular con la corteza perirrinal y el hipocampo (Nakashima et al., 2000; Sah et al., 2003; Tokita et al., 2010).

3.4. Neuroanatomía del sistema hipocampal.

La amnesia del paciente HM tras la extirpación de gran parte de su sistema hipocampal fue el germen de la investigación posterior que asoció los procesos de memoria con la función del sistema hipocampal. El sistema hipocampal, tal y como es utilizado el término en esta tesis, incluye la formación hipocampal y áreas corticales asociadas. La formación hipocampal está formada por el giro dentado, las zonas CA1, CA2 y CA3, y el subículo. Estas estructuras están organizadas en gran medida de manera unidireccional, de forma que el flujo de información sigue la dirección giro dentado, CA3, CA1 y subículo. El hipocampo tiene amplias proyecciones con la corteza parahipocampal, siendo la corteza entorrinal la principal fuente de aferencias y eferencias del hipocampo. La corteza perirrinal (PER) se considera, junto con la corteza entorrinal y postrrinal, parte integral de la corteza parahipocampal ya que su papel es fundamental en el procesamiento de información dentro y fuera de la región del hipocampo. En la rata, PER está localizada a lo largo del surco rinal, y está compuesta por las áreas 35 y 36 de Brodmann, lindando rostralmente con la corteza insular y el área visceral, caudalmente con la corteza postrrinal, dorsalmente con la corteza de asociación temporal ventral y ventralmente con la corteza entorrinal lateral (Burwell, Witter, & Amaral, 1995; Burwell, 2001; Kealy & Commins, 2011).

3.5. Sistema hipocampal y memoria de reconocimiento gustativa.

Aunque estudios tempranos informaron de la participación del hipocampo en la memoria gustativa aversiva (Reilly, Harley, & Revusky, 1993), su papel parece

ponerse de manifiesto únicamente en tareas que involucran relaciones complejas entre estímulos y efectos del contexto, tanto espacial como temporal, sin que su integridad sea necesaria para la adquisición y mantenimiento de aversiones gustativas condicionadas (véase Gallo, Ballesteros, Molero, & Morón, 1999 para una revisión). Por el contrario, se ha informado de que la inactivación temporal del hipocampo dorsal puede incluso mejorar la consolidación de este tipo de memoria (Stone, Grimes, & Katz, 2005).

Con respecto a la memoria de reconocimiento gustativa segura se ha demostrado que la inhibición de la síntesis de proteínas en el hipocampo deteriora la habituación de la neofobia gustativa, sin afectar a la memoria gustativa aversiva (De la Cruz, Rodriguez-Ortiz, Balderas, & Bermudez-Rattoni, 2008). En el mismo sentido, se ha descrito un aumento de la activación del gen CCAAT / enhancer binding protein (C / EBPb) en el hipocampo al presentar un sabor novedoso (sacarina 0,1% wt/vol) frente a la exposición de agua; además, la activación de C/EBPb aumentó 18 horas después de la exposición al sabor (Yefet et al., 2006).

Por otro lado, se ha informado de la participación crucial del hipocampo en inhibición latente, siempre y cuando se introduzcan cambios del contexto. Así, la lesión del hipocampo en ratas adultas elimina la dependencia del contexto que muestra este efecto (Molero et al., 2005) y facilita la aversión gustativa dependiente del contexto en ratas envejecidas (Manrique et al., 2009). En conjunto, los resultados apoyan un papel modulador del hipocampo sobre la memoria de reconocimiento gustativa, cuya relevancia depende de la tarea empleada.

La escasa investigación sobre la implicación de la corteza perirrinal en la formación de la memoria de reconocimiento gustativa muestra que la inactivación

temporal de la corteza perirrinal aplicando inyecciones locales de anisomicina, inhibidor de la síntesis proteica, puede impedir la habituación de la neofobia gustativa (De la Cruz et al., 2008). Asimismo, microinyecciones bilaterales en PER de escopolamina, antagonista de los receptores muscarínicos colinérgicos, impiden la formación de la memoria gustativa segura si se aplican inmediatamente después de la ingestión de una solución novedosa (Gutierrez, De la Cruz, Rodriguez-Ortiz, & Bermudez-Rattoni, 2004). Por otro lado, estudios previos realizados en nuestro laboratorio han evaluado la activación de PER en relación con la exposición a una solución gustativa empleando una solución de vinagre (3% vol/vol) mediante técnicas inmunohistoquímicas para el marcaje de la proteína c-Fos). Los resultados indican que la actividad de PER se modifica en relación con la novedad y la familiaridad del sabor (Gomez-Chacon, Gamiz, & Gallo, 2012).

3.6. Relevancia de la interacción entre el sistema gustativo y el sistema hipocampal para la formación de la memoria gustativa segura.

Los estudios neuroanatómicos indican que los dos sistemas descritos mantienen estrechas conexiones entre sí (Figura 1) y con otras áreas cerebrales implicadas en aprendizaje de preferencias gustativas, ocupando el tálamo una posición central en ésta interacción. Por un lado, a través de la vía tálamo-cortical, la corteza gustativa recibe información relacionada con el sabor principalmente desde el tálamo gustativo, con el cual mantiene conexiones recíprocas, aunque además envía axones descendentes directamente al primer y segundo nivel de relevo troncoencefálicos situados en el núcleo del tracto solitario y núcleo parabraquial, respectivamente (Lasiter, Glanzman, & Mensah, 1982; Lundy Jr et al.,

2004). Por otro lado, en la vía ventral, la corteza gustativa mantiene conexiones recíprocas con la amígdala y el hipotálamo lateral, los cuales envían a su vez proyecciones descendentes hacia NTS y PBN (Lundy Jr et al., 2004; Nakashima et al., 2000; Norgren, 1976; van der Kooy, Koda, McGinty, Gerfen, & Bloom, 1984). Asimismo, la corteza gustativa mantiene conexiones recíprocas tanto con la corteza prefrontal medial (Hoover & Vertes, 2007; Nunez-Jaramillo et al., 2010; Yamamoto, 2006) y el núcleo accumbens (Nunez-Jaramillo et al., 2010; Wright & Groenewegen, 1996; Yamamoto, 2006), implicados en el aprendizaje de preferencia de sabores (Yamamoto & Ueji, 2011), como con el hipocampo (Cenquizca & Swanson, 2007) y con la corteza perirrinal (Kealy & Commins, 2011; Sewards & Sewards, 2001), relacionados con la memoria de reconocimiento.

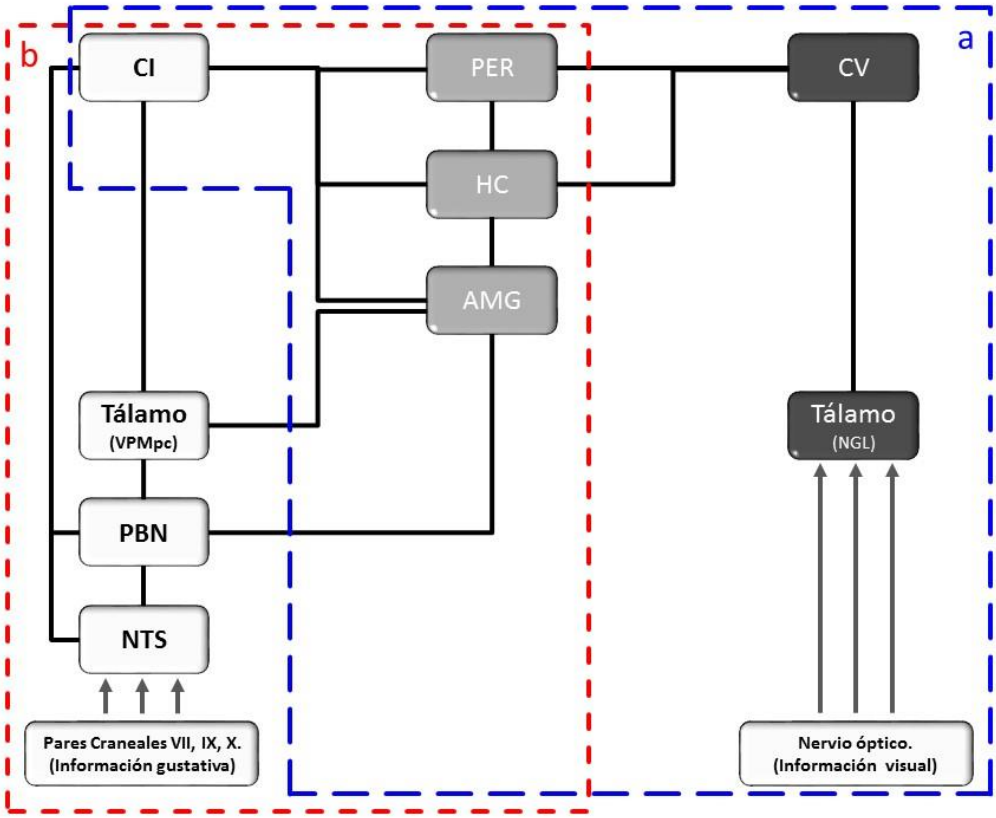

Figura 1. *Diagrama de las principales estructuras y conexiones implicadas en la memoria de reconocimiento visual y gustativa. a) circuito implicado en la memoria de reconocimiento visual. b) circuito implicado en la memoria de reconocimiento gustativa. NTS: núcleo del tracto solitario, PBN: núcleo parabraquial, VPMpc: área parvocelular del núcleo ventroposteromedial del tálamo, CI: corteza insular, AMG: amígdala, HC: hipocampo, PER: corteza perirrinal, NGL: núcleo geniculado lateral del tálamo, CV: corteza visual.*

Por su parte, la corteza perirrinal mantiene conexiones recíprocas con diferentes áreas subcorticales entre las que se encuentra la amígdala, además de otros núcleos de la línea media del tálamo, hipotálamo, ganglios basales, núcleo de raphé, y bulbo olfatorio (Mickley, Kenmuir, Yocom, Wellman, & Biada, 2005; Sah et al., 2003). Recibe proyecciones de la amígdala, el tálamo y el hipotálamo, entre otras zonas (Pikkarainen & Pitkanen, 2001; Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000). Desde la amígdala las principales proyecciones parten de los núcleos del tracto olfatorio lateral, el accesorio basal, y de los núcleos basal y lateral (Furtak, Wei, Agster, & Burwell, 2007; Pikkarainen & Pitkanen, 2001). A su vez la corteza

perirrinal envía eferencias a los núcleos lateral, basolateral y basomedial de la amígdala (Pitkanen et al., 2000). Dentro del tálamo tanto núcleos de la zona ventral como dorsal, así como los núcleos reuniens y rombiode de la línea media, mandan proyecciones hacia la corteza perirrinal (Vertes, Hoover, Do Valle, Sherman, & Rodriguez, 2006).

En cuanto a las aferencias corticales, PER recibe proyecciones desde la corteza precentral, cingulada, parietal, frontal, piriforme, insular, prelímbica, infralímbica, periamigdalina, y cortezas asociativas visual y auditiva. (Kealy & Commins, 2011; Majak & Pitkanen, 2003; Sewards & Sewards, 2001). De especial relevancia para la memoria de reconocimiento son las conexiones bidireccionales con la corteza frontal, insular, parietal y visual (Burwell & Amaral, 1998b). Dentro del sistema hipocampal, PER mantiene conexiones con la corteza entorrinal, postrrinal y el área CA1 del hipocampo (Cenquizca & Swanson, 2007; Kealy & Commins, 2011; Witter, Wouterlood, Naber, & Van Haeften, 2000). Así existen conexiones recíprocas con CA1, subículo y las cortezas entorrinal y postrrinal (Burwell & Amaral, 1998a; Kloosterman, van Haeften, & Lopes da Silva, 2004). A través de estas proyecciones, PER mantiene conexiones indirectas con los núcleos más importantes del sistema hipocampal, giro dentado y CA3 (para una visión más completa de las conexiones de la corteza perirrinal véase Kealy & Commins, 2011).

En definitiva, los conocimientos actuales dibujan un complejo circuito cerebral formado por el sistema gustativo y el sistema hipocampal, con especial relevancia del tálamo, la corteza perirrinal y áreas diencéfalicas. Este circuito es responsable de la asociación entre los estímulos gustativos y sus consecuencias dando lugar a las diversas modalidades de memoria de reconocimiento. Así, la lesión

de la amígdala basolateral interrumpe el patrón de actividad inducido en PER durante la habituación de la neofobia empleando una solución de vinagre de sidra (Gomez-Chacon et al., 2012). Asimismo, la habituación de la neofobia a una solución de sacarina sódica incrementa, tanto en PER como en la amígdala basolateral, la expresión génica de NSF (N-ethylmaleimide-sensitive factor), factor implicado en el tráfico de receptores AMPA asociado a plasticidad sináptica (Gomez-Chacon, Gamiz, Foster, & Gallo, 2016).

En este sentido se ha destacado el papel de PER en procesos perceptivos complejos, que permiten integrar diversos rasgos visuales o cuando existe superposición de características entre los objetos a discriminar o reconocer, lo que aumenta la ambigüedad de la tarea (Barense, Henson, & Graham, 2011; Burke et al., 2011). Así, se ha propuesto un modelo perceptual-mnésico de la actividad de PER, donde se integran ambas funciones (Bussey, Saksida, & Murray, 2003, 2005; Murray & Bussey, 1999). Según esta propuesta, PER cumple una función de puerta de entrada de la información sensorial hacia el hipocampo (Witter et al., 2000). Así, PER actuaría como un área de asociación de la información sensorial y de la información emocional procedente de la amígdala (Kajiwara, Takashima, Mimura, Witter, & Iijima, 2003). Más allá de mera estación de relevo, se le atribuyen importantes funciones perceptivas y mnésicas jugando un posible papel en el procesamiento perceptivo a alto nivel (Bussey et al., 2003, 2005; Murray & Bussey, 1999).

En conjunto, el conocimiento estructural del circuito neuronal relacionado con memoria de reconocimiento y de los cambios funcionales producidos en él por la exposición a sabores nos puede permitir avanzar en la comprensión del modo en que el procesamiento de un sabor determina el comportamiento posterior en una

tarea de aprendizaje. Los cambios en la familiaridad pueden generar diferentes fenómenos de aprendizaje en el estudio de preferencias condicionadas al sabor que pueden ser claves en el estudio de los mecanismos relacionados con el aprendizaje asociativo. Por ello, el planteamiento de esta tesis está basado en una aproximación multidisciplinar al estudio de los efectos de la exposición al sabor aplicando tanto un análisis comportamental como un análisis del Sistema Nervioso a nivel celular y a nivel de circuito.

CAPÍTULO 2

Justificación y objetivos

La memoria de reconocimiento gustativa puede estudiarse empleando una diversidad de procedimientos comportamentales. Todos ellos comparten la presentación de un sabor inicialmente novedoso para el organismo cuyo valor hedónico es modificado bien por las consecuencias positivas o negativas de su ingestión, bien por la ausencia de consecuencias.

En el caso de la adquisición de preferencias condicionadas por el sabor, los procedimientos habitualmente emplean como EC una variedad de sabores que son emparejados con un segundo sabor palatable o con un nutriente (o con una sustancia que posee ambas propiedades) que actúan como EI. Las propiedades positivas del EI son responsables de la adquisición de la preferencia condicionada. El uso de un nutriente palatable (p.ej., sacarosa) como EI puede inducir dos tipos de aprendizaje (Sclafani & Ackroff, 1994). Por un lado se propone que las propiedades nutritivas reforzantes de la sacarosa (i.e., aporte calórico) se asocian con el sabor del EC. Por otro, las propiedades positivas hedónicas del sabor del EI (i.e., dulzor) se asociarían con el sabor del EC, posibilitando una asociación sabor-sabor. Ambos tipos de aprendizaje obedecerían a leyes diferentes de manera que el aprendizaje "sabor-nutriente" se ajustaría a las leyes estándar del condicionamiento Pavloviano, y exhibiría una serie de fenómenos de aprendizaje tales como inhibición latente o extinción. Sin embargo, el aprendizaje "sabor-sabor" involucraría un mecanismo independiente asociado a cambios en el valor hedónico del EC, que no es sensible a tales fenómenos de aprendizaje (Campbell, Capaldi, Sheffer, & Bradford, 1988; De Houwer, Thomas, & Baeyens, 2001; Drucker et al., 1994; Pearce, 2002).

De esta forma, resulta de especial relevancia emplear procedimientos que permitan disociar el aprendizaje "sabor-sabor" del aprendizaje "sabor-nutriente"

cuando el procedimiento experimental se basa en el consumo de un EI que posee propiedades tanto nutritivas como hedónicas, como el caso de la sacarosa. Una posibilidad es que el estado motivacional del organismo en el momento de la prueba seleccione el tipo de aprendizaje, de los adquiridos durante el entrenamiento en el procedimiento estándar, que controlará la ejecución (i.e., la preferencia), puesto que las propiedades nutritivas parecen tener mayor relevancia en situación de privación de comida que en el caso de animales saciados, que se guiarían más por la palatabilidad. De acuerdo con ello, se ha demostrado que el aprendizaje de preferencias condicionadas al sabor inducido por sacarosa muestra fenómenos propios del aprendizaje "sabor-nutriente", tal como inhibición latente, cuando los sujetos están hambrientos (y sedientos) durante la prueba, pero no cuando solamente están sedientos (Garcia-Burgos, Gonzalez, & Hall, 2013).

El uso de reforzadores de diferente naturaleza nos ofrece otra posibilidad de disociar entre ambos tipos de aprendizaje. La comparación entre fructosa y maltodextrina como reforzadores ha sido previamente utilizada con cierto éxito en el aprendizaje de preferencias gustativas (Dwyer & Quirk, 2008). Hay razones para pensar que la maltodextrina (polímero formado por unidades de D-glucosa que es metabolizado de forma rápida en el organismo) puede generar un aprendizaje "sabor-nutriente", mientras que una preferencia generada por fructosa (monosacárido con la misma fórmula empírica que la glucosa pero con diferente estructura y respuesta glucémica inferior) estaría basada en aprendizaje "sabor-sabor". El uso de maltodextrina como reforzador genera preferencias gustativas cuando es presentada a través de infusión intragástrica (Ackroff & Sclafani, 1994). Sin embargo, aunque su sabor parece ser palatable (Dwyer & Quirk, 2008), hay poca

evidencia a favor de que su sabor por sí solo pueda generar una preferencia condicionada, como muestra el hecho de que el bloqueo de la digestión de carbohidratos a través de fármacos impide que la maltodextrina genere una preferencia condicionada (Elizalde & Sclafani, 1988). Por su parte, la fructosa posee un sabor dulce pero es metabolizada de forma más lenta por el organismo; consumida oralmente favorece el aprendizaje de preferencias condicionadas al sabor, pero administrada de forma intragástrica su poder como reforzador es menos efectivo (Ackroff, Touzani, Peets, & Sclafani, 2001; Sclafani, Cardieri, Tucker, Blusk, & Ackroff, 1993). Así, los efectos producidos por la preexposición al EC mediante el uso de ambos reforzadores en la adquisición de preferencias condicionadas pueden ser determinantes en la disociación entre aprendizaje "sabor-nutriente" y "sabor-sabor". En este sentido, cabría esperar que el aprendizaje de preferencias condicionadas al sabor inducido por maltodextrina muestre fenómenos como inhibición latente propios del aprendizaje "sabor-nutriente", cuando los sujetos son probados hambrientos; mientras que el uso de fructosa como reforzador no ocasionaría dichos fenómenos bajo las mismas condiciones al generar un aprendizaje "sabor-sabor".

Por otro lado, el tipo de sabor empleado como EC puede ser una variable crucial a la hora de inducir preferencias condicionadas, pudiendo su valor hedónico ejercer como variable moduladora. Así, la palatabilidad de un sabor determina la magnitud de la respuesta neofóbica, y por tanto la cantidad ingerida. La aversión condicionada al sabor puede ser modulada manipulando el valor hedónico de un sabor, viéndose disminuida la respuesta aversiva a medida que el valor hedónico del EC aumenta (Roll, 1994). Además, se ha demostrado que la mera exposición al EC

puede producir cambios en su palatabilidad (Garcia et al., 1972; Lin, Amodeo et al., 2012), y por tanto en su valor hedónico. Esta diferencia encontrada en los cambios en palatabilidad cuando un sabor es novedoso frente a cuando es familiar puede ser un factor relevante llegando a interferir en fenómenos de aprendizaje como la inhibición latente. Por ello, adquiere especial relevancia investigar el papel que la naturaleza del sabor empleado como EC adquiere en los procedimientos comportamentales dirigidos a comprender los mecanismos implicados en la adquisición y mantenimiento de preferencias por el sabor.

Dada la relevancia potencial de la naturaleza del sabor empleado como EC, resulta de interés profundizar en la investigación sobre el procesamiento neural del sabor y cómo es alterado por los cambios hedónicos producidos por la experiencia. Para ello, uno de los procedimientos comportamentales más sencillos consiste en la presentación repetida de un sabor. Este procedimiento permite explorar la habituación de la neofobia gustativa que tiene lugar a medida que el sabor se convierte en familiar y seguro. De hecho, se presenta como modelo de memoria de reconocimiento. Por otro lado, se trata de la fase inicial de preexposición cuando se induce inhibición latente. Adquirir mayor conocimiento sobre los circuitos cerebrales activados y los cambios que se producen durante la preexposición puede ayudar a comprender los procesos subyacentes a un fenómeno de aprendizaje de gran relevancia teórica en la investigación sobre los mecanismos responsables del aprendizaje de preferencias gustativas.

Resultados obtenidos con estudios de lesión parecen indicar que el procesamiento del sabor en el relevo talámico (VPMpc) del sistema gustativo es necesario para que se den una serie de fenómenos complejos de aprendizaje que

dependen de la evaluación del estímulo en función de cambios en sus propiedades discriminativas y hedónicas. La lesión de VPMpc interfiere con el fenómeno de contraste negativo anticipatorio (Reilly & Pritchard, 1996; Reilly, Bornovalova, & Trifunovic, 2004; Schroy et al., 2005) y el contraste sucesivo negativo (Reilly & Trifunovic, 1999, 2003; Sastre & Reilly, 2006). Asimismo, se ha descrito que la lesión deteriora al respuesta neofóbica (Arthurs & Reilly, 2013; Reilly & Trifunovic, 2003). Los resultados obtenidos con determinación inmunohistoquímica de la proteína c-Fos como índice de actividad neural, sin embargo, han relacionado la activación de VPMpc con el procesamiento tanto de un sabor familiar (Mungarndee et al., 2008), como de un sabor novedoso (Lin, Roman et al., 2012). Se requiere más investigación sobre los parámetros cruciales para determinar la participación de la zona en el proceso de habituación de la neofobia gustativa.

Estudios previos empleando la misma técnica de determinación de la actividad neural en el sistema hipocampal han mostrado que la actividad de la corteza perirrinal sufre cambios durante el proceso de habituación de la neofobia al sabor (Gomez-Chacon et al., 2012; Gomez-Chacon et al., 2015). Dichos cambios requieren la integridad de la amígdala (Gomez-Chacon et al., 2012), resultan afectados por el envejecimiento (Gomez-Chacon et al., 2015) y parecen estar asociados con procesos plásticos mediados por la interacción NSF/GluR2 similares a los descritos en el fenómeno de potenciación a largo plazo. Así, se han descrito incrementos en la expresión de NSF en la corteza perirrinal y la amígdala asociados a la familiarización del sabor durante la segunda exposición (Gomez-Chacon et al., 2016). De acuerdo con un papel de la corteza perirrinal en el proceso de familiarización, su inactivación mediante el inhibidor de la síntesis de proteínas

anisomicina (De la Cruz et al., 2008) o el antagonista muscarínico escopolamina (Gutierrez et al., 2004), impide la habituación de la neofobia gustativa cuando se aplica durante la segunda sesión de exposición. Sin embargo, se ha informado de que en ocasiones la lesión neurotóxica permanente de la corteza perirrinal parece deteriorar la respuesta neofóbica al sabor sin impedir por completo la habituación de la neofobia (Ramos, 2015). Dado que se trata de resultados contradictorios, resulta de interés investigar si estas discrepancias en torno al efecto de la lesión perirrinal sobre la memoria gustativa segura pueden ser atribuidas al tipo de intervención empleada o a las diferencias en el procedimiento comportamental aplicado. En este sentido, la naturaleza del sabor empleado puede jugar un papel importante en el tipo de proceso activado y la consiguiente implicación de circuitos neurales diferentes.

Por ello, los objetivos de la presente tesis doctoral son los siguientes:

1. Profundizar en la disociación de los dos tipos de aprendizaje "sabor-nutriente" y "sabor-sabor" que sustentan las preferencias condicionadas al sabor mediante el empleo como EIs de sustancias que en la literatura (Dwyer & Quirk, 2008) se informa producen aprendizaje "sabor-nutriente" pero no "sabor-sabor" (maltodextrina), o aprendizaje "sabor-sabor" pero no aprendizaje "sabor-nutriente" (fructosa); y explorar en ambos casos el fenómeno de inhibición latente en función del estado motivacional del organismo. Si la fructosa no da lugar a aprendizaje "sabor-nutriente", y es en éste donde únicamente ese observa inhibición latente (Garcia-Burgos et al.,

2013), probar a los animales hambrientos solo dará lugar al fenómeno en el caso de la maltodextrina; por otro lado, si probar a los animales sedientos, pero no hambrientos, favorece la expresión del aprendizaje "sabor-sabor", insensible a la inhibición latente, no se encontrará el fenómeno en ninguno de los dos grupos.

2. Explorar la contribución del valor hedónico del sabor empleado como EC en el aprendizaje de preferencias mediante la exploración del fenómeno de inhibición latente. El uso de sacarosa como EI en sujetos privados de comida durante la prueba nos permite valorar los efectos inducidos por ECs con diferente valor hedónico dentro de un tipo de aprendizaje predictivo "sabor-nutriente". Si el EC utilizado es un sabor neutro, y los animales están hambrientos durante la prueba, se observará inhibición latente en los animales preexpuestos al sabor. Por el contrario, bajo las mismas condiciones motivacionales, si el EC utilizado es un sabor "no-preferido", su preexposición puede producir cambios en su palatabilidad resultando en una facilitación de la respuesta condicionada.

3. Comparar la actividad de VPMpc, evaluada mediante la determinación inmunohistoquímica de la proteína c-Fos, durante la exposición a un sabor novedoso (primera presentación) y al mismo sabor con diversos grados de familiaridad (segunda y sexta presentación). Si la actividad talámica está relacionada con la respuesta neofóbica al sabor, entonces es de esperar mayor activación durante la primera exposición. Si por el contrario la

activación es mayor durante la segunda o la sexta exposición, entonces la actividad talámica estará relacionada con el proceso de familiarización o con el mantenimiento de la memoria consolidada del sabor, respectivamente.

4. Evaluar el efecto de la lesión neurotóxica de PER, provocada por microinyección bilateral de NMDA, sobre la respuesta neofóbica y su habituación empleando un procedimiento comportamental similar al utilizado en el objetivo anterior. Teniendo en cuenta los resultados previos obtenidos con determinación de la actividad en la zona durante el proceso de familiarización del sabor y con inactivación funcional de la zona, es de esperar que la lesión interfiera con la habituación de la neofobia. Ello solo puede evidenciarse si no resulta afectada la respuesta neofóbica, ya que no puede explorarse su atenuación si queda interrumpida dicha respuesta. Si la lesión interfiriera con la respuesta neofóbica, ello dificultaría o imposibilitaría extraer conclusiones acerca de su impacto sobre el proceso de familiarización.

CAPÍTULO 3

Latent inhibition in flavor-preference conditioning: Effects of motivational state and the nature of the reinforcer

Este capítulo corresponde al artículo González, F., Morillas, E., and Hall, G. (2015). "Latent inhibition in flavor-preference conditioning: Effects of motivational state and the nature of the reinforcer", *Learning & Behavior.* 43, 376-83.

1. Abstract

In two experiments, rats received pairings of the flavor of almond with either fructose or maltodextrin, and the conditioned preference for almond was then tested. In each experiment, half of the rats had received prior exposure to almond on its own, and half had received no preexposure. In Experiment 1, in which the rats were hungry during the test, the preference was greater in the nonpreexposed subjects, both for those trained with fructose and those trained with maltodextrin; that is, latent inhibition was obtained with both reinforcers. In Experiment 2, in which the rats were not food deprived prior to the test, not only was there no latent inhibition with either of the reinforcers, but, for both, the preference was greater for preexposed than for nonpreexposed subjects. These results give no support to the proposal that different types of reinforcer generate different types of learning. They are, however, consistent with the proposal that different types of learning control behavior when a rat is hungry and when it is not, and that the form that generates the preference in the latter case is not susceptible to the latent inhibition effect.

2. Introduction

Prior exposure to the event to be used as the conditioned stimulus (CS) in classical conditioning is usually found to retard acquisition of the conditioned response. This latent inhibition effect is robust and is readily obtained in a wide variety of conditioning procedures (see Lubow, 1989). An exception, however, is the flavor-preference conditioning procedure used in the experiments considered here. In this procedure, subjects (rats, in these experiments) are allowed to consume a neutral or nonpreferred flavor that is presented in compound with a substance of positive motivational value (such as a sucrose solution). After this training, rats given a choice between plain water and water containing the flavor show an increased tendency to consume the latter, an outcome that has been interpreted as an instance of conditioning, with the flavor serving as the CS and sucrose as the unconditioned stimulus (US). Exposure to the CS prior to such conditioning has produced varying results. De la Casa, Márquez, and Lubow (2009) found a reduction in the preference (i.e., latent inhibition), whereas Delamater (2011) found no effect (at least on initial testing; a difference emerged on a further test given after the rats had been given exposure to the US alone).

One factor that contributes to the outcome in this procedure appears to be the motivational state of the rat. In a series of experiments by Garcia-Burgos, González, and Hall (2013), rats were given preexposure to a solution of almond essence prior to pairing almond with sucrose[1]. In all of these experiments, the rats

[1] Almond is presumed to function principally as an odor; but as it may also have a taste component, we will refer to it as a flavor.

were water deprived throughout the procedure. In order to ensure that the stimuli, presented as fluids, would be consumed readily. In some experiments, they were also made hungry throughout the experiment or prior to the test, and in these the latent inhibition effect was evident. We note that in the experiments by De la Casa et al. (2009), in which latent inhibition was obtained, the rats were food deprived throughout. In the study by Delamater (2011) the rats were not food deprived, and Garcia-Burgos et al. found no latent inhibition when the animals were not food deprived before the test. In these circumstances a sizeable preference was still found, but this was as large (in fact, slightly larger) in subjects given preexposure to the CS flavor as in subjects not given preexposure. The absence of latent inhibition after CS preexposure is surprising and interesting, and the aims of the present study were to attempt to replicate this finding under different conditions and to investigate the processes that might underlie it.

A possible interpretation of these findings has come from the suggestion that more than one mechanism can contribute to the preference established with sucrose as the US. Sucrose has both a sweet taste and nutritive postoral consequences, and each of these properties is capable of supporting preference conditioning. A palatable but nonnutritive substance (such as saccharin) will serve as an effective US (e.g., Fanselow & Birk, 1982), a phenomenon referred to as flavor-taste learning. But a preference can also be formed when a nutrient US is delivered by intragastric infusion (e.g., Sclafani, Cardieri, Tucker, Blusk, & Ackroff, 1993), so that its taste properties are irrelevant. We refer to this as flavor-nutrient learning. The implication is that sucrose, when taken orally and subsequently metabolized, supports both forms of learning.

The motivational state of the subject appears to influence the contribution that each of these forms of learning makes to an observed preference (e.g., Fedorchak & Bolles, 1987; Harris, Gorrissen, Bailey, & Westbrook, 2000; Yiin, Ackroff, & Sclafani, 2005). Fedorchak and Bolles showed that both saccharin and sucrose supported a preference in rats that were not hungry when tested. Inducing a state of hunger was found to enhance the magnitude of the preference for those trained with sucrose, but not for those trained with saccharin. Thus, flavor-taste learning (supported by saccharin) is independent of the rat's motivational state. The results for sucrose may be interpreted as indicating that the preference based on flavor-taste learning (evident when the animals are not hungry) is supplemented (or even replaced) by a preference based on flavor-nutrient learning when the animals are hungry (see also Harris et al., 2000). If we assume that, after training with sucrose as the US, hungry rats principally show the effects of flavor-nutrient learning at test, whereas sated rats show principally the effects of flavor-taste learning, then the results reported by Garcia-Burgos et al. (2013) can be interpreted as indicating that flavor-nutrient learning is susceptible to latent inhibition, whereas flavor-taste learning is not. This interpretation accords with the widely held view that flavor-nutrient learning is a form of expectancy learning that will obey the standard laws of conditioning, but that flavor-taste learning involves a different mechanism (e.g., one that produces a change in the hedonic properties of the flavor) that operates according to different laws (see, e.g., Campbell, Capaldi, Sheffer, & Bradford, 1988; De Houwer, Thomas, & Baeyens, 2001; Drucker, Ackroff, & Sclafani, 1994; Pearce, 2002).

To test this interpretation of the results of Garcia-Burgos et al. (2013), it would be useful to have available procedures that allow for separate examinations of flavor-nutrient and flavor-taste learning. The former would be expected to show latent inhibition; the latter, not. A little information is already available, from experiments using somewhat unorthodox procedures. Weingarten and Kulikovsky (1989) reported results from a study investigating rats' response to sham-feeding, which they interpreted as supporting the proposal that preexposure to a flavor restricts the learning of an association between the flavor and the postingestive consequences of feeding. In contrast, no latent inhibition was found in a study by Galef and Durlach (1993), in which the training procedure involved allowing the subject rat to interact with another that had recently eaten food with a particular flavor. The enhanced flavor preference induced by this training (taken to reflect an association between the odor of the flavor and other cues produced by the demonstrator rat) was not prevented by preexposure to the flavor. Although these results are suggestive and consistent with the hypothesis under consideration, they come from complex procedures in which a range of factors would be operating, and alternative interpretations might be possible. Accordingly, in the experiments to be reported here, we have made use of the standard preference conditioning procedure and have attempted to isolate flavor-taste and flavor-nutrient learning by making use of substances other than sucrose as the USs.

In our experiments, the USs were the monosaccharide fructose and the polysaccharide maltodextrin. There are reasons to think that a preference supported by the former is based principally on flavor-taste learning, whereas the latter principally promotes flavor-nutrient learning. Specifically, fructose has a

sweet taste (there is good generalization between sucrose and fructose in rodents; Nissenbaum & Sclafani, 1987), but it is not readily metabolized; consumed orally, it will support flavor-preference learning, but intragastric infusions are much less effective (Ackroff, Touzani, Peets, & Sclafani, 2001; Sclafani et al., 1993). Maltodextrin, on the other hand, will support the conditioning of a flavor preference when it is presented by intragastric infusion (Ackroff & Sclafani, 1994), but although its taste appears to be palatable (e.g., Dwyer, 2008), there is little evidence to indicate that its taste alone would engender a conditioned preference. Elizalde and Sclafani (1988) reported that ingested maltodextrin did not support preference learning when carbohydrate digestion was blocked by a drug treatment.

We acknowledge that the effects of these different USs may well be more complex than this. As we have just noted, intragastric fructose can support a degree of preference learning (albeit rather less strong than that produced by sucrose), implying that flavor–nutrient learning will occur to some extent with fructose. And Myers and Sclafani (2001) have demonstrated, using a taste reactivity test, that rats show positive responses to a flavor that has been associated with intragastric infusion of a sugar, implying that flavor-nutrient learning (like flavor-taste learning) may be capable of changing the hedonic response to a flavor. None the less, the strategy of comparing fructose with maltodextrin has been used with some success to address other issues in flavor-preference learning (e.g., Dwyer & Quirk, 2008), and accordingly, we thought it worthwhile to look for latent inhibition in flavor-preference learning in rats trained with either fructose or maltodextrin as the US. Evidence of an effect with the latter but not the former would support the hypotheses (a) that different mechanisms underlie the preferences established by

these USs, and (b) that the mechanism engaged by fructose (presumed to be flavor–taste learning) is not susceptible to latent inhibition.

3. Experiment 1

We created four groups of rats: Two were given preexposure to the almond flavor that was to be used as the CS, and the other groups received only water at this stage. During conditioning, one pair of groups consumed a mixture of almond and fructose, and the other pair a mixture of almond and maltodextrin. In a final test, all subjects were given access to two bottles, one containing the almond solution, and the other, unflavored water. In order to ensure that they would drink the fluids offered, the rats were water deprived throughout training. They had free access to food during preexposure and conditioning, but, given that a preference based on flavor-nutrient learning (such as maltodextrin is likely to produce) may only be fully evident in hungry animals, access to food was denied all subjects prior to the test.

3.1. Method

Subjects and apparatus. The subjects were 32 naïve male Wistar rats (Janvier, France) with a mean body weight of 365 g at the start of the experiment. They were housed in individual home cages and kept in a colony room at the Biomedical Research Center of the University of Granada that was lit from 8:00 a.m. to 8:00 p.m. each day. Experimental procedures took place with the rats in their home cages and during the light period of the cycle. Inverted 50-ml plastic tubes equipped with stainless steel ball-bearing-tipped spouts were used to present fluids

in these cages. Consumption was estimated by weighing the tubes before and after fluid presentation to the nearest 0.1 g. Rats were maintained water deprived during preexposure and conditioning, but were both food and water deprived during the test. The solutions used were made up with tap water and consisted of 1 % (vol/vol) almond essence (Shepcote Distributors Ltd, Yorkshire , UK) and a compound of 1 % almond essence and 10 % (wt/vol) either maltodextrin (Maltodextrin white pure, Applichem, Darmstadt, Germany) or fructose (D[−]-Fructose, Panreac, Barcelona, Spain).

Procedure All of the experimental procedures were approved by the University of Granada Ethics Committee. To initiate the deprivation schedule, the water bottles were removed 24 h before the start of the experiment. The rats were then given three days to accommodate to a deprivation schedule, in which access to water was allowed twice a day for 30 min, at 9:00 a.m. and 1:30 p.m. Rats were randomly allocated to two weight-matched groups—group Pre (n = 16) and group NPre (n = 16)—for the flavor exposure phase. This phase consisted of one single daily trial (at 9:00 a.m.) across eight days. Each trial consisted of 10 min access to 10 ml of either almond (for animals in the Pre condition) or water (for animals in the NPre condition), followed by free access to water for 30 min. An additional 30-min period of access to water was given each day at 1:30 p.m. After the exposure phase, rats were divided into four groups for the conditioning phase, matched on either average almond (groups Pre) or water (groups NPre) consumption during the preexposure phase, Pre/M (n = 8), NPre/M (n = 8), Pre/F (n = 8), and NPre/F (n = 8).

Conditioning occurred over two days, with one trial each day during the morning session. In each trial, animals had 10 min access to 10 ml of either almond + maltodextrin (groups M) or almond + fructose (groups F). During this 10-min period, food was removed for all rats, in order to avoid any pairing of the flavor with the standard diet. After the last conditioning session, the rats had the second 30-min access to water at 5:30 p.m. instead of 1:30 p.m., and food was then removed from the cages at 6:00 p.m. On the next day, the animals were tested while food deprived; the test consisted of 15 min access to two bottles at 10:00 a.m., one containing 20 ml of the almond solution and the other 20 ml of water. The positions of the bottles were counterbalanced across groups.

3.2. Results and discussion.

All subjects drank readily during the preexposure phase. The mean consumptions (in grams) of almond over the course of this phase were 8.0 and 8.4 for groups Pre/M and Pre/F, respectively; the mean consumption of water was 8.4 for group NPre/M, and 8.3 for group NPre/F. An analysis of variance (ANOVA) with preexposure condition and reinforcer as variables yielded no significant main effects or interaction, largest $F(1, 28) = 1.62$, $p = .213$, $\eta p2 = .05$.

The mean consumptions (in grams) of the almond + maltodextrin compound over the two conditioning trials were 8.7 for group Pre/M and 8.4 for group NPre/M. For groups given the almond + fructose compound, the means were 8.9 for group Pre/F and 6.7 for group NPre/F. Thus, we observed no difference in average consumption during conditioning between the preexposed and nonpreexposed rats

when the reinforcer was maltodextrin, but preexposed animals conditioned with fructose drank more than the nonpreexposed ones. An ANOVA with Preexposure and Reinforcer as factors confirmed this impression, yielding a main effects of preexposure, $F(1, 28) = 6.48$, $p = .017$, $\eta p2 = .19$, and a Preexposure × Reinforcer interaction, $F(1, 28) = 4.36$, $p = .046$, $\eta p2 = .13$. The main effect of reinforcer was not significant, $F(1, 28) = 2.66$, $p = .114$, $\eta p2 = .09$. Post hoc comparison using Tukey's test showed that Pre/F rats drank significantly more than NPre/F rats. There was no difference between the preexposed and nonpreexposed animals when maltodextrin was the reinforcer.

Preference during the test was expressed as a ratio (consumption of almond/total consumption). Ratio scores are presented in Fig. 1 (left), where the groups are designated as TTH, to indicate that they were thirsty (T) during preexposure and conditioning, but also hungry (H) for the test. It is evident that both NPre groups showed a greater preference for almond than did the Pre groups (i.e., latent inhibition was obtained with both reinforcers). An ANOVA with preexposure and reinforcer as the variables showed only a significant main effect of preexposure, $F(1, 28) = 4.64$, $p = .040$, $\eta p2 = .10$; other $Fs < 1$. This difference is, in part, a consequence of the fact that the mean ratio scores for the Pre groups were less than .50. This should not be taken to imply that a learned aversion developed in these groups. With the stimuli and test procedures used here, rats given a choice between almond and water show a preference for water in the absence of any conditioning procedure (Garcia-Burgos et al., 2013, Exp. 3, reported a ratio score of about .40 in these circumstances). Thus, the rats in the Pre groups showed the slight aversion to almond that is found in untrained subjects, and this preference was reversed in the

NPre groups. Note also that although rats in the Pre/F group drank more of the compound during conditioning than did those in the NPre/F group, their preference score was lower; the difference between these groups at test cannot be attributed to differences in consumption during conditioning.

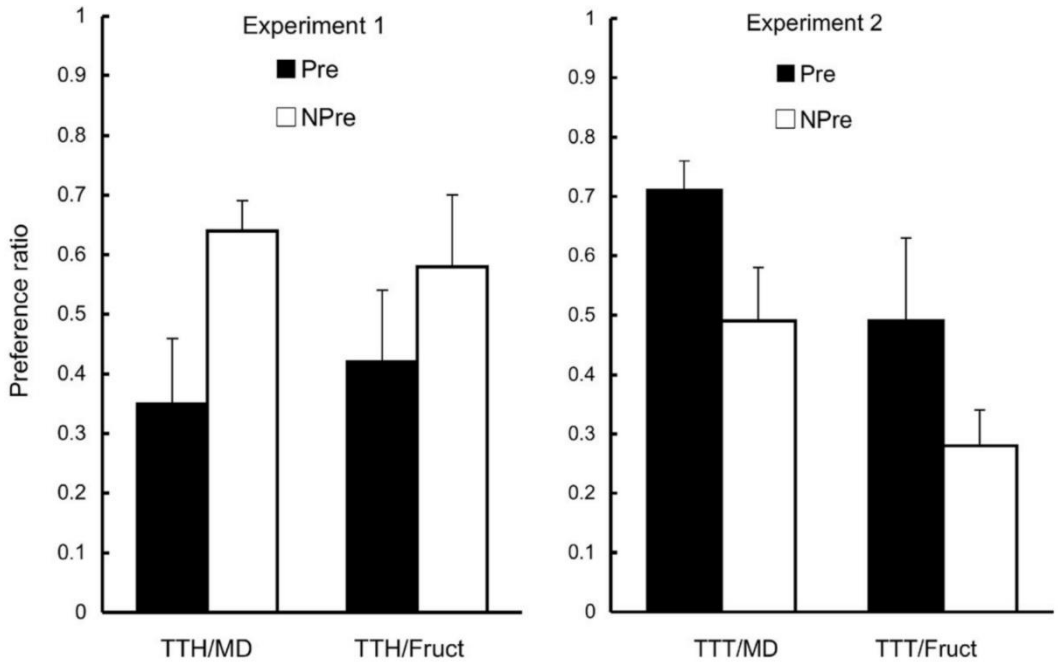

Figure 1. *Average preference ratios for almond over water for groups Pre (preexposed) and NPre (nonpreexposed) in Experiment 1 (left panel; groups TTH: T = thirsty, H = thirsty and hungry) and Experiment 2 (right panel; groups TTT), for the reinforcers maltodextrin (MD) and fructose (Fruct). Error bars represent SEMs.*

The absolute scores for the consumption of water and the almond solution, on which the ratios were based, are presented in the top panel of Fig. 2. The pattern is the same for both reinforcers, with both NPre groups drinking less water than almond, but both Pre groups drinking less almond than water. There was substantial within-group variability in the absolute consumption scores, and an ANOVA on those for the consumption of almond, with preexposure condition and reinforcer as the variables, showed only a marginally significant main effect of preexposure, F(1,

28) = 3.03, p = .09, ηp2 = .10; other Fs < 2. The water scores showed no significant differences (all Fs < 1).

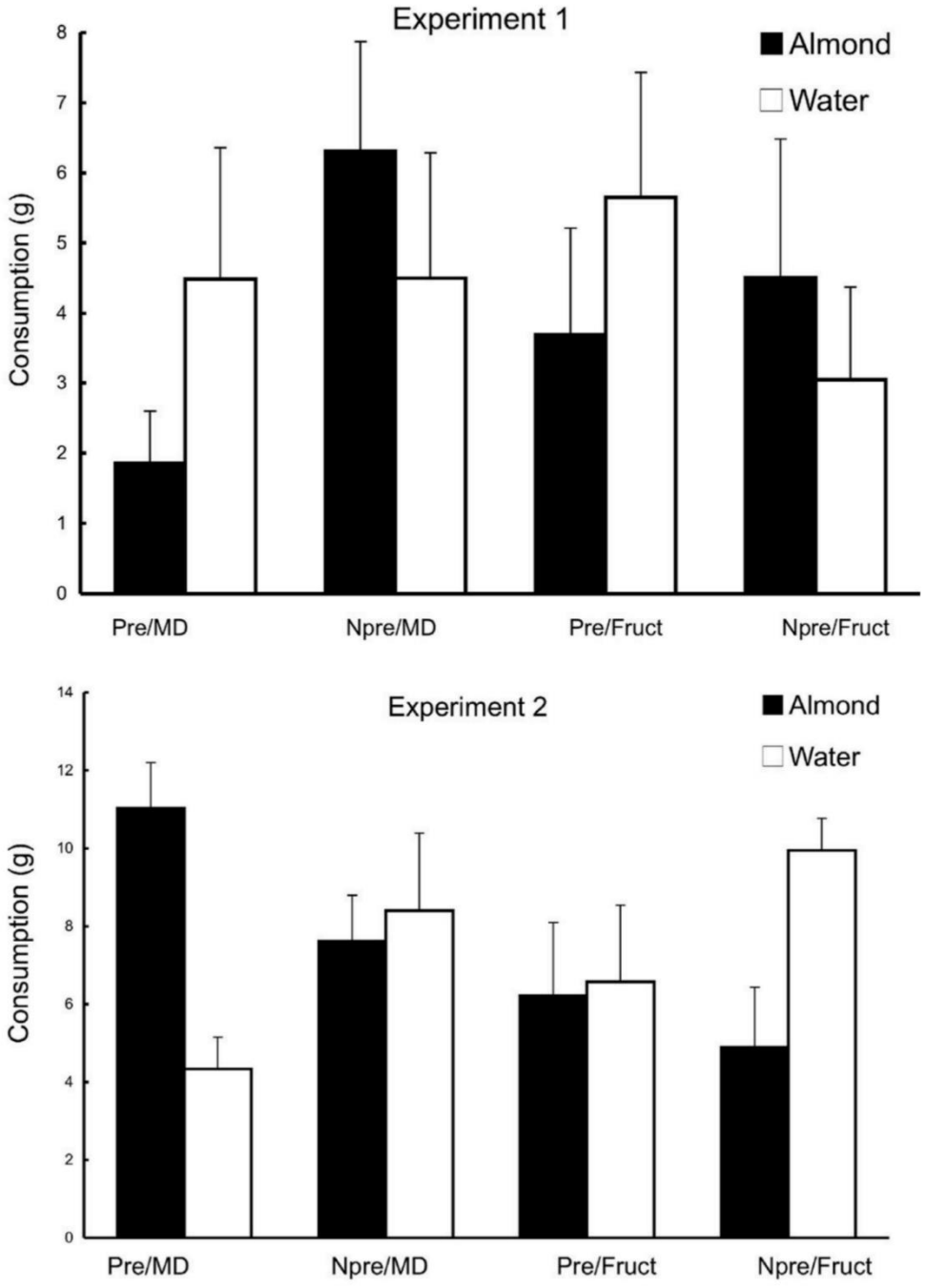

Figure 2. *Mean intakes (in grams) of almond and water for groups Pre (preexposed) and NPre (nonpreexposed) in Experiment 1 (top panel) and Experiment 2 (lower panel), for the reinforcers maltodextrin (MD) and fructose (Fruct). Error bars represent SEMs.*

The results of this experiment indicate that latent inhibition, shown by a lesser preference for almond in animals exposed to the flavor before conditioning, occurs with both fructose and maltodextrin as the US. If we accept that each reinforcer produced just one of the two types of learning considered so far (flavor–taste learning for fructose and flavor–nutrient for maltodextrin), we must conclude that the occurrence of latent inhibition does not depend of the content of learning. We found no support, therefore, for the hypothesis offered by Garcia-Burgos et al. (2013), on the basis of their experiments with sucrose as the US, that flavor–taste learning is immune to latent inhibition. The results of Garcia-Burgos et al., along with those reported so far, can be accommodated by the simpler hypothesis that making the animals hungry during the test increases its sensitivity, allowing latent inhibition to be obtained, whatever the properties of the reinforcer associated with the flavor.

Before discarding our original hypothesis, however, we should consider another possibility. Fructose is, after all, a sugar, and although its glycemic index is rather low, it is, nevertheless, capable of producing a degree of flavor–nutrient learning, and this might have been sufficient to generate a preference in our choice test procedure. If so, the results of this experiment could be taken to show, for fructose as for maltodextrin, that flavor–nutrient learning is susceptible to latent inhibition. The hypothesis that the rats needed to be hungry at test for the effects of flavor–nutrient learning to be seen suggests a possible test of this suggestion. If testing the rats when they are not hungry reveals a preference that depends chiefly on flavor–taste learning, then conditioning with fructose should still reveal a sizeable preference, but latent inhibition should be absent. Whether or not

maltodextrin would generate a preference under these test conditions remains to be determined, and no simple prediction about the effects of preexposure was possible. But if a preference were observed, this should, according to our hypotheses, be a product of flavor–taste learning, and thus, like that for fructose, should be insensitive to latent inhibition.

4. Experiment 2

In this experiment, we again studied the four conditions used in Experiment 1 (preexposed or not preexposed to the CS, and maltodextrin or fructose as the US). This experiment differed only in that the rats were not deprived of food at any stage.

4.1. Method

The subjects were 32 male naïve Wistar rats (Janvier, France) with a mean body weight of 355 g at the start of the experiment. The housing, general maintenance, solutions, and apparatus were the same as we described for Experiment 1, with the exceptions mentioned below.

As in Experiment 1, the subjects in the Pre groups received eight sessions of preexposure to almond, and the NPre subjects received unflavored water, prior to conditioning with either the almond + fructose compound or the almond + maltodextrin compound, and then the choice test. Food was available throughout, except for the 30-min duration of each conditioning and test session. In our previous experiments investigating preferences in nonhungry rats (Garcia-Burgos et al., 2013), we gave two conditioning–test cycles (i.e., after the first test, the rats received

two further sessions of conditioning, followed by another test), and this procedure was followed here.

4.2. Results and discussion

As in Experiment 1, the rats drank readily during preexposure, and we found no differences among the groups. The mean consumptions (in grams) of almond over the course of this phase were 8.2 for group Pre/M and 8.3 for group Pre/F. The mean consumptions of water were 8.4 for group NPre/M and 8.2 for group NPre/F. As we assessed by an ANOVA with preexposure and reinforcer as the variables, no significant effects were present, largest $F(1, 28) = 1.54$, $p = .225$, $\eta p2 = .05$.

Although the rats drank most of the fluids offered on the conditioning trials, some small differences did emerge among the groups. The mean consumptions (in grams) of the almond + maltodextrin compound for the two conditioning cycles were 9.3 and 9.5 for group Pre/M, and 7.7 and 9.6 for group NPre/M. The equivalent means for consumption of the almond + fructose compound were 8.4 and 8.7 for group Pre/F, and 6.4 and 7.7 for group NPre/F. An ANOVA with preexposure, reinforcer, and cycle as the variables yielded a significant main effect of reinforcer, $F(1, 28) = 15.80$, $p < .001$, $\eta p2 = .36$, confirming that maltodextrin was consumed more readily than fructose. We also found significant main effects of preexposure, $F(1, 28) = 13.12$, $p = .001$, $\eta p2 = .32$, and cycle, $F(1, 28) = 33.58$, $p < .001$, $\eta p2 = .54$, and a significant Cycle × Preexposure interaction, $F(1, 28) = 16.62$, $p < .001$, $\eta p2 = .37$. Analysis of the source of this interaction using post-hoc Tukey's tests showed that, whereas there were no differences in consumption over the two conditioning

cycles in the preexposed animals, nonpreexposed rats drank less on the first than on the second cycle. We interpret this as indicating that nonpreexposed subjects showed a degree of neophobia on the first test cycle that had habituated by the second. The analysis produced no other significant interactions, largest $F(1, 28) = 1.31$, $p = .263$, $\eta p2 = .04$.

Mean preference ratios pooled over the two tests for each group appear in Fig. 1 (right; the groups are now labeled TTT, for being only thirsty in all stages of the study). Scores were somewhat higher for maltodextrin than for fructose, and, in striking contrast to the results of Experiment 1, the preexposed groups showed a higher, rather than a lower, preference for almond, irrespective of the reinforcer. This reversal of the latent inhibition effect was quite unexpected. An ANOVA conducted on the average preference ratios, with preexposure and reinforcer as the variables, yielded significant main effects of preexposure, $F(1, 28) = 5.17$, $p = .031$, $\eta p2 = .16$, and of reinforcer, $F(1, 28) = 5.48$, $p = .027$, $\eta p2 = .16$; the interaction was not significant ($F < 1$).

Although the patterns of preference scores were the same for fructose and maltodextrin, inspection of the absolute consumption scores shows marked differences. The lower panel of Fig. 2 shows the group means for consumption of almond and water, pooled over both test trials. For maltodextrin, consumption levels of water and almond were approximately the same in subjects given no preexposure, but more almond than water was consumed by the preexposed animals. For fructose, on the other hand, the preexposed subjects were the ones to drink equal amounts, whereas the nonpreexposed subjects drank more water than almond. Rats conditioned with maltodextrin drank more almond than did those

conditioned with fructose. An ANOVA on the scores for consumption of almond, with preexposure condition and reinforcer as the variables, showed a significant effect of the nature of the reinforcer, $F(1, 28) = 6.48$, $p = .017$, $\eta p2 = .08$; the main effect of preexposure, $F(1, 28) = 2.57$, $p = .120$, and the interaction ($F < 1$) were not significant. Water consumption, by contrast, was sensitive to the effects of preexposure, with preexposed subjects drinking less than nonpreexposed subjects. An ANOVA showed a significant effect of preexposure, $F(1, 28) = 6.03$, $p = .021$, $\eta p2 = .18$; the main effect of reinforcer, $F(1, 28) = 1.54$, $p = .225$, and the interaction ($F < 1$) were not significant.

The results of the absolute scores from a choice test can be difficult to interpret, given that they are not independent (if only because drinking from one bottle necessarily limits the opportunity to drink from the other). With this caveat, we offer the following as a possible interpretation of the results in the lower panel of Fig. 2. First, we suggest that one effect of preexposure is to allow habituation of neophobia to almond; subjects given preexposure will drink almond more readily, and thus drink less water than nonpreexposed subjects. Next, maltodextrin is more effective as a reinforcer than is fructose, so that more almond is drunk by subjects given the maltodextrin US than by those given the fructose US. The interaction of these two factors could have generated the results obtained. It will be noted, however, that this analysis has no place for a latent inhibition effect. The implications of this are taken up in the General Discussion.

5. General discussion.

Latent inhibition is a powerful and well-documented effect, and any failure to observe retarded conditioning after extensive preexposure to the CS requires attention. The absence of latent inhibition in rats conditioned with sucrose as the US and tested when not hungry (Garcia-Burgos et al., 2013) prompted the hypothesis that a preference supported by flavor-taste learning is not susceptible to latent inhibition. In the present experiments, we attempted to test this hypothesis by making use of maltodextrin and fructose as the reinforcers and by testing preexposed and nonpreexposed subjects either in a state of hunger (Exp. 1) or when sated (Exp. 2). On the basis of the evidence presented in the Introduction, it was supposed that maltodextrin would engender flavor-nutrient learning, the effect of which would be evident in the preference shown by hungry animals, and that fructose would engender flavor-taste learning, capable of producing a preference even in nonhungry animals. The results of the experiments did not support these suppositions. We found, for subjects not given preexposure to the CS, that both USs were capable of generating a preference when the rats were hungry during the test, and when the rats were not hungry, neither produced a clear preference. If the proposed distinction between flavor-taste and flavor-nutrient learning is to be maintained, we must suppose that, with the stimuli and procedures used in our experiments, both USs are capable of producing flavor-nutrient learning, and that the effect of flavor-taste learning is weak for both reinforcers. This would impose limits on the strategy of using these two reinforcers to distinguish between two kinds of flavor-preference learning, suggesting that, at least under the conditions

used in the present experiments, they may be functionally equivalent (for related results in the case of blocking, see González, Garcia-Burgos, & Hall, 2014).

Nevertheless, the results for subjects given preexposure to the CS produced effects that are informative about the role of latent inhibition in flavor-preference conditioning. Experiment 1, in which the animals were hungry during the test, produced evidence of latent inhibition with both reinforcers; that is, we observed an effect of preexposure in the expected direction that did not depend on the nature of the reinforcer. This result is consistent with the suggestion that flavor-nutrient learning occurs with both reinforcers and is susceptible to latent inhibition. In Experiment 2, however, when the animals were not hungry during the test, not only was no latent inhibition effect obtained, but unexpectedly, for both USs the preference for the conditioned flavor (as assessed by preference ratio scores) was greater in subjects given preexposure to the flavor (i.e., the reverse of latent inhibition was found).

To an extent, therefore, these findings confirm those of Garcia-Burgos et al. (2013), from their experiments using sucrose as the US, in that latent inhibition was evident in flavor-preference conditioning when the subjects were hungry during the test, but not when they were sated. An obvious interpretation of this pattern of results, at least at first sight, is that hunger promotes the expression of a preference (whatever the US), allowing conditioning to be seen clearly, and thus, the effect of preexposure to be observed. It might be argued that the absence of latent inhibition in the study by Garcia-Burgos et al. was merely a consequence of a low sensitivity to appetitive conditioning in nonhungry animals. But this cannot explain the results of

the present Experiment 2, in which nonhungry rats showed a significant reversal of the latent inhibition effect. Other factors must be at work.

As we have noted, the results of Experiment 2 can be explained, in part, in terms of the rats' unconditioned response to the flavor. This factor could also have played a role in Experiment 1, but it seems reasonable to assume that the immediate response to the flavor of an ingested substance would be of more significance to a rat that is not in a state of hunger. The performance of those trained with fructose in Experiment 2 can be wholly explained in these terms. If we assume that fructose generated no substantial conditioned response in these test conditions, then the performance of the NPre group (a preference for water over almond) would be expected on the basis of a neophobic reaction to almond. Preexposure to almond, however, would allow habituation of neophobia, resulting in equal consumption of almond and water. The results for the maltodextrin groups are consistent with this analysis. These animals were more willing to drink almond, suggesting the operation of a conditioned preference. Such a preference could overcome neophobia in the NPre group, producing the result obtained, equal consumption of water and almond; and in the preexposed group, for which neophobia would not be factor, it could produce the marked preference for almond that was observed. This analysis does not require us to suppose that conditioning proceeded more readily in the Pre subjects. Critically, however, it does imply that the conditioning procedure was effective at producing a preference in the Pre group, and thus that latent inhibition was not effective in this condition. This is the outcome to be expected if flavor-taste learning is not subject to latent inhibition.

In summary, the use of maltodextrin and fructose as USs does not allow the clean separation of flavor–taste and flavor-nutrient learning that we had hoped for, but the manipulation of motivational state has a marked effect on latent inhibition. With both USs, preexposure retards the development of a conditioned preference for animals tested in a state of hunger, consistent with the conclusion that both generate flavor-nutrient learning that is susceptible to latent inhibition. When the subjects are not hungry during the test, there is an apparent reversal of latent inhibition. The source of this effect is complex and debatable, but we have offered a possible explanation that is consistent with the original proposal that flavor-taste learning is not susceptible to latent inhibition.

CAPÍTULO 4

The effect of CS preexposure in flavor preference learning seems to depend on the hedonic value of the flavor: Latent inhibition or facilitation

1. Abstract

In three experiments, rats received pairings of either a neutral (Experiments 1 and 3) or an unpreferred flavor (Experiments 2 and 3) with sucrose. In each experiment, half of the rats received prior exposure to the flavor and half received no preexposure. Conditioned preference was then assessed through two-bottle flavor vs. water choice tests under hunger condition. Latent inhibition was observed in experiments using a neutral flavor. However, with an unpreferred flavor, facilitation instead of latent inhibition was evident, either across acquisition trials or through extinction testing. The results from the present series of experiments indicate that, unlike other more conventional paradigms of Pavlovian conditioning, preexposure in flavor preference learning may produce either latent inhibition or facilitation in animals tested hungry, depending on the CS hedonic value.

2. Introduction

Selecting which foods to eat or reject is one of the most essential behaviors for survival. Food and fluid choices are influenced by innate and learned flavor preferences. Most animals (including humans) have innate predispositions to accept some foods (i.e., sweet tasting) and reject others (i.e., bitter tasting) (Birch, 1999), and they also acquire feeding responses on the basis of the postingestive consequences of foods (Sclafani, 2001). A preference for a flavor can be increased, for example, by repeatedly pairing it with a stimulus with positive properties such as sucrose (i.e., conditioned flavor preference, CFP). Flavor preference can enhance the hedonic value of food reward (Myers &Sclafani, 2006; Sclafani&Ackroff, 2006) and it is mediated, in part, by brain neurochemical systems implicated in innate taste preferences and drug reward (Touzani, Bodnar, & Sclafani, 2010).

Two different associations have been proposed to account for CFP (Fedorchak&Bolles, 1987; Sclafani&Ackroff, 1994): flavor–taste and flavor–nutrient learning. In flavor–taste learning, the critical association is between the flavor and the sensory properties of the taste of the Unconditioned stimulus (US). Flavor–nutrient learning is based on the motivational properties of the US generated by its postingestive consequences (i.e., calorific intake). Which of these forms of learning controls performance on test (i.e., preference) depends on motivational factors; flavor–nutrient preference is enhanced in hungry animals, but there is no evidence of this modulation on flavor–taste learning (Fedorchak & Bolles, 1987; Harris, Gorissen, Bailey, & Westbrook, 2000). In this sense, flavor–nutrient learning has been interpreted as a form of expectancy learning, depending on standard

associative mechanisms and, thus, will obey the standard laws of conditioning, being susceptible to latent inhibition and extinction procedures. On the other hand, flavor–taste learning has been proposed to involve a mechanism that produces a change in the hedonic properties of the flavor; this mechanism would operate according to different laws (Campbell, Capaldi, Sheffer, & Bradford, 1988; De Houwer, Thomas, &Baeyens, 2001; Drucker, Ackroff, &Sclafani, 1994; Pearce, 2002).

In classical conditioning, prior exposure to the to-be conditioned stimulus (CS) retards the acquisition process of the conditioned response during subsequent conditioning. This phenomenon is known as latent inhibition (LI), and it is readily obtained in a wide variety of conditioning procedures (Lubow & Weiner, 2010). However, in CFP, preexposure to the CS has produced varying results. De la Casa, Marquez, & Lubow (2009) found a reduction in the preference (i.e., latent inhibition) in hungry (and thirsty) rats, whereas Delamater (2011) found no effect in non-hungry animals on initial testing (a difference was later observed when rats were tested after they had been given exposure to the US alone). Therefore, one factor that contributes to the outcome in this procedure appears to be the motivational state of the rat.

In a series of experiments, Garcia-Burgos, Gonzalez, & Hall (2013) have examined the role of motivational factors in determining the effects of preexposure to the CS in CFP with sucrose as the reinforcer. LI was obtained when the subjects were food-deprived during the preference test, but it was consistently absent when they were given free access to food before the test. Moreover, LI was obtained when subjects were trained (i.e., during preexposure and conditioning) with free access to food and tested hungry (and thirsty), but it was absent in subjects that were food-

deprived during training and tested just thirsty (see Table 1, taken from Garcia-Burgos et al., 2013). This suggests that acquisition of LI is independent of the motivational state of the rats during training, but its expression depends on the animal's motivational state at the time of test, supporting the hypothesis that flavor-nutrient learning is susceptible to LI, whereas flavor-taste learning is immune to it.

Table 1
Experimental designs.

	Group	Preexp.	Condit.	Test
Experiment 1		*Hunger*	*Hunger*	
	PE/Th	8 A–	2 A+	*Th*: A vs. water
	PE/H			*H & Th*: A vs. water
	NPE/Th	8 water		*Th*: A vs. water
	NPE/H			*H & Th*: A vs. water
Experiment 2				2 cycles
		Non-hunger	*Non-hunger*	
	PE/Th	8 A–	2 A+	*Th*: A vs. water
	PE/H			*H & Th*: A vs. water
	NPE/Th	8 water		*Th*: A vs. water
	NPE/H			*H & Th*: A vs. water
Experiment 3				2 cycles
		Non-hunger	*Non-hunger*	*Non-hunger*
	PE/PA	8 A–	2 A+	A vs. water
	PE/UN		2 A/+	
	NPE/PA	8 water	2 A+	
	NPE/UN		2 A/+	

Note: Animals were maintained under a state of thirst throughout all of the stages of each experiment in this series. PE, preexposed; NPE, non-preexposed; PA, paired presentations of flavor and sucrose; UN, unpaired presentation of flavor and sucrose; A, almond; +, sucrose; –, nonreinforcement; Th, thirsty on test; H or H & Th, hungry & thirsty on test.

However, recent research suggests that CS preexposure may produce the opposite effect to latent inhibition among thirsty animals (i.e., facilitation) when the flavor is neophobic (Gonzalez, Morillas, & Hall, 2015). Using reinforcers that differed in palatability and post-oral calorific value (i.e., maltodextrin and fructose), preexposure to a solution of almond essence induced a reduced preference (as assessed by preference ratio scores) with both reinforcers in rats that were hungry during the test. However, when the animals were not hungry during the test, preference for the conditioned flavor was greater in subjects given preexposure to the flavor (i.e., facilitation) for both reinforcers. The consideration of the absolute consumption scores indicated that this facilitation effect could be explained, in part, in terms of the rats' unconditioned neophobic response to the flavor CS; this

response could be modulated by both preexposure and the effectiveness of the reinforcer to generate a conditioned preference (see Chapter 3).

As mentioned above, preexposure to the CS has produced varying results in CFP procedures, and the motivational state of the rat during testing seems to be crucial for LI expression. Nonetheless, it shall be noticed that, whereas sucrose has been frequently used as the US, a wide variety of flavors has been used as CSs. A conditioned taste aversion can be moderated by manipulating the hedonic value of the CS; for example, the severity of the aversion associated with a CS is attenuated as the hedonic value of the CS increase (Roll, 1994). Therefore, the qualities and the hedonic value of a flavorant could be of particular relevance in CFP procedures, especially in those using exposure to the flavor prior to conditioning. One of the main aspects to take into account is the concentration of a novel flavor solution. In the microstructural analysis of the licking response, the average cluster size (an index of palatability independent of the consumption measure) decreases as concentrations of naturally non-preferred aqueous stimuli such as quinine increases; and it becomes larger when the concentration of naturally preferred tastants, like polycose and sucrose, increases (Davis, 1989; Davis & Smith, 1992; Hsiao & Fan, 1993; Spector & St John, 1998). In addition, although there are unlearned preferences for certain taste qualities that influence their initial acceptability, the palatability of a new tastant can be increased when it is followed by no noxious state (Lin, Amodeo, Arthurs, & Reilly, 2012). Tasting a novel flavor induces an innate neophobic response consisting in an initial low consumption in order to prevent the ingestion of large amounts of potentially toxic food. Repeated presentations of the novel stimulus without negative consequences lead to a

reduction of the unconditioned response to that flavor (Jordan, Todd, Bucci, & Leaton, 2015), and consumption increases as it becomes recognized as safe (Bermudez-Rattoni, 2004). In a recent study, Lin et al. (2012) found that the cluster size for tastants with different innate hedonic value like saccharin and quinine, became larger (in addition to an increase in the amount consumed) as familiarity increased with mere exposure, suggesting that the pleasure of drinking increases (or the dislike decreases) as the novel tastant is repeatedly presented without consequences and becomes accepted as safe.

Taken together, these data suggest that flavors with different hedonic value can induce contrasted behavioral results and that prior exposure could modify the flavor's hedonic value. Therefore, the main goal of the present series of experiments was to compare the effects of CS preexposure on flavor preference learning using flavors with different hedonic value. LI can be expected to occur in flavor–nutrient learning, which is taken to depend on standard associative mechanisms. Therefore, LI was examined in hungry rats (at the time of the test; remind that flavor-nutrient learning controls performance when animals are hungry but no when they are just thirsty) using a CS that was either an unpreferred flavor (bitter almond in Exps. 2 & 3) or a neutral one (sweet almond or vanilla, in Exps. 1 & 3 respectively). In each experiment, half of the animals received prior exposure to the CS, and half received no CS preexposure (i.e., was exposed to water during this phase). Subsequently, all animals received pairings of the CS with sucrose. Preference was then assessed through two-bottle flavor vs. water choice tests (see Table 2). Using a neutral flavor as CS is expected to induce LI when the animals are hungry during the test, because flavor-nutrient learning controls performance and it is susceptible to LI. Using an

unpreferred flavor would allow the attenuation of a neophobic response to the flavor, weakening the retardation shown in LI or even inducing the facilitation effect observed in the previous chapter.

Table 2. Experimental designs

	Group	Preexposure	Conditioning cycles x3		
			Conditioning	***Test***	
		Hunger	*Hunger*	*Hunger*	
Experiment 1	Pre	8 SA-	1 SA+	SA vs water	
	NPre	Water	1 SA+	SA vs water	
	Group	**Preexposure**	**Conditioning cycles x3**		**Test (x3)**
			Conditioning	***Test***	
		Non-hunger	*Non-hunger*	*Hunger*	*Hunger*
Experiment 2	Pre	8 A-	2 A+	A vs water	A vs water
	NPre	Water	2 A+	A vs water	A vs water
	Group	**Preexposure**	**Conditioning**	**Test (x3)**	
		Hunger	*Hunger*	*Hunger*	
	Pre/N	8 V-	2 V+	V vs water	
Experiment 3	NPre/N	Water	2 V+	V vs water	
	Pre/U	8 A-	2 A+	A vs water	
	NPre/U	Water	2 A+	A vs water	

Note: Animals were maintained under a state of thirst throughout all of the stages of each experiment in this series, and hungry when indicated. Pre, preexposed; NPre, non-preexposed; SA, sweet almond; A, almond (bitter); V, vanilla; +, sucrose; –, nonreinforcement; N, neutral CS; U, unpreferred CS.

3. Experiment 1

Rats were divided in two groups: one receiving preexposure to a neutral flavor to be used as the CS (group Pre) and the other given no preexposure (group NPre). The flavorants used as CSs in this study are thought to function primarily as odors, but as they may also have a taste component, they will be considered as flavors. All subjects then received a phase of training in which the flavor was presented mixed with a sucrose solution. Afterwards, preference was assessed through a two-bottle choice test pitting the flavor against unflavored water. The conditioning and test phases were run in 3 cycles, providing the opportunity for testing the developing of the preference over the course of acquisition.

3.1. Method

Subjects and apparatus. The subjects were 16 naïve male Wistar rats (Janvier, France) with a mean body weight of 335g at the start of the experiment. They were housed in individual home cages and maintained in a temperature controlled room (21 ºC) on a 12:12 h light–dark cycle (lights on at 8:00 am) at the Biomedical Research Center of the University of Granada. Experimental procedures took place with the rats in their home cages and during the light period of the cycle. The drinking solutions were presented using inverted 50-ml plastic tubes equipped with stainless steel ball-bearing-tipped spouts. Consumption was estimated by weighing the tubes before and after fluid presentation to the nearest 0.1 g. Rats were maintained water and food deprived throughout all procedure. The solutions used

were made up with tap water at room temperature and consisted of 0.035% (vol/vol) solution of sweet almond essence (Manuel Riesgo, S.A., Spain) during preexposure and test phases, and a compound of 0.035 % sweet almond essence and 10% (w/v) sucrose (AB Azucarera Iberia S.L., Madrid, Spain) during conditioning.

Procedure. To initiate the deprivation schedule, food and water were removed 24 h before the start of the experiment. The rats were then given 3 days to accommodate to a deprivation schedule in which they had daily access to water for 30 min at 10:00 a.m. and access to water and food for 90 min at 1:30 p.m. Rats were randomly allocated to two weight-matched groups: Group Pre (n = 8) and group NPre (n = 8), for the flavor exposure phase. This phase consisted of one single daily trial (at 10:00 a.m.) across eight days. Each trial consisted of 10-min access to 10 ml of the flavor (for animals in the Pre condition) or water (for animals in the NPre condition) followed by free access to water for 30 min. An additional 90-min period of access to water and food was given each day at 1:30 p.m. After the exposure phase, the conditioning and test phases were run in 3 cycles. In each cycle, conditioning occurred on one trial at 10:00 a.m. in which animals had 10-min access to 10 ml of the sweet almond + sucrose compound. The test took place the following day. It consisted of 15-min access to two bottles at 10:00 a.m., one containing 20 ml of almond and the other 20 ml of water. The positions of the bottles during tests were counterbalanced across subjects and cycles. All the procedures were approved by the University of Granada Ethics Committee for Animal Research.

3.2. Results and Discussion

Over the course of the preexposure phase mean consumption (g) of almond for the Pre group was 3.6; and 3.7 of water for group NPre. A 2 (Preexposure) x 8 (Trial) mixed Analysis of Variance (ANOVA), with Preexposure as the between subject factor and Trial as the within subject factor, yielded no significant main effect of Preexposure, Trial; nor the interaction, all $F_s < 1$.

Three cycles, each one with one conditioning trial and one test, were run. Mean consumption (g) of the sweet almond + sucrose compound over the three conditioning trials were 9.3, 9.3 and 9.4 for group Pre; and 8.4, 8.9 and 9.2 for group NPre. A 2 (Preexposure) x 3 (Trial) ANOVA showed no significant main effect of Preexposure [$F(1, 14) = 3.22$, $p = .094$, $\eta_p^2 = .19$] or Trial [$F(2, 28) = 2.92$, $p = .071$, $\eta_p^2 = .17$]; nor interaction [$F(2, 28) = 2.18$, $p = .132$, $\eta_p^2 = .13$].

Preference during the tests was expressed as a ratio (consumption of the flavor/total consumption). Preference ratio scores of the three tests for both groups are presented in Figure 1. A 2 (Preexposure) x 3 (Test) mixed ANOVA showed a significant main effect of Preexposure [$F(1, 14) = 56.84$, $p < .001$, $\eta_p^2 = .80$]. Group NPre exhibited a greater preference for sweet almond than group Pre (i.e., latent inhibition was obtained). No difference was found among Tests [$F(2, 28) = 2.40$ $p = .109$, $\eta_p^2 = .15$], and the interaction was not significant [$F(2, 28) = 1.47$ $p = .247$, $\eta_p^2 = .09$]. Therefore, the greater preference showed by group NPre over group Pre was maintained over the course of the acquisition.

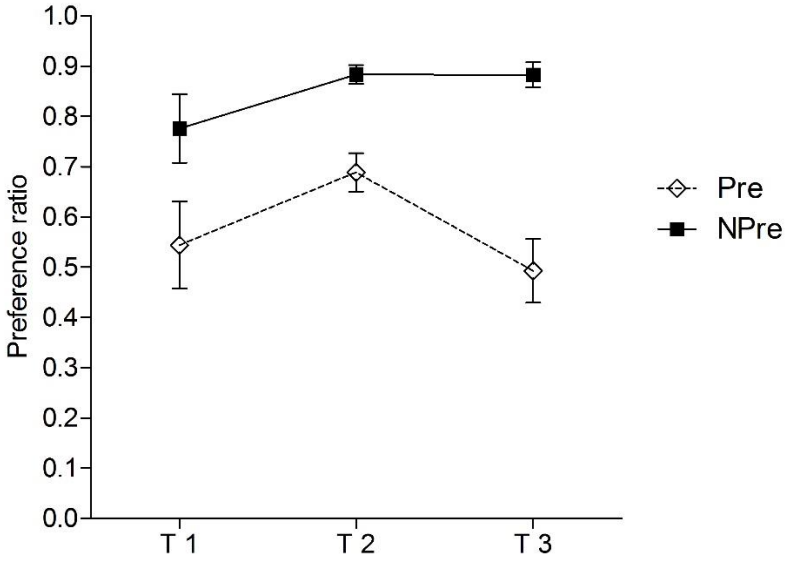

Figure 1. *Experiment 1. Mean preference ratios (±SEM) for the flavor vs. water choice test shown separately for the preexposed (Pre) and non-preexposed (NPre) during the tests along acquisition (Test 1, 2 and 3).*

These results demonstrate a strong preference in non-preexposed animals, and a corresponding reduced preference in the equivalent preexposed group. This greater preference exhibited by group NPre was observed even after just one conditioning trial, and lasted through further acquisition testing. The occurrence of a latent inhibition effect in the present experiment confirms the findings of previous reports (De la Casa et al., 2009; Garcia-Burgos et al., 2013) in which LI was found when rats were hungry throughout the whole procedure, supporting the hypothesis that flavor-nutrient learning is susceptible to LI.

4. Experiment 2

The aim of the following experiment was to assess the effect of preexposure to an unpreferred flavor (bitter almond) in rats that were hungry only during the

test. To better ensure the acquisition of a preference in not food-restricted animals during training, repeated testing cycles over the course of acquisition were run using two conditioning trials (instead of just one) for each cycle (see Experiment 2 in Garcia-Burgos et al., 2013).

4.1. Method

The subjects were 16 male naïve Wistar rats (Janvier, France) with a mean body weight of 336 g at the start of the experiment. The housing, general maintenance, and apparatus were the same as we described for Experiment 1, with the exceptions mentioned below.

All animals were maintained on a water deprivation schedule throughout the experiment; food was removed during the experimental sessions but was otherwise available, with the exception of the test sessions (see below). During accommodation to the deprivation schedule they had access to water for 30 min at 10:00 a.m. and at 2:00 p.m. Rats were assigned randomly to two weight-matched groups: Group Pre (n = 8) and group NPre (n = 8). Subjects in the Pre group received eight sessions of preexposure to a 1% (vol/vol) almond concentrate essence (Shepcote Distributors Ltd, Yorkshire, UK), while NPre subjects received unflavored water. As in Experiment 1, the conditioning and test phases were run in 3 cycles. In each cycle conditioning occurred over two days with one trial each day. In each conditioning trial, animals had 10-min access to 10 ml of 1 % almond + 10 % sucrose compound. As commented before, food was removed for all rats during this 10-min period to avoid any pairing of the flavor with the standard diet. After each second

conditioning session (the day before the test) the rats had the second 30-min access to water at 5:30 p.m., instead of at 2:00 p.m., and food was then removed from the cages at 6:00 p.m. Therefore they were conditioned while thirsty but tested both thirsty and hungry. The test phase consisted of 15-min access to two bottles at 10:00 a.m., one containing 20 ml of almond and the other 20 ml of water. After the three conditioning-testing cycles, three additional tests (one per day) were run under the same conditions (hunger) to explore extinction over test. The positions of the bottles during tests were counterbalanced across subjects and cycles.

4.2. Results and Discussion

Over the course of the preexposure phase, mean consumption (g) of almond for the Pre group was 8.5; for the NPre group the average water consumption was 8.9. A 2 (Preexposure) x 8 (Trial) mixed ANOVA yielded no significant main effect of Preexposure [$F(1, 14) = 1.73$, $p = .208$, $\eta_p^2 = .11$] or Trial [$F(7, 98) = 1.12$, $p = .354$, $\eta_p^2 = .01$]; nor interaction $F < 1$.

Regarding the three 2-trial conditioning-test cycles, mean consumption (g) of the almond + sucrose compound over the six conditioning trials was 9.6, 9.7, 9.8, 9.8, 9.8 and 9.8 for group Pre; and 6.4, 8.9, 9, 9.6, 9.7 and 9.8 for group NPre. A 2 (Preexposure) x 6 (Trial) ANOVA yielded a main effect of Trial [$F(5, 70) = 8.19$, $p < .001$, $\eta_p^2 = .37$] and a Preexposure x Trial interaction [$F(5, 70) = 6.19$, $p < .001$, $\eta_p^2 = .31$]. The main effect of Preexposure was not significant [$F(1, 14) = 4.48$, $p = .053$, $\eta_p^2 = .24$]. Post hoc comparison using Tukey's test showed that NPre rats drank significantly less amount of almond + sucrose compound on the first conditioning

trial than on the rests of trials (ps< .001), and also compared with the consumption on the first trial of the Pre group (p < .001). We interpret this as indicating that non-preexposed subjects showed a higher degree of neophobia on the first conditioning trial. No differences were found between groups on the second and further conditioning trials (ps> .7),

Preference ratio scores across the three tests during the acquisition cycles for both groups are presented in the left panel of the Figure 2. A 2 (Preexposure) x 3 (Test) mixed ANOVA showed a significant main effect of Preexposure [$F(1, 14) = 6.19$, $p = .026$, $\eta_p^2 = .31$] and Test [$F(2, 28) = 3.53$, $p = .043$, $\eta_p^2 = .20$]. The Pre group showed a greater preference for almond than the NPre group, and preference for the flavor was greater on the third than on the first test as shown by post-hoc Tukey's test ($p = .041$), meaning that preference increased through acquisition testing. Interaction Preexposure x Test was not significant [F < 1]

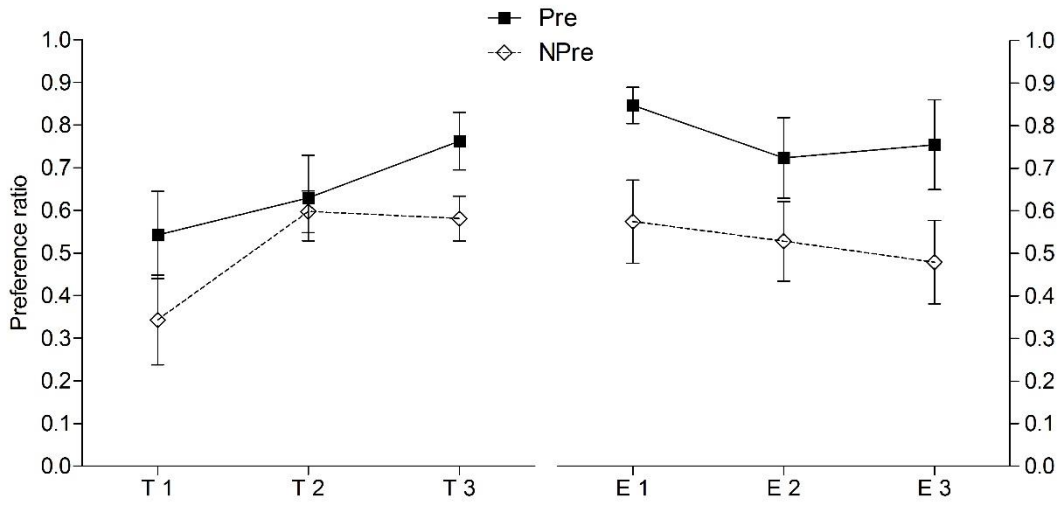

Figure 2. *Experiment 2. Mean preference ratios (±SEM) for the flavor vs. water choice test shown separately for the preexposed (Pre) and non-preexposed (NPre) groups for the test in acquisition (T1, T2 and T3) and test in extinction (E1, E2 and E3).*

Unexpectedly, preexposure to an unpreferred flavor not only did not induce latent inhibition, but rather produced its reverse effect. Preference for the conditioned flavor was greater in subjects given preexposure to the flavor, thus suggesting a facilitation effect of CS preexposure in CFP. It is possible that, in part, the facilitation effect found here could be related to a neophobic response to an unpreferred flavor in non-preexposed animals (as proposed in Chapter 3). Nonetheless, the NPre group still exhibited no clear preference for almond after six conditioning trials, and the difference in preference with the Pre group was maintained during acquisition testing. Thus, preexposure seems to enhance the acquisition of a conditioned preference for an unpreferred flavor.

Preference ratio scores of the three tests in extinction for both groups are presented in the right panel of the Figure 2. A 2 (Preexposure) x 3 (Test) mixed ANOVA showed a significant main effect of Preexposure [$F(1, 14) = 11.18$, $p = .004$, $\eta_p^2 = .44$]. The main effects of Trial and interaction were not significant, $Fs < 1$. Preference for the flavor in both groups remained through tests and differences between groups were maintained. These results indicate that the facilitation produced by preexposure to the CS was consistent and it was resistant to extinction, at least during three extinction tests.

5. Experiment 3

In Experiment 3 we looked at the effect of preexposure to two CSs with different hedonic value, in rats that were hungry throughout the experiment. This allowed us the possibility of replicating our results in the previous experiments now

using both CSs under the same training and testing conditions (Exps. 1 and 2 differed in some aspects). There were four groups of rats, two of which were given preexposure to the flavor to be used as the CS (the Pre groups) and two given no preexposure (the NPre groups). One of the Pre groups received an almond (bitter) solution as unpreferred CS and the other group a vanilla solution as neutral CS. During conditioning (see Table 2) half of the animals received pairings of almond and sucrose (those preexposed to almond and half of the exposed to water), and the other half pairings of vanilla and sucrose (those preexposed to vanilla and the other half of the ones exposed to water). All were hungry (as well as thirsty) during the whole procedure. As in the previous experiments, the final test phase consisted of a choice test between the flavor and unflavored water. The experiment was run in two replicas, the procedure differing in the flavor used as the CS, one replica was performed using a Neutral flavor (N condition), and the other using an Unpreferred flavor (U condition).

5.1. Method

Subjects and apparatus. The subjects were 32 (16 per replica) male naïve Wistar rats (Janvier, France). Mean body weight at the start of the experiment was 341 g for rats in the N condition, and 413 g for rats in the U condition. The housing, general maintenance, and apparatus were the same as we described for Experiment 1.

Procedure. All animals were maintained on a water and food deprivation schedule throughout the experiment. After 3 days to accommodate to the same deprivation schedule used in Experiment 1, rats were randomly (within each replica) allocated to two weight-matched groups: Group Pre (n = 16 in total) and group NPre (n = 16), for the flavor preexposure phase. This phase consisted of one single daily trial across eight days in which half of the animals in the Pre condition were exposed to the same unpreferred (U) almond solution used in Experiment 2, and the other half to a neutral (N) 0.035% (vol/vol) vanilla concentrate essence (Manuel Riesgo, S.A., Spain). Animals in the NPre condition were exposed to water. The two replicas differed in the maximum amount of liquid available during the preexposure phase, animals in the U condition had 10-min access to 10 ml of the flavor (for animals in the Pre condition) or water (for animals in the NPre condition), whereas animals in the N condition had 10-min access to 6 ml of the flavor (for animals in the Pre condition) or water (for animals in the NPre condition). Thus, after the exposure phase, the experiment consisted in four groups for the conditioning phase depending on preexposure and the hedonic value of the CS: Pre/N (n = 8), NPre/N (n = 8), Pre/U (n = 8) and NPre/U (n = 8). Conditioning occurred over two days with one trial each day at 10:00 a.m. In each trial, animals had 10-min access to 10 ml of either vanilla + sucrose (groups N), or almond + sucrose (groups U). The Test phase occurred over three days with one test each day consisting of 15-min access to two bottles at 10:00 a.m., one containing 20 ml of the flavor (vanilla for the N groups and almond for the U groups) and the other 20 ml of water. The positions of the bottles were counterbalanced across subjects and tests.

5.2. Results and Discussion

Mean consumption (g) of water across preexposure days was 2.6 for the NPre/N and 4.2 for the NPre/U group; this difference in water consumption is likely to be due to differences in average rats' weight between replicas, which turned out to be significant [$t(30) = 8.50$, $p < .001$, $d = 3.00$], difference that extended to CS consumption. Mean consumption of vanilla and almond was 3.1 and 4.2 respectively for the preexposed groups. A 2 (CS value) x 2 (Preexposure) x 8 (Trial) mixed ANOVA yielded a significant main effect of the CS hedonic value [$F(1, 28) = 12.61$, $p = .001$, $\eta_p^2 = .31$], indicating a higher fluid consumption for animals in the U condition, probably because rats were heavier and consumed more fluids generally. This was not an issue in the preference ratio used in the subsequent tests, which compared CS consumption over total consumption, thus avoiding the impact of this difference. In any case, as reported below, there were not differences in consumption between groups at the end of the conditioning phase Moreover, to anticipate the results of the test using preference ratio, LI was not observed in group U even if consumption during the preexposure phase was higher in absolute terms. The main effect of Trial [$F(7, 203) = 1.2$, $p = .303$, $\eta_p^2 = .04$] and the interaction [$F < 1$] were not significant.

Mean consumption (g) of the vanilla + sucrose compound over the two conditioning trials was 4.5 and 9.1, for group Pre/N; and 4.1 and 9.3 for group NPre/N. For groups given the almond + sucrose compound, mean consumption was 7.9 and 8.5 for group Pre/U; and 6.1 and 8.9 for group NPre/U. A 2 (CS value) x 2 (Preexposure) x 2 (Trial) ANOVA yielded a main effect of Trial [$F(1, 28) = 124.66$, p

< .001, η_p^2 = .82], a Trial x CS interaction [F(1, 28) = 28.72, p < .001, η_p^2 = .51] and Trial x Preexposure interaction [F(1, 28) = 6.08, p = .002, η_p^2 = .18]. The main effects of CS hedonic value [F(1, 28) = 2.93, p = .098, η_p^2 = .10] and Preexposure [F(1, 28) = 0.43, p = .516, η_p^2 = .02] were not significant. Post hoc comparison using Tukey's test showed that NPre/U, NPre/N and Pre/N rats drank significantly less on the first than on the second trial (ps< .01). No difference between trials was found in the Pre/U group (p = .973). Differences in consumption over conditioning trials for both of the NPre groups could be explained in terms of a neophobic reaction to the flavor + sucrose compound on the first conditioning trial. However, the Pre/N group also exhibited a lower consumption of the vanilla + sucrose compound on the first conditioning trial. It is possible that this unexpected neophobic response was related to the salience of sucrose over the vanilla solution. In any case no difference was found in consumption among groups during the second and final trial of conditioning (ps > .1).

The data of more interest appear in Figure 3 representing the mean preference ratios of the three consecutive tests collapsed; note that this is a relative measure, thus differences in absolute intakes are cancelled. An ANOVA with CS hedonic value and preexposure condition as factors showed a significant Preexposure x CS interaction [F(1, 28) = 21.85, p < .001, η_p^2 = .44]. The main effects of CS hedonic value [F(1, 28) = 3.91, p = .058, η_p^2 = .12] and Preexposure [F < 1] were not significant. Post hoc comparison using Tukey´s test indicated that group NPre/N showed a greater preference for the flavor than group Pre/N (p = .02) when a neutral flavor (i.e. vanilla) was used during preexposure and conditioning, that is, latent inhibition was observed. Nonetheless, when an unpreferred flavor was used

(i.e., almond), the NPre/U group showed a lesser preference for the flavor than the Pre/U, group ($p = .008$), showing a reversed effect to latent inhibition. Additionally, taking into account preference for the flavor in the two NPre groups, it is evident that preference ratio was greater for vanilla (NPre/N) than for almond (NPre/U) ($p < .001$), thus confirming the effect of the difference in hedonic value in control animals (those no preexposed to the CS before conditioning). No such difference was found between groups Pre ($p > .2$), presumably due to the differential effect of CS preexposure, which decreased preference in group Pre/N, and increased preference in group Pre/U, compared to their controls. This pattern of results suggests that latent inhibition occurred in the first case, and a reverse effect in the latter: a consumption facilitation that might have favored subsequent conditioning.

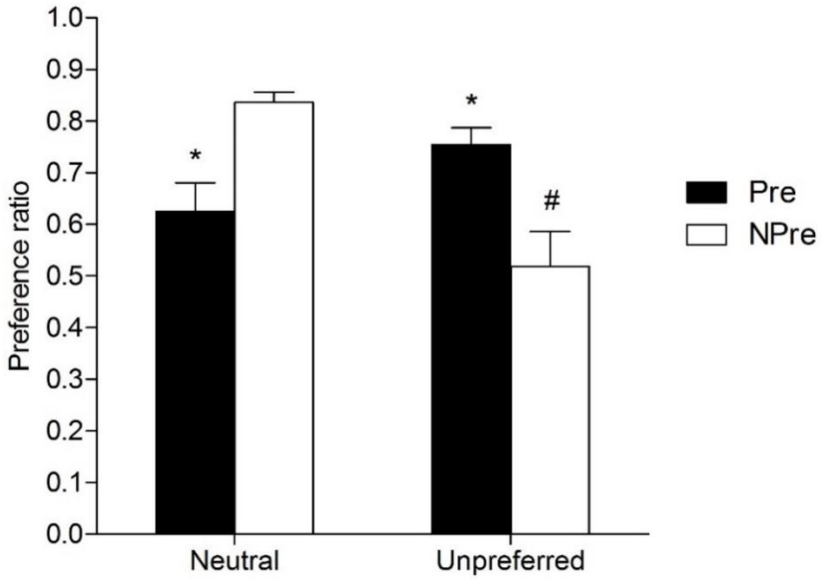

Figure 3. *Experiment 3. Mean preference ratios (±SEM) for the flavor vs. water choice test shown separately for the preexposed (Pre) and non-preexposed (NPre) groups when tested either with vanilla (Neutral) or almond (Unpreferred). * vs NPre ($p < .05$); # vs Neutral.*

Therefore these test results, this time using identical motivational conditions, confirm the findings of both Experiments 1 and 2. When trained and tested hungry,

preexposure to a neutral flavor produced a latent inhibition effect, whereas, under the same conditions, preexposure to an unpreferred flavor induced a facilitation effect. In addition, the difference found on mean preference ratio between NPre/N and NPre/U groups pointed out how flavor preference can be modulated using CSs with different hedonic value. While pairings of a neutral flavor with sucrose induced a strong preference for the flavor (over water), an unpreferred flavor paired with sucrose, produces no clear preference (see also González, Garcia-Burgos, Brugada, & Gil, 2010). Moreover, this modulation by the CS hedonic value extends to complex learning phenomena. Prior exposure to the unpreferred flavor facilitated, rather than retarded, conditioning. Possible explanations for this effect are discussed below.

6. General Discussion

The results of the present series of experiments demonstrated that previous exposure to a flavor stimulus affected its later conditioning, producing either retardation or facilitation of conditioning, as a function of the initial hedonic value of the flavor.

In Experiments 1 and 3, preexposure to a neutral flavor induced LI in hungry animals, which was expressed by a lower preference for the flavor in preexposed compared to non-preexposed animals. Although studies exploring the effect of preexposure in CFP are scarce, LI has been found using neutral flavors as the CS in rats that were hungry (and thirsty) either throughout all procedure (De la Casa et al., 2009; Garcia-Burgos et al., 2013, Experiment 1) or only during testing (Garcia-

Burgos et al., 2013, Experiment 2; Gonzalez et al., 2015, Experiment 1). By contrast, LI has not been found in rats tested just thirsty (Delamater, 2011, in the initial test; Garcia-Burgos et al., 2013, Experiments 1, 2, & 3; Gonzalez et al., 2015, Experiment 2). Thus, our results using a neutral flavor are consistent with previous studies and corroborate the hypothesis that flavor-nutrient learning preference is enhanced by a motivational shift to hunger and that it is susceptible to LI.

Nevertheless, the most relevant finding in our study is that preexposure to an unpreferred flavor induced the opposite effect to LI: conditioning facilitation (Experiments 2 and 3). A facilitating effect of preexposure has been observed in infant rats in olfactory aversion conditioning (Hoffmann & Spear, 1989), and in taste aversion paradigms (Chotro& Alonso, 2001; Gaztañaga, Aranda-Fernandez, Diaz-Cenzano, &Chotro, 2015). It has usually been explained in terms of a deficient processing of the stimulus based in the immaturity of the sensory system of the infant rat; previous experience with the CS would allow a better processing of the stimulus. A facilitation of conditioned taste aversion learning after stimulus preexposure has also been found in adult rats when increasing the relative difficulty of the task by reducing the amount of the CS during conditioning and by increasing the complexity of the taste stimuli (Bennett, Tremain, & Mackintosh, 1996). In this case, facilitation of conditioning was also attributed to a better processing of the CS induced by preexposure that complete the otherwise deficient processing of the CS during conditioning. However, our procedure does not support this account; the flavor stimuli used in our experiments were similar in complexity and were presented to the subjects under the same conditions during preexposure. A facilitating effect of preexposure in CFP was also previously found in our lab using

fructose and maltodextrin as reinforcers in non-hungry subjects (Gonzalez et al., 2015). It was proposed that the flavor used as CS induced a neophobic reaction in animals tested non-hungry, and that the effect of this neophobic response on conditioning was modulated by the effectiveness of the substance used as US during conditioning, as well as by preexposure to the CS (see Chapter 3). In the present series, we discarded the differential effect of the effectiveness of the US in by using the same across conditions, and one whose effectiveness has proved to be high in previous experiments (i.e., sucrose). Therefore here we focused on the interaction between the CS hedonic value (unpreferred flavors are neophobic), the effect of CS preexposure and their interaction.

In order to explore the developing preference for an unpreferred flavor over the course of acquisition, thirsty rats were subjected to three cycles of conditioning + test in Experiment 2, and three additional tests were given in extinction. Although NPre animals showed no clear preference for almond, both Pre and NPre group's preference increased over acquisition testing. Figure 2 suggests that the NPre group's preference increased on the second test, resulting in a similar preference shown by the Pre group on the first test. This increase in preference may be related to the habituation of a neophobic response to almond in non-preexposed animals (and could explain, in part, the results of the previous Chapter). In any case, in those experiments, almond preference for the Pre group was greater than for the NPre group during all acquisition testing, indicating that the observed facilitation effect was not only based on a neophobic reaction to an unpreferred flavor by non-preexposed rats on an initial test. Further extinction tests corroborated that; after

six conditioning trials, difference between groups remained even when the flavor was presented alone without sucrose.

The results from Experiment 3 demonstrated that, while pairings of vanilla and sucrose induced a strong preference in non-preexposed animals, pairings of almond with sucrose induced no clear preference, if preference at all. The low preference ratios for almond observed both in Experiments 2 (although it was slightly increased by training) and 3 increased by previous exposure to the EC. Thus, acquisition of a preference can be modulated by experience; being familiar seems to make the unpreferred flavor more susceptible to changes in preference by associative learning than being novel.

When a new tastant is followed by no noxious state the palatability of the stimulus increases (Lin et al., 1972). Repeated exposure without negative consequences would allow the flavor to be categorized as familiar and safe (Bermudez-Rattoni, 2004), thereby resulting in a greater palatability. In a recent study monitoring the cluster size as a primary index of taste palatability during the initial occurrence and habituation of the neophobic reactions to saccharin and quinine, Lin et al. (2012) found that the cluster size for both saccharin and quinine became larger as familiarity increased. This result suggests that the palatability increases as the novel tastant becomes accepted as safe, even for a naturally non-preferred aqueous stimuli like quinine. In this sense, studies using *in vivo* optical imaging have shown that the primary gustatory cortex, the insular cortex, is organized in spatial maps according to specific sensory features of the stimuli and its hedonic value (Accolla, Bathellier, Petersen, & Carleton, 2007), and that these cortical maps can be susceptible to plastic rearrangements. Accolla & Carleton

(2008) found that inducing conditioned taste aversion to a sweet and pleasant stimulus prompted plastic rearrangement of its cortical representation, becoming more similar to a bitter and unpleasant taste representation. Likewise, optical imaging revealed that sweet and bitter representations became dissimilar again after CTA extinction. Taken together, these results suggest that experience can influence the perception of a stimulus and its hedonic value, inducing changes in palatability and cortical rearrangements.

This account may well be of special relevance when an unpreferred flavor is used as CS in CFP procedures. The flavor's initial acceptability could be modified by previous experience inducing changes in its palatability and hedonic value as it becomes recognized as familiar and safe. These changes would make the flavor more prone to associative learning resulting in a greater preference after conditioning. In any case, the current procedure does not allow us to explore this hypothesis and further research is needed to clarify this matter.

In summary, the results of the present series of experiments indicate that using flavors with different innate hedonic value as the CS induce strong differences in flavor preference, and thus, prior exposure to the CS produce contrasted behavioral effects. On one hand, the results obtained when a neutral flavor is used as CS and animals are hungry during testing support flavor–nutrient learning, which depends upon a standard associative learning process and is prone to latent inhibition. On the other hand, using an unpreferred flavor also in hungry animals produced no clear preference after conditioning. However, preexposure in this latter case facilitated the acquisition of the conditioned response, resulting in a greater preference for the unpreferred flavor. This facilitation effect was consistent

and the difference in preference between Pre and NPre animals was maintained through acquisition and extinction testing, suggesting that previous experience might induce changes in the flavor's perception that would increase its associability with the US.

CAPÍTULO 5

Increased thalamic activity related to taste familiarity assessed by Fos immunohistochemistry

1. Abstract

The parvocellular portion of the ventral posteromedial nucleus of the thalamus (VPMpc) is a relevant structure for processing both sensory and hedonic taste information. In order to investigate the potential relevance of VPMpc in safe taste memory c-Fos immunoreactivity was examined as an index of neural activity in attenuation of neophobia. Fos-positive cells were quantified in Wistar male rats that were exposed to either water (Control) or a vinegar solution during the first (Novel), second (Familiar I) or sixth (Familiar II) exposure. The Control group exhibited lower Fos-positive neurons than the three groups exposed to vinegar. An increased number of Fos-positive cells was found in the Familiar II compared with the Novel and the Familiar I groups. The results indicated that the neural activity of VPMpc was modified by the degree of familiarity to a taste stimulus, corroborating the role of VPMpc in taste recognition memory.

2. Introduction

Intake of novel foods elicits an innate defensive response known as gustatory neophobia (Domjan & Gillan, 1976). It consists in a low consumption of this novel food to prevent the ingestion of large amounts of potentially toxic food. If no negative gastrointestinal consequences follow the intake of the novel food, this innate defensive response dissipates and consumption increases as the taste becomes recognized as safe (Bermudez-Rattoni, 2004). This particular effect is known as attenuation of neophobia (Domjan & Gillan, 1976). Attenuation of neophobia is a non-associative learning process based on the repeated presentation of a taste stimulus. Conversely, if ingestion is followed by negative consequences the taste will become aversive and it will be avoided in later encounters. Both aversive and safe taste memory involve learning and memory processes of great relevance for an adaptive selection of the diet, especially in omnivorous species such as humans and rats.

The parvocellular portion of the ventral posteromedial nucleus of the thalamus (VPMpc), also known as gustatory thalamus, is a relay nucleus considered one of the most relevant areas for processing both sensory and hedonic taste information (Lundy Jr, Norgren, & George, 2004; Sewards, 2004). However, its role in taste memory remains under discussion. Early research found that VPMpc electrolytic and excitotoxic lesions induced a marked attenuation of taste aversive learning (Lasiter, Deems, & Glanzman, 1985; Loullis, Wayner, & Jolicoeur, 1978; Yamamoto, Fujimoto, Shimura, & Sakai, 1995). However, recent studies propose that VPMpc integrity is not relevant for the acquisition of conditioned taste aversions

(Arthurs & Reilly, 2013; Grigson, Lyuboslavsky, & Tanase, 2000; Mungarndee, Lundy, & Norgren, 2006; Reilly, 1998; Reilly, Bornovalova, Dengler, & Trifunovic, 2003). Nonetheless, it has been shown that excitotoxic lesions of VPMpc induce a decreased neophobic response to a novel taste (Arthurs & Reilly, 2013; Reilly et al., 2003), indicating the relevance of VPMpc integrity for taste neophobia.

In addition, VPMpc is also involved in more complex tasks that require assessing the relative value of a gustatory stimulus. Lesions of the VPMpc nucleus abolish anticipatory negative contrast (Reilly & Pritchard, 1996; Reilly, Bornovalova, & Trifunovic, 2004; Schroy et al., 2005) and consummatory successive negative contrast (Reilly & Trifunovic, 1999, 2003; Sastre & Reilly, 2006), thus suggesting a role of VPMpc in the preparatory phase of taste-guided behaviors.

The use of c-Fos protein labeling as a marker of neural activity has been widely used to investigate the neural substrates of taste related learning (Ferreira, Ferry, Meurisse, & Levy, 2006; Gomez-Chacon, Morillas, & Gallo, 2015; Koh & Bernstein, 2005; Lin, Roman, Arthurs, & Reilly, 2012; Wilkins & Bernstein, 2006). Although this technique has been seldom employed in the VPMpc nucleus, it has been shown that sham consumption of a sodium saccharine solution after five exposures induces a higher number of Fos-positive cells than sham intake of water (Mungarndee, Lundy, & Norgren, 2008), revealing taste-related activity in VPMpc. Furthermore, a study using c-Fos labelling in VPMpc found a higher number of Fos-positive cells in the VPMpc nucleus of rats that were exposed to a novel saccharin solution (0.5% wt/vol) compared with rats that were exposed to a highly familiar saccharin solution after six exposures (Lin et al., 2012). The authors suggest that VPMpc has a role in processing taste novelty during the neophobic response, but not

in the attenuation of taste neophobia. However, since the attenuation of neophobia is a learning process that proceeds along various stages, it cannot be discarded that VPMpc activity could change during different phases of the familiarization process.

The aim of the present study was to explore the effect of familiarity on VPMpc activity during three different phases of taste recognition memory. Thus, we have assessed the attenuation of the neophobic reaction to a vinegar solution over a period of 6 days and quantified VPMpc c-Fos activity in rats during the first, the second and the sixth exposure to the tastant. Additional assessments of c-Fos activity in the ventral posterolateral nucleus of the thalamus (VPL), an area non-related with taste processing, were used as control. VPL is part of the ascending pathway for pain (Gauriau & Bernard, 2002) and visceral sensory information (Cechetto & Saper, 1987) and it is involved in the discriminative component of nociceptive and visceral processing.

3. Methods

3.1. Subjects

Twenty-eight adult Wistar rats served as subjects. They were housed individually and maintained in a temperature controlled room (21 ºC) on a 12:12 h light–dark cycle (lights on at 8:00 am) with food and water ad libitum until the experiment started. Rats were water deprived and subjected to daily 15 min drinking sessions at 10 a.m. Twenty one of the rats were assigned to either of three experimental groups Novel (n=7), Familiar I (FI, n=7) and Familiar II (FII, n=7) depending on the number of exposures to a novel tastant.

3.2. Behavioral procedure

After the water baseline was stabilized (5 days), a 3% (vol/vol) vinegar solution was available instead of water during the following six days. Depending on the group they belonged the rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde 90 min after drinking the vinegar solution, either during the first vinegar session on day 6 (Novel), the second session on day 7 (FI) or the session on day 11 (FII) (Table 1). In order to assess the basal activity level the remaining rats were assigned to the Control group (n=7). These rats were subjected to 15 min water session during the whole procedure and were sacrificed after the experimental groups drank the vinegar solution (Day 12).

Table 1. Timeline depicting the experimental procedure. W

Groups	*Days*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Control		W	W	W	W	W	W	W	W	W	W	W	Wª
Novel		W	W	W	W	W	VINª						
Familiar I		W	W	W	W	W	VIN	VINª					
Familiar II		W	W	W	W	W	VIN	VIN	VIN	VIN	VIN	VINª	

Note: Water, VIN: 3% vinegar solution; ªSacrifice 90 min after the drinking period.

3.3. Fos immunohistochemistry

The brains were removed and placed in 4% paraformaldehyde solution for 4 hours before being transferred to 30% sucrose solution until they sank for cryoprotection. Tissue sections (20µm) were rinsed in phosphate-buffered saline

(PBS; 0.01M, pH 7.4), incubated for 15 minutes with 3% hydrogen peroxide (H2O2), rinsed again, and incubated in a solution of 3% normal goat serum and 0.4% Triton X-100 in PBS for 60 min. Slices were transferred to c-Fos primary antibody (1:10000; Calbiochem) for 48 hours at 4ºC. After being rinsed with PBS, they were incubated in a secondary antibody (biotinylated goat anti-rabbit IgG, 1:500; Calbiochem) for 120 min at room temperature. Primary and secondary antibody solutions were mixed in a solution of 2% normal goat serum, 0.4% Triton x-100 and PBS. The sections were rinsed, processed using the ABC kit (Vector Laboratories, Burlingame, CA) and the reaction was visualized using peroxidase substrate kit (DAB) (vector laboratories, Burlingame, CA). Finally, they were rinsed, mounted on gelatine-subbed slides, rehydrated with ethanol and xylene and cover-slipped.

3.4. Quantification of c-Fos

Slides were coded so that the investigator was blind to the treatment groups during analysis. The Neurolucida system (Micro Bright Field Inc., Williston, USA) was used to count the number of Fos-positive cells using a light microscope (Olympus BX41) with a motorized stage interfaced to a computer. This program allowed us to delineate the VPMpc nucleus using an X2 objective, and count manually the number of cells identified in this area with an X40 objective. VPMpc was identified by using the convergence of the fasciculus retroflexus and the medial lemniscus as a reference point (Figure 1). Fos-positive cells were also quantified in an additional control area, the VPL nucleus, located at the same rostro-caudal level according to Paxinos & Watson (2009): -3,6mm to -4,2mm from bregma. Counts

were taken bilaterally from 2 sections within these A-P coordinates. Mean values of both hemispheres and the two sections counted were calculated for each brain area. All the procedures were approved by the University of Granada Ethics Committee for Animal Research.

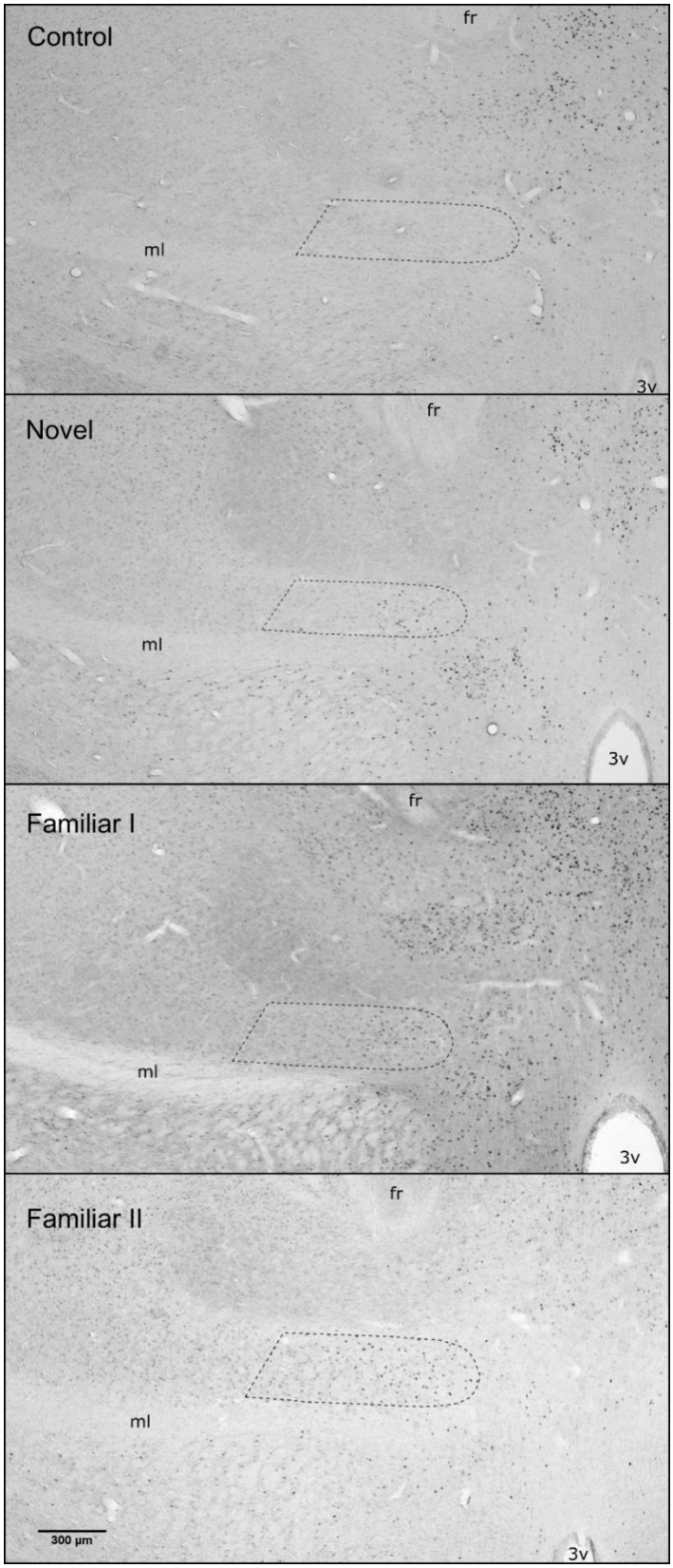

Figure 1. *Representative photomicrographs showing stained Fos-positive cells within VPMpc of the Control, Novel, Familiar I (FI) and Familiar II (FII) groups. Abbreviations are fr, fasciculus retroflexus; ml, medial lemniscus; and 3v, third ventricle.*

4. Results

4.1. Behavioral results

Since the FII group was euthanized after the end of the behavioral procedure, only a within-subject analysis of consumption by the FII group was performed in order to evaluate the attenuation of vinegar neophobia curve. A one-way repeated-measure ANOVA revealed a significant main effect of Session [$F(5, 30) = 6.82$; $p < .001$; $\eta_p^2 = .53$]. Post-hoc comparisons by Newman-Keuls tests revealed that intake on Session 1 was significantly lower than on Sessions 2–6 (ps < .05), thus indicating attenuation of neophobia (Fig. 2A). There was no significant intake differences between Sessions 2–6 (ps > .1), meaning that the consumption of vinegar solution was fully attenuated on the second Session.

Water intake during the last baseline day did not differ among groups [$F(3, 27) = 0.65$; $p > .59$; $\eta_p^2 = .07$]. Figure 2B shows the mean (± SEM) water intake of the Control group and vinegar intake of Novel, FI and FII groups on the day they were perfused for c-Fos labelling. A between-group one-way ANOVA of the vinegar consumption revealed a significant main effect of group [$F(3, 27) = 12.64$; $p < .001$; $\eta_p^2 = .58$]. Post-hoc comparisons by Newman-Keuls test showed that the Novel group drank a significant lower volume of vinegar solution than the FI, FII and Control groups (ps < .05). Moreover, there were no differences between these latter three groups (ps > .1). These results were consistent with the mentioned within-subject data of the FII group along the attenuation of neophobia.

4.2. c-Fos quantification

Counting on the VPMpc nucleus was not possible in three brains, thus the final number of subjects for c-Fos quantification was: Control = 7, Novel = 6, FI = 6 and FII = 6. No differences in Fos-positive cells were found in the control VPL nucleus [$F < 1$]. Figure 2C shows the mean (±SEM) number of Fos-positive cells in the VPMpc nucleus for the four groups. A one-way ANOVA analysis yielded a main significant effect of group [$F(3, 21) = 16.76$; $p < .0001$; $\eta_p^2 = .71$]. Post-hoc analyses by Newman-Keuls tests showed a lower number of Fos-positive cells in the Control group than in the Novel, FI and FII groups (ps < .05), indicating that either one, two or six exposures to vinegar elicited a greater level of activation in VPMpc than water. Furthermore, the number of Fos-positive cells was higher after receiving six exposures to the vinegar solution than after receiving one ($p < .01$) or two exposures ($p < .01$). Thus, the highest level of activation was found in the FII group when the taste was more familiar, but no differences were found between the Novel and FI groups ($p > .1$).

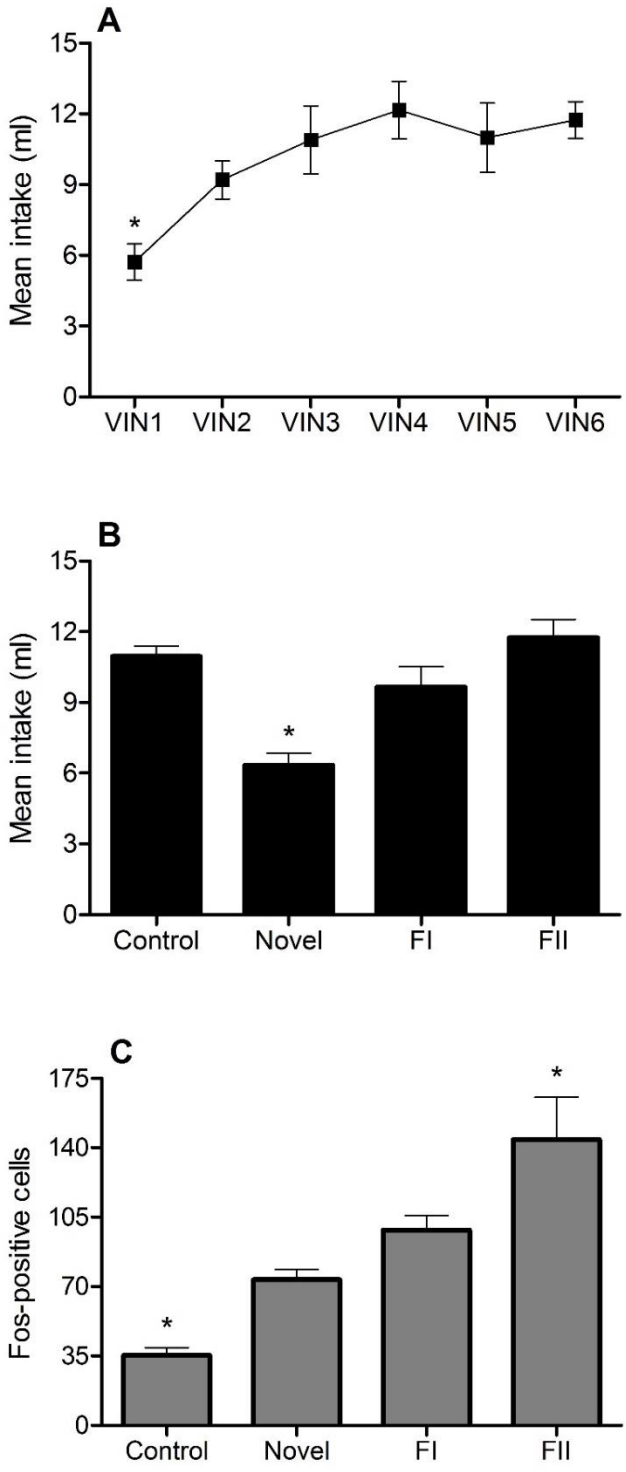

***Figure 2**. (A) Mean (±SEM) intake of Familiar II group during the six cider-vinegar solution (VIN) exposures. * vs VIN 6 ($p < 0.05$); (B) Mean (±SEM) water intake of the Control group and vinegar intake of Novel, Familiar I (FI) and Familiar II (FII) groups on the day they were perfused for c-Fos labelling. * vs rest of the groups ($p < 0.05$); (C) Mean (±SEM) number of c-Fos positive cells in the VPMpc nucleus for Control, Novel, Familiar I (FI) and Familiar II (FII) groups. * vs rest of the groups.*

5. Discussion

Our behavioral data support the widely studied phenomenon of attenuation of neophobia (Bermudez-Rattoni, 2004; De la Cruz, Rodriguez-Ortiz, Balderas, & Bermudez-Rattoni, 2008; Domjan & Gillan, 1976; Gomez-Chacon et al., 2015; Gomez-Chacon, Gamiz, Foster, & Gallo, 2016). Exposure to a novel vinegar solution induced an initial reduced vinegar consumption that increased after a second exposure as the tastant was recognized as safe. Our results indicated that a single exposure was enough to induce attenuation of vinegar neophobia, since there was no significant increase during additional exposures. The main contribution of the present study is to examine the neural activity in the VPMpc nucleus during different phases of safe taste memory recognition. While no differences between groups were found in the control area VPL, our results revealed that either one, two or six exposures to vinegar induced a higher number of Fos-positive cells in VPMpc compared with exposure to water. This data support the implication of VPMpc in taste processing given the fact that consumption of a taste solution induced a greater level of neural activity than the consumption of water. Similar results were found by Mungarndee et al. (2008) in a previous study where sham ingestion of a familiar saccharin solution induced a higher number of Fos-positive cells than sham ingestion of water in VPMpc and other areas of the gustatory circuit like the rostral nucleus of the solitary tract, the parabrachial nucleus and the insular cortex.

The most relevant finding in our study is that the activity of VPMpc increased as the degree of familiarity was higher. In fact, FII group showed a higher number of Fos-positive cells than the Novel and FI groups. Although the behavioral data point

to no differences between FI and FII in the level of taste familiarity since no statistical differences were found in the vinegar intake, the significant increase in the number of Fos-positive cells in the FII group in comparison with FI indicates a more gradual change in the VPMpc activation. This suggests that the activity of VPMpc was related with the attenuation of vinegar neophobia.

To our knowledge there is only one study that has explored the changes of the neural activity on VPMpc during the attenuation of neophobia (Lin et al., 2012). This study reported a different pattern of activation since the number of VPMpc Fos-positive cells was higher following consumption of a novel 0.5% saccharin solution than following the familiar solution after six exposures, thus suggesting that VPMpc activity was related with the taste neophobia instead of with the attenuation of neophobia. However, various procedural differences can explain this discrepancy. First, regarding the taste solution applied, while Lin et al. (2012) used a saccharin solution we applied a 3% cider vinegar solution. Experiments using single-unit recordings have reported that gustatory neurons discharge rates change depending on the taste stimuli applied. Moreover, previous results indicate that VPMpc can encode the hedonic value of tastes (Liu & Fontanini, 2015; Verhagen, Giza, & Scott, 2003). Thus, differences in encoding saccharin and vinegar could explain the discrepancy between c-Fos activation patterns. Second, while in the present experiment all the rats were allowed to ad libitum intake for 15 min during the vinegar sessions, in the study of Lin et al (2012) the intake of the Familiar group was limited at 5 ml on the sixth exposure to the taste to match the amount of saccharin consumed by the Novel group. Although the behavioral procedure applied by Lin et al. (2012) has the great advantage of discarding any potential unspecific effect of an

increased intake of the familiar taste, it is possible that limited and ad libitum access to the liquid solution could elicit different c-Fos activation in VPMpc. In fact, it has been proposed that changes in the amount that the animal is allowed to drink, can increase the salience of the taste (Manrique et al., 2004). It can be conceived that this would increase novelty and prevent the neural activity associated with safe memory. In any case our data allow us to discard that the increase of VPMpc activity would be related with an increased volume of liquid drank since the lowest activation was found in the Control group being the consumption of this group similar to the FII group. Last, in our procedure the FII group received consecutive vinegar sessions (1 per day) until they were perfused for C-Fos labelling on the sixth session. In the study of Lin et al. (2012) the animals in the Familiar group received saccharin every third day and water access on the intervening days. It has been proposed that VPMpc is involved in the preparatory (i.e. food-seeking) rather than the consummatory (i.e. food-eating) aspects of taste-guided behavior (Reilly, 1998). Lesions of VPMpc impair anticipatory contrast (Reilly & Pritchard, 1996, 1997; Reilly et al., 2004; Schroy et al., 2005) and consummatory successive negative contrast (Reilly & Trifunovic, 1999, 2003; Sastre & Reilly, 2006) suggesting an involvement of the thalamus in the anticipation and expectation of taste. Consecutive exposure to a vinegar solution at the same hour every day could generate a general expectation state towards the taste solution. A previous single-unit recording study in alert rats receiving multiple tastants reported changes in VPMpc neurons activity depending on the taste being expected or unexpected (Liu & Fontanini, 2015), indicating that thalamic coding of taste is dynamic and modulated by general expectation. Thus it is possible that taste expectation might

be responsible for the increased number of Fos-positive cells in the FII after six sessions of vinegar. This line of argumentation also could explain the lack of differences in neural activity of the Novel and FI groups, suggesting that neither of these groups received enough vinegar exposures to generate taste expectation. In this way, discrepancies with our results and those obtained by Lin et al. (2012) regarding the thalamic neural activity of the Familiar groups could be explained by different taste expectation states since rats receiving one per day consecutive taste solution exposures more readily generate taste expectation than rats receiving taste solution exposures every third day. Therefore, further research is needed in order to clarify the role of the behavioral procedure used in the pattern of VPMpc activity in taste-guided behaviors and taste memory.

In summary, our data confirm the involvement of VPMpc in taste processing since drinking a taste solution elicited an activation greater than the basal level during water exposure. Furthermore, the activity changes depending on the degree of taste familiarity support a role of VPMpc in the processing of safe taste memory.

CAPÍTULO 6

Taste and object recognition memory impairment by excitotoxic lesions of the perirhinal cortex

1. Abstract

Recognition memory is based on the ability to assess the familiarity of a previously encountered stimulus. It can be approached using test for different sensorial modalities. Excitotoxic lesions of the perirhinal cortex (Prh) were performed in order to assess the relevance of its integrity for object and taste recognition memory. Object recognition memory was impaired with a 24 h retention interval. Taste neophobia attenuation was prevented on a second encounter with the tastant. These results support a role of the perirhinal cortex in mediating the transition from novel to familiar both in object and taste recognition memory.

2. Introduction

The perirhinal cortex (Prh) has been extensively related to recognition memory, known as the ability to determine that something has been previously experienced (Brown & Aggleton, 2001; Brown, Warburton, & Aggleton, 2010; Warburton & Brown, 2010). Recognition memory can be studied using different sensorial modalities. In rodents, one of the most widely used behavioral test for recognition memory is the spontaneous object recognition (SOR) task (Ennaceur & Delacour, 1988) based on the innate preference of the rodents to explore novel objects compared with previously encountered ones. Several studies have shown that Prh lesions impair the spontaneous discrimination of a novel object from a familiar object (Brown, Barker, Aggleton, & Warburton, 2012; Dere, Huston, & De Souza Silva, 2007; Warburton & Brown, 2015; Winters, Saksida, & Bussey, 2008), pointing out the need of the Prh integrity for visual recognition memory.

Another approach to the study of recognition memory using a different sensory modality is taste recognition. It refers to the ability to assess the familiarity of a previously ingested taste. While a novel tastant induces a neophobic response, as the taste is classified as safe (no negative consequences) consumption increases, thus indicating attenuation of neophobia. Lesion studies exploring the Prh involvement in taste neophobia and its attenuation are scarce. It has been reported that Prh reversible inactivation by blocking both the protein synthesis (De la Cruz, Rodriguez-Ortiz, Balderas, & Bermudez-Rattoni, 2008) and the cholinergic neurotransmission (Gutierrez, De la Cruz, Rodriguez-Ortiz, & Bermudez-Rattoni, 2004) impair the stabilization of taste memories, preventing the habituation of

neophobia to 0.3% and 0.5% sodium saccharin solutions respectively. However, a study using Prh neurotoxic permanent lesions has found a disruption of the initial neophobic response to 0.3% and 0.5% sodium saccharin solutions, but no effect was found on its attenuation (Ramos, 2015). Thus, in spite of the consistent evidence on the effect of Prh lesions in visual recognition memory, there are scarce evidence and controversial findings regarding taste recognition memory.

In order to further explore the effect of Prh permanent damage on both visual and taste recognition memory we have assessed the performance of lesioned and SHAM rats in both SOR and attenuation of neophobia tasks. This allows us to establish comparisons between the effects of the same lesion on both tasks, thus reducing variability. In addition, we have used a vinegar solution previously in our lab (Gomez-Chacon, Gamiz, & Gallo, 2012; Gomez-Chacon, Morillas, & Gallo, 2015).

3. Method

3.1. Subjects

Twenty five adult Wistar rats were assigned to one of two surgical groups receiving i.c. bilateral infusions of either NMDA (lesioned PERx group, n=15) or saline solution (SHAM lesions group, n=10). One SHAM rat died during surgery and one lesioned rat showed no damage in Prh being removed from all the analyses. Thus the final number of subjects was: PERx = 14, SHAM = 9. Rats were housed individually and maintained in a temperature controlled room (21 ºC) on a 12:12 h light–dark cycle (lights on at 8:00 am) with food and water ad libitum until the

behavioral procedure started. All the procedures were approved by the University of Granada Ethics Committee for Animal Research.

3.2. Surgery

All the rats were anaesthetized (mixture of 5 mg/ml of xylacine and 50 mg/ml of ketamine in saline solution 0.9%) and mounted on a stereotaxic apparatus (Stoelting Co.Instruments, Word Dale, IL, USA). They were randomly assigned to one of two groups. The lesioned group (PERx) received bilateral injections (three per hemisphere) of N-methyl-D-aspartic acid (NMDA, Sigma–Aldrich, 0.077 M) through injection cannulae (30 gauge) connected to 10 µl Hamilton microsyringes, so that 0.4 µl of NMDA solution were infused in each injection site for 2 min at a rate of 0.2 µl/min using an injection pump (Harvard, USA). The cannulae were left in situ for an additional 2 min before being withdrawn. The stereotaxic coordinates from bregma according to Paxinos & Watson (2009) were: AP = -3.3, ML = ±6.6, DV = -7.5; AP = -4.8, ML = ±6.8, DV = -7.4; AP = -6.3, ML = ±7, DV = -6.8. The SHAM group received equivalent volumes of phosphate-buffered saline (PBS) infused into the same coordinates.

3.3. Behavioral procedure

3.3.1. Spontaneous object recognition

Two weeks after surgery, a standard SOR task was performed in an open box made of black-painted wood (52 x 52 x 40 cm) placed in a dim-light illuminated room. Three geometrical simple objects (duplicated copies) with different shapes

and colors were used: a blue pentagonal-based pyramid, a green triangular prism and a yellow cube (Figure 1). The objects were fixed to the floor with Velcro and thoroughly cleaned with 70% ethanol before each trial to avoid olfactory cues. Objects and their relative positions were counterbalanced.

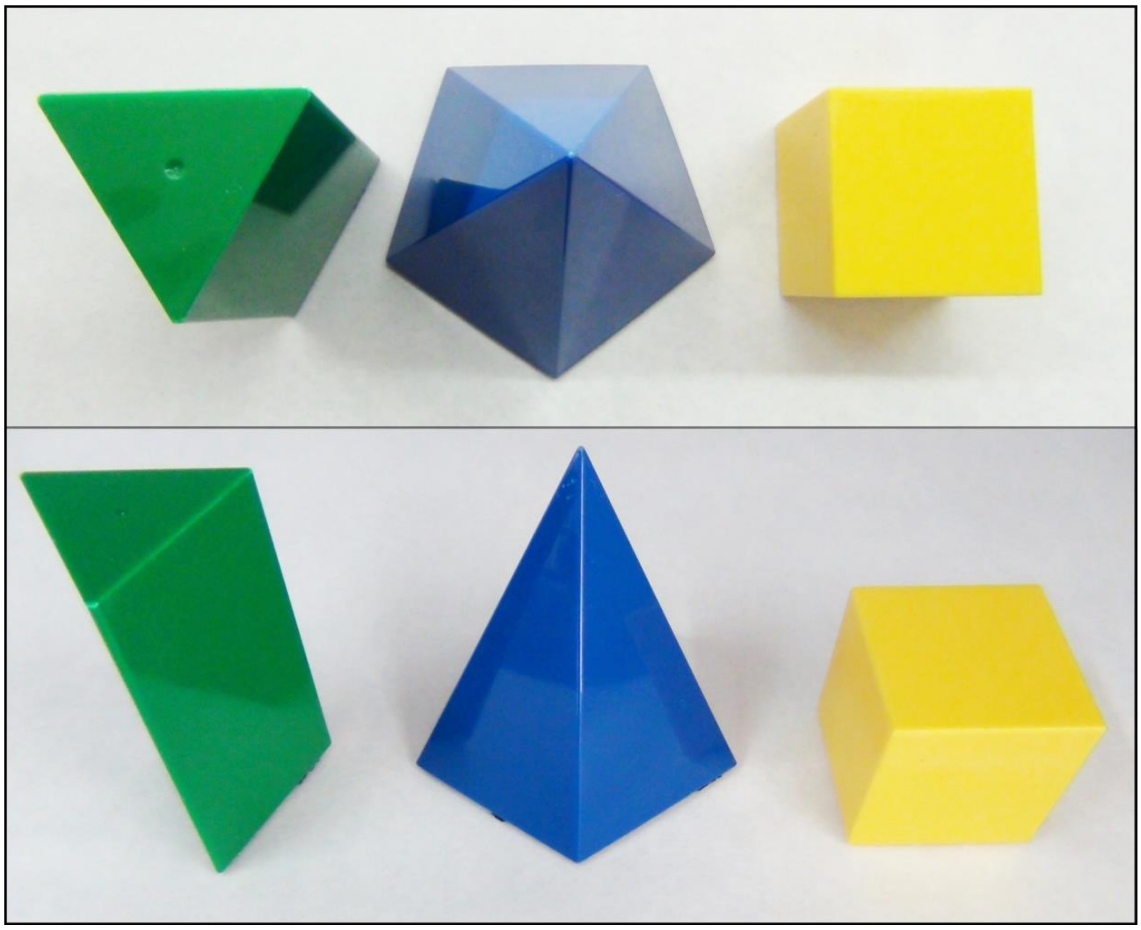

Figure 1. *Photographs showing a perspective from different angles of the corresponding objects' pairs used in the SOR experiments. Simple objects consisting of a green triangular prism, a blue pentagonal-based pyramid and a yellow cube.*

On days 1, 2 and 3 all the rats were given a daily habituation session in which they were placed individually into the empty box for 5 min. In the sample phase, rats were placed in the box facing the wall opposite to two identical objects (A1 and A2) and they were allowed to freely explore them. This phase ended either after the time

spent exploring both objects reached a total of 40 s duration or after 4 min had elapsed. Test 1 took place 1 min after the sample phase. The rats were placed again in the box and one copy of the object from the sample phase (A) together with a new object (B) were presented for 2 min. Twenty four hours later a second test (Test 2) was applied. The rats were placed once again in the box containing one copy of the familiar object (A) together with a different new object (C) for 2 min in order to test memory consolidation. Both the sample phase and tests were video recorded and the total time spent exploring each of the two objects was recorded by the experimenter with two stop watches.

3.3.2. Attenuation of neophobia

The attenuation of neophobia procedure started one week after completing the SOR task. For this task the number of animals was reduced since a number of them was assigned to a preliminary inmunohistochemical experiment. Thus, 8 lesioned and 7 SHAM rats were used. Animals were water deprived and subjected to daily 15 min drinking sessions. After 6 days of water baseline habituation, a 3% (vol/vol) cider vinegar solution was available instead of water during the following four days. The drinking solutions were presented using inverted 50-ml plastic tubes equipped with stainless steel ball-bearing-tipped spouts. Consumption (ml) was recorded after each session.

3.4. Histology

Once the behavioral testing was completed, the rats were deeply anaesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were then removed and placed in 4% paraformaldehyde solution for 4 hours before being transferred for cryoprotection to 30% sucrose solution until they sank. Coronal sections (40 μm) were cut on a cryostat (Leica CM 1900) and stained with cresyl violet. The Neurolucida system (Micro Bright Field Inc., Williston, USA) was used to quantify the extension of the damage in each lesioned rat using a light microscope (Olympus BX41) with a motorized stage interfaced to a computer. Regions of cell loss and gliosis were identified and every coronal section was digitalized.

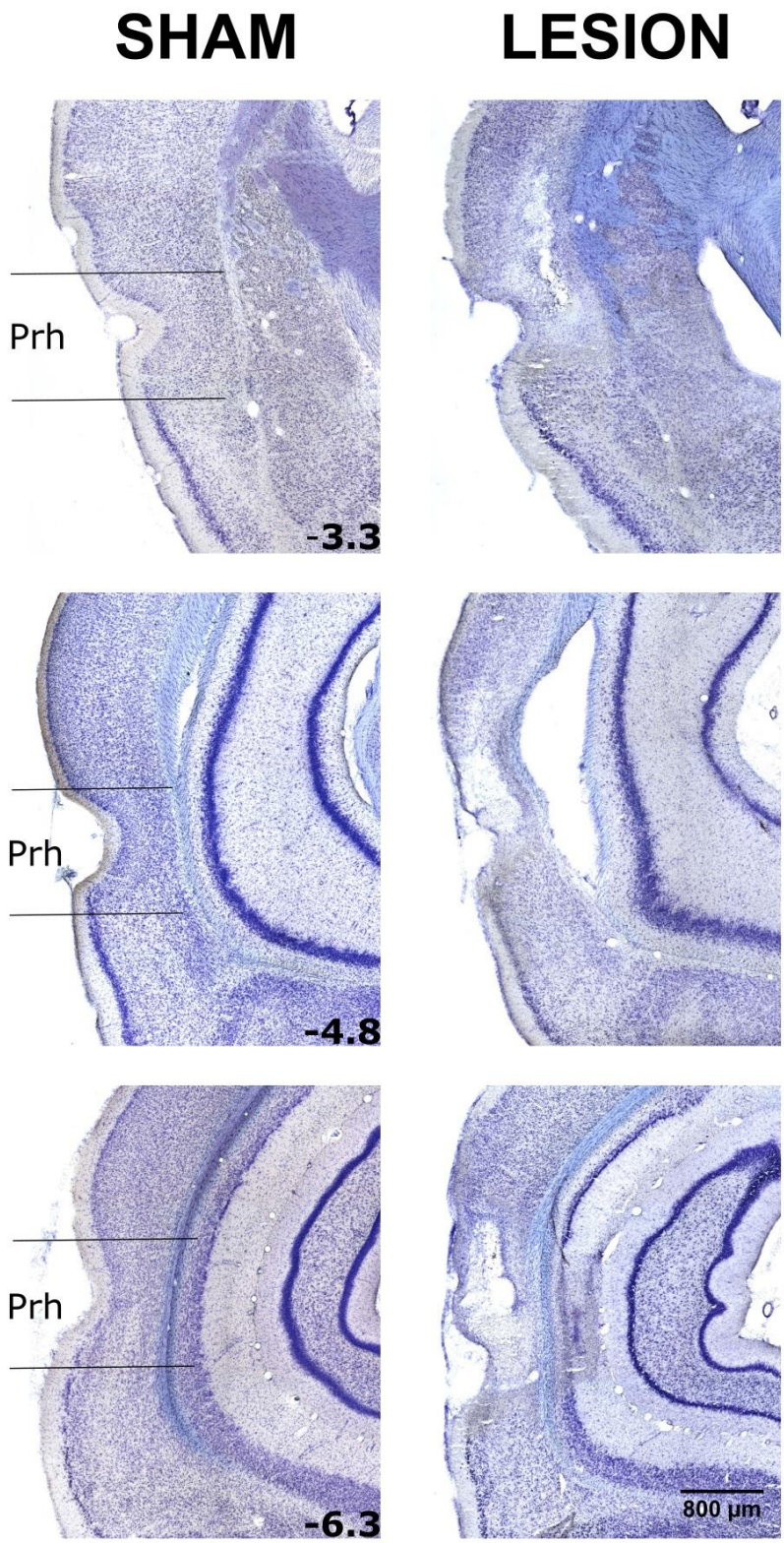

Figure 2. *Photomicrographs of coronal sections stained with cresyl violet from a representative SHAM and perirhinal-lesioned rat. Coordinates indicate the distance (mm) posterior to bregma. Prh: perirhinal cortex.*

4. Results

4.1. Histology

As shown in Fig. 2, the histological analysis confirmed that NMDA infusion induced neuronal loss as well as large changes in the cytoarchitecture of the Prh in the PERx group. Most of the lesions involved the entire rostro-caudal extent of the perirhinal cortex bilaterally, although in one case most of the damage was found unilaterally. In another case there was limited sparing of the perirhinal cortex at its most rostral border.

Adjacent regions to the Prh were largely preserved. Nonetheless, in the two largest lesions the cell loss extended ventrally to involve dorsal and superficial parts of the piriform cortex, in one of them the basolateral amygdala was also damaged at its dorsal part unilaterally. Also, the CA1 field of the hippocampus was affected minimally and always unilaterally in three cases. The insular cortex was intact in all the brains.

4.2. Object recognition

During the sample phase of the SOR task, rats explored both copies of the similar objects equally and there were no differences in exploration time (ET) among the groups [$F < 1$]. A 2 x 2 (Lesion x Novelty) ANOVA analysis of the ET in Test 1 (1 min retention interval) yielded a significant main effect of Novelty [$F(1, 21) = 16.44$, $p < .001$; $\eta_p^2 = .44$], revealing a longer exploration of the novel object for both groups (Fig. 3a). That is, with a short retention interval, both groups performed the task equally, exploring longer a novel object than a familiar one, thus showing

intact short-term memory in the lesioned group. The same ANOVA analysis of the performance in test 2 after a 24-hour retention interval revealed a significant effect of Novelty [$F(1, 21) = 4.78$, $p < .05$; $\eta_p^2 = .19$] and Lesion x Novelty interaction [$F(1, 21) = 4.78$, $p < .05$; $\eta_p^2 = .19$]. Analyses of the interaction by Newman-Keuls tests showed that, whereas the SHAM group explored longer the novel object ($p < .05$), the PERx group explored equally both novel and familiar objects ($p = 1$) (Fig. 3b). Moreover, the ET of the familiar object was significantly higher in the lesioned group than in the SHAM group ($p < .01$), thus indicating an impairment of object recognition memory by Prh lesions.

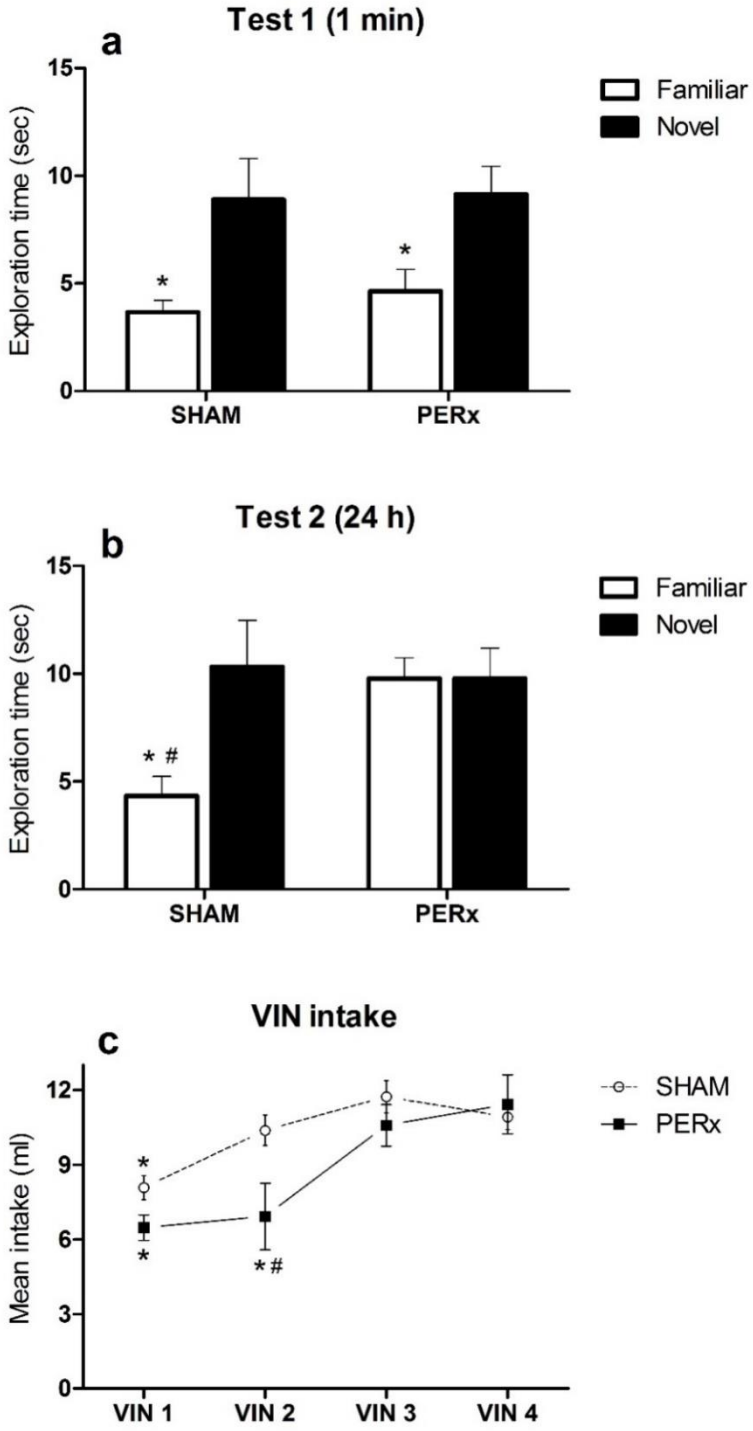

Figure 3. *(a & b) Spontaneous object recognition (SOR) test performance at different retention intervals. (a) Mean (+ SEM) ET of novel and familiar object for SHAM and PERx groups in Test 1 (1 min after sample). * vs Novel (p<0.05). (b) Mean (+ SEM) ET of novel and familiar object for SHAM and PERx groups in Test 2 (24 h after sample). * vs Novel (p<0.05); # SHAM vs PERx groups (p<0.05). (c) Attenuation of neophobia test performance. Mean (±SEM) intake in SHAM and PERx groups during the four cider vinegar solution (VIN) exposures. * vs VIN 4 (p<0.05); # SHAM vs PERx groups (p<0.05).*

4.3. Attenuation of neophobia

Fig. 3c shows the mean (±SEM) intake of vinegar solution by the PERx and SHAM groups in the attenuation of neophobia test. Water intake during the last baseline day did not differ between the groups [$t(13) = 1.44$, $p > .1$, $d = .76$]. A 2 × 4 (Lesion × Session) mixed ANOVA analysis of the amount ingested by the different groups during the vinegar drinking sessions revealed a significant effect of the within-subject factor Session [$F(3, 39) = 16.72$, $p < .0001$; $\eta_p^2 = .56$], thus indicating habituation of neophobia; and a significant Lesion x Session interaction [$F(3, 39) = 2.99$, $p < .05$; $\eta_p^2 = .19$]. Analyses of the interaction by Newman-Keuls tests showed that the SHAM group drank a lower amount of the novel vinegar solution on Session 1 than on Sessions 2, 3 and 4 (ps < .05). Furthermore, there were no differences in vinegar intake among Sessions 2, 3 and 4 (ps > .1). This means that the neophobic response of SHAM rats was fully attenuated on the second session reaching an asymptote of vinegar consumption on the following trials. However there were no differences between the amount ingested by the PERx group, in sessions 1 and 2 (p > .5), thereby indicating absence of attenuation of the neophobic response in the second exposure to vinegar. Consumption on trials 1 and 2 was lower than on trials 3 and 4 (ps < .05), showing that the lesioned group attenuated the neophobic response on the third trial, reaching the asymptote of consumption in the last two trials (p > .5). Consistently there was a significant difference between both groups intake in session 2 (p < .05), since the SHAM group exhibited attenuation of the neophobic response but the PERx group did not. Therefore, Prh lesions had no influence on the magnitude of the neophobic response and had no influence on the

final, asymptotic level of vinegar intake, but they did delay the attenuation of the neophobic response, preventing it on a second encounter with the taste.

5. Discussion

The main contribution of the present study is to examine the impact of Prh lesions on the exploration of novel and familiar objects and flavors in the same group of animals. Our results regarding object recognition memory confirm previous reports showing that Prh lesions impair the performance in the SOR memory task (Brown et al., 2012; Dere et al., 2007; Warburton & Brown, 2015; Winters et al., 2008). When tested one minute after the sample phase (Test 1), object recognition memory was not impaired by Prh lesions. PERx group performance was similar to the SHAM group. They spent more time exploring the novel object, thus, recognizing the familiar object. This is consistent with previous studies using short delays between the sample and test phases which confirmed that lesioned animals do not show perceptual or short-term memory deficits (Norman & Eacott, 2005; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). However, several reports have found deficits in SOR memory tasks with retention intervals of 15 min or longer after Prh damage (Albasser, Davies, Futter, & Aggleton, 2009; Mumby, Glenn, Nesbitt, & Kyriazis, 2002; Mumby, Piterkin, Lecluse, & Lehmann, 2007; Winters et al., 2004). In our study, the memory impairment was found after a 24 hours delay (Test 2). The PERx group spent significantly more time exploring the familiar object than the SHAM group. The fact that the lesioned group explored the familiar object the same amount of time than the novel object indicates that the lesioned group behaved as if

the familiar object were novel. These results support an impairment of object recognition memory by Prh lesions which affected to the familiarity of the object but not to novelty detection.

Our results with respect to the effect of Prh lesions on taste recognition memory showed no effect on taste neophobia but a delayed habituation of the neophobic response. While the SHAM group exhibited a complete attenuation of taste neophobia in the second exposure, the lesioned group showed a similar level of neophobia during the first and the second encounter with the vinegar solution. The fact that the lesioned group showed neophobia indicates that Prh lesions did not affect to taste novelty detection. The lack of attenuation on the second trial revealed an impairment of taste recognition memory. This could be interpreted as if the transition from novel to familiar was not accomplished or that the categorization of the vinegar solution as a safe taste was prevented.

These results are in accordance with previous reports where the microinfusion into the Prh of either the muscarinic receptor antagonist scopolamine (Gutierrez et al., 2004) or the protein synthesis inhibitor anisomycin (De la Cruz et al., 2008) prevented the attenuation of gustatory neophobia on the second encounter with saccharin. Additionally, our results showed that on the third exposure to vinegar the lesioned group was able to attenuate the initial neophobia, reaching an asymptote on consumption similar to the SHAM group. The ability to exhibit attenuation of taste neophobia, although delayed, can be attributed to the fact that there are other regions involved on the formation and consolidation of taste memories like the insular cortex (Bermudez-Rattoni, 2014; Nunez-Jaramillo, Ramirez-Lugo, Herrera-Morales, & Miranda, 2009) or the basolateral amygdala

(Gomez-Chacon et al., 2012; Nunez-Jaramillo et al., 2009) that might have played an important role.

To our knowledge, there is only one previous study using permanent neurotoxic lesions of Prh in taste neophobia (Ramos, 2015). The author found that the lesioned group drank during the first session a significantly greater amount of the novel saccharin solution than the control group, thus reporting an impairment of the neophobic response by the lesion. This impairment was found when using 0.3% and 0.5% saccharin solutions, but not a 0.7% saccharin solution. However, no effect was found on the attenuation of taste neophobia assessed in 3 or 5 exposure sessions, respectively. It has to be taken into account that the disruption of taste neophobia using two exposures to 0.3% saccharin solutions prevents the assessment of neophobia attenuation. Taking into account the existing knowledge at the time the author proposed that the critical difference between his finding and the opposite findings reported with pharmacological manipulations was the type of inactivation, so that permanent lesions prevented the neophobic response while reversible interventions disrupted the attenuation of neophobia (Ramos, 2015). Our data do not support this explanation, because permanent Prh lesions affect to the habituation of neophobia but not to the neophobic response. Rather other various procedural differences can explain this discrepancy. First, with respect to the neurotoxic lesions although the NMDA dosage was identical in both studies, the number of injection sites and coordinates used differed. While we used 3 injection sites, Ramos (2015) added a fourth injection point at -2.5 mm from bregma. Therefore, it can be envisaged that the lesion performed by Ramos (2015) affected to more rostral areas than our lesion, thus it cannot be discarded that the neophobia

impairment reported by Ramos (2015) could be attributed to a potential damage induced by larger lesions. Second, regarding the taste solution applied, while Ramos (2015) used saccharin solutions lacking odor cues, we applied a 3% cider vinegar solution with a clear olfactory component. Given the fact that the piriform cortex is adjacent to the lesioned area and that it is even damaged in some of the brains in the present study as mentioned in the histological report, it could be conceived that the Prh lesion could have a greater impact on the habituation of neophobia using a taste with an odor component such as vinegar than a taste without odor, such as saccharin. Although we did not find bilateral lesions of the piriform cortex in most of the brains, it cannot be discarded the role of a potential functional deficit of the area. However, this explanation is not supported by the effects of Prh pharmacological reversible inactivation either by scopolamine (Gutierrez et al., 2004) or anisomycin (De la Cruz et al., 2008) on the attenuation of saccharin neophobia. These results are consistent with ours since they prevented the attenuation of neophobia on the second encounter with saccharin. Moreover, our results are also consistent with the delayed habituation of taste neophobia described in aged rats (Gomez-Chacon et al., 2015), which has been related with a decay of the Prh function at advanced age (Gamiz & Gallo). Therefore, more research is needed to clarify this issue.

In all, the impairment of both object and taste recognition memories suggests that the Prh might have an important role in the transition from novel to familiar. However, its integrity seems not to be critical for novelty detection and for the consolidation of a long term safe taste memory.

DISCUSIÓN GENERAL

El planteamiento de esta tesis está basado en una aproximación multidisciplinar al estudio de los efectos de la exposición al sabor, aplicando tanto un análisis comportamental como un análisis del Sistema Nervioso en el nivel celular y de circuito. Esta combinación de aproximaciones permite avanzar en la comprensión del modo en que el procesamiento de un sabor determina el comportamiento posterior en una tarea de aprendizaje y en el conocimiento del circuito neuronal subyacente que sufre cambios funcionales inducidos por la mera presentación.

En el **Capítulo 3** se exploró la disociación de los dos tipos de aprendizaje "sabor-nutriente" y "sabor-sabor" que sustentan las preferencias condicionadas al sabor, además de mediante la manipulación del estado motivacional, mediante el empleo como EIs de fructosa y maltodextrina. La exposición al sabor (EC) previa al condicionamiento produce un retraso en el aprendizaje de preferencias (IL), que es observado selectivamente en el aprendizaje "sabor-nutriente". Nuestra hipótesis de partida supone que la fructosa no da lugar a un aprendizaje "sabor-nutriente", por lo que probar a los animales hambrientos solo dará lugar al fenómeno de IL en el caso de la maltodextrina. Por otro lado, si probar a los animales sedientos, pero no hambrientos, favorece la expresión del aprendizaje "sabor-sabor", insensible a la IL, no se encontrará el fenómeno en ninguno de los dos grupos. En contra de la primera hipótesis, los resultados del Experimento 1, en el que los animales están hambrientos durante el test, indican que la IL es evidente tanto empleando maltodextrina como fructosa. Ambos reforzadores fueron capaces de generar una preferencia cuya adquisición fue retrasada por la exposición previa al EC. Por tanto, este resultado indica que la fructosa y la maltodextrina son funcionalmente

equivalentes como reforzadores bajo las condiciones utilizadas en este experimento, siendo capaces de generar un aprendizaje "sabor-nutriente".

Por otra parte, los resultados del Experimento 2 de este capítulo, en el que los animales no están hambrientos durante ninguna de las fases, parecen apoyar la segunda hipótesis. En efecto, la exposición previa al EC no induce inhibición latente independientemente del EI empleado, ya sea maltodextrina o fructosa. Ello es congruente con resultados previos empleando sacarosa como EI donde se obtuvo inhibición latente sólo en animales privados de comida pero no en animales saciados durante la prueba (Garcia-Burgos, Gonzalez, & Hall, 2013). Sin embargo, la exposición previa al sabor induce en este experimento un efecto de facilitación, que se traduce en un aumento de la preferencia condicionada al sabor debido a la experiencia previa con el EC. Este efecto permite descartar una posible interpretación alternativa de los resultados de investigaciones previas (Garcia-Burgos et al., 2013) en términos de una falta de sensibilidad del procedimiento de condicionamiento apetitivo aplicado en animales saciados. La explicación de este efecto de facilitación no está clara pero el análisis del consumo del sabor (almendra) y de agua durante la prueba indica que parece deberse a una posible respuesta neofóbica ante el sabor que es modulada por la preexposición al EC y por el tipo de reforzador utilizado durante el condicionamiento previo a la prueba. Así, si asumimos que la fructosa en las condiciones empleadas en este trabajo no produce una preferencia condicionada sustancial cuando las animales no están hambrientos sino solo sedientos, el consumo observado en animales no-preexpuestos durante la prueba (un mayor consumo de agua) es compatible con una reacción de neofobia al sabor, que puede ser atenuada mediante la preexposición al EC, resultando entonces

en un consumo por igual de la solución de almendra y de agua. En el caso de la maltodextrina, cuyo poder reforzante parece ser mayor, se genera una preferencia condicionada que hace superar la respuesta neofóbica en animales no-preexpuestos, y que induce una marcada preferencia en animales preexpuestos.

En resumen, los resultados del Capítulo 3 indican que el estado motivacional juega un papel crucial en el efecto de la exposición previa al sabor en una tarea de aprendizaje de preferencia por el sabor. Además, los reforzadores utilizados no permiten diferenciar entre los dos tipos de aprendizaje de preferencias en animales probados hambrientos, aunque parecen generar distintas respuestas en animales probados sedientos. Por último, los resultados señalan la posible relevancia de la neofobia desencadenada por el sabor empleado y su habituación durante la preexposición a la hora de explicar el efecto de facilitación encontrado en el segundo experimento con animales saciados.

Dado que la naturaleza hedónica del sabor puede determinar la magnitud de la neofobia durante la primera presentación y, por tanto, el efecto de la preexposición, en el **Capítulo 4** se comparó el efecto de la exposición a sabores con diverso valor hedónico sobre la adquisición de preferencias condicionadas inducida por sacarosa en animales hambrientos durante la prueba. Se utilizó una solución de almendra amarga como sabor no-preferido y soluciones de almendra dulce y de vainilla como sabores neutros.

El Experimento 1 de este capítulo confirmó la presencia del fenómeno de inhibición latente cuando se empleó un sabor neutro como EC en animales hambrientos durante todo el procedimiento, lo cual es consistente con resultados previos (De la Casa, Marquez, & Lubow, 2009; Garcia-Burgos et al., 2013), y apoya la

hipótesis de que el aprendizaje "sabor-nutriente" es sensible a la IL y es observado es un estado motivacional de hambre durante la prueba.

Sin embargo, los resultados del Experimento 2 mostraron que al emplear un sabor no-preferido como EC, no sólo no se produjo inhibición latente, sino que tuvo lugar el efecto contrario (facilitación) en animales hambrientos durante la prueba. Los animales preexpuestos exhibieron mayor preferencia por el sabor que los animales no-preexpuestos. Esta diferencia en preferencia fue además mantenida tanto durante pruebas a lo largo del curso de adquisición, como durante tests en extinción posteriores, lo que no es compatible con una explicación del efecto de facilitación en términos de una reacción neofóbica al sabor durante un test inicial.

En el Experimento 3 se replicaron los resultados de los dos experimentos anteriores, esta vez en animales bajo las mismas condiciones motivacionales (hambrientos durante todo el procedimiento). La preexposición a un sabor neutro indujo IL, mientras que la preexposición a un sabor no-preferido produjo una facilitación de la respuesta condicionada. Una explicación del efecto de facilitación podría basarse en cambios en la representación del sabor producidos por la experiencia previa. Un sabor novedoso se convierte en familiar y seguro a través de la exposición repetida sin consecuencias negativas (Bermudez-Rattoni, 2004). Datos previos han mostrado que la familiarización con un sabor sin consecuencias negativas incrementa su palatabilidad (Lin, Amodeo, Arthurs, & Reilly, 2012), pudiendo modificar su valor hedónico. Los cambios en el valor hedónico de un sabor pueden modificar su representación neural, induciendo una reorganización funcional en áreas corticales (Accolla & Carleton, 2008). Una explicación de este tipo requiere más investigación empleando una aproximación psicobiológica que

permita registrar la actividad neural inducida por el sabor e intervenir sobre los circuitos implicados en su procesamiento.

Por ello, en el **Capítulo 5** se estudian los cambios que induce la exposición a un sabor neofóbico sobre la actividad neural desencadenada en el sistema gustativo. Utilizando la determinación inmunohistoquímica de la proteína c-Fos como índice de actividad neural, se cuantifica la actividad relacionada con cambios en la familiaridad del sabor en el tálamo gustativo (VPMpc), una estación central de procesamiento que mantiene conexiones recíprocas con áreas corticales y subcorticales. El procedimiento empleado permite estudiar neofobia gustativa y su atenuación como consecuencia de la familiarización sin consecuencias negativas. La elección de una solución de vinagre de sidra se debe a que se trata de uno de los sabores básicos (ácido) empleado previamente en estudios sobre los mecanismos cerebrales implicados en la atenuación de la neofobia (Gomez-Chacon, Gamiz, & Gallo, 2012; Gomez-Chacon, Morillas, & Gallo, 2015) que tiene en común con el extracto de almendra ser una solución no preferida.

Los resultados confirmaron que la solución de vinagre empleada induce una respuesta neofóbica que consiste en la reducción de su ingestión con respecto a la línea base de agua previa. La exposición repetida de vinagre sin consecuencias negativas produjo un incremento significativo en la segunda y ulteriores exposiciones. Una exposición fue suficiente para que se produjera la habituación de la respuesta neofóbica inicial. Por su parte, el marcaje de la proteína c-Fos mostró un incremento selectivo en la actividad de VPMpc durante la exposición al sabor, tal y como se había descrito previamente en relación con otros sabores (Mungarndee, Lundy, & Norgren, 2008). Además, dicho incremento estuvo relacionado con el

grado de familiaridad del sabor, de manera que el número de células activadas fue superior cuando el sabor era más familiar tras 6 exposiciones (Lin, Roman, Arthurs, & Reilly, 2012). Es de destacar que la actividad de VPMpc no refleja los cambios en el consumo, sino que se producen posteriormente, ya que el incremento de la ingesta se produce en la segunda presentación y la cantidad ingerida no difiere de la consumida en la sexta presentación. Ello permite descartar que se trate de un incremento inespecífico de la actividad talámica asociado con movimientos orofaciales o con una mayor exposición al sabor. Por el contrario, parece tratarse de un cambio en la representación del sabor inducido por la ausencia de consecuencias negativas o bien por las consecuencias positivas de reducción de sed en animales privados de agua.

El único estudio previo que ha investigado los cambios en la actividad de VPMpc durante la exposición repetida a un sabor (Lin, Roman et al., 2012) empleó una solución de sacarina y obtuvo resultados discrepantes de los nuestros, mostrando un mayor número de células activadas durante la primera exposición en comparación con la sexta . Dado que registros neurofisiológicos han demostrado la posibilidad de que VPMpc codifique el valor hedónico del sabor (Liu & Fontanini, 2015; Verhagen, Giza, & Scott, 2003), la distinta naturaleza hedónica de la sacarina y el vinagre pueden haber influido en el resultado del efecto de la exposición. Ello sería congruente con la explicación propuesta en el capítulo 4 para los diferentes efectos de la exposición al sabor sobre el condicionamiento posterior en función del valor hedónico del estímulo. Otras explicaciones relacionadas con diferencias en el procedimiento de acceso al agua durante las sesiones experimentales aplicado por Lin, Roman et al. (2012) no pueden ser descartadas. El hecho de que se limitara el

consumo a 5 ml durante la sexta exposición exclusivamente para igualar la cantidad a la consumida durante la primera exposición pudo haber añadido novedad incrementando la saliencia del sabor, tal y como se ha descrito en estudios previos (Manrique et al., 2004).

En definitiva, los resultados del Capítulo 5 apoyan la participación del tálamo gustativo en el proceso de habituación de la neofobia, mostrando la mayor activación durante la exposición a un sabor inicialmente neofóbico que se ha convertido en seguro gracias a un proceso de familiarización bien consolidado. Otras áreas cerebrales muestran cambios asociados a la habituación de la neofobia gustativa. Entre ellas, la corteza perirrinal (PER) ha sido relacionada tanto con la memoria de reconocimiento gustativa como con la memoria de reconocimiento visual. Así, la lesión de PER interfiere con la memoria de reconocimiento de objetos (Dere, Huston, & De Souza Silva, 2007; Warburton & Brown, 2015; Winters, Saksida, & Bussey, 2008) y su inactivación reversible impide la habituación de la neofobia gustativa (De la Cruz et al. 2008; Gutierrez et al. 2004). Sin embargo, se ha informado de que la lesión neurotóxica permanente de esta estructura puede interferir de manera específica con la respuesta neofóbica producida por diversas concentraciones de sacarina sódica (Ramos, 2015), viéndose alterada en bajas concentraciones.

Así, en el **Capítulo 6** se exploró el efecto de la lesión neurotóxica de PER inducida por inyección intracerebral de NMDA, lesión que afecta selectivamente a los somas de las neuronas en la zona pero no a las fibras de paso, sobre la memoria de reconocimiento visual y la memoria gustativa segura en el mismo grupo de animales. A diferencia de las investigaciones previas que exploran el papel de PER

en memoria gustativa mediante la exposición a sacarina sódica, los experimentos incluidos en este capítulo arrojan nuevos resultados empleando una solución de vinagre de sidra como sabor con un valor hedónico claramente diferenciado que desencadena una evidente reacción neofóbica, tal y como se describe en el Capítulo 5. Los resultados confirmaron que la integridad de PER es necesaria para reconocer un objeto como familiar frente a uno novedoso, de acuerdo con los resultados obtenidos previamente en otros laboratorios (Dere et al., 2007; Warburton & Brown, 2015; Winters et al., 2008). El efecto solo fue evidente en la prueba de retención a largo plazo, lo que concuerda con informes previos (Mumby, Glenn, Nesbitt, & Kyriazis, 2002; Mumby, Piterkin, Lecluse, & Lehmann, 2007; Winters, Forwood, Cowell, Saksida, & Bussey, 2004).

Con respecto a la habituación de la neofobia, la lesión de PER indujo un retraso significativo en el proceso de familiarización, aunque no lo impidió, y tampoco afectó a la habituación de la neofobia gustativa. El máximo efecto se detectó en la segunda exposición en la que, a diferencia del grupo control falso-lesionado, el grupo con lesión no incrementó el consumo con respecto a la primera exposición. Ello es congruente con los resultados obtenidos en los estudios previos utilizando inactivación temporal de PER bien por el antagonista muscarínico escopolamina (Gutierrez, De la Cruz, Rodriguez-Ortiz, & Bermudez-Rattoni, 2004), bien por el inhibidor de la síntesis de proteínas anisomicina (De la Cruz, Rodriguez-Ortiz, Balderas, & Bermudez-Rattoni, 2008). En ambos casos la interrupción de la habituación de la neofobia se obtuvo en la segunda exposición. Del mismo modo, los resultados son acordes con datos previos que muestran un incremento de la actividad Fos en PER durante la segunda presentación de la solución de vinagre

(Gomez-Chacon et al., 2012). Con respecto al estudio de Ramos (2015) en el que la lesión permanente de PER no afecta a la habituación de la neofobia, pero sí a la respuesta neofóbica, se requiere más investigación sobre el papel que han podido jugar las diferencias en la extensión de la lesión y/o en la naturaleza del sabor empleado.

En conjunto, los resultados obtenidos en los Capítulos 5 y 6 plantean un panorama en el que PER parece jugar un rol importante en el proceso de familiarización durante la transición de lo novedoso a lo familiar, mientras que el tálamo gustativo parece estar implicado en una fase más avanzada cuando la memoria o categorización del sabor como familiar y seguro está bien consolidada. En cualquier caso, las dos áreas parecen de especial relevancia ante los cambios comportamentales producidos por la mera exposición a un sabor sin consecuencias negativas (habituación) relacionados con el paso del sabor de novedoso a familiar. La familiaridad de un sabor puede modular el aprendizaje, retrasando la adquisición de respuestas condicionadas (IL). Además, el uso de sabores con distinto valor hedónico puede generar resultados contrapuestos en procedimientos experimentales orientados a generar preferencias condicionadas como se muestra en el Capítulo 4. La preexposición a un sabor neutro genera un efecto de IL del condicionamiento de preferencias gustativas en animales hambrientos durante la prueba, mientras que la preexposición a un sabor no-preferido puede producir el efecto opuesto (facilitación). Este resultado se debe tener en cuenta en procedimientos orientados a profundizar en la disociación de los dos tipos de aprendizaje "sabor-nutriente" y "sabor-sabor" que sustentan las preferencias condicionadas al sabor. Un aspecto fundamental para la disociación de los dos tipos

de aprendizaje parece ser el estado motivacional durante la prueba como se muestra en el Capítulo 3. La exposición previa a un sabor puede generar efectos comportamentales diferenciados dependiendo del estado motivacional durante la prueba, animales privados de comida muestran IL tanto con el uso de fructosa como de maltodextrina como reforzadores, mientras que animales no privados muestran un efecto de facilitación que parece ser modulado por el tipo de reforzador utilizado.

A modo de conclusión, la exposición repetida a sabores puede producir cambios comportamentales que pueden estar relacionados con la actividad neuronal de áreas cerebrales implicadas en memoria de reconocimiento. Estos cambios comportamentales pueden depender de variables como el valor hedónico del sabor, y pueden generar diferentes fenómenos de aprendizaje, como inhibición latente o facilitación, fenómenos que pueden ser claves en el estudio de los mecanismos relacionados con el aprendizaje asociativo.

CONCLUSIONES

1. La manipulación del estado motivacional durante la prueba tiene un marcado efecto sobre la aparición del fenómeno de inhibición latente. Éste se observa en animales privados de comida durante la prueba, indicando una preferencia basada en aprendizaje "sabor-nutriente"; mientras el aprendizaje "sabor-sabor" parece operar cuando el animal no se encuentra privado, resultando en una ausencia de inhibición latente bajo estas condiciones.

2. El uso de fructosa y maltodextrina como reforzadores no permite una disociación clara entre aprendizaje "sabor-nutriente" y "sabor-sabor". Ambos son funcionalmente equivalentes como reforzadores en animales probados hambrientos, siendo capaces de generar un aprendizaje "sabor-nutriente", aunque parecen diferir en su efectividad en animales probados sedientos.

3. La preexposición a sabores con diverso valor hedónico sobre la adquisición de preferencias condicionadas inducida por sacarosa puede generar efectos comportamentales contrapuestos en animales hambrientos durante la prueba. La preexposición a un sabor neutro produce un retraso en el aprendizaje de preferencias (inhibición latente), mientras que la

preexposición a un sabor no-preferido induce una facilitación de la respuesta condicionada.

4. El tálamo gustativo y la corteza perirrinal son componentes de un circuito neuronal relevante ante los cambios comportamentales relacionados con la transición de un sabor de novedoso a familiar, producidos por la mera exposición a un sabor sin consecuencias negativas (habituación).

5. La actividad del tálamo gustativo está relacionada con la familiaridad de un sabor producida por el proceso de habituación de la neofobia, mostrando éste la mayor activación durante la exposición a un sabor que se ha convertido en seguro gracias a un proceso de familiarización bien consolidado.

6. La corteza perirrinal parece jugar un rol importante en el proceso de familiarización durante la transición de lo novedoso a lo familiar. Sin embargo, su integridad no parece necesaria para la detección de la novedad o para la consolidación de una memoria gustativa segura.

PUBLICACIONES RELACIONADAS

Morillas E, Gámiz F and Gallo M. "Neurotanatomía funcional de la memoria de reconocimiento visual y gustativa" En: F. Gámiz, M. Gallo & F. Vives (eds.) *Encuentros en Neurociencias Vol II*, 2013, 2, 151-180. Ediciones Ciber, S.C.: Granada (Spain). ISBN: 978-84-96876-87-7.

González F, Morillas E. and Hall, G. "Latent inhibition in flavor-preference conditioning: Effects of motivational state and the nature of the reinforcer" *in Learning & Behavior.* 2015 Dec;43(4):376-83.

González F, Morillas E and Hall G. "The extinction procedure modifies a conditioned flavor preference in nonhungry rats only after revaluation of the unconditioned stimulus" *in J Exp Psychol Anim Learn Cogn. 2016 Oct;42(4):380-390.*

Morillas E, González F and Hall G. "The effect of CS preexposure in flavor preference learning seems to depend on the hedonic value of the flavor: Latent inhibition or facilitation" (To be submitted).

Morillas E, Gómez-Chacón B and Gallo M. "Increased thalamic activity related to taste familiarity assessed by Fos immunohistochemistry" (To be submitted).

Morillas E, Gallo M. "Taste and object recognition memory impairment by excitotoxic lesions of the perirhinal cortex" (To be submitted).

BIBLIOGRAFÍA

Accolla R, Bathellier B, Petersen CC, et al.: Differential spatial representation of taste modalities in the rat gustatory cortex. J Neurosci 27:1396-1404, 2007.

Accolla R, Carleton A: Internal body state influences topographical plasticity of sensory representations in the rat gustatory cortex. Proc Natl Acad Sci U S A 105:4010-4015, 2008.

Ackroff K, Sclafani A: Flavor preferences conditioned by intragastric infusions of dilute polycose solutions. Physiol Behav 55:957-962, 1994.

Ackroff K, Touzani K, Peets TK, et al.: Flavor preferences conditioned by intragastric fructose and glucose: differences in reinforcement potency. Physiol Behav 72:691-703, 2001.

Ackroff K: Learned flavor preferences. The variable potency of post-oral nutrient reinforcers. Appetite 51:743-746, 2008.

Aggleton JP, Brown MW: Contrasting hippocampal and perirhinal cortex function using immediate early gene imaging. Q J Exp Psychol B 58:218-233, 2005.

Albasser MM, Davies M, Futter JE, et al.: Magnitude of the object recognition deficit associated with perirhinal cortex damage in rats: Effects of varying the lesion extent and the duration of the sample period. Behav Neurosci 123:115-124, 2009.

Albasser MM, Poirier GL, Aggleton JP: Qualitatively different modes of perirhinal-hippocampal engagement when rats explore novel vs. familiar objects as revealed by c-Fos imaging. Eur J Neurosci 31:134-147, 2010.

Albasser MM, Amin E, Iordanova MD, et al.: Separate but interacting recognition memory systems for different senses: The role of the rat perirhinal cortex. Learn Mem 18:435-443, 2011.

Arthurs J, Reilly S: Role of the gustatory thalamus in taste learning. Behav Brain Res 250:9-17, 2013.

Asok A, Schreiber WB, Jablonski SA, et al.: Egr-1 increases in the prefrontal cortex following training in the context preexposure facilitation effect (CPFE) paradigm. Neurobiol Learn Mem 106:145-153, 2013.

Barense MD, Henson RN, Graham KS: Perception and conception: temporal lobe activity during complex discriminations of familiar and novel faces and objects. J Cogn Neurosci 23:3052-3067, 2011.

Bennett CH, Tremain M, Mackintosh NJ: Facilitation and retardation of flavour aversion conditioning following prior exposure to the CS. Q J Exp Psychol B 49:220-230, 1996.

Bermudez-Rattoni F: Molecular mechanisms of taste-recognition memory. Nat Rev Neurosci 5:209-217, 2004.

Bermudez-Rattoni F: The forgotten insular cortex: its role on recognition memory formation. Neurobiol Learn Mem 109:207-216, 2014.

Birch LL: Development of food preferences. Annu Rev Nutr 19:41-62, 1999.

Brown MW, Aggleton JP: Recognition memory: what are the roles of the perirhinal cortex and hippocampus? Nat Rev Neurosci 2:51-61, 2001.

Brown MW, Warburton EC, Aggleton JP: Recognition memory: material, processes, and substrates. Hippocampus 20:1228-1244, 2010.

Brown MW, Barker GR, Aggleton JP, et al.: What pharmacological interventions indicate concerning the role of the perirhinal cortex in recognition memory. Neuropsychologia 50:3122-3140, 2012.

Burke SN, Wallace JL, Hartzell AL, et al.: Age-associated deficits in pattern separation functions of the perirhinal cortex: a cross-species consensus. Behav Neurosci 125:836-847, 2011.

Burwell RD, Witter MP, Amaral DG: Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. Hippocampus 5:390-408, 1995.

Burwell RD, Amaral DG: Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. J Comp Neurol 391:293-321, 1998.

Burwell RD, Amaral DG: Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. J Comp Neurol 398:179-205, 1998b.

Burwell RD: Borders and cytoarchitecture of the perirhinal and postrhinal cortices in the rat. J Comp Neurol 437:17-41, 2001.

Bussey TJ, Saksida LM, Murray EA: Impairments in visual discrimination after perirhinal cortex lesions: testing 'declarative' vs. 'perceptual-mnemonic' views of perirhinal cortex function. Eur J Neurosci 17:649-660, 2003.

Bussey TJ, Saksida LM, Murray EA: The perceptual-mnemonic/feature conjunction model of perirhinal cortex function. Q J Exp Psychol B 58:269-282, 2005.

Campbell DH, Capaldi ED, Sheffer JD, et al.: An examination of the relationship between expectancy learning and preference conditioning. Learning and Motivation 19:162-182, 1988.

Cechetto DF, Saper CB: Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. J Comp Neurol 262:27-45, 1987.

Cenquizca LA, Swanson LW: Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain Res Rev 56:1-26, 2007.

Clouard C, Jouhanneau M, Meunier-Salaun MC, et al.: Exposures to conditioned flavours with different hedonic values induce contrasted behavioural and brain responses in pigs. PLoS One 7:e37968, 2012.

Chotro MG, Alonso G: Some Parameters of Stimulus Preexposure that Affect Conditioning and Generalization of Taste Aversions in Infant Rats. International Journal of Comparative Psychology 14, 2001.

Davis JD: The microstructure of ingestive behavior. Ann N Y Acad Sci 575:106-119, 1989.

Davis JD, Smith GP: Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. Behav Neurosci 106:217-228, 1992.

De Houwer J, Thomas S, Baeyens F: Associative learning of likes and dislikes: a review of 25 years of research on human evaluative conditioning. Psychol Bull 127:853-869, 2001.

De la Casa LG, Marquez R, Lubow RE: Super-latent inhibition of conditioned taste preference with a long retention interval. Learning and Motivation 40:329-342, 2009.

De la Cruz V, Rodriguez-Ortiz CJ, Balderas I, et al.: Medial temporal lobe structures participate differentially in consolidation of safe and aversive taste memories. Eur J Neurosci 28:1377-1381, 2008.

Delamater AR: Partial reinforcement and latent inhibition effects on stimulus-outcome associations in flavor preference conditioning. Learn Behav 39:259-270, 2011.

Dere E, Huston JP, De Souza Silva MA: The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev 31:673-704, 2007.

Domjan M, Gillan D: Role of novelty in the aversion for increasingly concentrated saccharin solutions. Physiol Behav 16:537-542, 1976.

Drucker DB, Ackroff K, Sclafani A: Nutrient-conditioned flavor preference and acceptance in rats: effects of deprivation state and nonreinforcement. Physiol Behav 56:701-707, 1994.

Dunn LT, Everitt BJ: Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. Behav Neurosci 102:3-23, 1988.

Dwyer DM: Microstructural analysis of conditioned and unconditioned responses to maltodextrin. Learn Behav 36:149-158, 2008.

Dwyer DM, Quirk RH: Context conditional flavor preferences in the rat based on fructose and maltodextrin reinforcers. J Exp Psychol Anim Behav Process 34:294-302, 2008.

Elizalde G, Sclafani A: Starch-based conditioned flavor preferences in rats: influence of taste, calories and CS-US delay. Appetite 11:179-200, 1988.

Ennaceur A, Delacour J: A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res 31:47-59, 1988.

Fanselow MS, Birk J: Flavor-flavor associations induce hedonic shifts in taste preference. Animal Learning & Behavior 10:223-228, 1982.

Fedorchak PM, Bolles RC: Hunger enhances the expression of calorie- but not taste-mediated conditioned flavor preferences. J Exp Psychol Anim Behav Process 13:73-79, 1987.

Ferreira G, Ferry B, Meurisse M, et al.: Forebrain structures specifically activated by conditioned taste aversion. Behav Neurosci 120:952-962, 2006.

Figueroa-Guzman Y, Kuo JS, Reilly S: NMDA receptor antagonist MK-801 infused into the insular cortex prevents the attenuation of gustatory neophobia in rats. Brain Res 1114:183-186, 2006.

Figueroa-Guzman Y, Reilly S: NMDA receptors in the basolateral amygdala and gustatory neophobia. Brain Res 1210:200-203, 2008.

Furtak SC, Wei SM, Agster KL, et al.: Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. Hippocampus 17:709-722, 2007.

Galef BG, Durlach PJ: Absence of blocking, overshadowing, and latent inhibition in social enhancement of food preferences. Animal Learning & Behavior 21:214-220, 1993.

Gallo M, Roldan G, Bures J: Differential involvement of gustatory insular cortex and amygdala in the acquisition and retrieval of conditioned taste aversion in rats. Behav Brain Res 52:91-97, 1992.

Gallo M, Ballesteros MA, Molero A, et al.: Taste aversion learning as a tool for the study of hippocampal and non-hippocampal brain memory circuits regulating diet selection. Nutr. Neurosci. 2:277-302., 1999.

Gamiz F, Gallo M: Spontaneous object recognition memory in aged rats: Complexity versus similarity. Learn Mem 19:444-448.

Garcia-Burgos D, Gonzalez F, Hall G: Motivational control of latent inhibition in flavor preference conditioning. Behav Processes 98:9-17, 2013.

Garcia J, Hankins WG, Robinson JH, et al.: Bait shyness: tests of CS-US mediation. Physiol Behav 8:807-810, 1972.

Gauriau C, Bernard JF: Pain pathways and parabrachial circuits in the rat. Exp Physiol 87:251-258, 2002.

Gaztañaga M, Aranda-Fernandez PE, Diaz-Cenzano E, et al.: Latent inhibition and facilitation of conditioned taste aversion in preweanling rats. Dev Psychobiol 57:96-104, 2015.

Gomez-Chacon B, Gamiz F, Gallo M: Basolateral amygdala lesions attenuate safe taste memory-related c-fos expression in the rat perirhinal cortex. Behav Brain Res 230:418-422, 2012.

Gomez-Chacon B, Morillas E, Gallo M: Altered perirhinal cortex activity patterns during taste neophobia and their habituation in aged rats. Behav Brain Res 281:245-249, 2015.

Gomez-Chacon B, Gamiz F, Foster TC, et al.: Increased N-Ethylmaleimide-Sensitive Factor Expression in Amygdala and Perirhinal Cortex during Habituation of Taste Neophobia. Neural Plast 2016:2726745, 2016.

Gonzalez F, García-Burgos D, de Brugada I, et al.: Learned preference for a hedonically negative flavor is observed after pairings with positive post-ingestion consequences rather than with a palatable flavor. Learning and Motivation 41:141-149, 2010.

Gonzalez F, Garcia-Burgos D, Hall G: Analysis of blocking of flavor-preference conditioning based on nutrients and palatable tastes in rats. Appetite 80:161-167, 2014.

Gonzalez F, Morillas E, Hall G: Latent inhibition in flavor-preference conditioning: effects of motivational state and the nature of the reinforcer. Learn Behav 43:376-383, 2015.

Grigson PS, Lyuboslavsky P, Tanase D: Bilateral lesions of the gustatory thalamus disrupt morphine- but not LiCl-induced intake suppression in rats: evidence against the conditioned taste aversion hypothesis. Brain Res 858:327-337, 2000.

Grill HJ, Norgren R: The taste reactivity test. II. Mimetic responses to gustatory stimuli in chronic thalamic and chronic decerebrate rats. Brain Res 143:281-297, 1978.

Gutierrez R, Tellez LA, Bermudez-Rattoni F: Blockade of cortical muscarinic but not NMDA receptors prevents a novel taste from becoming familiar. Eur J Neurosci 17:1556-1562, 2003.

Gutierrez R, De la Cruz V, Rodriguez-Ortiz CJ, et al.: Perirhinal cortex muscarinic receptor blockade impairs taste recognition memory formation. Learn Mem 11:95-101, 2004.

Harris JA, Gorissen MC, Bailey GK, et al.: Motivational state regulates the content of learned flavor preferences. J Exp Psychol Anim Behav Process 26:15-30, 2000.

Harris JA, Shand FL, Carroll LQ, et al.: Persistence of preference for a flavor presented in simultaneous compound with sucrose. J Exp Psychol Anim Behav Process 30:177-189, 2004.

Hill WF: Effects of mere exposure on preferences in nonhuman mammals. Psychol Bull 85:1177-1198, 1978.

Hoffmann H, Spear NE: Facilitation and impairment of conditioning in the preweanling rat after prior exposure to the conditioned stimulus. Animal Learning & Behavior 17:63-69, 1989.

Holman EW: Immediate and delayed reinforcers for flavor preferences in rats. Learning and Motivation 6:91-100, 1975.

Hoover WB, Vertes RP: Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149-179, 2007.

Hsiao S, Fan RJ: Additivity of taste-specific effects of sucrose and quinine: microstructural analysis of ingestive behavior in rats. Behav Neurosci 107:317-326, 1993.

Iwasaki S, Sakaguchi T, Ikegaya Y: Brief fear preexposure facilitates subsequent fear conditioning. Neurosci Res 95:66-73, 2015.

Jordan WP, Todd TP, Bucci DJ, et al.: Habituation, latent inhibition, and extinction. Learn Behav 43:143-152, 2015.

Kajiwara R, Takashima I, Mimura Y, et al.: Amygdala input promotes spread of excitatory neural activity from perirhinal cortex to the entorhinal-hippocampal circuit. J Neurophysiol 89:2176-2184, 2003.

Kealy J, Commins S: The rat perirhinal cortex: A review of anatomy, physiology, plasticity, and function. Prog Neurobiol 93:522-548, 2011.

Kesner RP, Berman RF, Tardif R: Place and taste aversion learning: role of basal forebrain, parietal cortex, and amygdala. Brain Res Bull 29:345-353, 1992.

Kloosterman F, van Haeften T, Lopes da Silva FH: Two reentrant pathways in the hippocampal-entorhinal system. Hippocampus 14:1026-1039, 2004.

Koh MT, Wilkins EE, Bernstein IL: Novel tastes elevate c-fos expression in the central amygdala and insular cortex: implication for taste aversion learning. Behav Neurosci 117:1416-1422, 2003.

Koh MT, Bernstein IL: Mapping conditioned taste aversion associations using c-Fos reveals a dynamic role for insular cortex. Behav Neurosci 119:388-398, 2005.

Kolakowska L, Larue-Achagiotis C, Le Magnen J: [Comparative effects of lesion of the basolateral nucleus and lateral nucleus of the amygdaloid body on neophobia and conditioned taste aversion in the rat]. Physiol Behav 32:647-651, 1984.

Lasiter PS, Glanzman DL, Mensah PA: Direct connectivity between pontine taste areas and gustatory neocortex in rat. Brain Res 234:111-121, 1982.

Lasiter PS: Thalamocortical relations in taste aversion learning: II. Involvement of the medial ventrobasal thalamic complex in taste aversion learning. Behav Neurosci 99:477-495, 1985.

Lasiter PS, Deems DA, Glanzman DL: Thalamocortical relations in taste aversion learning: I. Involvement of gustatory thalamocortical projections in taste aversion learning. Behav Neurosci 99:454-476, 1985.

Lin JY, Roman C, St Andre J, et al.: Taste, olfactory and trigeminal neophobia in rats with forebrain lesions. Brain Res 1251:195-203, 2009.

Lin JY, Amodeo LR, Arthurs J, et al.: Taste neophobia and palatability: the pleasure of drinking. Physiol Behav 106:515-519, 2012.

Lin JY, Roman C, Arthurs J, et al.: Taste neophobia and c-Fos expression in the rat brain. Brain Res 1448:82-88, 2012.

Liu H, Fontanini A: State Dependency of Chemosensory Coding in the Gustatory Thalamus (VPMpc) of Alert Rats. J Neurosci 35:15479-15491, 2015.

Loullis CC, Wayner MJ, Jolicoeur FB: Thalamic taste nuclei lesions and taste aversion. Physiol Behav 20:653-655, 1978.

Lubow, R. E. (1989). Latent inhibition and conditioned attention theory. New York, NY: Cambridge University Press.

Lubow RE, Moore AU: Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. J Comp Physiol Psychol 52:415-419, 1959.

Lubow R E, Weiner I. (2010). Issues in latent inhibition research and theory: an overview. En R. E. Lubow y I. Weiner (Eds.), *Latent inhibition: Cognition, Neuroscience and Applications to Schizophrenia (*pp. 531-557). Cambridge: Cambridge University Press.

Lundy Jr RF, Norgren R, George P: Chapter 28 - Gustatory System, in The Rat Nervous System (Third Edition). Edited by. Burlington, Academic Press, 2004, pp. 891-921.

Majak K, Pitkanen A: Projections from the periamygdaloid cortex to the amygdaloid complex, the hippocampal formation, and the parahippocampal region: a PHA-L study in the rat. Hippocampus 13:922-942, 2003.

Manrique T, Molero As, Ballesteros MA, et al.: Time of day-dependent latent inhibition of conditioned taste aversions in rats. Neurobiology of Learning and Memory 82:77-80, 2004.

Manrique T, Moron I, Ballesteros MA, et al.: Hippocampus, aging, and segregating memories. Hippocampus 19:57-65, 2009.

McDonald AJ: Cortical pathways to the mammalian amygdala. Prog Neurobiol 55:257-332, 1998.

Mickley GA, Kenmuir CL, Yocom AM, et al.: A role for prefrontal cortex in the extinction of a conditioned taste aversion. Brain Res 1051:176-182, 2005.

Mishkin M, Delacour J: An analysis of short-term visual memory in the monkey. J Exp Psychol Anim Behav Process 1:326-334, 1975.

Molero A, Moron I, Angeles Ballesteros M, et al.: Hippocampus, temporal context and taste memories. Chem Senses 30 Suppl 1:i160-161, 2005.

Montag-Sallaz M, Welzl H, Kuhl D, et al.: Novelty-induced increased expression of immediate-early genes c-fos and arg 3.1 in the mouse brain. J Neurobiol 38:234-246, 1999.

Moron I, Gallo M: Effect of previous taste experiences on taste neophobia in young-adult and aged rats. Physiol Behav 90:308-317, 2007.

Mumby DG, Glenn MJ, Nesbitt C, et al.: Dissociation in retrograde memory for object discriminations and object recognition in rats with perirhinal cortex damage. Behav Brain Res 132:215-226, 2002.

Mumby DG, Piterkin P, Lecluse V, et al.: Perirhinal cortex damage and anterograde object-recognition in rats after long retention intervals. Behav Brain Res 185:82-87, 2007.

Mungarndee SS, Lundy RF, Jr., Norgren R: Central gustatory lesions and learned taste aversions: unconditioned stimuli. Physiol Behav 87:542-551, 2006.

Mungarndee SS, Lundy RF, Jr., Norgren R: Expression of Fos during sham sucrose intake in rats with central gustatory lesions. Am J Physiol Regul Integr Comp Physiol 295:R751-763, 2008.

Murray EA, Bussey TJ: Perceptual-mnemonic functions of the perirhinal cortex. Trends Cogn Sci 3:142-151, 1999.

Myers KP, Sclafani A: Conditioned enhancement of flavor evaluation reinforced by intragastric glucose. II. Taste reactivity analysis. Physiol Behav 74:495-505, 2001.

Myers KP, Sclafani A: Development of learned flavor preferences. Dev Psychobiol 48:380-388, 2006.

Nachman M, Ashe JH: Effects of basolateral amygdala lesions on neophobia, learned taste aversions, and sodium appetite in rats. J Comp Physiol Psychol 87:622-643, 1974.

Nakashima M, Uemura M, Yasui K, et al.: An anterograde and retrograde tract-tracing study on the projections from the thalamic gustatory area in the rat: distribution of neurons projecting to the insular cortex and amygdaloid complex. Neurosci Res 36:297-309, 2000.

Nissenbaum JW, Sclafani A: Qualitative differences in polysaccharide and sugar tastes in the rat: a two-carbohydrate taste model. Neurosci Biobehav Rev 11:187-196, 1987.

Nomura T, Ogawa H: The taste and mechanical response properties of neurons in the parvicellular part of the thalamic posteromedial ventral nucleus of the rat. Neurosci Res 3:91-105, 1985.

Norgren R: Taste pathways to hypothalamus and amygdala. J Comp Neurol 166:17-30, 1976.

Norman G, Eacott MJ: Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. Behav Neurosci 119:557-566, 2005.

Nunez-Jaramillo L, Ramirez-Lugo L, Herrera-Morales W, et al.: Taste memory formation: latest advances and challenges. Behav Brain Res 207:232-248, 2009.

Nuñez-Jaramillo L, Ramirez-Lugo L, Herrera-Morales W, et al.: Taste memory formation: latest advances and challenges. Behav Brain Res 207:232-248, 2010.

Ottersen OP, Ben-Ari Y: Afferent connections to the amygdaloid complex of the rat and cat. I. Projections from the thalamus. J Comp Neurol 187:401-424, 1979.

Paxinos G, Watson C: The Rat Brain in Stereotaxic Coordinates: Academic, 2009.

Pearce JM: Evaluation and development of a connectionist theory of configural learning. Anim Learn Behav 30:73-95, 2002.

Pérez C, Lucas Fo, Sclafani A: Carbohydrate, fat, and protein condition similar flavor preferences in rats using an oral-delay procedure. Physiology & Behavior 57:549-554, 1995.

Pikkarainen M, Pitkanen A: Projections from the lateral, basal and accessory basal nuclei of the amygdala to the perirhinal and postrhinal cortices in rat. Cereb Cortex 11:1064-1082, 2001.

Pisano MV, Ferreras S, Krapacher FA, et al.: Re-examining the ontogeny of the context preexposure facilitation effect in the rat through multiple dependent variables. Behav Brain Res 233:176-190, 2012.

Pitkanen A, Savander V, LeDoux JE: Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci 20:517-523, 1997.

Pitkanen A, Pikkarainen M, Nurminen N, et al.: Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Ann N Y Acad Sci 911:369-391, 2000.

Ramos JM: Differential contribution of perirhinal cortex and hippocampus to taste neophobia: effect of neurotoxic lesions. Behav Brain Res 284:94-102, 2015.

Reilly S, Harley C, Revusky S: Ibotenate lesions of the hippocampus enhance latent inhibition in conditioned taste aversion and increase resistance to extinction in conditioned taste preference. Behav Neurosci 107:996-1004, 1993.

Reilly S, Pritchard TC: Gustatory thalamus lesions in the rat: II. Aversive and appetitive taste conditioning. Behav Neurosci 110:746-759, 1996.

Reilly S, Pritchard TC: Gustatory thalamus lesions in the rat: III. Simultaneous contrast and autoshaping. Physiol Behav 62:1355-1363, 1997.

Reilly S: The role of the gustatory thalamus in taste-guided behavior. Neurosci Biobehav Rev 22:883-901, 1998.

Reilly S: The parabrachial nucleus and conditioned taste aversion. Brain Res Bull 48:239-254, 1999.

Reilly S, Trifunovic R: Gustatory thalamus lesions eliminate successive negative contrast in rats. Behav Neurosci 113:1242-1248, 1999.

Reilly S, Bornovalova M, Dengler C, et al.: Effects of excitotoxic lesions of the gustatory thalamus on latent inhibition and blocking of conditioned taste aversion in rats. Brain Res Bull 62:117-128, 2003.

Reilly S, Trifunovic R: Gustatory thalamus lesions eliminate successive negative contrast in rats: evidence against a memory deficit. Behav Neurosci 117:606-615, 2003.

Reilly S, Bornovalova M, Trifunovic R: Excitotoxic lesions of the gustatory thalamus spare simultaneous contrast effects but eliminate anticipatory negative contrast: evidence against a memory deficit. Behav Neurosci 118:365-376, 2004.

Reilly S, Bornovalova MA: Conditioned taste aversion and amygdala lesions in the rat: a critical review. Neurosci Biobehav Rev 29:1067-1088, 2005.

Robinson-Drummer PA, Stanton ME: Using the context preexposure facilitation effect to study long-term context memory in preweanling, juvenile, adolescent, and adult rats. Physiol Behav 148:22-28, 2016.

Roll JM: The effect of manipulating CS hedonic value on the magnitude of a conditioned taste aversion. Current Psychology 13:226-232, 1994.

Rozin P, Zellner D: The role of Pavlovian conditioning in the acquisition of food likes and dislikes. Ann N Y Acad Sci 443:189-202, 1985.

Sah P, Faber ES, Lopez De Armentia M, et al.: The amygdaloid complex: anatomy and physiology. Physiol Rev 83:803-834, 2003.

Sastre A, Reilly S: Excitotoxic lesions of the gustatory thalamus eliminate consummatory but not instrumental successive negative contrast in rats. Behav Brain Res 170:34-40, 2006.

Scalera G, Grigson PS, Norgren R: Gustatory functions, sodium appetite, and conditioned taste aversion survive excitotoxic lesions of the thalamic taste area. Behav Neurosci 111:633-645, 1997.

Sclafani A, Cardieri C, Tucker K, et al.: Intragastric glucose but not fructose conditions robust flavor preferences in rats. Am J Physiol 265:R320-325, 1993.

Sclafani A, Ackroff K: Glucose- and fructose-conditioned flavor preferences in rats: taste versus postingestive conditioning. Physiol Behav 56:399-405, 1994.

Sclafani A: Post-ingestive positive controls of ingestive behavior. Appetite 36:79-83, 2001.

Sclafani A, Ackroff K: Nutrient-conditioned flavor preference and incentive value measured by progressive ratio licking in rats. Physiol Behav 88:88-94, 2006.

Schroy PL, Wheeler RA, Davidson C, et al.: Role of gustatory thalamus in anticipation and comparison of rewards over time in rats. Am J Physiol Regul Integr Comp Physiol 288:R966-980, 2005.

Sewards TV, Sewards MA: Cortical association areas in the gustatory system. Neurosci Biobehav Rev 25:395-407, 2001.

Sewards TV: Dual separate pathways for sensory and hedonic aspects of taste. Brain Res Bull 62:271-283, 2004.

Spector AC, St John SJ: Role of taste in the microstructure of quinine ingestion by rats. Am J Physiol 274:R1687-1703, 1998.

Stehberg J, Moraga-Amaro R, Simon F: The role of the insular cortex in taste function. Neurobiol Learn Mem 96:130-135, 2011.

Stone ME, Grimes BS, Katz DB: Hippocampal inactivation enhances taste learning. Learn Mem 12:579-586, 2005.

Tokita K, Inoue T, Boughter JD, Jr.: Subnuclear organization of parabrachial efferents to the thalamus, amygdala and lateral hypothalamus in C57BL/6J mice: a quantitative retrograde double labeling study. Neuroscience 171:351-365, 2010.

Touzani K, Bodnar RJ, Sclafani A: Neuropharmacology of learned flavor preferences. Pharmacol Biochem Behav 97:55-62, 2010.

Turner BH, Herkenham M: Thalamoamygdaloid projections in the rat: a test of the amygdala's role in sensory processing. J Comp Neurol 313:295-325, 1991.

Van der Kooy D, Koda LY, McGinty JF, et al.: The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat. J Comp Neurol 224:1-24, 1984.

Verhagen JV, Giza BK, Scott TR: Responses to taste stimulation in the ventroposteromedial nucleus of the thalamus in rats. J Neurophysiol 89:265-275, 2003.

Vertes RP, Hoover WB, Do Valle AC, et al.: Efferent projections of reuniens and rhomboid nuclei of the thalamus in the rat. J Comp Neurol 499:768-796, 2006.

Warburton EC, Brown MW: Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. Neuropsychologia 48:2262-2272, 2010.

Warburton EC, Brown MW: Neural circuitry for rat recognition memory. Behav Brain Res 285:131-139, 2015.

Weingarten HP, Kulikovsky OT: Taste-to-postingestive consequence conditioning: is the rise in sham feeding with repeated experience a learning phenomenon? Physiol Behav 45:471-476, 1989.

Wilkins EE, Bernstein IL: Conditioning method determines patterns of c-fos expression following novel taste-illness pairing. Behav Brain Res 169:93-97, 2006.

Winters BD, Forwood SE, Cowell RA, et al.: Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. J Neurosci 24:5901-5908, 2004.

Winters BD, Saksida LM, Bussey TJ: Object recognition memory: neurobiological mechanisms of encoding, consolidation and retrieval. Neurosci Biobehav Rev 32:1055-1070, 2008.

Witter MP, Wouterlood FG, Naber PA, et al.: Anatomical organization of the parahippocampal-hippocampal network. Ann N Y Acad Sci 911:1-24, 2000.

Wright CI, Groenewegen HJ: Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. Neuroscience 73:359-373, 1996.

Yamamoto T, Shimura T, Sako N, et al.: Neural substrates for conditioned taste aversion in the rat. Behav Brain Res 65:123-137, 1994.

Yamamoto T, Fujimoto Y, Shimura T, et al.: Conditioned taste aversion in rats with excitotoxic brain lesions. Neurosci Res 22:31-49, 1995.

Yamamoto T: Neural substrates for the processing of cognitive and affective aspects of taste in the brain. Arch Histol Cytol 69:243-255, 2006.

Yamamoto T, Ueji K: Brain mechanisms of flavor learning. Front Syst Neurosci 5:76, 2011.

Yasui Y, Itoh K, Mizuno N: Projections from the parvocellular part of the posteromedial ventral nucleus of the thalamus to the lateral amygdaloid nucleus in the cat. Brain Res 292:151-155, 1984.

Yefet K, Merhav M, Kuulmann-Vander S, et al.: Different signal transduction cascades are activated simultaneously in the rat insular cortex and hippocampus following novel taste learning. Eur J Neurosci 24:1434-1442, 2006.

Yiin YM, Ackroff K, Sclafani A: Food deprivation enhances the expression but not acquisition of flavor acceptance conditioning in rats. Appetite 45:152-160, 2005a.

Yiin YM, Ackroff K, Sclafani A: Flavor preferences conditioned by intragastric nutrient infusions in food restricted and free-feeding rats. Physiol Behav 84:217-231, 2005b.

Zhu XO, Brown MW, McCabe BJ, et al.: Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. Neuroscience 69:821-829, 1995.

Zhu XO, McCabe BJ, Aggleton JP, et al.: Mapping visual recognition memory through expression of the immediate early gene c-fos. Neuroreport 7:1871-1875, 1996.

ÍNDICE DE ABREVIATURAS

A: "Almond"

BLA: "Basolateral Amygdala"

BNST: núcleo de la base de la estría terminal (Bed Nucleus of the Stria Terminalis)

CFP: Conditioned flavor preference.

CS: Conditioned stimulus.

EC: Estímulo condicionado (in English, CS).

EI: Estímulo incondicionado (in English, US).

Exp.: Experiment.

FI: "Familiar I"

FII: "Familiar II"

g: Gramos

H2O2: "Hydrogen peroxide"

H: "Thristy and hungry"

IL: Inhibición latente (in English, LI)

i.p.: Intra-peritoneal (inyección)

LI: Latent inhibition.

mg/Kg: Miligramos/kilogramos

MD: "maltodextrin"

ml: Mililitros

N: "Neutral"

NMDA: N-metil-D-aspartato

NPre: "Non-preexposed"

NSF: "N-ethylmaleimide-sensitive factor"

NTS: Núcleo del tracto solitario

PBN: Núcleo parabraquial

PBS: "Phosphate-buffered saline"

PCS: Preferencia condicionada al sabor (in English, CFP).

PER: Corteza perirrinal (In English, Prh)

PERx: "Perirhinal cortex lesioned"

Pre: "Preexposed"

Prh: "Perirhinal Cortex"

SA: "Sweet almond"

SEM: "Standard error of the mean"

SOR: "Spontaneous Object Recognition"

T: "Thirsty"

U: "Unpreferred"

US: Unconditioned stimulus.

V: "Vanilla"

VPM: Núcleo ventroposteromedial del tálamo

VPMpc: Área parvocelular del núcleo ventroposteromedial del tálamo

VPL: Núcleo ventroposterolateral del tálamo

Vol/vol: volumen/volumen

Wt/vol: Peso (weight)/volumen

µl: Microlitros

µm: Micras

+: "Reinforcement"

-: "Nonreinforcement"

ÍNDICE DE FIGURAS Y TABLAS

CAPÍTULO 1.

CAPÍTULO 3.

CAPÍTULO 4.