11281

Universidad Nacional Autónoma de México

Programa de Doctorado en Ciencias biomédicas

Instituto de Fisiología Celular

Formación de la memoria gustativa: papel de la Corteza Insular

Tesis que para obtener el grado de
Doctor en Ciencias

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2004

Ciudad universitaria 2003

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Resumen

La decisión de ingerir o no un alimento familiar está basada en las consecuencias gástricas de su consumo previo y esta conducta ya sea de rechazo o ingesta depende de procesos de aprendizaje y memoria bastante robustos y duraderos. La corteza insular así como el sistema colinérgico y glutamatérgico participan en la adquisición del condicionamiento aversivo a los sabores (CAS), tarea en la cual un animal desarrolla aversión a un sabor debido a que su consumo fue seguido por malestar gástrico. Aún se desconoce la participación de esta corteza en la atenuación de la neofobia (AN). Para esto decidimos utilizar microinyecciones de antagonistas de receptores muscarínicos y receptores tipo NMDA dirigidas directamente a corteza insular para evaluar la participación del sistema colinérgico y glutamatérgico en la conducta neofóbica y su consecuente disminución. Neofobia se refiere a la cautela que los organismos muestran al consumir un sabor o alimento novedoso, esto implica que conforme se familiarizan con el sabor, los animales consumen más del sabor (AN). Ninguno de estos antagonistas afectó la percepción del sabor ya que los animales mostraron neofobia a sacarina 0.5%. Sin embargo, el bloqueo de receptores muscarínicos previno de manera consistente y robusta la atenuación de la neofobia lo que sugiere que los animales no recordaron haber bebido este sabor un día antes (a pesar de haberlo percibido) o que no recordaron que

consecuencias gástricas conllevó su consumo y por tal lo trataron nuevamente con cautela como si fuera nuevo otra vez. Los resultados no permitieron distinguir entre estas dos alternativas. El bloqueo de receptores glutamatérgicos tipo NMDA no tuvo efecto ni en la neofobia ni en su atenuación lo que sugiere que este es un aprendizaje independiente de receptores tipo NMDA al menos en corteza insular. Los resultados de esta tesis señalan a la corteza insular como una región de suma importancia para ambas conductas (aversión aprendida o atenuación de la neofobia). Pero ¿la corteza insular es la única región cortical involucrada en aprendizaje gustativo? recientemente se encontró que la corteza perirrinal esta involucrada en la formación del trazo de memoria del sabor pero no en la asociación con malestar. Por esto decidimos investigar si la actividad colinérgica y glutamatérgica desempeñan papeles diferenciales en el CAS y en la AN. Los resultados indican que la aplicación de antagonistas de receptores NMDA y AMPA no interfieren con la aversión aprendida al sabor, lo cual, contrasta con los efectos observados en corteza insular. Los efectos de escopolamina (antagonista de receptores muscarínicos), fueron muy similares a los observados en corteza insular, es decir, cuando se inyectó antes de la presentación del sabor, degradó la adquisición de ambas conductas CAS y AN mientras que la aplicación después del sabor interfirió únicamente con la AN, lo que reafirma que la corteza perirrinal está involucrada en procesos de aprendizaje gustativo, principalmente en la formación de la memoria del sabor, y en la consolidación de la AN pero de manera importante sugiere que a nivel cortical, la escopolamina (antagonista de receptores muscarínicos) podría interferir en la formación del TMS en distintas regiones corticales pero no en su asociación con malestar. Los resultados indican que cuando el TMS es formado éste es procesado por al menos dos mecanismos el de preferencia y el de aversión probablemente de manera independiente en la misma región cortical (corteza insular) y de forma paralela en distintas regiones corticales (corteza insular y perirrinal).

Abstract

The decision to ingest or not a familiar edible is based upon its previous post-ingestional consequences and depends on very robust and long lasting learning and memory processes. The insular cortex as well as the cholinergic and glutamatergic system is involved in the acquisition of conditioned taste aversion. (CTA), in which, an animal develops aversion to a taste since its intake was followed by gastric malaise. It is still unknown the participation of this cortex on attenuation of neophobia (AN). Therefore, we decide to use microinjections of muscarinic and NMDA receptor antagonists aimed at insular cortex, in order to evaluate the participation of cholinergic and glutamatergic system on neophobia and its subsequent attenuation. Neophobia means the caution that organisms display when eating novel edibles, once that the taste becomes familiar, the animal increase its intake (AN). Neither of these antagonists disrupted taste perception since all animals displayed neophobia to 0.5% saccharin solution. However, blockade of muscarinic receptors prevents AN. These results suggest that animals did not remember the taste or they did not remember the gastric consequences of its ingestion and hence they displayed neophobia again. The results did not allow us differentiate between these two alternatives. Blockade of NMDA receptors did disrupt neither neophobia nor AN suggesting that this learning is independent of NMDA receptors at least in insular cortex. These results indicate that insular cortex is involved in both behaviors (learned aversion and AN). But, is the insular cortex the only cortical region involved in gustatory

learning?. Recently, it was found that perirrinal cortex is involved in the formation of the taste memory trace (TMT) but not in the association with malaise. We decide to investigate if cholinergic and glutamatergic cortical activity plays a differential participation on CTA and AN. Our results indicate that infusions of NMDA and AMPA antagonists did not disrupt acquisition of CTA which contrast with the effects observed in insular cortex. The amnesic effects induced by scopolamine (muscarinic receptors antagonist) were very similar to those obtained in the insular cortex. That is, when it was microinjected before taste presentation, impaired acquisition of both CTA and AN whereas its infusion after novel taste presentation only prevented AN. These results confirm the participation of the perirrinal cortex on gustatory learning and memory, mainly in the TMT formation and in AN consolidation. Importantly, these results suggest that infusion of scopolamine in cortex could interfere in the TMT formation in different cortical regions but not in its association with malaise. In summary once that the TMT is formed this is processed for at least two mechanisms (preference and aversion) probably in and independent manner in the same cortical region (insular cortex) and in parallel in different cortical structures (insular and perirrinal cortex).

Antecedentes

Quizá una de las actividades más importantes para cualquier organismo es la obtención e ingesta de alimentos. Debido a las cualidades intrínsecas de éstos, algunos nutritivos otros letales, el sistema gustativo entre otras cosas debe de aprender y recordar que alimentos son tóxicos y cuales nutritivos. La elección de alimentos es desencadenada en primera instancia por el sistema olfativo, visual y en ocasiones auditivo, pero el gusto es quien debe decidir si se continua ingiriendo un alimento, con base en que el gusto provee información acerca de la sustancia ingerida mucho más rápido de lo que lo hace el sistema gastrointestinal (por el tiempo que tarda el proceso de digestión). El sistema

gustativo es en forma de metáfora la última barrera para filtrar los alimentos nutritivos de los tóxicos. El sentido del gusto es responsable de detectar y distinguir entre sabores dulces, amargos, ácidos y salados (aunque también detecta el umami, un aminoácido). Este poder discriminatorio provee al animal de información sensorial vital, la recepción a lo dulce y a aminoácidos permiten el reconocimiento de fuentes alimenticias nutritivas mientras que la percepción de lo amargo desencadena respuestas aversivas a estímulos nocivos y tóxicos (Zhang et al., 2003). No es de extrañarse que las ratas beban con menos avidez las sustancias amargas más toxicas con respecto a las menos tóxicas (Scott y Mark, 1987). Por otra parte, el sistema gustativo al igual que los otros sistemas sensoriales pueden ser modulados por el sistema gastrointestinal, por ejemplo, si un alimento intoxicó a un animal éste lo rechazará en subsecuentes encuentros no solamente con base en la información gustativa sino también visual y/o olfativa. Esto se demostró por una serie de trabajos realizados por Carl Gustavson (1977) en donde alimentó a lobos con un cordero repleto de cápsulas con LiCl (irritante gástrico), después de lo cual, los lobos no solo no comieron más corderos sino que también suprimieron la frecuencia de ataque, desplegando conducta de juego hacia ellos. La relevancia de las pistas visuales también se ha reportado en primates no humanos (Johnson et al., 1975), los cuales adquieren y mantienen aversión a pistas visuales apareadas con el alimento aún después de tres años (Johnson et al., 1979) aunque en aves la aversión basada en pistas visuales no dura más de 14 días (Hayne et al., 1996). Respecto al cambio en el sistema olfativo lo demuestran los trabajos de aversión al olor potenciada por el sabor, es bien conocido que el apareamiento de un olor con malestar en ausencia de un sabor no induce aversión al olor; sin embargo cuando el olor es administrado en conjunto con un sabor, los animales desarrollan aversión a ambos olor y sabor (Garcia et al., 1985). Estos resultados dejan abierta la posibilidad de que en el cerebro, cortezas secundarias o terciarias visuales u olfativas además de corteza gustativa podrían también participar en este aprendizaje "gustativo".

El sistema gustativo como modelo de aprendizaje y memoria

La sobrevivencia de cualquier organismo está basada entre otras cosas en la capacidad de aprender y recordar que un alimento fue seguido por malestar y con base a las características del mismo predecir el potencial dañino de otros alimentos con atributos similares, para así poderlos rechazar sin tener que averiguar directamente sus consecuencias post-ingestionales. Esto quedó ilustrado por Frank, et al (1985) cuando dió a beber una solución con sacarosa a un grupo de ratas y tiempo después les indujo malestar. Como resultado las ratas no solamente dejaron de consumir sacarosa sino también rechazaron otras sustancias con sabor dulce entre ellas sacarina o fructuosa, sin dejar de consumir sustancias con sabor salado. En este sentido, Nachman et al., (1963) descubrieron que la aversión condicionada a cloruro de litio ingerido (que en humanos se detecta como sabor salado) se generaliza a cloruro de sodio y que la presentación repetida de cloruro de sodio, extingue la aversión a cloruro de litio. Estos resultados sugieren que las ratas pueden utilizar la cualidad de un sabor para predecir el potencial de envenenamiento de otros sabores (Smith y Theodore, 1984). Por otra parte, también se ha demostrado que las ratas pueden utilizar la temperatura (Nachman et al., 1970) o la textura de un alimento como atributos para predecir la ocurrencia de malestar (Sako et al., 2002).

Esta habilidad de los roedores ha sido ampliamente estudiada en el laboratorio desde que John García describió por primera vez que las ratas desarrollan aversión a soluciones con sabor dulce cuando estas son seguidas por la aplicación de rayos gamma (Garcia et al., 1955). A esta conducta de rechazo basada en aprendizaje se le denominó Condicionamiento Aversivo a los Sabores (CAS). El CAS describe un cambio en el valor hedónico de un sabor

cambiándolo de preferido a aversivo, aunque en realidad aún no se sabe con exactitud si el estímulo gustativo después del CAS tiene un sabor desagradable o el animal no lo consume porque tiene "conocimiento" de que produce malestar, aunque siga teniendo un sabor "agradable". El término condicionamiento refleja las similitudes de este aprendizaje con el condicionamiento clásico, en donde el sabor sirve como estímulo condicionado y el malestar como estímulo incondicionado, de modo que el estímulo gustativo, en un principio neutro, adquiere la capacidad de inducir una respuesta condicionada (predecir la ocurrencia de malestar). Resulta interesante remarcar que aunque el CAS sigue muchos de los principios del condicionamiento clásico, la conducta de rechazo a un sabor inducida el día de la evocación es completamente operante (la conducta de la rata tiene el control del reforzador), quizá en un futuro se reconozca al CAS como un modelo para estudiar las interacciones entre estas dos formas básicas de aprendizaje.

Principios generales del CAS:

1. Si un animal consume un alimento con sabor y subsecuentemente sufre malestar gástrico, en los siguientes encuentros ese animal evitará o reducirá drásticamente su consumo. Este principio es bastante homogéneo entre especies ya sean invertebrados (moluscos y celenterados) o vertebrados (primates, osos, caninos, felinos, aves, roedores, reptiles, y peces)(Garcia et al., 1985;Gustavson, 1977).
2. La fuerza de la aversión aprendida está directamente relacionada con la intensidad del sabor y del malestar, y es inversamente relacionada al intervalo temporal entre la presentación del sabor y la inducción de malestar. Esta escala temporal se mide en horas mientras que en la mayoría de modelos de aprendizaje, se mide en segundos o minutos (Domjan, 1985).
3. Los estímulos gustativos se asocian más fácilmente a estímulos gástricos (Garcia y Koelling, 1966) (ver figura 1).

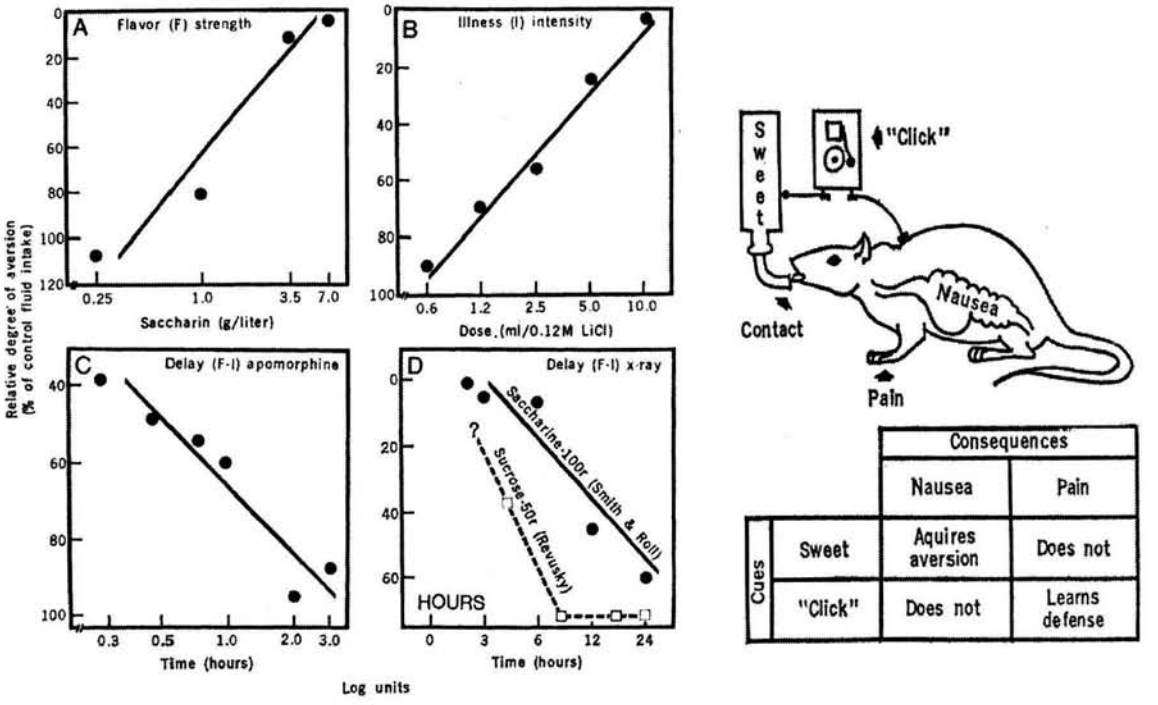

Figura 1. Esquemas no modificados de (Garcia et al., 1974). En los cuatro paneles izquierdos se resume en "A" la influencia que ejerce la intensidad del sabor (concentración) y en "B" la intensidad del malestar en la adquisición del CAS. Se puede observar que a mayor concentración mayor aversión aprendida (porcentajes de ingesta bajos reflejan mayor aversión). Mientras que en "C y D "se observa la influencia de la demora entre la presentación del sabor y la inducción de malestar, se extrae de estos resultados que el intervalo máximo de asociación es variable pero siempre en escala de horas y éste es resultado de la interacción entre el tipo de sabor y clase de malestar así como de sus respectivas intensidades (en este caso apomorfina en "C" y radiación en "D") ver el segundo artículo de esta tesis figura 1 para un ejemplo con sacarina y LiCl (Gutierrez et al., 2003a). Por otra parte en el lado derecho se ilustra el experimento de (Garcia y Koelling, 1966) que en su momento fue motivo de controversia porque sus resultados argumentan en contra de la idea en ese entonces en boga de que todos los estímulos tienen la misma capacidad asociativa. Como se puede observar García observó que una solución con sabor se asociaba más fácilmente a la inducción de náusea que a la aplicación de choques eléctricos en las patas mientras que para un estímulo auditivo la relación era inversa.

Los animales pueden aprender CAS si la solución con sabor es ingerida espontáneamente, pero también lo aprenden cuando el estímulo gustativo es inyectado por vía intraperitoneal (Bures y Buresova, 1989) o intravenosa (Bradley y Mistretta, 1971). Como ya se mencionó, en este aprendizaje el valor hedónico de un comestible cambia de positivo a negativo debido a la inducción de malestar presumiblemente gástrico aunque pueden existir varias formas para inducir aversión al sabor por ejemplo rotación (Arwas et al., 1989) o incluso en humanos se puede inducir CAS con palabras (Garb y Stunkard, 1974). Aunque es lógico pensar que exista una relación entre el grado de toxicidad del agente inductor de malestar y el grado de aversión aprendida, resulta increíble que la estricnina (veneno altamente tóxico) no sirva como agente inductor de CAS (Nachman y Hartley, 1975). Hay que aclarar que la estricnina no actúa a través del sistema gastrointestinal por lo cual aun está en pie la idea de que la característica más importante para la inducción de CAS es la presencia de malestar gástrico y no el grado de toxicidad *per se* (Gamzu, 1977).

Para una revisión más detallada sobre el condicionamiento aversivo a los sabores se recomiendan las siguientes revisiones (Garcia et al., 1974;Welzl et al., 2001;Bures et al., 1998)

Fases del Condicionamiento Aversivo a los Sabores

La aversión al sabor se desarrolla aún cuando el malestar es inducido horas después de la presentación del estímulo gustativo (Garcia et al., 1966), lo que permite a los animales formar una relación causal entre las cualidades de los alimentos aun cuando sus consecuencias gástricas ocurran tiempo después. Esta característica hace del CAS un modelo único para el estudio de la neurobiología del aprendizaje y memoria, ya que permite diferenciar los efectos de algún fármaco en el procesamiento del estímulo gustativo o el estímulo visceral así como en su asociación o consolidación.

Como se puede ver en la figura (2) las etapas del CAS son (1) procesamiento a nivel periférico y central del estímulo gustativo ver más adelante la anatomía del sistema gustativo. (2) Formación de la representación central del sabor que de ahora en adelante denominaré trazo de memoria del sabor (TMS), las evidencias de la existencia del TMS son básicamente dos: como ya se mencionó el CAS soporta intervalos de tiempo de horas, en muchos de los casos tiempo suficiente para que el estímulo gustativo haya sido digerido por completo(Domjan, 1985) en especial cuando se usa sacarina como estímulo gustativo(Smith y Roll, 1967). En este sentido, la segunda evidencia proviene de un trabajo realizado por Schafe et al., (1995) en donde demuestra que las ratas no aprenden CAS cuando el sabor y el malestar tienen una separación de 10 segundos pero sí cuando están separados por 10 min., lo que indica que contrario a otros paradigmas de condicionamiento clásico la contigüidad entre el estimulo gustativo físicamente real y el malestar no permite el desarrollo de aversión al sabor. Con base en esto se puede especular que la formación del TMS debe de llevarse a cabo en ese lapso de tiempo y el TMS es condición necesaria para la formación del CAS (Bures J, 1998). (3) Presencia física del malestar gástrico las características del CAS permiten afirmar que es improbable la existencia de una representación central del malestar que perdure más allá de la duración de sus síntomas, ya que si se induce malestar antes de la presentación del sabor los animales no desarrollan aversión. (4) Asociación del TMS con malestar: hasta el momento se desconocen las regiones del cerebro responsables de este proceso, pero la asociación puede ocurrir en ausencia de actividad cortical(Buresova y Bures, 1973) lo que sugiere que estructuras subcorticales pueden también estar involucradas. Swank, (2000) encontró activación de fosfo CREB y fosfo ERK en la amígdala lateral únicamente cuando ambos sabor y malestar estuvieron presentes pero no por separado lo que sugiere que esta estructura podría estar involucrada en el proceso de asociación. (5) Consolidación de la memoria aversiva. (6) Retención en donde la memoria consolidada es almacenada de manera pasiva o tal vez activa por grandes periodos de tiempo.

Como se muestra en la figura 2 inmediatamente después de que la asociación TMS-malestar ha sido establecida, la aversión adquirida al sabor puede ser evocada(Spector et al., 1988;Smith y Roll, 1967), se puede evocar memoria a corto plazo cuando se reexpone el sabor pocas horas después de la inducción del malestar entre dos o cuatro horas o si la prueba es dada días después se puede medir memoria a largo plazo (Ferreira et al., 2002). Se recomiendan los siguientes artículos (Bures J, 1998;Tassoni et al., 2000) ver también parte última de la discusión del segundo artículo de esta tesis (Gutierrez et al., 2003a).

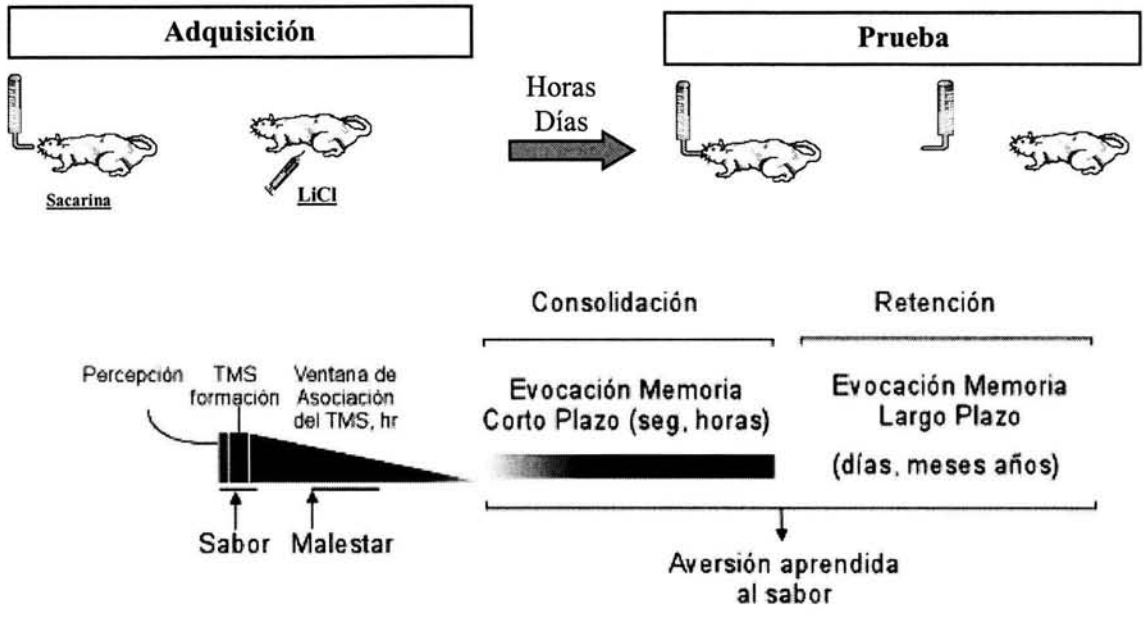

Figura 2. Protocolo para inducir aversión condicionada al sabor. En la parte superior se dibuja el consumo de un sabor nuevo que es seguido de la inyección de cloruro de litio (agente inductor de malestar). En el siguiente encuentro con el sabor la rata evoca la aversión adquirida anteriormente. En la parte inferior se esquematizan las posibles fases del condicionamiento aversivo a los sabores. Ver texto por explicación.

La novedad como punto de partida

El mundo de lo comestible para cualquier organismo se puede dividir en dos, los alimentos nuevos y los familiares. Los primeros inducen conductas de precaución e.g. neofobia, que indican el completo desconocimiento de las consecuencias gástricas que podría conllevar su consumo, mientras que los alimentos familiares inducen conductas mas certeras que reflejan el recuerdo de sus consecuencias previas, así puede haber sabores familiares seguros o aversivos (Rozin, 1977). La novedad del sabor es la variable con mayor influencia sobre la habilidad para aprender CAS en un solo ensayo ya sea en el laboratorio o en la vida salvaje (Kalat, 1974;Bower, 1997).

En un ejemplo más sobre la influencia de la novedad en el CAS, Revusky y Bedarf (1967) la presentación simultánea de dos sabores (uno nuevo y otro familiar), ambos seguidos por malestar, encontraron que los animales siempre desarrollaban mayor aversión al estímulo gustativo nuevo aun cuando el sabor familiar haya estado mas cerca en tiempo a la inducción de malestar. A esta dificultad para desarrollar aversión a un sabor familiar se le ha denominado Inhibición Latente, que es importante porque implica que un sabor familiar necesita más ensayos para desarrollar montos similares de aversión a los inducidos por un sabor nuevo en un apareamiento. En ese mismo estudio los autores notaron que el consumo de los sabores nuevos esta acompañado por neofobia (rechazo a lo nuevo), así de acuerdo con estos autores esta neofobia podría explicar porque los sabores nuevos desarrollan aversión más fácilmente(Revusky y Bedarf, 1967). La neofobia es causada entre otras cosas por la novedad del estímulo gustativo y la función que clásicamente se le atribuye es la defensa en contra de la administración oral de grandes cantidades de sustancias altamente toxicas, por medio de la reducción en el consumo de sabores nuevos y por lo tanto potencialmente letales, hasta que sus consecuencias post-ingestionales hayan sido determinadas(Domjan, 1976;Stewart y Reidinger, 1984;Siegel, 1974). En este sentido la neofobia y el condicionamiento aversivo a los sabores tienen una función similar, evitar el

contacto con sustancias tóxicas, la primera limitando el consumo de sustancias nuevas y el segundo evitando el consumo repetido de sustancias letales. Sin embargo, se han reportado algunos resultados contradictorios. Por ejemplo, Hayne et al., (1996) encontró que pollos pueden beber grandes cantidades en ocasiones letales de LiCl, por lo que concluyó que la neofobia por si sola es insuficiente para prevenir la ingesta de grandes cantidades de toxinas, ver también (Miller y Holzman, 1981a).

Otra posible función de la neofobia ha sido estudiada por Galef (1977) quien está interesado en la transmisión social de las preferencias alimenticias, cuando una población de ratas se les induce aversión a un tipo de alimento ellas cambian de tipo de alimentación cuando existe otra alternativa, al nacer las nuevas crías y después de un tiempo de convivencia con las ratas adultas, las crías prefieren el alimento que comen los adultos aun cuando los dos tipos de alimentos ya no estén contaminados. Cuando las crías son separadas de la influencia de los padres ellas continúan comiendo el alimento opuesto al que las ratas adultas aprendieron aversión hasta por 7 días, posteriormente se observa un incremento gradual en el consumo de la otra alternativa alimenticia (atenuación de la neofobia). Estos datos apoyan que la función de la neofobia y del CAS puede ser la transmisión de las preferencias alimenticias de una generación a otra evitando consumir un gran número de alimentos y reforzando el consumo de alimentos que no han matado a las generaciones pasadas.

¿Cual es el papel que desempeña la neofobia en los seres humanos? Rozin (1977) propone que la cultura culinaria ha usurpado el papel de la neofobia. En alguna parte del mundo existen seres humanos que tienen en su dieta alimentos que son el martirio de otras culturas. Me atrevo a decir que los seres humanos podemos comer casi cualquier cosa, sin embargo, también es un hecho que ningún ser humano come alimentos nuevos todos los días de su vida. La gran mayoría de los alimentos que come un hombre está determinado culturalmente, por ejemplo, las personas en Brasil sienten repulsión con la "idea" de comer aguacate con sal mientras que disfrutan comerlo con azúcar y leche cuya idea por sí misma no es muy agradable para muchos mexicanos. La

cultura, entre otras cosas, se encarga de transmitir a sus miembros cuales alimentos deben consumir, cómo se deben de preparar y cómo se deben de condimentar. Gran parte de los alimentos que una persona no ingiere, no ha sido con base en las cualidades aversivas de esos alimentos, en su mayoría ni siquiera los ha probado, debido a que los consideran como un alimento fuera de los hábitos alimenticios dictados por su cultura(Koivisto y Sjoden, 1996). Por otra parte, un exceso en el nivel de neofobia podría resultar contraproducente y en casos extremos puede llevar a malos hábitos alimenticios (Falciglia et al., 2000) y mal nutrición en niños e interacción social limitada en adultos, ya que el comer en humanos es un acto social(Marcontell et al., 2002).

Neofobia y su atenuación como modelo para estudiar la memoria del sabor en ausencia de malestar

La neofobia en animales a sustancias comestibles fue observada por primera vez por exterminadores que intentaron eliminar plagas de ratas distribuyendo carnadas envenenadas. Robert Smith, el exterminador de la princesa Amelia de Inglaterra, en 1768 notó que era necesario presentar cierta cantidad de un alimento nuevo antes de que lo aceptaran (Domjan, 1977). Estudios de laboratorio posteriores han confirmado que las ratas tienden a comer menos de alimentos nuevos que de familiares (Barnett, 1958;Mitchell, 1976;Nachman y Jones, 1974). La neofobia a comida ha sido observada en muchas especies entre ellas se encuentran el puerco de guinea (Miller y Holzman, 1981a), conejo (Dogterom y van, 1988), hámster (Cornwell y Kovanic, 1981), gerbo (Wong y McBride, 1993), tortugas (Burghardt y Hess, 1966), peces (Mackay, 1974), aves (Franchina y Slank, 1989), perro (Maslow, 1932), cordero (Burritt y Provenza, 1989) primates no humanos (Johnson et al., 1975;Johnson, 2000) y humanos (Pliner et al., 1993;Pliner et al., 1998;Rozin, 1977)

La rata presenta neofobia a varios alimentos y soluciones con sabor pero su neofobia es bastante homogénea y robusta a concentraciones altas de sacarina, la cual, comienza a atenuarse desde el primer consumo de sacarina consiguiendo su máximo nivel en al menos dos presentaciones adicionales. En

la figura 3 se puede observar que todos los animales muestran neofobia e incrementan su consumo en la segunda presentación, en su mayoría duplicando el valor de su primer consumo.

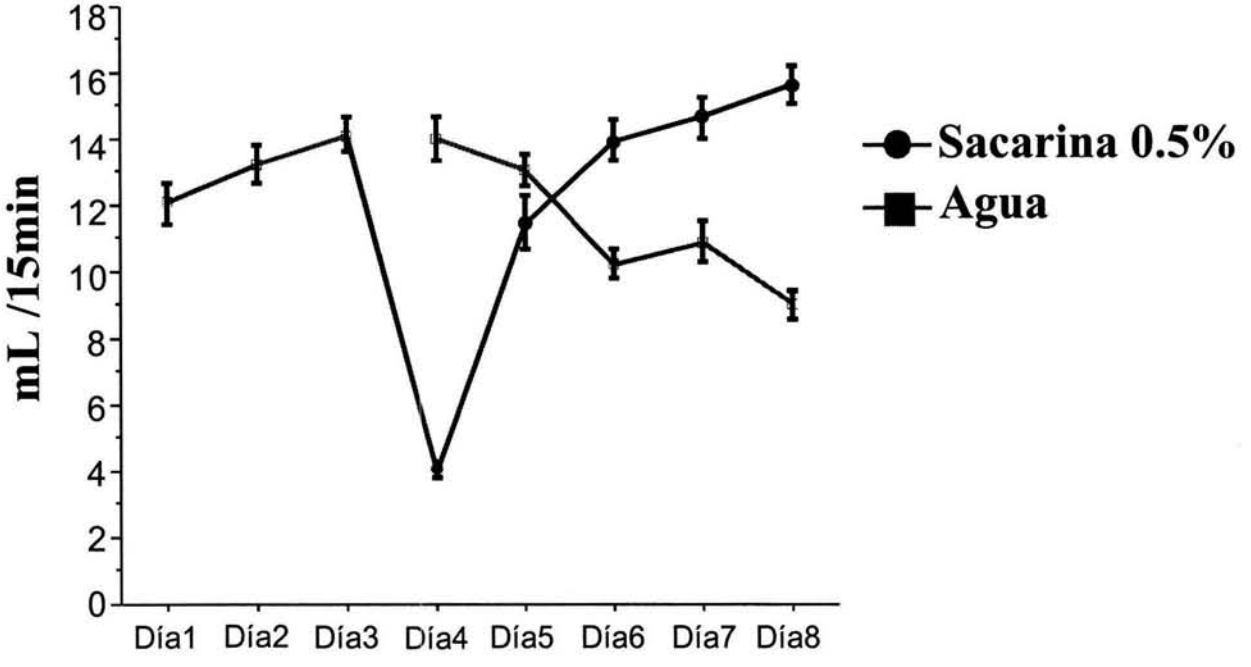

Figura 3. Muestra el consumo de agua (día 1-3) en la línea base y los primeros cinco consumos de sacarina (día 4-8) de 84 animales intactos, se puede observar lo robusto de la respuesta neofóbica a esta solución (día 4). Los animales reducen drásticamente su consumo de sacarina con respecto a su consumo de agua en la línea base, debido a este bajo consumo, fue necesario implementar un consumo adicional de agua para mantener un nivel adecuado de hidratación, este consumo también sirvió como un indicador de la motivación para beber. No obstante a la respuesta neofóbica tan marcada, en las subsecuentes presentaciones ésta se va atenuando.

El consumo de menos de 5 ml de sacarina 0.5% es suficiente para inducir AN la cual debe de ser almacenada en una memoria a largo plazo ya que la AN puede ser evocada 24 ó 72 hr después del primer consumo (ver figura 2 Gutiérrez 2003b; Buresova y Bures, 1980). Un trabajo realizado por Best et al., (1978) encontró que 48 días después del primer consumo de sacarina el nivel de

AN comenzaba a disminuir aunque los animales no volvían a mostrar la conducta neofóbica tan robusta como la observada en el primer consumo, estos resultados sugieren que consumir un sabor por primera vez modifica drásticamente y a largo plazo varios parámetros conductuales implicando cambios plásticos a nivel neuronal.

En esta tesis utilizamos esta conducta neofóbica como un marcador de novedad. Debido a que la neofobia se atenúa en las subsecuentes presentaciones se puede utilizar esta conducta como un indicador de memoria de reconocimiento al sabor (ver el tercer artículo de esta tesis).

Características de la neofobia a sacarina y de su atenuación

La concentración del sabor es la variable con mayor influencia sobre la neofobia a sacarina y por ende en su atenuación. Como se puede observar en la figura 4, el volumen del primer consumo de sacarina (neofobia) está inversamente relacionado con la concentración de la solución con sacarina. Asimismo, en todos los casos se observó un incremento en el consumo en la segunda presentación del sabor (AN), sin embargo, este incremento es menor en concentraciones altas.

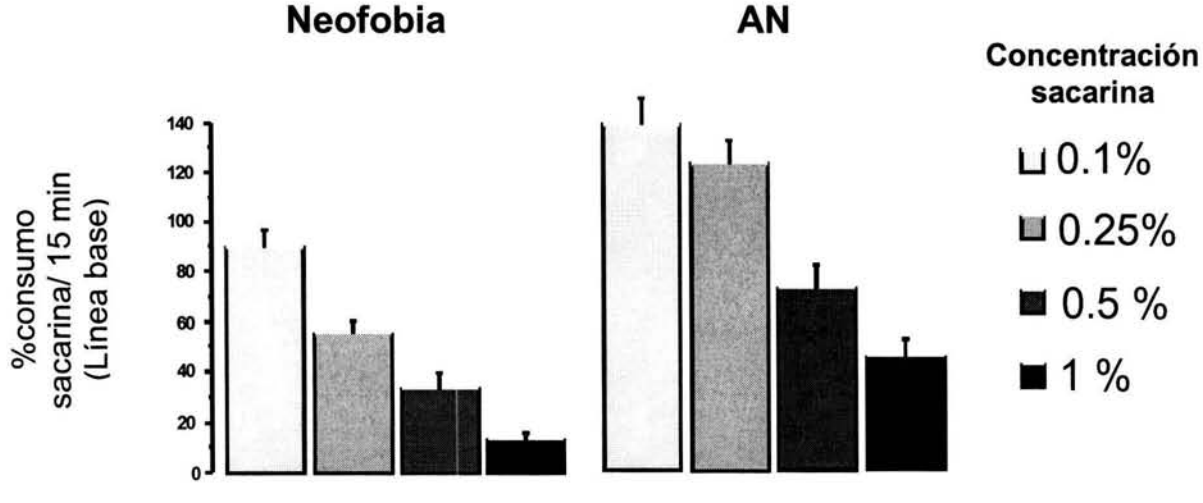

Figura 4. Muestra los dos primeros consumos (24 hrs separados) de una solución con sacarina. Cada uno de los cuatro grupos fue estimulado por 15 minutos con la misma concentración de sacarina, n=5.

En una demostración de que la variable más importante para la neofobia es la novedad, Gilley y Franchina (1985) encontraron que la presentación por varios días de una concentración baja de sacarina 0.25% induce atenuación de la neofobia a una concentración alta de sacarina 1.5%. En un estudio piloto comprobamos que este efecto puede ocurrir incluso con una sola presentación de una concentración baja de sacarina, aunque el efecto no fue tan robusto como el que observó Gilley (1985) con varias presentaciones, ver figura 6. Estos resultados sugieren que la novedad del sabor y no la concentración *per se* es la variable más importante para inducir atenuación de la neofobia.

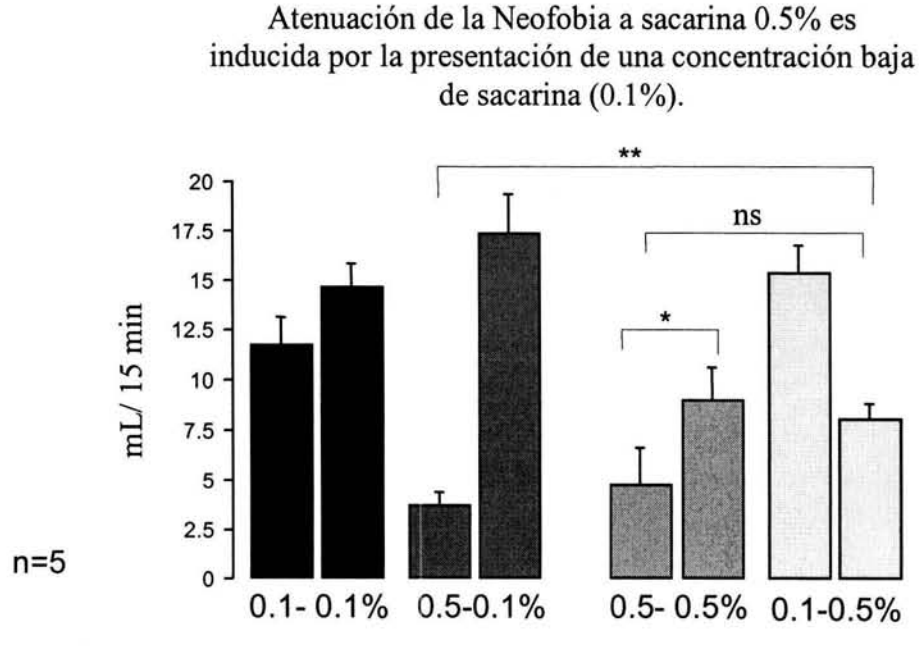

Figura 6. Efecto de la concentración del sabor en la inducción de atenuación de la neofobia. A cuatro grupos de animales (0.1-0.1%, 0.1-0.5%, 0.5-0.5% y 0.5-0.1%) se les dió de beber en dos ocasiones la misma concentración de sacarina o una concentración diferente en cada día, así el grupo 0.5-0.5% bebió en ambas ocasiones

una solución de sacarina 0.5% mientras que el grupo 0.1-0.5%, primero bebió sacarina 0.1% y al siguiente día sacarina 0.5%. Se puede observar en el grupo (0.1-0.5%) que la ingesta de una concentración baja de sacarina (0.1%) desencadena AN a una concentración alta de sacarina (0.5%), **$p<0.05$.

Ya que todo aprendizaje gustativo depende en primera instancia en la percepción del sabor, antes de continuar con la neurobiología del aprendizaje gustativo conviene describir la anatomía del sistema gustativo.

Anatomía del sistema gustativo

Todos los vertebrados detectan el sabor de los alimentos a través de la activación de células receptoras del gusto (taste receptor cells en inglés) que responden a moléculas o iones disueltos en la saliva. Por tanto, pertenecen a la categoría de quimiorreceptores. La agrupación de estas células aceptadoras del gusto junto con células basales y de soporte forman las yemas gustativas (taste buds). Las yemas gustativas son los órganos sensoriales periféricos del gusto, como se puede ver en la figura 7, en mamíferos tienen forma ovalada y en su parte apical presentan un poro (taste pore) por donde las células aceptadoras del gusto proyectan sus microvellosidades. Cada yema gustativa contiene entre 50 a 150 células receptoras del gusto.

Dependiendo de la especie cada yema gustativa se encuentra conformada por diversos tipos celulares. Aquí solo se describirá la anatomía de las yemas gustativas en mamíferos para una revisión en todas las clases de vertebrados (ver Reutter y Witt, 1993). Las yemas gustativas de la rata están conformadas por tres tipos de células, células tipo I, tipo II y células básales, las cuales se pueden observar en la figura 7.

Las células tipo I presentan puntos electro-densos por lo que también se les conoce como células negras (dark cells). Ellas son alargadas y se localizan en las orillas de las yemas gustativas, son las células más comunes llegando a

ser el 55 al 75% de las células en la yema gustativa. En su parte apical se extienden de 30 a 40 microvellosidades (de 0.1 a 0.2 µm de ancho) a través del poro gustativo. Se piensa que su función no está directamente relacionada con la detección del sabor porque no presentan sinapsis con las neuronas sensoriales, sin embargo, no se ha podido descartar una posible participación en procesos de quimiorecepción. Por otra parte, se cree que su función es más de soporte y nutrición.

Las Células tipo II, son menos electro-densas por lo que se les conoce como células claras (light cells). Aunque menos numerosas que las tipo I (15-30%), estas células también son alargadas y en su porción apical proyectan microvellosidades a través del poro. Se les localiza principalmente en el centro de la yema gustativa. Debido a que estas células presentan contactos sinápticos con nervios sensoriales se cree que su función principal es de quimiorecepción. Importante, este tipo de célula solo hace contacto con un solo tipo de neurona sensorial, sin embargo una misma neurona sensorial puede presentar contactos sinápticos con varias células tipo II provenientes de distintas yemas gustativas. Por lo cual una misma fibra nerviosa puede transmitir la información de las cinco clases de sabores, sin embargo una fibra nerviosa por lo general responde a una cualidad más que a otra (Mierson, 1995), por lo que se dice que a este nivel el procesamiento de la información gustativa es ampliamente sintonizado (broadly tuned). Este tipo de procesamiento también ocurre a nivel de las yemas gustativas, cuando se estimula con diferentes sabores una misma yema gustativa puede responder a distintos sabores (Mierson, 1995).

Existe un tercer tipo celular que conforma las yemas gustativas en conejos, ratón, monos y humano, pero no en rata, se les conoce como células tipo III ellas comparten algunas características de las células tipo I (presentan puntos electro-densos), pero hacen contactos sinápticos como hacen las tipo II por lo cual también son llamadas células intermedias. De hecho, en las especies en donde están presentes con excepción del ratón son las únicas que presentan contactos sinápticos con los nervios periféricos.

Las células tipo IV o células básales como su nombre lo indica se encuentran en la lámina basal de la yema gustativa en contacto directo con el epitelio de la lengua, generalmente se observan de 1 a 2 de estas células por cada yema, ellas no proyectan microvellosidades al poro gustativo y no presentan contactos sinápticos, por lo que no participan en la detección del sabor.

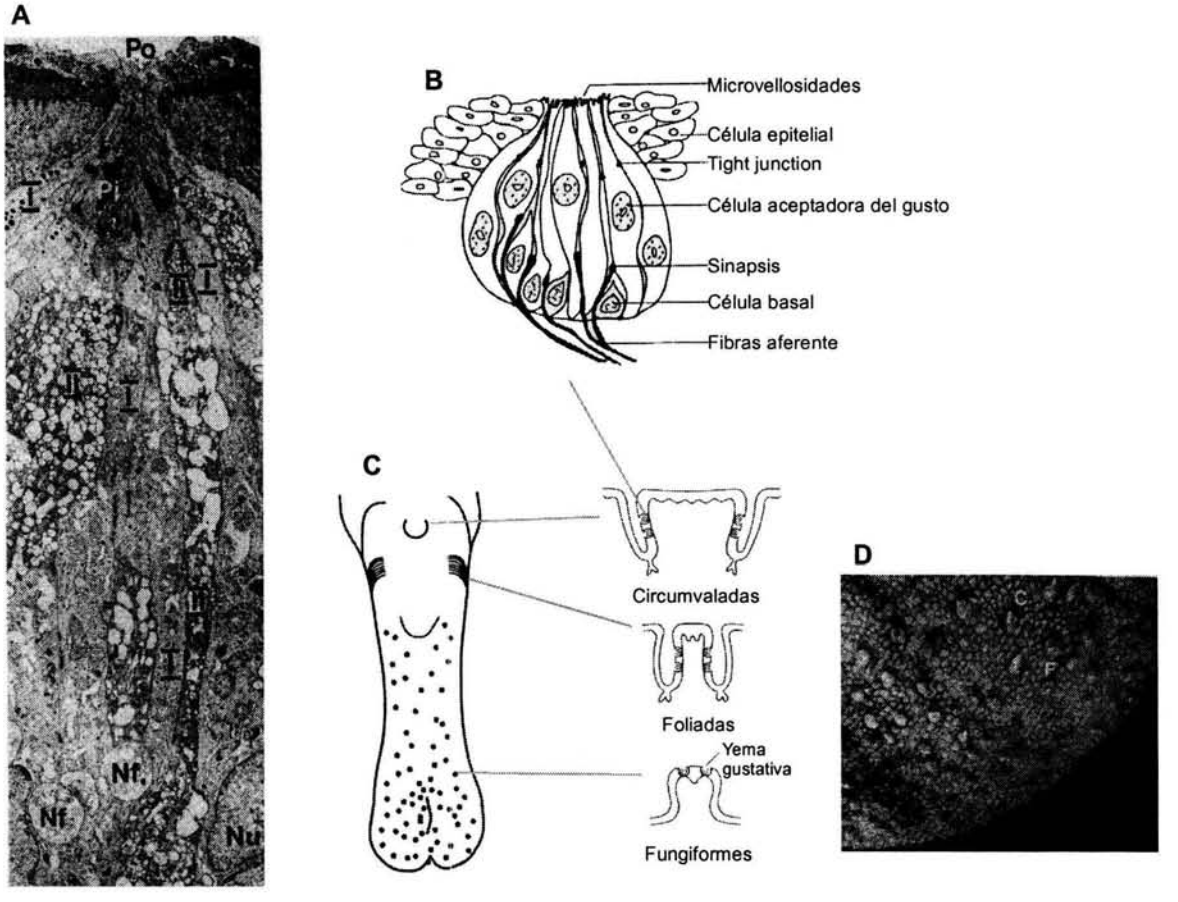

Figura 7. En "A" se muestran las células tipo I y II que conforman una yema gustativa, extraída de una papila circunvalada de mamífero, se observa también el poro gustativo (Po, taste pore) y el pozo gustativo (Pit, taste pit) así como dos contactos sinápticos de las fibras aferentes (Nf, nerve fibers). Sacado de (Reutter y Witt, 1993). En "B" se muestran los componentes de una yema gustativa y en "C" la distribución de los tres tipos de papilas gustativas en la lengua de rata. En "D" se muestra una fotografía de la parte anterior de lengua humana en donde se pueden observar la variedad de formas de las papilas fungiformes entre C se observan dos papilas cónicas y entre F se encuentran dos papilas con forma fungiforme, sacado de (Miller, 1995).

Las yemas gustativas están presentes en todos los grupos de vertebrados, sin embargo su localización en el cuerpo varía dependiendo del habitat por ejemplo en peces se localizan además de en la boca en varias partes del cuerpo(Reutter y Witt, 1993). En todos los vertebrados terrestres su presencia está limitada a la cavidad oral localizándose en pequeñas protuberancias del epitelio de la lengua denominadas papilas. Se pueden distinguir por su forma y localización tres clases de papilas gustativas circunvaladas, foliadas y fungiformes que se encuentran distribuidas en lugares específicos de la lengua, paladar y faringe. Importante, las yemas gustativas también se encuentran en la epiglotis y en la parte superior del esófago, las yemas gustativas que no están en la lengua no se encuentran en papilas.

Como su nombre lo indica las papilas fungiformes tienen forma de hongo, aunque paradójicamente éstas presentan una gran variedad de formas, cónicas y filiformes pero por convención se les denomina como fungiformes ver figura 7D (Miller, 1995). Cada papila puede contener de una a cinco yemas gustativas, pero solo la mitad de las papilas fungiformes contienen yemas gustativas, existen varios cientos de papilas fungiformes en los dos tercios anteriores de la lengua. Las papilas foliadas presentan dobleces en forma de hoja localizadas en el borde posterior de la lengua. Las papilas circunvaladas (rodeado por una pared) son grandes estructuras redondeadas por surcos, estas papilas se localizan en la tercera porción posterior de la lengua(Miller, 1995).

Inervación periférica de las yemas gustativas: los pares craneales numero VII (facial), IX (glosofaríngeo) y X (vago) inervan las yemas gustativas de la cavidad oral. Como se puede ver en la figura 8 una ramificación del nervio facial, la *chorda tympani* (CT) inerva a las yemas gustativas localizadas en la papilas fungiformes, por lo que esta ramificación cubre la parte anterior de la lengua(Miller, 1995). Otra ramificación del nervio facial, greater superficial petrosal (GSP) inerva las yemas gustativas localizadas en el paladar y en el ducto nasoincisor que se encuentra éste último únicamente en mamíferos

inferiores como la rata, Un dato interesante es que las neuronas del núcleo del solitario responden más intensamente cuando se estimula el ducto del nasoincisor que cuando se estimula la parte anterior de la lengua con sustancias dulces(Travers y Norgren, 1991). Los axones de la *chorda tympani y del greater superficial petrosal* provienen del ganglio geniculado en el oído medio, por lo que se debe de tener cuidado de no romper el tímpano al momento de hacer una cirugía en ratas, es recomendable usar barras atraumáticas.

El nervio glosofaríngeo inerva a las papilas foliadas y circunvaladas de la parte posterior de la lengua, los axones de este nervio provienen del ganglio inferior petrosal. Por último una ramificación del nervio vago, el nervio superior laríngeo (SLN) inerva las yemas gustativas de la epiglotis, laringe y esófago cuyos somas se localizan en el ganglio nodoso. Por otra parte, el nervio del trigémino inerva la periferia de las yemas gustativas, este nervio transmite información principalmente somatosensorial textura y temperatura de los alimentos(Reutter y Witt, 1993;Reutter y Witt, 1993).

Procesamiento de la información gustativa y visceral en el cerebro: Aunque las células aceptadoras del gusto tienen origen epitelial, ellas pueden generar potenciales de acción y liberar neurotransmisores de manera similar a una neurona(Mierson, 1995). De esta forma, cuando una molécula activa los receptores de las células aceptadoras del gusto se genera un potencial de acción que en última instancia termina en la liberación de neurotransmisores y en la activación de las neuronas sensoriales que inervan las yemas gustativas. Las fibras nerviosas transmiten la información a la parte rostral del núcleo del tracto del solitario (NTS){Torvik, 1955 1061 /id;Halsell, 1996 1062 /id}, el NTS presenta conexiones recíprocas con el núcleo del hipogloso (XII) en donde proyecta el nervio hipogloso que inerva la lengua, asimismo también envía y recibe información de la formación retícular (FR) en donde se encuentra el generador de patrones motores encargado de generar los movimientos rítmicos de la lengua necesarios para beber (lengüetear), (Travers et al., 1997). De aquí en adelante las diferencias del sistema gustativo entre la rata respecto a

primates y humanos se intensifica. En la rata las fibras de segundo orden proyectan ipsilateralmente y hacen sinapsis obligatorias con el núcleo parabraqueal (PBN) {Norgren, 1971 1063 /id}, las neuronas del PBN que responden vigorosamente a sabores proyectan al núcleo del tálamo ventroposteriomedial en su división parvo celular (VPMpc){Norgren, 1973 1064 /id}, desde donde los axones de cuarto orden proyectan a la corteza gustativa localizada en la parte anterior de la corteza insular (CI). Además, el PBN también presenta una vía ventral que inerva el hipotálamo lateral (HL), sustancia inominata, núcleo central de la amígdala (Ami) y el núcleo de la base estría terminales(BST){Norgren, 1976 1065 /id}}.

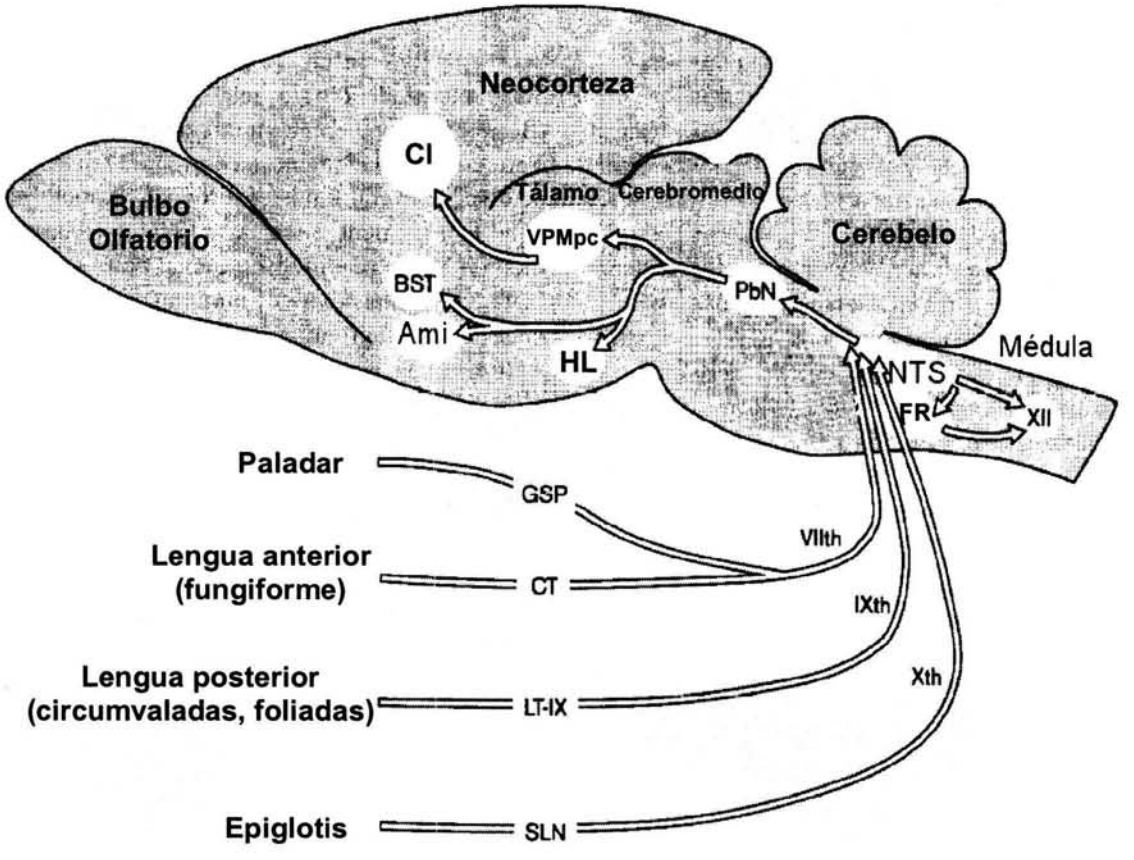

Figura 8. Se muestran las vías de procesamiento del estímulo gustativo en el cerebro de rata, ver texto para explicación, petrosal superficial superior (GSP), corda timpani (CT), superior laringeo (SLN), núcleo del tracto del solitario (NTS), núcleo parabraquial (PBN), formación reticular (FR), tálamo ventroposteromedial parte parvocelular (VPMpc),

amígdala (Ami), hipotálamo lateral (HL), núcleo de la cama estría terminalis (BST), corteza insular (CI). Tomado de (Scott y Giza, 1996).

En primates y humanos las fibras de segundo orden provenientes del NTS proyectan rostralmente hacia el PBN sin embargo no hacen sinapsis hasta el tálamo (VPMpc) que a su vez transmite la información a la ínsula anterior y al operculum frontal (AI/FO) considerado como corteza gustativa primaria {Pritchard, 1986 1066 /id;Kinomura, 1994 1067 /id}. AI/FO envía proyecciones al núcleo central de la amígdala de donde la información gustativa es enviada a la parte ventral del cerebro anterior y al hipotálamo. AI/FO también envía proyecciones a la corteza gustativa secundaria localizada en la parte caudolateral de la corteza orbitofrontal {Rolls, 1981 1068 /id;Critchley, 1996 1079 /id}, interesantemente, la frecuencia de disparo de neuronas de corteza orbitofrontal que responden ávidamente a un alimento disminuye conforme los macacos son alimentados con el mismo alimento hasta saciarse, sin embargo ante un alimento distinto esta misma neurona no disminuye su frecuencia de disparo a este fenómeno se le ha denominado saciedad sensorial específica y se ha demostrado también en humanos {Rolls, 1981 1068 /id}, quizá este fenómeno podría explicar porque después de una comida fuerte siempre hay espacio para un postre.

Por otra parte muchas de las estructuras involucradas en el procesamiento de la información gustativa también procesan estímulos viscerales cuya información es transmitida por dos vías periféricas esto es por el nervio vago y por activación de área postrema a través de torrente sanguíneo(Yamamoto et al., 1992). Basados en estudios con marcadores de actividad cerebral como genes de expresión temprana (c-Fos, FosB, JunB, Zif/268 y CREM) inducidos por LiCl se ha reportado activación de NTS, PBN y núcleo central de la amígdala(Yamamoto et al., 1992;Lamprecht y Dudai, 1995;Swank, 1999) y un cambio en Fos, Zif/268 en el núcleo paraventricular hipotalámico(Swank, 1999). Un estudio electrofisiológico demostró activación de

corteza insular 5 minutos después de la estimulación con LiCl (Yamamoto et al., 1989; Weiner, I et al., 1994)

Cualidades de los sabores

Existen cuatro sabores básicos salado, dulce, amargo y agrio sin embargo recientemente se ha arrojado información que postula la existencia de un quinto sabor: umami, sabor inducido por glutamato monosódico y algunos otros L-aminoácidos cuyo mecanismo de transducción fue recientemente descrito(Zhang et al., 2003;Chaudhari et al., 1996), no obstante a estas cinco cualidades aun no se sabe con exactitud todos los mecanismos por los cuales un compuesto induce alguna de estas cualidades y mucho menos cuando se usan mezclas de sabores. No existe una relación sencilla entre la estructura química de un compuesto y el sabor que induce. En este sentido no es de extrañarse encontrar que estructuras químicas tan diversas como la de carbohidratos (monosacáridos y disacáridos), algunos D- aminoácidos así como edulcorantes artificiales (sacarina y aspartamen), proteínas de plantas (monellin y taumatin), cloroformo y algunas sales (de berilio o de plomo) producen todas ellas la sensación de sabor dulce en humanos(Margolskee, 1995) y lo mismo sucede con el resto de las cualidades. En algunos casos como sucede con el sabor ácido existe una correlación entre la cantidad de protones y la percepción de este sabor sin embargo, cuando se compara un ácido orgánico versus uno inorgánico a concentraciones equimolares el ácido orgánico induce un sabor mas intenso, lo que sugiere que el pH no es la única característica que deben de cumplir ciertos compuestos para inducir esta cualidad. No es el objetivo de esta tesis describir todos los mecanismos de transducción de cada sabor pero en la caja 1 se describen brevemente los más importantes, se recomiendan las siguientes revisiones(Margolskee, 1995;Herness, 1999;Gilbertson et al., 2000).

Mundos gustativos: Se han reportado diferencias en la percepción del sabor entre especies, de especial interés en esta tesis son las diferencias entre rata y humano. Por ejemplo las ratas no beben fenilalanina presumiblemente

porque les induce un sabor amargo mientras que en humanos este compuesto es utilizado como edulcorante artificial(Mierson, 1995). Por otra parte, las proteínas taumatin y monelin que son ~100,000 veces mas dulce que sacarosa basándose en la molaridad solo son percibidas como dulces por humanos y monos del viejo mundo; mientras que en la rata, hámster, perro y conejo no exhiben ninguna respuesta neuronal (Brouwer et al., 1973;Hellekant et al., 1976), así también ratas no perciben aspartamen que es utilizado como edulcorante artificial en humanos(Nelson et al., 2002).

Caja 1: Mecanismos de transducción del sabor

Dulce: dos mecanismos de transducción se han propuesto, el primero localizado en la parte apical de la célula del gusto, que utiliza canales de Na^+ independiente de voltaje, ya que la despolarización inducida por sacarosa es inhibida por amiloride, antagonista de canales de cationes. El segundo mecanismo es mediado por activación de receptores transmembranales, a través de la activación de proteína G_s y adenilato ciclasa, esta última genera un incremento en AMPc que termina activando PKA que a su vez fosforila canales de K^+ dependientes de voltaje induciendo el cerrado de estos canales y un cambio en el potencial de membrana. Edulcorantes artificiales como sacarina presentan un mecanismo de transducción completamente diferente éste es vía ionositol trifosfato (IP_3) e incremento de Ca^{2+} intracelular. Recientemente se reportó que una familia de receptores acoplados a proteína G T1R podrían ser los receptores para el sabor dulce en especifico dos miembros de esta familia T1R2 + T1R3 que al formar un heterodimero se convierten en un receptor funcional, que solo se expresa en las células aceptadoras del gusto. Este receptor T1R2+3 responde a sustancias dulces tan diversas como sacarosa, sacarina, dulcina, y acesulfamima-K y algunos D-aminoácidos con sabor dulce(Nelson et al., 2001).

Amargo: el sabor amargo es inducido por compuestos bastante heterogéneos por lo que se han propuesto varios mecanismos ver (Mierson, 1995) sin embargo se cree que el mecanismo principal es a través de incremento de Ca^{2+} proveniente de depósitos intracelulares vía activación de inositol trifosfato (IP_3), el incremento en Ca^{2+} se piensa que causa la liberación de neurotransmisores que activan las fibras nerviosas, también sea propuesto la activación de gustducin que es una proteina G especifica de células aceptadoras del gusto, sin embargo ratones knockout de gustducin mantienen un poco de sensibilidad a lo amargo por lo que se cree debe de existir otros mecanismos(Wong et al., 1996). Recientemente se reportó una familia de ~30 miembros de receptores acoplados a proteína-G, llamados T2R1 como probables receptores para sustancias amargas. Muchos de sus miembros se coexpresan en una misma célula aceptadora del gusto (Chandrashekar et al., 2000), por lo que aunque existe una gran diversidad de compuestos amargos el organismo no puede discriminarlos porque todos ellos terminan activando a la misma célula aceptadora del gusto.

Ácido: los compuestos ácidos inducen este sabor, al parecer de manera directa penetrando a la membrana sin necesidad de receptores de membrana. La entrada de protones en la célula bloquea canales de Na^+, Ca^{2+} y K^+ dependientes de voltaje.

Salado: se ha propuesto que uno de los mecanismos de transducción del sabor salado está dado por la entrada directa de sodio por canales de sodio sensibles a amiloride este último un diurético aparentemente insípido que cuando es mezclado en agua con sal disminuye la percepción de lo salado, sin embargo en algunas especies se cree que debe de existir un segundo mecanismo aún no identificado ya que la aplicación de amiloride solo reduce pero no elimina por completo el sabor salado en humanos. La rata parece tener ambos mecanismos en distintas partes de la lengua.

Umami: el sabor de monosodio L-glutamato es mediado vía activación de receptores mRGlut4 (Chaudhari et al., 1996), Recientemente se reportó la existencia de otro receptor para L-aminoácidos, T1R1 que en combinación con T1R3 forman un receptor heterodimero funcional que responde a casi todos los L–aminoácidos interesantemente este receptor T1R1+3 no responde a D-aminoácidos que normalmente tienen sabor dulce estos últimos activan el receptor T1R2+3 que se piensa media la percepción del sabor dulce(Nelson et al., 2002).

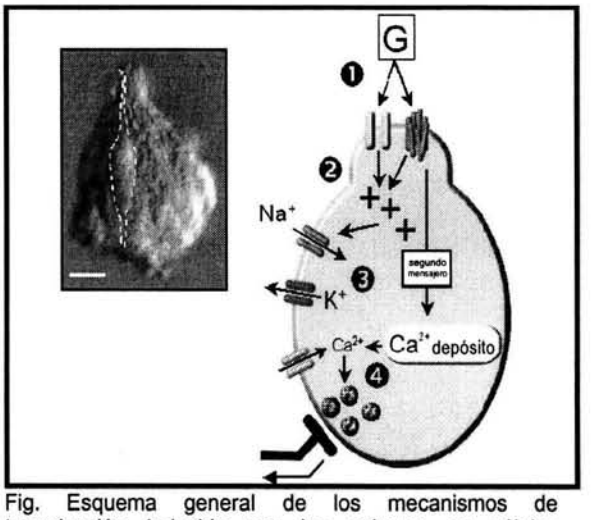

Fig. Esquema general de los mecanismos de transducción inducido por los sabores en células gustativas de vertebrados. Los estímulos gustativos (G) interactúan con canales iónicos o receptores transmembranales en la parte apical de la célula gustativa (1). Donde generalmente produce una despolarización del receptor (2). Esta despolarización activa canales iónicos dependientes de voltaje (3), incluyendo canales de Na^+ y K^+ que genera un potencial de acción durante la estimulación del sabor. Potenciales de acción pueden ser necesarios para abrir canales de Ca^{2+} dependientes de voltaje que incrementan los niveles de Ca^{2+} intracelular (4) lo que conlleva un cambio en la liberación de neurotransmisor en las fibras aferentes. Un incremento en el nivel de Ca^{2+} intracelular también puede ser mediado por activación de receptores transmembranales. Que incrementan el nivel de segundos mensajeros. El incremento en segundos mensajeros libera Ca^{2+} de depósitos intracelulares. En el recuadro se muestra una yema gustativa de rata, también está delineada una célula gustativa. Tomado de (Gilbertson, 2003)

Neurobiología del CAS y AN

Se han descrito diversas regiones cerebrales involucradas en el condicionamiento aversivo a los sabores en su mayoría las mismas que están involucradas en el procesamiento de la información gustativa. Lesiones del PBN afectan drásticamente la adquisición y retención del CAS(Spector et al., 1992;Sakai y Yamamoto, 1998) así también reduce la conducta neofóbica(Reilly y Trifunovic, 2001). Utilizando la expresión de c-Fos como marcador de actividad celular se encontró que sacarina normalmente induce activación del subnúcleo lateral central del PBN, la cual, después de la adquisición de un CAS la sacarina ahora activa el subnúcleo lateral ventral del PBN sugiriendo que el CAS modifica el procesamiento del sabor (Yamamoto, 1993). Cambios semejantes también se han reportado en el NTS(Swank et al., 1995). Con base en esto Yamamoto propuso al PBN como uno de los lugares responsables de la asociación del trazo de memoria del sabor con el malestar(Yamamoto et al., 1994). Sin embargo, ratas crónicamente descerebradas a nivel supracolicular son incapaces de adquirir y recordar la aversión aprendida al sabor (Grill y Norgren, 1978), lo que contrario a la propuesta de Yamamoto indica, que el tallo cerebral por si solo no es suficiente para aprender CAS. No obstante, los animales descerebrados no beben ni se alimentan voluntariamente, pero, si se aplica un sabor directamente a la cavidad bucal por medio de cánulas intraorales se puede observar que aún son capaces de rechazar soluciones con sabor amargo como quinina y también rechazan una solución de sacarosa después de saciarse. Por tanto, el cerebro anterior es responsable de los procesos de memoria involucrados en el CAS y el cerebro anterior puede controlar una serie de respuestas motoras (lengüetear) esas si localizadas a nivel del tallo cerebral cuando el sabor ha sido apareado con malestar. De las estructuras del cerebro anterior responsables del CAS pueden ser muchas tálamo ventroposteriomedial (Lasiter et al., 1985a;Reilly y Pritchard, 1996), núcleo basal magnocelular (Lopez et al., 1993;Gutierrez et al., 1999a), pero de especial importancia son la amígdala y corteza insular.

La participación de la amígdala y de sus subnúcleos en el CAS ha sido motivo de debate, algunos investigadores reportaron no efecto(Bermudez y McGaugh, 1991a) mientras que otros aseguran que la lesión de la amígdala deteriora la adquisición del CAS(Aggleton et al., 1981;Yamamoto et al., 1994), El grupo de Bernstain demostró que el método utilizado para condicionar cambia dramáticamente la participación de la amígdala, así lesiones de esta estructura destruyen completamente un CAS adquirido con cánulas intraorales en contraste un CAS adquirido con una sola botella solo se atenúa pero no se elimina(Cubero et al., 1999). No obstante a esta propuesta recientemente ha quedado claro que la amígdala es indispensable para la adquisición del CAS (Lamprecht et al., 1997;Lamprecht y Dudai, ;Yasoshima y Yamamoto, 1997). Los receptores glutamatérgicos, tanto los metabotrópicos como los del tipo AMPA y NMDA, son indispensables para la adquisición del CAS en esta estructura(Yasoshima et al., 2000). La amígdala también ha sido involucrada en la expresión de la neofobia ya que su lesión la disminuye(Aggleton et al., 1981). Por otra parte la inyección de norepinefrina previene la atenuación de la neofobia a jugo de uva (Ellis y Kesner, 1981).

En la década de los 70 se descubrió que animales con lesiones de la corteza insular (CI) que contiene la corteza gustativa(Braun, 1995) no desarrollaban aversión a sacarina 0.1% pero sí a quinina 10^{-4} M, interesante ambas soluciones sápidas fueron discriminadas respecto a agua(Braun et al., 1972), a partir de entonces numerosos experimentos han demostrado que lesiones bilaterales de la CI realizadas antes o después de la adquisición del CAS deterioran su aprendizaje y/o retención-evocación. Otros estudios han confirmado que estas lesiones, no parecen afectar la percepción del sabor, ya que los animales con lesiones de corteza insular pueden discriminar entre diferentes concentraciones de sacarosa o NaCl (Lasiter et al., 1985b) y categorizar entre sabores aceptables e inaceptables(Lorden, 1976). Por otra parte, también presentan niveles de reactividad al malestar similares a aquellos mostrados por animales intactos, i.e. el tiempo en que aparecen los síntomas de

malestar inducidos por beber LiCl fueron similares entre animales lesionados y controles(Lasiter, 1983), con base en estos resultados y en muchos otros se ha concluido que los animales sin corteza insular pueden percibir normalmente el sabor y el malestar, pero presentan un deterioro en la capacidad para aprender aversión a un sabor(Lasiter, 1983). Sin embargo, la corteza insular no inhabilita a las ratas para aprender aversión a todos los sabores(Lorden, 1976), por ejemplo, animales sin corteza insular pueden adquirir un CAS cuando el estímulo gustativo es muy intenso(Braun et al., 1972;Lorden, 1976) o cuando varios apareamientos son dados pero probablemente esto se deba a la utilización de pistas olfativas porque la lesión simultánea de corteza insular y bulbo olfativo reduce drásticamente (pero no elimina por completo) la capacidad para aprender aversión a sacarosa aún después de seis inyecciones de LiCl, la bulboctomía por si sola no afecta la adquisición del CAS(Braun, 1995).

Braun, 1995 llamó a este efecto agnosia para la aversión aprendida al sabor, proponiendo que la ablación de corteza insular induce los siguientes síntomas, primero este tratamiento no parece alterar la capacidad asociativa en general ya que estos animales aprenden aversión al olor potenciada por el sabor(Kiefer et al., 1984). "Lo que si parece afectarse es la habilidad para asociar cierta información gustativa con nuevos patrones conductuales, así como, las memorias del **significado aprendido del estímulo gustativo** formadas antes de la lesión. Todos los niveles de la relación conducta-sabor quedan prácticamente intactas, con excepción de la saliencia asociativa, sugiriendo que la corteza gustativa está esencialmente involucrada en **todas** las conductas aprendidas que son explícitamente desencadenadas por la estimulación gustativa de la lengua"(Braun, 1995). Aunque esta es una propuesta bastante amplia, un gran número de evidencias han apoyado esta conclusión. La ablación de corteza insular deteriora también la inhibición latente (Braun et al., 1972) y acelera la extinción del CAS (Braun, 1995) y la inhibición de síntesis de proteínas la inhibe (Berman y Dudai, 2001).

Se ha descubierto que lesiones electrolíticas de corteza insular ya sea antes o después de la fase de adquisición deterioran la aversión aprendida al

sabor, sin importar que método de condicionamiento se haya utilizado (Cubero et al., 1999;Schafe y Bernstein, 1998). Lesiones excitotóxicas que no destruyen fibras de paso(Bermudez y McGaugh, 1991b), así como la inhibición de potenciales de acción por medio de microinyecciones de tetrodotoxina directamente en corteza insular(Gallo et al., 1992) y la inhibición de síntesis de proteínas (Rosenblum et al., 1993) deterioran la adquisición del CAS.

Estudios posteriores utilizando microinyecciones de antagonistas de receptores muscarínicos y tipo NMDA han reafirmado que la participación de corteza insular en el CAS esta mediada por receptores metabotrópicos de acetilcolina y receptores ionotrópicos de ácido glutámico, por ejemplo antagonistas de receptores muscarínicos (escopolamina) y de receptores tipo NMDA (AP5) deterioran la adquisición del CAS cuando son inyectados antes de la presentación del estímulo gustativo novedoso (Naor y Dudai, 1996a;Berman et al., 2000).

Cuando estos fármacos son inyectados tiempo después de la presentación del sabor novedoso solamente AP5 sigue interfiriendo con el CAS probablemente impidiendo la señalización del malestar(Ferreira et al., 2002;Gutierrez et al., 1999b). El hecho, que escopolamina solo tenga efectos cuando es inyectada antes de la presentación del sabor novedoso y no después ha sido utilizado como argumento para decir que el sistema colinérgico en corteza insular está involucrado únicamente en la formación del trazo de memoria del sabor pero no en su asociación (Naor y Dudai, 1996a), resultados similares fueron encontrados con inyecciones sistémicas de atropina otro antagonista de receptores muscarínicos (Deutsch et al., 1976).

Planteamiento del problema

Solo tres regiones del sistema nervioso central han sido involucradas en la atenuación de la neofobia, se ha reportado que la estimulación eléctrica de la formación reticular del cerebro medio (Kesner y Berman, 1977), infusión de norepinefrina en la amígdala (Ellis y Kesner, 1981) e inactivación de todo el

manto cortical (Buresova y Bures, 1980) después de beber un sabor novedoso previene la AN. Sin embargo, aún falta por definir que regiones corticales, en específico, están contribuyendo con la AN así como descubrir en que sistemas de neurotransmisión a nivel cortical subyace este aprendizaje. Con base en que la AN es una conducta que depende en la capacidad de los animales para aprender y recordar que el sabor es seguro para ingerirlo (Nachman y Jones, 1974) y la idea de que ratas con lesión de la corteza gustativa parecen perder "la memoria del significado aprendido del sabor" predicen la participación de corteza gustativa en la AN muy probablemente a través de la activación de receptores muscarínicos y tipo NMDA como lo es en el caso del CAS. Por lo anterior decidimos investigar los efectos de antagonistas de estos receptores inyectados en corteza insular sobre la AN a sacarina.

Objetivo general

Determinar la participación de receptores metabotrópicos de acetilcolina y de los receptores ionotrópicos para glutamato en corteza insular en la AN y CAS.

Objetivos específicos

1. Retomar a la AN como un modelo conductual para estudiar la memoria del sabor en ausencia de malestar.
2. Demostrar que la AN y CAS dependen de diferentes mecanismos celulares en la corteza insular.

Resultados

Artículo 1: Blokade of cortical muscarinic but not NMDA receptors prevents a novel taste from becoming familiar.

Artículo 2: Cholinergic dependence of taste memory formation: Evidence of two distinct processes

European Journal of Neuroscience, Vol. 17, pp. 1556–1562, 2003

Blockade of cortical muscarinic but not NMDA receptors prevents a novel taste from becoming familiar

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Keywords: AP-5, conditioned taste aversion, insular cortex, neophobia, novelty, rats, scopolamine

Abstract

Exposure to a novel taste solution in the rat is followed by a decrease in its intake known as neophobia. This effect gradually disappears, and consumption increases from the second presentation of the taste (attenuation of neophobia), reflecting that the animal learned that it is safe to drink it. Conversely, if gastric malaise is induced after first intake, the rat will develop a long-lasting aversion (conditioned taste aversion). Previous attempts to elucidate the physiological nature of taste memory trace stems only from procedures that require malaise to measure taste memory. Here we assess the relevance of both muscarinic and *N*-methyl-D-aspartate receptors, known to be involved in conditioned taste aversion, on taste memory using a nonaversive procedure (attenuation of neophobia learning). Attenuation of neophobia was impaired by the muscarinic receptor antagonist, scopolamine, microinjected 20 min before, immediately after or up to 2 h after the first taste experience, suggesting that muscarinic receptors are involved in the acquisition and consolidation of attenuation of neophobia learning. However, the *N*-methyl-D-aspartate receptor antagonist, D,L-2-amino-5-phosphonovaleric acid, did not affect attenuation of neophobia even when the same dose of the drug was able to disrupt conditioned taste aversion learning, which suggests that attenuation of neophobia learning would be independent of *N*-methyl-D-aspartate receptors activity in the insular cortex. The neophobic response induced by strong saccharin presentation was not affected by either of the treatments given, which rules out any impairment in taste perception. These results indicate that while cortical muscarinic receptors are important in the formation and consolidation of safe memory trace, *N*-methyl-D-aspartate receptor activity appears to be noncritical.

Introduction

When animals drink a novel taste solution they innately hesitate to drink it, thereby reducing its consumption (neophobia) until its post-digestive consequence has been assessed (Siegel, 1974; Domjan, 1977). Thus, if a novel taste (conditioned stimulus, CS) is associated with malaise (unconditioned stimulus, US), animals will reject it in the next presentation, developing a long-lasting taste aversion, i.e. the taste cue will become a familiar aversive signal and this is referred to as conditioned taste aversion (CTA) (Garcia *et al.*, 1966; for review see Bures, 1998). Conversely, when taste is followed by absence of malaise, increased consumption is observed, and is termed as attenuation of neophobia (AN) (Domjan, 1976). The neophobic response is an innately protective behaviour that would be useful to avoid the intake of great amounts of toxic edibles. The reduction of this neophobic response has an important function on survival, probably as important as neophobia itself and taste aversion learning. Therefore, the study of AN would be useful to gain insight into the understanding of the complexity of the gustatory memory system. This avidity to ingest familiar solutions not paired with malaise suggests a learning process. Contrary to a nonassociative explanation, the learned safety theory (Rozin & Kalat, 1971; Kalat & Rozin, 1973) proposes that AN depends on the association of a taste cue with internal nonaversive consequences, i.e. the absence of malaise could be used to predict if it is 'safe' to consume a taste solution.

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Received 30 January 2003, revised 19 February 2003, accepted 21 February 2003

doi:10.1046/j.1460-9568.2003.02608.x

A number of studies have demonstrated the importance of the insular cortex (IC) (gustatory neocortex) in the acquisition and long-term storage of visceral and aversively motivated learning tasks like CTA learning (Bermudez & McGaugh, 1991; Braun, 1995; Bermudez-Rattoni & Yamamoto, 1998). In addition, it is well known that muscarinic and *N*-methyl-D-aspartate (NMDA) receptor antagonists microinjected into the IC disrupt CTA (Naor & Dudai, 1996; Rosenblum *et al.*, 1997; Gutierrez *et al.*, 1999b; Berman *et al.*, 2000). However, even though AN is a long-lasting behaviour (Best *et al.*, 1978) and does not require the induction of malaise to measure taste memory, there is little information about the neural substrates and molecular mechanisms involved in this learning. Therefore, we designed a series of experiments to assess the effects of muscarinic and NMDA receptor antagonists microinjected into the IC on attenuation of saccharin neophobia. Some of these results have previously been presented in abstract form (Gutierrez *et al.*, 2001).

Materials and methods

Subjects

Two-hundred and seventy-six male Wistar rats, weighing between 260 and 300 g at the beginning of the experiments were used. They were individually caged and kept in a 12 h light : 12 h dark cycle phase. All behavioural manipulations were performed in the light cycle phase. Rats received lab chow throughout the experiment. Experiments were performed in accordance with the Rules in Health Matters (Ministry of Health, Mexico) and with approval of the local Animal Care Committee.

Reagents

D,L-2-Amino-5-phosphonovaleric acid (AP-5; RBI, Natick, MA, USA) and scopolamine hydrobromide (Sigma, St. Louis, MO, USA) were used. All other chemicals were of analytical grade or the highest grade available (J.T. Baker, Xalostoc, Mexico City).

Surgery and microinjection

Animals were anaesthetized with sodium pentobarbital (65 mg/kg) and mounted in a stereotaxic apparatus. A midline incision was made to expose the skull, and two holes were made at the following coordinates: AP +1.2 mm relative to Bregma; Lateral ±5.5 mm (Paxinos & Watson, 1998). Two stainless steel guide cannulae were inserted bilaterally 3 mm below bregma aimed 2.5 mm above the IC: the cannulae were fixed with acrylic dental cement, using two stainless steel screws attached to the skull bone as anchors. A stylus was then inserted into the guide cannula to prevent clogging. Rats were allowed to recover from surgery for at least 5 days before the beginning of behavioural training.

Drugs were dissolved in Ringer (in mM: NaCl, 118; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 19; $C_6H_{12}O_6$, 3.3). The concentrations of the drugs used were 60 μg/μL for scopolamine, and 10 μg/μL for AP-5, according to previous reports (Naor & Dudai, 1996; Rosenblum *et al.*, 1997; Gutierrez *et al.*, 1999a; Gutierrez *et al.*, 1999b).

Microinjections were given bilaterally via a 30-gauge stainless steel injector 2.5 mm larger than tips of guide cannulae in order to reach the IC. The injector was connected via Teflon tubing to a 10-μL glass microsyringe attached to a microinfusion pump (Carnegie Medicin, Stockholm, Sweden). Infusions of 0.5 μL volume were given per hemisphere over 1 min (Myers, 1966). The injector was left in place for another minute to allow a complete diffusion. All intracortical infusions were given to hand-restrained conscious animals.

Histology and confirmation of injections site

At the end of the experiments, all animals were killed by an overdose of pentobarbital and perfused with saline followed by 0.4% paraformaldehyde. The brains were removed and placed in 30% sucrose/phosphate buffer (PB) 0.1 M solutions, and then sectioned and stained with Cresyl violet to establish the place of microinjection.

Behavioural procedure

Neophobia and attenuation of neophobia

We used a modified version of Domjan's (1976) protocol with a highly concentrated saccharin solution, that enhances the robustness of neophobia (Domjan & Gillan, 1976). Briefly, 5 days after surgery, rats were water deprived for 24 h. Afterwards, as baseline they were given 3 days of water in their home cages every 23.5 h for 15 min, and volume consumption was registered. On day 4, the animals were counterbalanced by weight and sorted in their corresponding group, as described in experimental design, and the neophobic response was tested by the presentation of 0.5% (w/v) sodium saccharin solution (Sigma) for 15 min. Following this, the rats received access to water for 15 min in order to ensure that all animals consumed their daily fluid requirement regardless of their consumption of saccharin. The same procedure was repeated from the fifth to the eighth days for AN test. Thus, the neophobic response was analysed in terms of the reduction in the intake of a novel taste solution relative to baseline intake of water. AN was then observed by the increased consumption of saccharin in the following presentations. Liquid intake was recorded with 0.5-mL accuracy.

Conditioned taste aversion

As with AN baseline measurement, rats were water deprived for 24 h and then given water in their home cage every 23.5 h for 15 min, for 4 days. The next day, the acquisition trial was performed by presentation for 15 min of a 0.1% (w/v) solution of saccharin as CS in distilled water, and 15 min later a malaise-inducing drug (LiCl, 0.4 M; 7.35 mL/kg) as an US was injected intraperitoneally. Two subsequent drinking sessions were performed with water only, and on the third day the test was conducted. The subjects were presented with 0.1% saccharin solution for the second time, and the decrease in consumption compared with the baseline was used as a measure of the strength of aversion.

Experimental design

Drugs injected before taste presentation

The animals were divided into four groups that received the behavioural procedure of neophobia and AN. Three groups were cannulated as described above, and 20 min before presentation of novel 0.5% saccharin solution (day 4) they received bilateral microinjections in the IC of either scopolamine (Scop, $n = 13$), AP-5 (AP-5, $n = 13$) or Ringer solution (Veh, $n = 10$), which was used as vehicle control. One group remained unoperated as intact control (Con, $n = 13$).

Drugs injected after taste presentation

To ascertain the drug effects in AN consolidation, the microinjections were made 15 min after novel taste presentation (day 4). One group received scopolamine (Scop-Post, $n = 13$), while the second group received AP-5 (AP-5-Post, $n = 8$) and the last group received Ringer solution (Veh-Post, $n = 12$). Additionally, in order to discard an unspecific suppression of intake of saccharin solution induced by scopolamine, one group (Scop-Post 5, $n = 8$) received a single microinjection of scopolamine 30 min after the second taste presentation (day 5).

Time-curve of scopolamine effects on AN

To determine the time-dependent effect of scopolamine on AN, seven independent groups were used. Each group received a single microinjection of scopolamine at only one of the following times: 15 min ($n = 8$), 30 min ($n = 8$), 2 h ($n = 5$), 4 h ($n = 9$), 8 h ($n = 9$) or 12 h ($n = 9$) after novel saccharin presentation (day 4). Twenty-four hours after the first saccharin intake, all groups received a second presentation with saccharin solution (day 5). It should be noted that the 12-h group had the shorter interval between microinjection and second saccharin intake. The remaining group received Ringer solution 30 min after saccharin presentation (day 4) and was used as vehicle control (Veh, $n = 8$).

Repeated scopolamine microinjections

In order to assess whether scopolamine induced a state-dependency phenomenon, two groups received a microinjection of scopolamine (D-Scop-Pre, $n = 9$) or Ringer solution (D-Veh-Pre, $n = 10$) 20 min before saccharin presentation twice, once on day 4 and once on day 5. Two other groups were treated similarly, but using scopolamine (D-Scop-Post, $n = 8$) or Ringer solution (D-Veh-Post, $n = 10$) injected 30 min after the 1st and 2nd (days 4 and 5) taste presentation, to test for consolidation.

Effect of scopolamine and AP-5 on CTA

To evaluate whether the volumes and concentrations of the drugs used in the present study impaired CTA, as would be expected from previous reports, we studied the effects of AP-5 and scopolamine

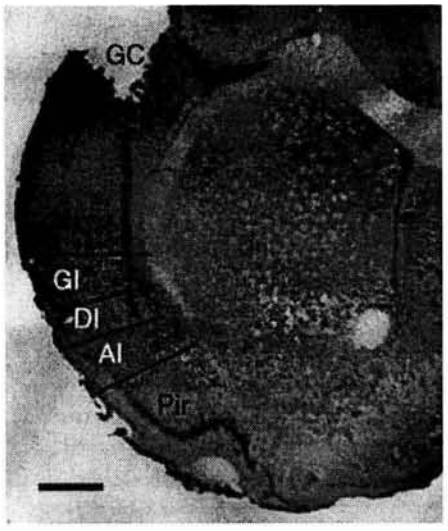

FIG. 1. Photomicrograph of coronal section of rat brain, stained with Cresyl violet, showing the guide cannula, the needle tract and the injection tip placement in the insular cortex. GI, DI and AI, granular, disgranular and agranular part of the insular cortex, respectively; Pir, piriform cortex; GC, guide cannula. Scale bar, 0.5 mm.

on CTA learning with 0.1% saccharin, which is known to be blocked by microinjections of both drugs into the IC (Berman *et al.*, 2000). Six groups were implanted with bilateral guide cannulae and received microinjections of Ringer solution (Veh-Pre, $n=9$; Veh-Post, $n=7$), scopolamine (Scop-Pre, $n=7$; Scop-Post, $n=9$) or AP-5 (AP-5-Pre, $n=7$; AP-5-Post, $n=8$). Groups 'Pre' were injected 20 min before presentation of saccharin, and groups 'Post' received the injection immediately after taste, 15 min before induction of malaise.

Can scopolamine be used as an US?

To demonstrate if scopolamine can be used as an US, we reduced the concentration of saccharin to 0.1%, as this is the concentration commonly used in CTA learning studies (Bures, 1998). Two groups of experimental animals received a single microinjection of scopolamine 20 min before (Scop-Pre, $n=8$) or 15 min after (Scop-Post, $n=9$) the first presentation of 0.1% of saccharin solution. In addition, two control groups received Ringer solution at the same time before (Veh-Pre, $n=9$) or after (Veh-Post, $n=9$). Therefore, if scopolamine induced CTA, the animals would show a significant rejection in a second saccharin presentation.

Results

Confirmation of cannulae location

The injection sites overlapped the granular and disgranular portions of the IC. Figure 1 shows a typical cannula placement in the IC; 11 animals were not considered for further analysis due to misplaced cannulae.

AN is prevented by scopolamine, but not by AP-5 when injected prior to novel taste presentation

In all experiments described herein, there were no significant differences in weight (data not shown), or water baseline consumption. The average baseline means (± SEM) of water intake (days 1–3) were (in mL): 13.7 ± 1, 14.1 ± 0.7, 14.3 ± 0.6 and 13.6 ± 0.4 for each of the Con, Veh, Scop and AP-5 groups, respectively.

All the groups showed a strong neophobic response, measured by the reduction in the consumption of a strong saccharin solution regardless of the treatment (see day 4, Fig. 2). Although the activity of scopolamine is less than 24 h (Sipos *et al.*, 1999) in the second taste experience (day 5), the group treated with scopolamine still showed a neophobic response compared with all other groups ($F_{3,45}=13.9$; $P<0.001$; Fisher, $P<0.05$). In the third taste experience (day 6), there were still significant differences between the scopolamine group and all the others ($F_{3,45}=3.7$; $P<0.05$); however, despite these differences, the animals displayed a normal AN because they drank a similar amount as the control animals did in the second saccharin intake (day 5). This indicates that scopolamine does not induce a permanent effect on AN. It is noteworthy that the total fluid intake (saccharin + water) on the day of microinjection (day 4) was not affected. Mean total intake was (in mL): 17.5 ± 1.1, 16.6 ± 1.3, 15.07 ± 0.8 and 15.5 ± 0.8 for the Con, Veh, Scop and AP-5 groups, respectively.

AN is prevented by scopolamine but not by AP-5 infusion after novel taste presentation

Scopolamine prevented AN even though it was microinjected 15 min after the first saccharin presentation (Fig. 3, day 5; $F_{3,37}=11.8$; $P<0.001$). A Fisher *post hoc* analysis showed that Scop-Post was significantly different from all other groups ($P<0.001$). Therefore, animals injected with scopolamine immediately after the novel taste drank a similar amount of saccharin as in the previous presentation. The mean values of saccharin solution intake for the AP-5-Post group were (in mL): 3.5 ± 0.6, 10.3 ± 1.6, 13.5 ± 1 and 15.6 ± 0.8 for days 4–7, respectively.

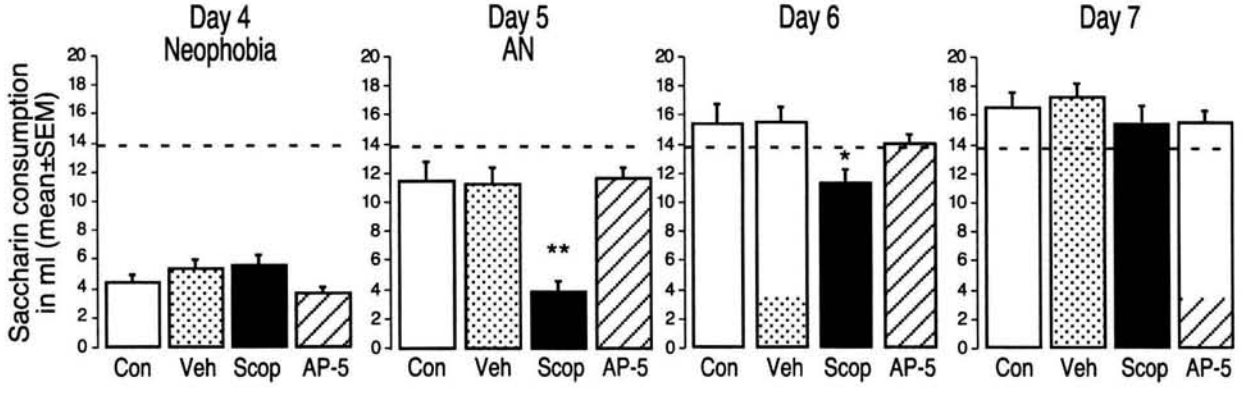

FIG. 2. Effect of the muscarinic receptor antagonist, scopolamine (Scop), NMDA receptor antagonist (AP-5), and vehicle (Veh) microinjected bilaterally into the insular cortex 20 min before presentation of saccharin on day 4. The 'Con' group remained without treatment as naïve control. Mean daily consumption (± SEM) of 0.5% sodium saccharin solution (days 4–7). Dashed line indicates average of water baseline consumption. $^{*}P<0.05$ or $^{**}P<0.01$, significantly different from the other groups.

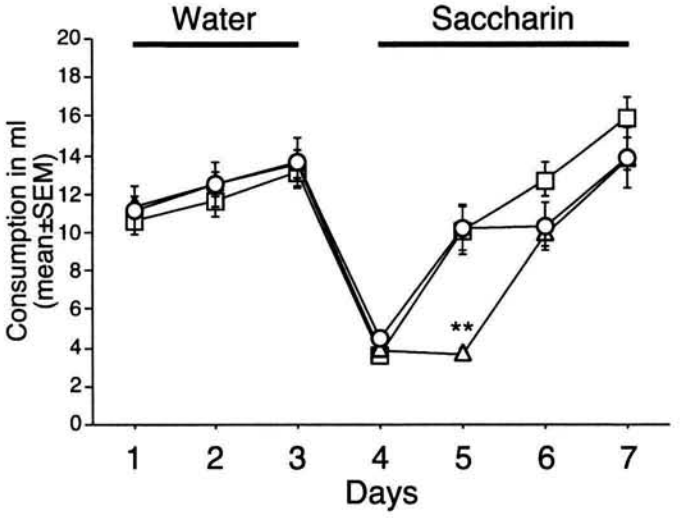

FIG. 3. Each point represents mean (± SEM) volume of daily fluid intake of water (days 1–3) and 0.5% saccharin solution (days 4–7). Scopolamine (△, Scop-Post) or vehicle (□, Veh-Post) was microinjected into insular cortex immediately after the presentation of novel saccharin (day 4). Scop-Post 5 group (○) received microinjection of scopolamine immediately after the second saccharin intake (day 5). $^{**}P < 0.001$ vs. corresponding control group.

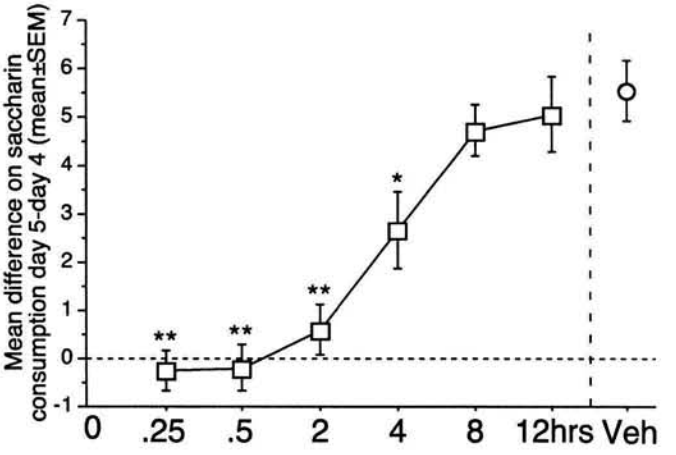

FIG. 4. Time-dependent effects of scopolamine on AN. AN is expressed as mean (± SEM) of the difference of saccharin consumed on day 5 minus saccharin consumption on day 4. Zero value indicates a similar amount consumed on both days. Each square point represents an independent group and the time when scopolamine was microinjected on day 4. The open circles represent vehicle injection group (Veh). $^{*}P < 0.05$ or $^{**}P < 0.01$, significantly different from the Veh group.

In addition, scopolamine by itself does not induce an unspecific decrease in the intake of a familiar saccharin solution (see Fig. 3); an unpaired *t*-test did not show significant differences on day 6 between Scop-Post 5 and Veh-Post groups ($t_{17} = 1.69$; $P > 0.05$). A paired *t*-test between days 5 and 6 indicated that the Scop-Post 5 group does not increase saccharin intake ($t_6 = 0.57$; $P > 0.05$), whereas the Veh-Post group did ($t_{11} = -3.1$; $P < 0.05$).

Scopolamine prevents AN for up to 2 h

To determine maximum duration of the scopolamine effect, independent groups received a single bilateral microinjection of scopolamine into the IC at several times after the presentation of a novel taste. In the first taste presentation, a strong neophobic response was observed in all the groups (data not shown). Conversely, significant differences among groups were found by subtracting the first saccharin consumption from the second ($F_{6,49} = 9.4$; $P < 0.001$). Those groups that received microinfusion of scopolamine during the first 2 h after the neophobia test (day 4) did not increase saccharin solution intake in the subsequent taste presentation (day 5). A *post hoc* pair-wise Fisher test showed that the 15-min, 30-min and 2-h groups were significantly different from all other groups ($P < 0.001$). The 4-h group still showed a significant impairment of AN, but to a lesser degree than the previous groups. No disruptive effects were observed when scopolamine was given over periods longer than 8 h (Fig. 4).

Repeated scopolamine administration prevents AN

To assess if scopolamine produced a state dependency, we administered it on the first and second exposure to the saccharin solution (days 4 and 5) as described in experimental design. Scopolamine-treated animals, either microinjected pre- or post-saccharin presentation, did not show an increase in consumption, as did their respective controls. Repeated analysis of variance (ANOVA, days 4–8 × treatment) showed significant differences among groups ($F_{3,33} = 11.3$; $P < 0.001$), a significant effect on days ($F_{4,132} = 126.4$; $P < 0.001$), and interaction among days and treatment ($F_{12,132} = 13$; $P < 0.001$), indicating that the effect of scopolamine is not permanent (Fig. 5).

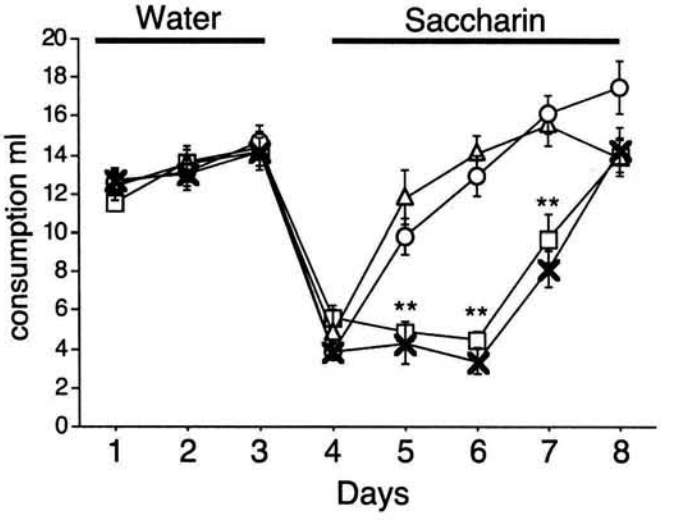

FIG. 5. Each point represents mean (± SEM) volume of daily fluid intake of water (days 1–3) and 0.5% saccharin solution (days 4–8). The 'D' indicates a double microinjection of scopolamine (Scop) or vehicle (Veh). Groups 'Pre' were injected before and groups 'Post' after the first (day 4) and second (day 5) taste presentation. (□, D-Scop-Pre, △, D-Veh-Pre, ×, D-Scop-Post and ○, D-Veh-Post group). $^{**}P < 0.01$ vs. corresponding control group.

Scopolamine and AP-5 disrupt long-term memory of CTA learning

There were no significant differences among groups in water baseline. As noted in Fig. 6, two-way ANOVA [three drugs (Scop, AP-5 and Veh) × 2 injection times (before and after taste)], showed significant differences in terms of drugs ($F_{2,41} = 5.6$; $P < 0.05$), time of injection ($F_{1,41} = 27.9$; $P < 0.05$), and there were significant interactions ($F_{1,41} = 6.7$; $P < 0.05$). The *post hoc* Fisher test showed that the Scop-Pre and AP-5-Pre groups impaired CTA learning, which indicates that scopolamine and AP-5 impaired CTA learning when they were applied before, but not after, taste presentation (see Fig. 6).

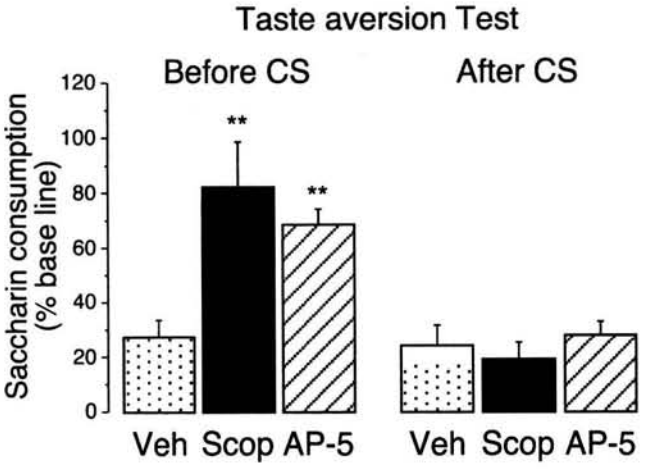

FIG. 6. Effect of scopolamine and AP-5 on conditioned taste aversion. Aversion is expressed as a percentage of the 0.1% saccharin solution intake with respect to water consumed at baseline (mean ± SEM). Drugs were injected before taste (CS), or between taste and malaise (US), $^{**}P < 0.01$ vs. Veh group.

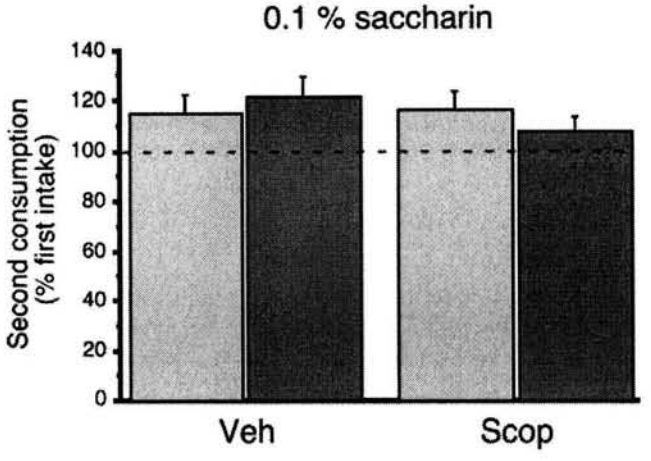

FIG. 7. The bars indicate the second saccharin consumption expressed as a percentage of the first saccharin intake (mean ± SEM). The dashed line shows the mean intake of first saccharin consumption. The effect of microinjection of scopolamine (Scop) or vehicle (Veh) into the IC 20 min before (grey bars) or immediately after (dark bars) the first intake of 0.1% saccharin solution is also shown.

Scopolamine is not an US

When scopolamine was used as an US, there were no significant differences in the first saccharin intake. The means (± SEM) of saccharin solution intake were (in mL): for vehicle before 13.1 ± 0.6, after 11.8 ± 0.9; and for scopolamine before 11.2 ± 0.5, after 11.2 ± 0.9. As noted in Fig. 7, none of the groups drank less saccharin solution relative to the first saccharin solution intake.

Discussion

The purpose of this study was to determine the participation of cortical muscarinic and NMDA receptors on taste memory formation using a task that does not require malaise to measure taste memory. Our results indicate that the blockade of muscarinic, but not NMDA receptors in the IC disrupts AN, i.e. the animals consumed a similarly reduced amount of saccharin solution on the first and second day. This suggests that they still showed neophobia. Moreover, this effect is not permanent, as once the animals were exposed to the taste in the absence of scopolamine, normal AN was observed on their next exposure to the taste.

From these findings, it is tempting to conclude that in their second taste presentation the rats perceived the familiar taste as 'novel' again. However, at least three additional explanations need to be assessed before ascribing to scopolamine the induction of a cognitive deficit, i.e. impairment in taste perception, state dependency and scopolamine as US. First, the scopolamine impairments on AN were not due to a deficit in taste perception, as the drug treatment did not disturb the neophobic response. This result is also in agreement with the failure of scopolamine to impair the retrieval of taste aversion learning (Naor & Dudai, 1996). A second possibility is that scopolamine might induce state dependency that would predict that AN must be observed under the same pharmacological context in the first and second taste presentations (Morilak *et al.*, 1983). The results presented herein do not support this prediction, as the animals with a double microinjection did not show AN in the second and third presentations. Therefore our results not only ruled out the state-dependency explanation, but also suggest that the activation of the muscarinic receptors in the IC is a requirement for AN induction. The third alternate explanation, that scopolamine may induce aversion, stems from data that show that intraperitoneal administration of scopolamine after taste induces CTA, but this is due to direct or indirect stimulation of the area postrema (Ossenkopp *et al.*, 1986; Ossenkopp & Giugno, 1990). This explanation does not apply here, because we used microinjections directly in the IC, thus reducing the possibility that scopolamine activates the area postrema. Additionally, we did not find any evidence that scopolamine induced aversion to 0.1% saccharin solution when used as US, which is in accordance with earlier reports (Naor & Dudai, 1996; Berman *et al.*, 2000). In this regard, scopolamine did not reduce the volume intake of a familiar taste. Therefore, we conclude that our results are best explained by an involvement of muscarinic receptors in AN. If true, it may be reasonable to ask what part of the taste memory trace is affected by scopolamine.

Recently, we demonstrated that microinjections of scopolamine into the IC before, but not after, novel taste impairs both short- and long-term memory for CTA (Ferreira *et al.*, 2002). These results, in conjunction with the results showed herein, indicate that in the early stages of taste processing the blockade of muscarinic receptors in the IC interferes with both CTA and AN learning, probably by disrupting the formation of the taste memory trace. In this regard, scopolamine-induced impairments have been found in the formation of several nongustatory memory traces; in humans (Petersen, 1977), monkeys (Aigner *et al.*, 1991) and rats (Bohdanecky & Jarvik, 1967; Aigner *et al.* 1991) it was reported that systemic injections of scopolamine disrupt a visual recognition memory task when applied before, but not immediately after, acquisition of the task.

It has been reported in several CTA-based studies that functional inactivation of the cortex (Buresova & Bures, 1973; Gallo *et al.*, 1992; Roldan & Bures, 1994) or infusion of muscarinic antagonists immediately after the novel taste presentation (Deutsch, 1978; Naor & Dudai, 1996) did not disrupt CTA learning. Conversely, our results suggest that when the taste stimulation is no longer present, the taste memory trace continues at cortical levels in the IC, as AN was prevented when scopolamine was applied immediately after or up to 2 h after the novel saccharin presentation. Our observations are in agreement with those of Buresova & Bures (1980), where functional decortications after apple juice intake prevented AN. In addition, because scopolamine induced a temporally graded retrograde amnesia on AN, we may conclude that muscarinic receptors are involved in the

consolidation of the AN, confirming that this learning is gradually consolidated during the first 6–8 h after novel taste experience, as suggested in several behavioural studies (Nachman & Jones, 1974; Green & Parker, 1975).

The paradigm used in the present study allowed us to selectively measure two behavioural components: one that requires detection of the novelty (neophobia); while the second requires detection of familiarity (AN, taste recognition). By using this protocol, we found that neither scopolamine nor the NMDA receptor blocker, AP-5, applied in the IC, disrupted the neophobic response to 0.5% saccharin solution, which suggests that these drugs did not affect novelty discrimination. Moreover, our results demonstrate a clear-cut dissociation between AN and neophobia, suggesting that the gustatory memory trace processed both behaviours in an independent way and was probably carried out in different structures. In this regard, significant impairments have been found in taste neophobia induced by lesions of either the parabrachial nucleus (Reilly & Trifunovic, 2001) or amygdala (Nachman & Ashe, 1974). In addition, the infusion of AP-5 into nucleus accumbens decreases food neophobia (Burns *et al.*, 1996), and disrupts the detection of spatial and object novelty (Usiello *et al.*, 1997). These results contrast with our failure to find effects on neophobia. A possible explanation is that taste neophobia would be mediated mainly by subcortical and limbic structures, whereas AN would require both subcortical (Kesner & Berman, 1977; Ellis & Kesner, 1981; Hernadi *et al.*, 1997) and cortical components (Buresova & Bures, 1980).

Earlier reports indicate that the cortical cholinergic system becomes activated when animals are stimulated with a novel tone/light (Acquas *et al.*, 1996), a novel environment (Giovannini *et al.*, 2001) and a novel taste experience (Miranda *et al.*, 2000). Recently, we found that novel saccharin solution intake induces a significant increment in acetylcholine (ACh) release in the IC in free-moving rats. On this basis, we proposed that cortical ACh would encode taste novelty (Miranda *et al.*, 2000), and the results presented here are in agreement with this idea. Furthermore, our results also suggest that ACh through muscarinic receptors would trigger molecular events involved in AN. Because a novel taste stimulus induces phosphorylation of 2B subunit of NMDA receptors (Rosenblum *et al.*, 1997) and ERK I/II (Berman *et al.*, 1998) in the IC, presumably by activation of muscarinic receptors (Rosenblum *et al.*, 1996; Rosenblum *et al.*, 2000), both molecular events might be good candidates for the mediation of AN learning. The relevance of these molecules in this learning paradigm is currently being evaluated in our laboratory.

It has been shown that NMDA receptors play an important role in the consolidation of a variety of learning tasks, including CTA (Rosenblum *et al.*, 1997; Gutierrez *et al.*, 1999b; Yasoshima *et al.*, 2000). However, the participation of the NMDA receptor does not seem to be involved in the formation and consolidation of the AN. This result indicates that blockade of NMDA receptors in the IC does not interfere with the taste memory trace, in agreement with the failure of AP-5 microinjected before novel taste to disrupt short-term memory for CTA (Ferreira *et al.*, 2002). This suggests that under AP-5 treatment, the taste memory trace was formed and associated to aversive visceral inputs, but this association did not consolidate into a more stable memory. Recently, a lack of change in glutamate release in the parietal cortex during exposure to a novel environment has been described (Giovannini *et al.*, 2001), and Miranda *et al.* (2002) reported no changes in glutamate release in the IC after presentation of a novel taste. These results suggest that cortical glutamate activity is not modulated by the novelty or familiarity dimension of these stimuli. Thus, it was proposed that glutamatergic activity in the amygdala and IC is modulated by visceral input (malaise) rather than novel taste input during CTA acquisition (Miranda *et al.*, 2002). Therefore, the failure of AP-5 to affect AN, in a concentration and volume sufficient to impair long-term memory of CTA, is consistent with the literature, in which it has been demonstrated that systemic injections of ketamine, a noncompetitive NMDA receptor antagonist, block CTA learning but not AN (Aguado *et al.*, 1994). Overall, these data suggest that the taste memory trace could be relying on NMDA-independent receptor activity until the onset of US visceral input modifies the memory trace, making it aversive and then NMDA receptor dependent (Ferreira *et al.*, 2002).

In summary, to our knowledge this study constitutes the first demonstration that muscarinic receptors in the IC play an important role in the AN, which is an appropriate model for investigating the molecular and cellular mechanisms involved in the gustatory memory trace without interference of malaise.

Acknowledgements

This work was supported by CONACYT-Mexico grants MRI 35806-N and 31842-N DGAPA-IN215001. We acknowledge the technical assistance of Federico Jandete, Raul Zárate Zarza, Oreste Carbajal, Francisco Pérez Eugenio, Yolanda Díaz de Castro, and give thanks to Shaun Harris, Violeta Ortega Cuevas, Paul Afolabi Remi and Christopher Ormsby for the English revision of the final text.

Abbreviations

ACh, acetylcholine; AN, attenuation of neophobia; ANOVA, analysis of variance; AP-5, D,L-2-amino-5-phosphonovaleric acid; CS, conditioned stimulus; CTA, conditioned taste aversion; IC, insular cortex; NMDA, *N*-methyl-D-aspartate; PB, phosphate buffer; US, unconditioned stimulus.

References

Acquas, E., Wilson, C. & Fibiger, H.C. (1996) Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation, and fear. *J. Neurosci.*, **16**, 3089–3096.

Aguado, L., San Antonio, A., Perez, L., del Valle, R. & Gomez, J. (1994) Effects of the NMDA receptor antagonist ketamine on flavor memory: conditioned aversion, latent inhibition, and habituation of neophobia. *Behav. Neural Biol.*, **61**, 271–281.

Aigner, T.G., Walker, D.L. & Mishkin, M. (1991) Comparison of the effects of scopolamine administered before and after acquisition in a test of visual recognition memory in monkeys. *Behav. Neural Biol.*, **55**, 61–67.

Berman, D.E., Hazvi, S., Neduva, V. & Dudai, Y. (2000) The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste: activation of ERK1-2 and formation of a memory trace. *J. Neurosci.*, **20**, 7017–7023.

Berman, D.E., Hazvi, S., Rosenblum, K., Seger, R. & Dudai, Y. (1998) Specific and differential activation of mitogen-activated protein kinase cascades by unfamiliar taste in the insular cortex of the behaving rat. *J. Neurosci.*, **18**, 10,037–10,044.

Bermudez Rattoni, F. & McGaugh, J.L. (1991) Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion. *Brain Res.*, **549**, 165–170.

Bermudez-Rattoni, F. & Yamamoto, T. (1998) Neuroanatomy of CTA: lesion studies. In Bures, J., Bermudez-Rattoni, F. & Yamamoto, T. (Eds), *Conditioned Taste Aversion. Memory of a Special Kind.* Oxford Science Publications, New York, pp. 28–44.

Best, M.R., Domjan, M. & Haskins, W.L. (1978) Long-term retention of flavor familiarization: effects of number and amount of prior exposures. *Behav. Biol.*, **23**, 95–99.

Bohdaneckyy, Z. & Jarvik, M.E. (1967) Impairment of one-trial passive avoidance learning in mice by scopolamine, scopolamine methylbromide, and physostigmine. *Int. J. Neuropharmacol.*, **6**, 217–222.

Braun, J.J. (1995) Gustatory cortex: definition and function. In Kolb, B. & Tees, R.C. (Eds), *The Cerebral Cortex of the Rat.* MIT Press, Cambridge, pp. 407–430.

Bures, J. (1998) The CTA paradigm: terminology, methods, and conventions. In Bures, J., Bermudez-Rattoni, F. & Yamamoto, T. (Eds), *Conditioned*

Taste Aversion. Memory of a Special Kind. Oxford University Press, Oxford, pp. 14–25.

Buresova, O. & Bures, J. (1973) Cortical and subcortical components of the conditioned saccharin aversion. *Physiol. Behav.*, **11**, 435–439.

Buresova, O. & Bures, J. (1980) Post-ingestion interference with brain function prevents attenuation of neophobia in rats. *Behav. Brain Res.*, **1**, 299–312.

Burns, L.H., Annett, L., Kelley, A.E., Everitt, B.J. & Robbins, T.W. (1996) Effects of lesions to amygdala, ventral subiculum, medial prefrontal cortex, and nucleus accumbens on the reaction to novelty: implication for limbic–striatal interactions. *Behav. Neurosci.*, **110**, 60–73.

Deutsch, R. (1978) Effects of atropine on conditioned taste aversion. *Pharmacol. Biochem. Behav.*, **8**, 685–694.

Domjan, M. (1976) Determinants of the enhancement of flavored-water intake by prior exposure. *J. Exp. Psychol. Anim. Behav. Process*, **2**, 17–27.

Domjan, M. (1977) Attenuation and enhancement of neophobia for edibles substance. In Barker, L.M., Best, M.A. & Domjan, M. (Eds), *Learning Mechanisms in Food Selection.* Baylor University Press, TX, pp. 151–179.

Domjan, M. & Gillan, D. (1976) Role of novelty in the aversion for increasingly concentrated saccharin solutions. *Physiol. Behav.*, **16**, 537–542.

Ellis, M.E. & Kesner, R.P. (1981) Physostigmine and norepinephrine: effects of injection into the amygdala on taste associations. *Physiol. Behav.*, **27**, 203–209.

Ferreira, G., Gutierrez, R., De la Cruz, V. & Bermudez-Rattoni, F. (2002) Differential involvement of cortical muscarinic and NMDA receptors in short- and long-term taste aversion memory. *Eur. J. Neurosci.*, **16**, 1139–1145.

Gallo, M., Roldan, G. & Bures, J. (1992) 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.

Garcia, J., Ervin, F.R. & Koelling, R.A. (1966) Learning with prolonged delay of reinforcement. *Psychon. Sci.*, **5**, 121–122.

Giovannini, M.G., Rakovska, A., Benton, R.S., Pazzagli, M., Bianchi, L. & Pepeu, G. (2001) Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. *Neuroscience*, **106**, 43–53.

Green, K.F. & Parker, L.A. (1975) Gustatory memory: incubation and interference. *Behav. Biol.*, **13**, 359–367.

Gutierrez, H., Gutierrez, R., Silva, G., Estrada, J., Miranda, M.I. & Bermudez-Rattoni, F. (1999a) Differential effects of 192IgG-saporin and NMDA-induced lesions into the basal forebrain on cholinergic activity and taste aversion memory formation. *Brain Res.*, **834**, 136–141.

Gutierrez, H., Hernandez-Echeagaray, E., Ramirez-Amaya, V. & Bermudez-Rattoni, F. (1999b) Blockade of N-methyl-D-aspartate receptors in the insular cortex disrupts taste aversion and spatial memory formation. *Neuroscience*, **89**, 751–758.

Gutierrez, R., Tellez, L. & Bermudez-Rattoni, F. (2001) Attenuation of taste neophobia depends on activation of muscarinic receptors in the insular cortex. *Soc. Neurosci. Abstr.*, **27**, 854.6.

Hernadi, I., Karadi, Z., Faludi, B. & Lenard, L. (1997) Disturbances of neophobia and taste-aversion learning after bilateral kainate microlesions in the rat pallidum. *Behav. Neurosci.*, **111**, 137–146.

Kalat, J.W. & Rozin, P. (1973) Learned safety as a mechanism in long-delay taste-aversion learning in rats. *J. Comp. Physiol. Psychol.*, **83**, 198–207.

Kesner, R.P. & Berman, R.F. (1977) Effects of midbrain reticular formation, hippocampal, and lateral hypothalamic stimulation upon recovery from neophobia and taste aversion learning. *Physiol. Behav.*, **18**, 763–768.

Miranda, M.I., Ramirez, L. & Bermudez-Rattoni, F. (2000) Cortical cholinergic activity is related to the novelty of the stimulus. *Brain Res.*, **882**, 230–235.

Miranda, M.I., Ferriera, G., Ramirez-Lugo, L. & Bermudez-Rattoni, F. (2002) Glutamatergic activity in the amygdala signals visceral input during taste memory formation. *Proc. Natl Acad. Sci. USA*, **99**, 11,417–11,422.

Morilak, D.A., Orndoff, R.K., Riccio, D.C. & Richardson, R. (1983) Persistence of flavor neophobia as an indicator of state-dependent retention induced by pentobarbital, stress, and estrus. *Behav. Neural Biol.*, **38**, 47–60.

Myers, R. (1966) Injection of solutions into cerebral tissue: relation between volume and diffusion. *Physiol. Behav.*, **1**, 171–174.

Nachman, M. & Ashe, J.H. (1974) Effects of basolateral amygdala lesions on neophobia, learned taste aversions, and sodium appetite in rats. *J. Comp. Physiol. Psychol.*, **87**, 622–643.

Nachman, M. & Jones, D.R. (1974) Learned taste aversions over long delays in rats: the role of learned safety. *J. Comp. Physiol. Psychol.*, **86**, 949–956.

Naor, C. & Dudai, Y. (1996) Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. *Behav. Brain Res.*, **79**, 61–67.

Ossenkopp, K.P. & Giugno, L. (1990) Nicotine-induced conditioned taste aversions are enhanced in rats with lesions of the area postrema. *Pharmacol. Biochem. Behav.*, **36**, 625–630.

Ossenkopp, K.P., Sutherland, C. & Ladowsky, R.L. (1986) Motor activity changes and conditioned taste aversions induced by administration of scopolamine in rats: role of the area postrema. *Pharmacol. Biochem. Behav.*, **25**, 269–276.

Paxinos, G. & Watson, C. (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th Edn, CD-ROM. Academic Press, San Diego.

Petersen, R.C. (1977) Scopolamine induced learning failures in man. *Psychopharmacology (Berl.)*, **52**, 283–289.

Reilly, S. & Trifunovic, R. (2001) Lateral parabrachial nucleus lesions in the rat: neophobia and conditioned taste aversion. *Brain Res. Bull.*, **55**, 359–366.

Roldan, G. & Bures, J. (1994) Tetrodotoxin blockade of amygdala overlapping with poisoning impairs acquisition of conditioned taste aversion in rats. *Behav. Brain Res.*, **65**, 213–219.

Rosenblum, K., Berman, D.E., Hazvi, S. & Dudai, Y. (1996) Carbachol mimics effects of sensory input on tyrosine phosphorylation in cortex. *Neuroreport*, **7**, 1401–1404.

Rosenblum, K., Berman, D.E., Hazvi, S., Lamprecht, R. & Dudai, Y. (1997) NMDA receptor and the tyrosine phosphorylation of its 2B subunit in taste learning in the rat insular cortex. *J. Neurosci.*, **17**, 5129–5135.

Rosenblum, K., Futter, M., Jones, M., Hulme, E.C. & Bliss, T.V. (2000) ERKI/II regulation by the muscarinic acetylcholine receptors in neurons. *J. Neurosci.*, **20**, 977–985.

Rozin, P. & Kalat, J.W. (1971) Specific hungers and poison avoidance as adaptative specializations of learning. *Psychol. Rev.*, **78**, 459–486.

Siegel, S. (1974) Flavor preexposure and learned safety. *J. Comp. Physiol. Psychol.*, **86**, 674–678.

Sipos, M., Burchnell, V. & Galbicka, G. (1999) Dose–response curves and time-course effects of selected anticholinergics on locomotor activity in rats. *Psychopharmacology*, **147**, 250–256.

Usiello, A., Sargolini, F., Roullet, P., Ammassari-Teule, M., Passino, E., Oliverio, A. & Mele, A. (1997) N-Methyl-D-aspartate receptors in the nucleus accumbens are involved in detection of spatial novelty in mice. *Psychopharmacology (Berl.)*, **137**, 175–183.

Yasoshima, Y., Morimoto, T. & Yamamoto, T. (2000) Different disruptive effects on the acquisition and expression of conditioned taste aversion by blockades of amygdalar ionotropic and metabotropic glutamatergic receptor subtypes in rats. *Brain Res.*, **869**, 15–24.

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Cholinergic dependence of taste memory formation: Evidence of two distinct processes

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Received 20 May 2003; revised 13 June 2003; accepted 17 June 2003

Abstract

Learning the aversive or positive consequences associated with novel taste solutions has a strong significance for an animal's survival. A lack of recognition of a taste's consequences could prevent ingestion of potential edibles or encounter death. We used conditioned taste aversion (CTA) and attenuation of neophobia (AN) to study aversive and safe taste memory formation. To determine if muscarinic receptors in the insular cortex participate differentially in both tasks, we infused the muscarinic antagonists scopolamine at distinct times before or after the presentation of a strong concentration of saccharin, followed by either an i.p. injection of a malaise-inducing agent or no injection. Our results showed that blockade of muscarinic receptors before taste presentation disrupts both learning tasks. However, the same treatment after the taste prevents AN but not CTA. These results clearly demonstrate that cortical cholinergic activity participates in the acquisition of both safe and aversive memory formation, and that cortical muscarinic receptors seem to be necessary for safe but not for aversive taste memory consolidation. These results suggest that the taste memory trace is processed in the insular cortex simultaneously by at least two independent mechanisms, and that their interaction would determine the degree of aversion or preference learned to a novel taste.

Keywords: Memory trace; Conditioned taste aversion; Neophobia; Muscarinic receptor; Novelty; Scopolamine; Insular cortex

1. Introduction

Perception of novelty depends on two aspects of the stimuli, the detection of its physical attributes and the lack of experience with the novel cue. Taste neophobia (caution to consume a novel flavor) (Bures, 1998a) is an innate behaviour, probably useful in avoiding the ingestion of great amounts of a toxic edible (Domjan, 1976). The neophobic response triggers cellular events involved in learning and remembering the occurrence or not of aversive consequences. Thus, if a novel taste experience is followed by gastrointestinal discomfort, animals will reject it on the next presentation and develop a long lasting taste aversion, i.e., the taste cue would become a familiar aversive signal, namely conditioned taste aversion (CTA) (Bures, 1998b; Garcia, Kimeldorf, & Koelling, 1955). In contrast, when the novel neophobic taste is not followed by internal discomfort, in subsequent presentations the animal will increase its consumption; this is termed attenuation of neophobia (AN) (Domjan, 1977). In other words, the novel taste cue becomes a familiar safe signal (Rozin, 1977).

It is thought that both CTA and AN learning depend upon a neural representation of the taste that remains active probably in several brain regions in parallel, even when the taste stimulus is not present anymore (Buresova & Bures, 1980). This representation has been called the gustatory memory trace (Welzl, Alessandri, & Bättig, 1990) or gustatory short-term memory (Bures, 1998a). Hereafter, we refer to it as taste memory trace (TMT). Thus, the TMT may be the conjunction of cellular activities induced by taste stimulation that occur in parallel and in distributed assemblies of neurons through the brain. It is also thought that the TMT,

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doi:10.1016/S1074-7427(03)00066-2

rather than the physical presence of the taste itself, is directly associated with gastric illness or with safe signals (thirst reduction, illness recovery, etc.) (Bures, 1998a; Green & Garcia, 1971). Hence, the TMT could have two different long lasting traces, the first for aversion and the second for safe learning; both memories from now on could well be independent, but may interact to a certain degree.

It is well known that the insular cortex participates in the formation of the TMT. This is supported by the disruptive effects of CTA learning induced by ablations (Braun, Slick, & Lorden, 1972), excitotoxic lesions (Bermudez-Rattoni & McGaugh, 1991), and by temporal inactivation of the insular cortex (Buresova & Bures, 1973; Gallo, Roldan, & Bures, 1992). Moreover, it has been demonstrated that blockade of insular cortex muscarinic receptors before taste presentation impairs CTA (Naor & Dudai, 1996; Ramirez-Lugo, Miranda, Escobar, Espinosa, & Bermudez-Rattoni, 2003). Recently, we have demonstrated that these receptors are also important for AN learning (Gutierrez, Tellez, & Bermudez-Rattoni, 2003). Thus, microinjection of a muscarinic receptor antagonist, scopolamine, into the insular cortex prevented the novel taste from becoming a familiar safe signal. However, the same treatment applied immediately after novel taste presentation prevented AN but not CTA learning. These results strongly suggest that muscarinic receptors participate at specific times after taste stimulation depending on the presence or absence of aversive signals. However, these results might not be conclusive, since there were differences in the time of the second presentation of the taste (72 h vs. 24 h), and in the concentration of saccharin used (0.1% vs. 0.5%) (Ferreira, Gutierrez, De La Cruz, & Bermudez-Rattoni, 2002; Gutierrez et al., 2003; Naor & Dudai, 1996). Thus, the purpose of this study was to investigate the temporal characteristics of the TMT, and to determine the time-dependent participation of cortical muscarinic receptors on the formation and associative processing of the TMT, keeping constant those variables.

2. Materials and methods

2.1. Subjects

Male Wistar rats, weighing between 260 and 300 g at the beginning of the experiments were used. They were individually caged and kept in a 12 h light/dark cycle. All behavioral manipulations were performed in the light phase. Rats received ad libitum lab chow throughout the experiment. Experiments were performed in accordance with Rules in Health Matters (Ministry of Health, Mexico) and with approval of the local Animal Care Committee.

2.2. Behavioral procedures

2.2.1. Neophobia and attenuation of neophobia

We used the same protocol as in previous studies (Gutierrez et al., 2003) with a highly concentrated saccharin solution, which enhances the robustness of neophobia (Domjan & Gillan, 1976). Briefly, rats were water deprived for 24 h and then they were given access to water for 15 min on each of three days in their home cages. Volume consumption was registered with 0.5 ml accuracy. On day four, the animals were counterbalanced by weight and sorted in their corresponding group, as described in Section 2.3 design and the neophobic response was tested by presentation of 0.5% (w/v) sodium saccharin solution (Sigma, St. Louis, MO). In order to ensure that all animals consumed their daily fluid requirements regardless of their consumption of saccharin, an additional 15 min of water access were given unless otherwise indicated. The same saccharin procedure was repeated for the attenuation of neophobia (AN) test depending on the Section 2.3 as described below. Thus, the neophobic response was analyzed in terms of the reduction in the intake of a novel taste solution relative to water baseline intake. AN was then observed by the increased consumption of saccharin in the following presentations.

2.2.2. Conditioned taste aversion

As with AN baseline measurement rats were water deprived for 24 h and then were given 15 min water access in their home cage every day for three days. The next day, the acquisition trial was performed by presentation of 0.5% (w/v) saccharin in distilled water as a conditioned stimulus and 15 min later a malaise-inducing drug (LiCl 0.4 M; 2.94 mEq/kg) as an unconditioned stimulus was injected intraperitoneally. Two subsequent drinking sessions were performed with water only and 72 h after the acquisition trial the test trial was conducted. The test trial consisted in the presentation of 0.5% saccharin solution for a second time, and the decrease in consumption compared to the baseline was used as a measure of the strength of aversion. In some groups as indicated in Section 2.3, the same procedure used in the test trial was repeated in order to measure extinction.

2.2.3. Reagents

Scopolamine hydrobromide (Sigma, St. Louis, MO) was used. All other chemicals were of analytical grade or the highest grade available (J.T. Baker, Xalostoc, México). Drugs was dissolved in Ringer (in mM, 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 19 $NaHCO_3$, and 3.3 $C_6H_{12}O_6$). The concentration of scopolamine was 60 μg/μl according to our previous reports (Ferreira et al., 2002; Gutierrez et al., 2003).

2.2.4. Surgery and microinjection

Animals were anesthetized with sodium pentobarbital (65 mg/kg) and mounted in the stereotaxic apparatus. A midline incision was made to expose the skull, and two holes were made at the following coordinates: AP + 1.2 mm; lateral, ±5.5 mm relative to Bregma (Paxinos & Watson, 1986). The tips of two stainless steel guide cannulae aimed at insular cortex were inserted bilaterally 3 mm below Bregma. Two screws were implanted and served as anchors in the skull bone. The whole assembly was attached on the skull with acrylic dental cement. A stylus was inserted into the guide cannula to prevent clogging. Rats were allowed to recover from surgery for at least 5 days before the beginning of behavioral training.

Microinjections were given bilaterally via a 30 gauge stainless steel injector 3 mm larger than the tips of the guide cannulae, connected via Teflon tubing to a 10 μl glass microsyringe attached to a microinfusion pump (Carnegie Medicin, Stockholm, Sweden). Infusions of 0.5 μl were given per hemisphere over one minute. The injector was left in the guide cannula one additional minute to allow complete diffusion. Intracortical infusions were given to hand restrained conscious animals.

2.2.5. Histology

At the end of the experiments, all rats were sacrificed by an overdose of pentobarbital and perfused with saline followed by 0.4% paraformaldehyde. The brains were removed and placed in 30% sucrose/PB 0.1 M solution. Brains were sectioned and stained with cresyl violet to establish the place of microinjection.

2.3. Experimental design

2.3.1. Experiment 1

To ascertain the properties of the behaviors triggered by a single presentation of 0.5% saccharin, one of two groups of animals were trained as mentioned above for attenuation of neophobia task; (AN, $n = 11$) (Fig. 1A), whereas the other for conditioned taste aversion task (CTA, $n = 12$).

To study the time that the animals require to consolidate AN memory, 52 animals were divided into six groups ($n = 8$ to 9) and trained in AN protocol, and the second presentation of saccharin was given once at the following times 1, 2, 4, 6, 8, or 24 h after the onset of the first presentation of saccharin, and their consumption was recorded (Fig. 1B). In the first group, the animals did not receive water just after the first presentation of saccharin to avoid satiety during the next presentation of saccharin 1 h later.

In order to study the CS-US delay that reliably induces a taste aversion to 0.5% saccharin solution, the interval between taste and abdominal irritation (LiCl injection) was manipulated in 8 groups of rats ($n = 10$,

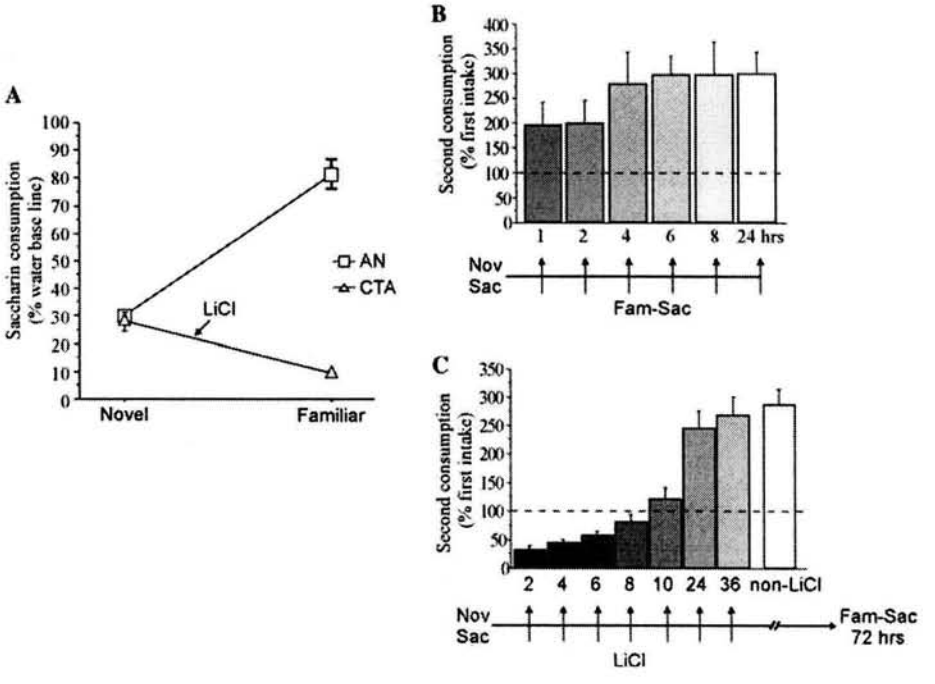

Fig. 1. (A) Percentage of baseline consumption (means ± SEM) during the first (novel) and second (familiar) sodium saccharin (sac) presentation. The triangles group received an i.p. injection of LiCl 15 min after first intake; hence this group developed conditioned taste aversion (CTA), whereas the squares group showed attenuation of neophobia (AN) because it did not receive the LiCl injection. In B and C are plotted the percentage of second saccharin intake with respect to the first saccharin intake. In B, consumption of familiar saccharin intake as a function of time elapsed from the first presentation (see bottom of figure) is plotted for animals that had not been injected with LiCl. In C, familiar saccharin consumption 72 h after the first taste intake in animals that had been injected with LiCl at different times after the first saccharin intake is shown.

each). Animals underwent CTA training as mentioned above, except that the intraperitoneal injection of LiCl was given 2, 4, 8, 10, 12, 24, or 36 h after the first taste presentation (Fig. 1C). It is known that the optimal CS-US association interval depends on two factors: the intensity of the taste (Braun & Rosenthal, 1976) and the degree of malaise factor (Nachman & Ashe, 1973). Therefore, in this experiment we increased the amount of LiCl injected to 0.4 M (3.74 mEq/kg).

2.3.2. Experiment 2

In order to determine if the muscarinic receptors in the insular cortex are involved at different times in AN or CTA, we used the following design: For each learning paradigm, four groups were used, one received insular cortex scopolamine microinjections 20 min before presentation of the taste and one received the microinjections immediately after the taste (for AN task, Scop-b, $n = 7$; Scop-a, $n = 7$; and for CTA task, Scop-b, $n = 22$; Scop-a, $n = 8$, respectively). Each scopolamine injected group had its corresponding vehicle (Ringer) control (for AN task, Veh-b, $n = 10$; Veh-a, $n = 12$; and for CTA task, Veh-b, $n = 23$; Veh-a, $n = 6$). The larger number of subjects in the groups Scop-b and Veh-b for CTA task is due to the fact that a replica was made of these groups, since in the first experiment we observed a significantly decreased learning in the scopolamine treated group compared to the vehicle group, but still with a strong taste aversion. Since the results were very similar and there were no significant differences, they were pooled together. The procedure consisted of measuring base line water consumption and injecting scopolamine or vehicle before or after the presentation of the taste solution on the fourth day as described in the design.

The CTA animals received an i.p. injection of LiCl (0.4 M; 2.94 mEq/kg) 15 min after the taste solution was retired, and AN animals had no treatment. Two more days of water baseline measurements were taken, and on the third day saccharin was again presented to all animals, followed by water as described in methods, and again on the two consecutive days after.

Statistical analysis. A one-way ANOVA or repeated measures ANOVA was used to compare means (±SEM) of ml consumed water or saccharin among groups, presented as a percentage of the water consumed during base line. The Fisher pairwise test was used for post hoc analysis with $p < .05$ considered significant.

3. Results

3.1. Experiment 1

Fig. 1A shows the normal behavior induced by stimulation with novel 0.5% saccharin solution. As it can be seen, all animals displayed a strong neophobic response to saccharin, reflected by the low intake of the solution with respect to the water baseline intake. The neophobic response disappeared by 24 h; the animals that did not receive a LiCl injection (AN) drank more saccharin during the second taste presentation than in the previous intake (see squares). Conversely, the animals that received a malaise inducing agent (LiCl) showed strong aversion to the familiar taste, this is observed by the low solution intake during their second taste presentation (familiar, see triangles). These results indicate that a novel taste might become depending on its consequences, in a familiar aversive or a familiar safe taste. With these results, we asked which is the critical period of time after the presentation of a novel taste that is required to allow it to become familiar safe or familiar aversive. In order to answer this question, naive rats were presented with novel saccharin, and a second saccharin presentation was given at distinct intervals as described in Section 2.3. As it can be seen in Fig. 1B the rats exhibited an attenuation of neophobia as early as 1 h and reached an asymptotic level at 6 h. Repeated measures ANOVA showed no significant differences among groups ($F_{(5,46)} = 0.966$, $p > .05$), but showed a significant effect with respect to the presentation day (1st vs. 2nd taste presentation; $F_{(1,46)} = 124$, $p < .0001$) and a significant interaction among groups and days ($F_{(1,46)} = 2.9$, $p < .05$), which indicates that all the groups increased their saccharin intake in the second presentation. This was not gradual, although it occurred with a clear tendency (see Fig. 1B).

Conversely, since the induction of malaise triggers the familiar aversive long-lasting trace, we systematically analyzed the effect of the interval between taste and malaise by means of a single i.p. injection of LiCl at seven different times after the first intake (Fig. 1C). As expected, the delay of LiCl onset gradually decreased the ability of rats to learn aversion ($F_{(7,72)} = 19.9$, $p < .0001$). Remarkably, there was an absence of learned an aversion in the groups 24 and 36 h, which despite LiCl injection, developed a preference for saccharin comparable to animals without malaise (compare Fig. 1B, 24 h). The post hoc analysis showed a gradual reduction of the learned aversion, i.e., the groups 2 and 4 h were different from those groups injected during the 10–36 h interval and control group (non-LiCl injection), whereas the 6–10 h groups were significantly different with respect to the 24–36 h and control group. Neither weight nor water baseline consumption were significantly different among groups (data not shown).

3.2. Experiment 2

To assess the role of muscarinic receptors in the insular cortex on both forms of processing (safe/aversive) of the TMT, scopolamine was microinjected either be-

fore or after TMT formation. All the animals showed similar levels of neophobia indicating an intact taste novelty perception (see first intake Figs. 2A, B, and D).

Regarding attenuation of neophobia, scopolamine microinjected before or after the novel taste prevented the attenuation of neophobia, i.e., in the second intake both scopolamine treated groups (Scop-b and Scop-a) drank similar amounts of saccharin such as those observed in the first trial, and were significantly different to those receiving vehicle ($F_{(3,32)} = 11.8$, $p < .0001$) Veh-b and Veh-a (Fig. 2A). On the second taste presentation the animals drank the saccharin solution in the absence of muscarinic receptor blockade. Hence, the animals enhanced their intake and showed a normal expression of AN in the third presentation (see Fig. 2A). These results indicate that the infusion of scopolamine into the insular cortex did not interfere permanently with the ability of animals to learn the safety of a taste solution.

The learned taste aversion was significantly reduced in subjects treated with scopolamine before (Scop-b), but not after the taste presentation (Scop-a) when compared with their respective control groups ($F_{(3,55)} = 5.2$, $p < .01$); (Figs. 2B and D). These differences persisted even in the first extinction trial ($F_{(3,55)} = 12.4$, $p < .001$ see Fig. 2B).

The histological results showed that the injection sites overlapped the granular and disgranular part of the insular cortex. Fig. 2C shows a typical cannula placement; 4 animals were excluded due to misplacement of the cannulae and 2 more subjects from the groups scop-b were eliminated on the day of microinjection due to a strong adipsia induced by scopolamine (Vila & Miranda, 1994; Poulos & Hinson, 1984).

4. Discussion

It is known that the sensory properties of tastants influence ingestive behavior, e.g., the degree of the innate neophobic response depends upon the concentration used (Domjan & Gillan, 1976), but learning is also critical. The results of Experiment 1 confirm that the central representation of the sensory characteristics of saccharin acquires predictive properties based on the post-ingestive consequences of their ingestion (Mattes, 2003). Hence, with the presentation of 0.5 % of saccharin to two groups of animals depending on the consequences, the learned behaviors ranged from taste aversions to preference. However, it is not clear if the aversion or preference learning share similar mechanisms, and if there is some interaction between them.

The main results of this study are that AN and CTA have a differential temporal sensitivity to muscarinic receptor blockade in the insular cortex. Usually, CTA studies use a low concentration of saccharin (0.1%) to avoid the presence of robust neophobia (Bures, 1998b) and eliminate a possible unspecific interaction with the taste aversion learning. However, we used a high concentration of saccharin solution sufficient to induce a robust neophobia in order to ascertain the role that the

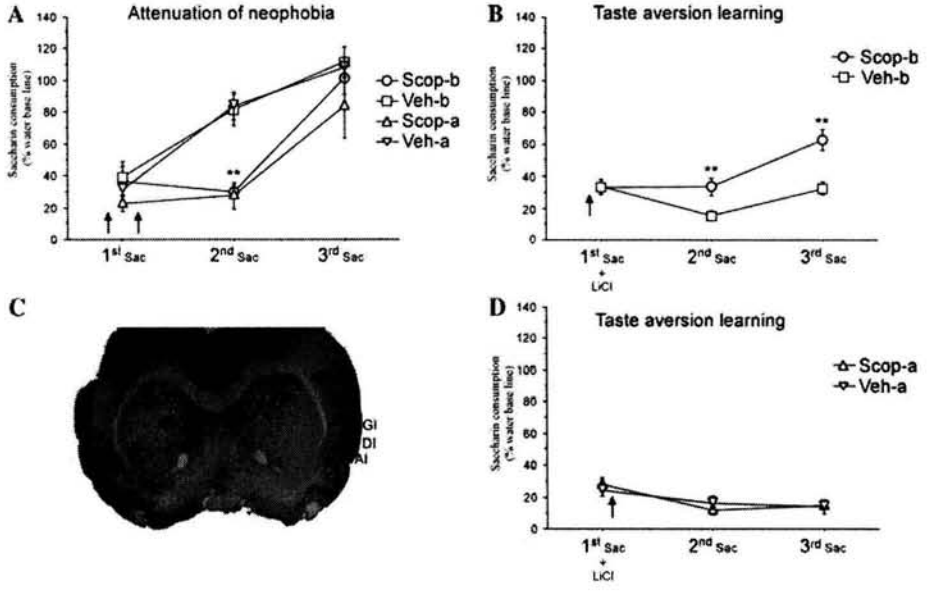

Fig. 2. Effect of scopolamine (Scop) or vehicle (Veh) microinjected bilaterally into the insular cortex 20 min before "b" or immediately after "a" the presentation of novel 0.5% saccharin solution in conditioned taste aversion (B and D) or in attenuation of neophobia (A). In A, B, and D are plotted the percentage of the mean (±SEM) of 15 min intake of saccharin relative to water baseline. (**$p < 0.01$) significantly different among groups. The interval between the first and the second saccharin intake was 72 h, while 24 h for the second to the third intake. (C) A photomicrograph of a coronal section of a rat brain stained with cresyl violet, showing the needle tract and the injection tip placement in the insular cortex. GI, DI, and AI; granular, disgranular, and agranular insular cortices, respectively.

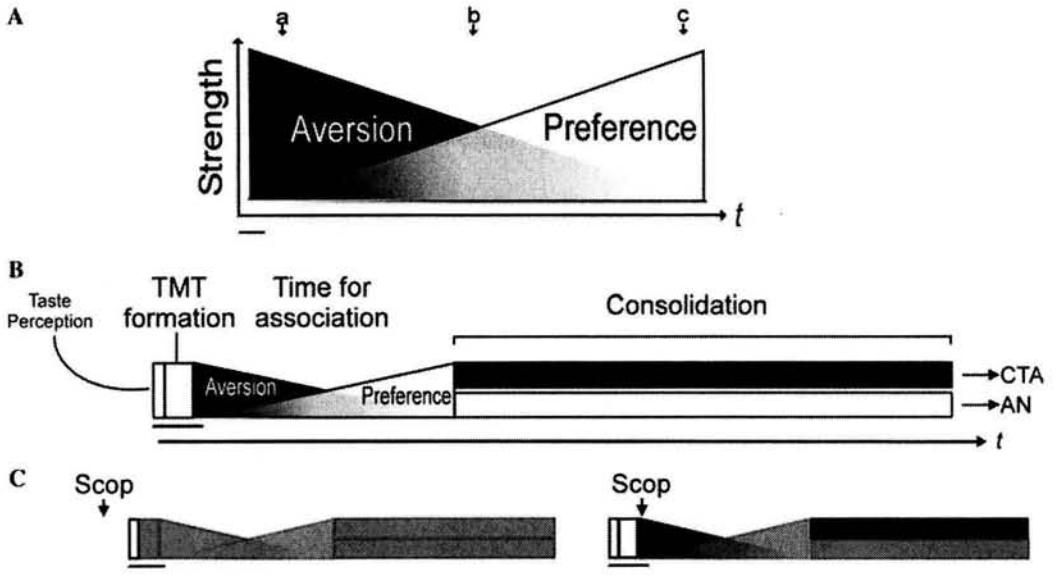

Fig. 3. (A) Graphical representation of the learned safety theory, which proposes that when a rat ingests a harmless novel substance (*bottom short line*), it gradually learns over time that the substance is safe to consume. The longer the time period after the ingestion, more taste safety is learned and hence less aversion will be manifested if the animal is made sick (Nachman & Jones, 1974). *Arrows* represent possible onset of malaise, e.g., if the malaise is induced at time "a" the rat will learn a greater aversion than in time "b"; however once that associable period for aversion is over, (*black triangle*), animals will develop attenuation of neophobia, (*white triangle*), even when malaise occurred in time "c." (B) The time course of events that would occur after novel taste stimulation (*bottom short line*). Briefly, the taste perception first triggers taste memory trace (TMT) formation, after which a transient "associable" period is opened. If malaise is induced during this interval then the association is formed and a consolidation process begins (*Black rectangle*). On the other hand, if malaise did not occur in the associable period, then a consolidation of the learned safety inhibits the development of CTA. (C) Schematic representation of the proposed scopolamine effects in the insular cortex on CTA (*black shapes*), and AN (*white shapes*). Grey shapes indicate disrupting effects, e.g., infusion of scopolamine before taste (*left*) degraded the TMT formation which is probably shared by both CTA and AN. The scopolamine infusion after the taste (*right*) prevented the consolidation of the learned safety, but left intact CTA association and consolidation (see text for alternative hypothesis).

cortical muscarinic receptors play in both aversive and safe familiar taste memory formation (Gutierrez et al., 2003; Nachman & Jones, 1974).

The results presented herein show that the neophobic response was not affected with the muscarinic receptor blockade, which indicates a normal taste perception of the saccharin solution, in accordance with previous results (Gutierrez et al., 2003; Naor & Dudai, 1996). Nevertheless, the infusion of scopolamine before and after taste prevented the attenuation of neophobia. It seems that the animals perceived the familiar taste as "novel" again as we proposed elsewhere (Gutierrez et al., 2003). We previously demonstrated that this effect could not be explained by state dependency or because scopolamine induced aversion, since application of scopolamine after novel 0.1% saccharin presentation did not produce noticeable CTA (Gutierrez et al., 2003; Naor & Dudai, 1996). In addition, these results extend our previous observations indicating that the blockade of AN by scopolamine is also observable with an interval of three days between first and second taste presentation. In addition, scopolamine left intact the ability to learn the safety of a taste solution during the subsequent days, since in the absence of scopolamine, the animals showed AN in their third saccharin presentation (Fig. 2A).

Previous studies using a low concentration of saccharin (0.1%) have demonstrated that the infusion of scopolamine in the insular cortex before but not after a novel taste impaired the CTA learning, leaving a certain portion of residual learning (see below) (Ferreira et al., 2002; Berman, Hazvi, Neduva, & Dudai, 2000; Naor & Dudai, 1996). Our results are in agreement with those observations even with the use of a highly concentrated saccharin solution. However, since this solution induces a robust neophobic response special considerations must be stressed. First, despite the evidence with low concentrations of a saccharin solution (Berman et al., 2000), if we suppose that scopolamine injected before the taste completely disrupted the CTA, the animals would not learn the aversive consequence of the taste. If this were true, then the expected behavior in their second taste experience would be an attenuation of neophobia, since that is the usual behavior that the non-LiCl groups show. However, the animals drank a similar amount of saccharin solution during the second saccharin presentation, as if it were the first presentation. These results would still be consistent with the first supposition, since scopolamine also prevents AN when the taste is not paired with malaise. These results suggest that perhaps the animals perceived the familiar taste as "novel" again, as the non-LiCl plus scopolamine treatment

groups presumably did. Nevertheless, this conclusion could not be completely true, since using a low saccharin concentration the scopolamine infused in the insular cortex does not eliminate the CTA completely; hence, it is less probable that the scopolamine's effect improves using a high taste concentration since the learned aversion's strength increases with the augment of the taste concentration (Garcia, Hankins, & Rusiniak, 1974; Lorden, 1976). Therefore, it is more probable that these animals displayed less aversion in their second intake and a fast extinction in the third saccharin presentation rather than "neophobia and AN," respectively.

Interestingly, when animals were microinjected immediately after the taste presentation, they developed strong taste aversion almost undistinguishable from vehicle groups, as both showed a lower saccharin intake during the test, and this behavior remained until the last extinction trial. Similar results have been traditionally used to argue that acetylcholine activity in the insular cortex is not involved in the association with aversive consequences (Deutsch, 1978) (Miranda & Bermudez-Rattoni, 1999; Naor & Dudai, 1996). Our results confirm that statement with the use of a highly concentrated saccharin solution. A similar result, but using functional decortication, was used by Buresova and Bures (1973) to hypothesize that the formation of the TMT requires cortical participation. Once the TMT has been formed, the aversive processing of the TMT may persist in subcortical structures, where it could be associated with aversive gastrointestinal consequences probably in the lateral amygdala (Swank, 2000). However, we find arguments against to this view, since our present results and former studies (Gutierrez et al., 2003) demonstrated that the preference (safety) processing of the TMT seems to be muscarinic dependent and remains at cortical level for at least 2 h.

Altogether, these results not only suggest that AN requires muscarinic receptor activity from the insular cortex after taste stimulation, but more importantly, it supports the idea that CTA and AN initially share a similar component of the TMT (Buresova & Bures, 1980). Nevertheless, both behaviors could use different pathways for long-lasting memory traces. We have recently proposed that the safe processing responsible for AN depends on cortical muscarinic receptor but not on NMDA receptor activity (Gutierrez et al., 2003). In contrast, the aversive processing responsible for CTA seems to be muscarinic receptor independent, once the TMT has been formed (see Fig. 3C right). Our results confirm behavioral (Braveman & Jarvis, 1978; Domjan, 1976), pharmacological (Buresova & Bures, 1980; Aguado, San Antonio, Perez, del Valle, & Gomez, 1994; Ellis & Kesner, 1981) and electrical stimulation studies (Kesner & Berman, 1977) that suggested that after TMT formation, AN and CTA learning depend upon different brain mechanisms. In this regard, Kesner and Berman (1977) found that electrical stimulation of midbrain reticular formation or infusion of norepinephrine in the basolateral amygdala (Ellis & Kesner, 1981) after novel grape juice intake, disrupt AN, but leave CTA intact. Therefore, it is possible that safe and aversive processing of the TMT could be carried out in an independent way through subcortical areas like the midbrain reticular formation, amygdala and cortical regions like the insular cortex. It should be pointed out that the exact role of muscarinic receptors in these subcortical structures still remains to be determined in both CTA and AN learning.

It becomes clear that cells of the insular cortex expressing muscarinic receptors could not be participating in novelty perception, since the rats showed strong neophobic response under blockade of these receptors. The results presented here suggest that these cells encode the taste stimulus as already experienced and it would be recognized in future presentations as familiar. However, as noted, there are at least two types of familiar tastants, aversive/safe (Rozin, 1977), and our results indicate differential effects of muscarinic receptor blockade before and after stimulation. Therefore, before the TMT formation these cells seem to be important to both kinds of familiar tastes, but once the TMT presumably has been formed; their participation is restrained to the safe familiar taste consolidation.

Nevertheless, It should be pointed out that several cholinergic drugs change their efficiency under stress situation, e.g., foot shock (Kaneto, 1997; Kant et al., 2001). It would be possible that the arousal of sickness induced by LiCl injection also induces stress. If so, it would be an alternative explanation to the absence of impairment found when scopolamine was administered immediately after taste in the CTA task. Against this hypothesis, it has been demonstrated that an i.p. injection of LiCl did not induce a significant release of ACh in the insular cortex (Miranda & Bermudez-Rattoni, 1999). Therefore, if LiCl-stress reduces the efficacy of scopolamine it could be by an independent cortical cholinergic mechanism (Smythe, Murphy, & Costall, 1996). Since it seems almost impossible to separate the sickness and the stress induced by LiCl injection, further investigations of the scopolamine efficiency in the attenuation of neophobia with or without stress (induced, e.g., by food shock) are necessary to assess the viability of this hypothesis.

It is possible to summarize that the encoding of a novel taste starts with the perception of its physical attributes that stimulates the taste receptor cells within the oral cavity. The taste perception processes should take around 200 ms, since in this timeframe rats can discriminate tastants from water (Halpern & Tapper, 1971), but should require less than 3 sec according to electrical recordings of the insular cortex (Katz, Simon, & Nicolelis, 2001; Katz, Nicolelis, & Simon, 2002). One

of the results of this perceptual analysis is the formation of the TMT, which would be formed probably between 10 s and 10 min, since shorter intervals between taste and malaise do not induce CTA learning (Schafe, Sollar, & Bernstein, 1995). The results of Experiment 1 suggest that the TMT has a transient ability to be associated with gastric malaise, which decays slowly (see Fig. 3A). Conversely, the association with preference signals is gradually enhanced as predicted by the learned safety theory (Nachman & Jones, 1974). Accordingly, the TMT would have two associative phases that process the TMT in two independent ways: one related with positive (preference) and the other related with the processing of aversive consequences. These phases would continue for a critical time after the intake, usually hours depending on the tastant, (Kalat & Rozin, 1971). Herein, we found that saccharin 0.5% has an interval of less than 24 h for taste aversion learning whereas requiring less than 6 h for safety association. We found that AN could recruits the participation of cortical muscarinic receptors at different times than those used in conditioned taste aversion. We conclude that the taste memory trace is not a unitary process but rather seems to have at least two dissociable components, with (safe/aversive) being the first and as dependent on cortical muscarinic activity for consolidation and the second seems to be independent.

Acknowledgments

This work was supported by CONACYT-Mexico Grants MRI W8072-35806-N and DGAPA-IN215001. We acknowledge the technical assistance of Federico Jandete, Oreste Carbajal, Francisco Pérez Eugenio, Yolanda Díaz de Castro and give thanks Christopher Ormsby and Jennifer Stapleton for English revision of the final text.

References

Aguado, L., San Antonio, A., Perez, L., del Valle, R., & Gomez, J. (1994). Effects of the NMDA receptor antagonist ketamine on flavor memory: conditioned aversion, latent inhibition, and habituation of neophobia. *Behavioral and Neural Biology, 61*, 271–281.

Berman, D. E., Hazvi, S., Neduva, V., & Dudai, Y. (2000). The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste: activation of ERK1-2 and formation of a memory trace. *Journal of Neuroscience, 20*, 7017–7023.

Bermudez-Rattoni, F., & McGaugh, J. L. (1991). Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion. *Brain Research, 549*, 165–170.

Braun, J. J., & Rosenthal, B. (1976). Relative salience of saccharin and quinine in long-delay taste aversion learning. *Behavioral Biology, 16*, 341–352.

Braun, J. J., Slick, T. B., & Lorden, J. F. (1972). Involvement of gustatory neocortex in the learning of taste aversions. *Physiology & Behavior, 9*, 637–641.

Braveman, N. S., & Jarvis, P. S. (1978). Independence of neophobia and taste aversion learning. *Animal Learning & Behavior, 6*, 406–412.

Bures, J. (1998a). Ethology, physiological psychology, and neurobiology of CTA. In J. Bures, F. Bermudez-Rattoni, & T. Yamamoto (Eds.), *Conditioned taste aversion: memory of a special kind* (pp. 1–10). New York: Oxford science publications.

Bures, J. (1998b). The CTA paradigm: terminology, methods, and conventions. Memory of a special kind. In J. Bures, F. Bermudez-Rattoni, & T. Yamamoto (Eds.), *Conditioned taste aversion* (pp. 14–25). New York: Oxford science publications.

Buresova, O., & Bures, J. (1973). Cortical and subcortical components of the conditioned saccharin aversion. *Physiology & Behavior, 11*, 435–439.

Buresova, O., & Bures, J. (1980). Post-ingestion interference with brain function prevents attenuation of neophobia in rats. *Behavioural Brain Research, 1*, 299–312.

Deutsch, R. (1978). Effects of atropine on conditioned taste aversion. *Pharmacology Biochemestry & Behavior, 8*, 685–694.

Domjan, M. (1977). Attenuation and enhancement of neophobia for edibles substance. In L. M. Barker, M. A. Best, & M. Domjan (Eds.), *Learning mechanisms in food selection* (pp. 151–179). TX: Baylor University Press.

Domjan, M. (1976). Determinants of the enhancement of flavored-water intake by prior exposure. *Journal of Experimental Psychology Animal behavior processes, 2*, 17–27.

Domjan, M., & Gillan, D. (1976). Role of novelty in the aversion for increasingly concentrated saccharin solutions. *Physiology & Behavior, 16*, 537–542.

Ellis, M. E., & Kesner, R. P. (1981). Physostigmine and Norepinephrine: effects of injection into the amygdala on taste associations. *Physiology & Behavior, 27*, 203–209.

Ferreira, G., Gutierrez, R., De La Cruz, V., & Bermudez-Rattoni, F. (2002). Differential involvement of cortical muscarinic and NMDA receptors in short- and long-term taste aversion memory. *European Journal of Neuroscience, 16*, 1139–1145.

Gallo, M., Roldan, G., & Bures, J. (1992). Differential involvement of gustatory insular cortex and amygdala in the acquisition and retrieval of conditioned taste aversion in rats. *Behavioural Brain Research, 52*, 91–97.

Garcia, J., Hankins, W. G., & Rusiniak, K. W. (1974). Behavioral regulation of the milieu interne in man and rat. *Science, 185*, 824–831.

Garcia, J., Kimeldorf, D. J., & Koelling, R. A. (1955). Conditioned aversion to saccharin resulting for exposure to gamma radiation. *Science, 122*, 157–158.

Green, K. F., & Garcia, J. (1971). Recuperation from illness: flavor enhancement for rats. *Science, 20*, 749–751.

Gutierrez, R., Tellez, L. A., & Bermudez-Rattoni, F. (2003). Blockade of cortical muscarinic but not NMDA receptors prevents a novel taste from becoming familiar. *European Journal of Neuroscience, 17*, 1556–1562.

Halpern, B. P., & Tapper, D. N. (1971). Taste stimuli: quality coding time. *Science, 171*, 1256–1258.

Kalat, J. W., & Rozin, P. (1971). Role of interference in taste-aversion learning. *Journal Comparative and Physiological Psychology, 77*, 53–58.

Kaneto, H. (1997). Learning/memory processes under stress conditions. *Behavioural Brain Research, 83*, 71–74.

Kant, G. J., Bauman, R. A., Feaster, S. R., Anderson, S. M., Saviolakis, G. A., & Garcia, G. E. (2001). The combined effects of pyridostigmine and chronic stress on brain cortical and blood acetylcholinesterase, corticosterone, prolactin and alternation performance in rats. *Pharmacology, Biochemistry and Behavior, 70*, 209–218.

Katz, D. B., Nicolelis, M. A., & Simon, S. A. (2002). Gustatory processing is dynamic and distributed. *Current Opinion in Neurobiology, 12*, 448–454.

Katz, D. B., Simon, S. A., & Nicolelis, M. A. (2001). Dynamic and multimodal responses of gustatory cortical neurons in awake rats. *Journal of Neuroscience, 21*, 4478–4489.

Kesner, R. P., & Berman, R. F. (1977). Effects of midbrain reticular formation, hippocampal, and lateral hypothalamic stimulation upon recovery from neophobia and taste aversion learning. *Physiology & Behavior, 18*, 763–768.

Lorden, J. F. (1976). Effects of lesions of the gustatory necortex on taste aversion learning in the rat. *Journal Comparative and Physiological Psychology, 90*, 665–679.

Mattes, R. (2003). Taste: It's more than meets the tongue. *Chemo Sense, 5*, 9–14.

Miranda, M. I., & Bermudez-Rattoni, F. (1999). Reversible inactivation of the nucleus basalis magnocellularis induces disruption of cortical acetylcholine release and acquisition, but not retrieval, of aversive memories. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 6478–6482.

Nachman, M., & Ashe, J. H. (1973). Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. *Physiology & Behavior, 10*, 73–78.

Nachman, M., & Jones, D. R. (1974). Learned taste aversions over long delays in rats: The role of learned safety. *Journal Comparative and Physiological Psychology, 86*, 949–956.

Naor, C., & Dudai, Y. (1996). Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. *Behavioural Brain Research, 79*, 61–67.

Paxinos, G., & Watson, C. (1986). *The rat brain in stereotaxic coordinates* (3rd ed.). San Diego: Academic press.

Poulos, C. X., & Hinson, R. E. (1984). A homeostatic model of Pavlovian conditioning: tolerance to scopolamine-induced adipsia. Journal of experimental psychology. *Animal behavior processes, 10*, 75–89.

Ramirez-Lugo, L., Miranda, M. I., Escobar, M. L., Espinosa, E., & Bermudez-Rattoni, F. (2003). The role of cortical cholinergic pre- and post-synaptic receptors in taste memory formation. *Neurobiology of Learning and Memory, 79*, 184–193.

Rozin, P. (1977). The significance of learning mechanisms in food selection: somebiology, psychology and sociology of science. In L. M. Barker, M. A. Best, & M. Domjan (Eds.), *Learning mechanisms in food selection* (pp. 557–589). TX: Baylor University Press.

Schafe, G. E., Sollar, S. I., & Bernstein, I. L. (1995). The CS-US Interval and taste aversion learning: a brief look. *Behavioral Neuroscience, 109*, 799–802.

Smythe, J. W., Murphy, D., & Costall, B. (1996). Benzodiazepine receptor stimulation blocks scopolamine-induced learning impairments in a water maze task. *Brain Research Bulletin, 41*, 299–304.

Swank, M. W. (2000). Phosphorilation of MAP kinase and CREB in mouse cortex and amygdala during taste aversion learning. *Learning and Memory, 11*, 1625–1630.

Vila, J., & Miranda, F. (1994). Pavlovian inhibitory conditioning and tolerance-reversal to scopolamine-induced adipsia in rats. *Behavioral Neural Biology, 61*, 177–180.

Welzl, H., Alessandri, B., & Bättig, K. (1990). The formation of a new gustatory memory trace in rats is prevented by the noncompetitive NMDA antagonist ketamine. *Psychobiology, 18*, 43–47.

Research

Perirhinal Cortex Muscarinic Receptor Blockade Impairs Taste Recognition Memory Formation

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The relevance of perirhinal cortical cholinergic and glutamatergic neurotransmission for taste recognition memory and learned taste aversion was assessed by microinfusions of muscarinic (scopolamine), NMDA (AP-5), and AMPA (NBQX) receptor antagonists. Infusions of scopolamine, but not AP5 or NBQX, prevented the consolidation of taste recognition memory using attenuation of neophobia as an index. In addition, learned taste aversion in both short- and long-term memory tests was exclusively impaired by scopolamine. These data provide neurochemical support for the theory that cholinergic activity of the perirhinal cortex participates in the formation of the taste memory trace and that it is independent of the NMDA and AMPA receptor activity. These results support the idea that cholinergic neurotransmission in the perirhinal cortex is also essential for acquisition and consolidation of taste recognition memory.

Perirhinal cortex has been defined as a higher-order polymodal associational cortex because it receives both unimodal input from all sensory modalities as well as afferents from polymodal association areas (Burwell 2001). The principal pathways come from frontal, parietal, temporal, cingulate, occipital, and subicular cortices (Deacon et al. 1983). In addition, perirhinal cortex sends direct and indirect (through entorhinal cortex) projections to the hippocampus (Burwell and Amaral 1998), a structure involved in plasticity and learning (Martin and Morris 2002). Moreover, it should be noted that the perirhinal cortex is interconnected with the amygdala and the insular cortex (McDonald and Jackson 1987; Burwell and Amaral 1995), brain regions responsible for gustatory learning and memory in the rat (Lamprecht and Dudai 1996; Yasoshima and Yamamoto 1997; Bermudez-Rattoni and Yamamoto 1998).

Lesions to the perirhinal cortex induce several cognitive deficits that range from difficulties recognizing an item that has been previously presented (recognition memory; Mandler 1980), to impairments in cross-modal associative memories (for review, see Murray and Richmond 2001). For example, damage to the perirhinal and entorhinal cortices (referred to as rhinal cortex) impairs cross-modal tactual–visual recognition memory (Goulet and Murray 2001) as well as cross-modal flavor–visual associative memory in which the monkey must use an association between a food cue and a visual object to get a food reward (Parker and Gaffan 1998). In addition, injury to the perirhinal cortex (Meunier et al. 1993; Mumby and Pinel 1994; Malkova et al. 2001) as well as perirhinal microinjections of the cholinergic muscarinic receptor blocker scopolamine impair visual recognition memory (Tang et al. 1997), whereas intraperitoneal administration of physostigmine, a cholinesterase inhibitor, improves it (Aigner and Mishkin 1986). These results indicate the relevance of the perirhinal cortex cholinergic activity for normal visual recognition memory. In addition, diminished performance on tactual (Buffalo et al. 1999) and olfactory recognition memory tasks has been reported in animals lacking perirhinal cortex (Otto and Eichenbaum 1992). However, auditory recognition memory is not affected by lesions of the perirhinal cortex (Kowalska et al. 2001).

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Article and publication are at http://www.learnmem.org/cgi/doi/10.1101/lm.69704.

Scarce information about the participation of perirhinal cortex on taste recognition memory is available in the literature (Brown and Xiang 1998). Because eating and drinking are multimodal integration processes, that is, the aspect (visual), odor (olfactory), texture (somatosensory), and taste of a foodstuff must be integrated in specific brain regions to predict how tasty or disgusting an edible might be, and because it is known that the perirhinal cortex integrates almost all sensory modalities to identify an object as the same even in different contexts or perspectives (Murray and Richmond 2001), the perirhinal cortex emerges as a plausible integration region of taste information with that of other sensory systems. In this regard, it was reported that perirhinal cortex is involved in conditioned taste aversion (CTA), in which a palatable taste changes from positive to aversive because of a malaise-inducing agent injection. Thus, Tassoni et al. (2000), using a sodium channel blocker (TTX), reported that temporal inactivation of the perirhinal cortex when rats drink a novel taste solution impairs CTA, but the impairment was not observed when inactivation was induced between taste exposure and induction of malaise (association phase) or before the retrieval test. These results indicate that the perirhinal cortex is involved in the formation of the taste memory trace (TMT; for definition, see Gutierrez et al. 2003b), but not in the association or retrieval of learned taste aversion.

Therefore, in this study we assessed the participation of cholinergic and glutamatergic receptors of the perirhinal cortex on taste recognition memory and learned taste aversion. We used the innate neophobic behavior as a novelty index. This behavior consists of a reduced fluid consumption that rats show when exposed to a novel taste solution. This response is attenuated in subsequent presentations (Nachman and Jones 1974; Domjan 1976); that is, rats drink more when the taste is familiar. Attenuation of neophobia (AN) was therefore used as an index of taste recognition memory. It should be stressed that AN is a long lasting behavior that persists for days (Buresova and Bures 1980) or even months (Best et al. 1978), and it is dependent on muscarinic receptor activity of the insular cortex (gustatory cortex; Gutierrez et al. 2003a,b).

To evaluate in more detail the participation of perirhinal cortex on learned taste aversion, microinjections of scopolamine were performed in this area. It has been previously demonstrated that scopolamine impairs specifically the formation of the TMT

when it is injected in the insular cortex (Naor and Dudai 1996; Ferreira et al. 2002; Gutierrez et al. 2003b). In addition, NMDA and AMPA receptor blockers were also applied because previous reports indicate that glutamatergic activity is required in the insular cortex for CTA long-term memory but not for taste memory trace formation (Ferreira et al. 2002; Gutierrez et al. 2003a,b).

RESULTS

Taste Recognition Memory

To assess the role of muscarinic, NMDA, and AMPA receptors in the perirhinal cortex on taste recognition memory, respective antagonists were microinjected either before or after novel taste presentation. A highly concentrated saccharin solution (0.5%) was used, which is known to increase the robustness of neophobia; that is, rats innately drink much less saccharin solution in comparison with their normal water consumption (cf. Fig. 1, first sacch vs. Fig. 2, acquisition). All animals showed comparable neophobic response to saccharin ($F_{(3, 82)} = 1.1$; $p > 0.05$), drinking on average only 31% (3.7 ± 0.25 mL) relative to water baseline (Fig. 1A,B). On the second taste experience (Fig. 1A, second sacch), the group treated with scopolamine drank similar levels of saccharin solution as in the previous presentation, whereas all the other groups increased their saccharin intake, showing the expected attenuation of neophobia ($F_{(3, 47)} = 14$; $p < 0.0001$). In the third taste experience (third sacch), there were still significant differences between the scopolamine group and the vehicle group ($F_{(3, 47)} = 5$, $p < 0.01$). However, despite these differences, rats that received scopolamine displayed a normal attenuation of neophobia because they drank as the control group did during their second saccharin intake. These results indicate that scopolamine does not induce a permanent effect on taste recognition. It should be noted that the activity of scopolamine lasts less than 24 h (Sipos et al. 1999), and it was only injected on the first saccharin presentation.

The results were similar when scopolamine was microinjected immediately after novel taste presentation; that is, there were no significant differences on the first saccharin intake ($F_{(1, 35)} = 2.8$, $p > 0.05$). However, on the second taste experience, scopolamine prevented attenuation of neophobia ($F_{(1, 35)} = 83.5$, $p < 0.0001$). Moreover, on the third taste presentation, there were still significant differences among groups ($F_{(1, 35)} = 7.8$, $p > 0.01$; Fig. 1B).

As can be seen in Figure 1A, the inactivation of AMPA or NMDA receptors before taste presentation did not impair neophobia or attenuation of neophobia, which indicates that taste recognition memory can persist even in the absence of activity of these receptors in the perirhinal cortex.

Long-Term Memory of Learned Taste Aversion

Microinjection of scopolamine, AP-5 or NBQX in the perirhinal cortex, was performed to assess their effect on conditioned taste aversion using a 15-min long-term memory test 72 h after the acquisition trial (as described below in "Behavioral Procedures and Experimental Design"). In all the experiments described herein, neither weight nor baseline water intake was significantly different among the groups (data not shown).

The four groups that received either vehicle, scopolamine or AP-5 or NBQX microinjection, 20 min before the acquisition trial (see Materials and Methods) showed similar consumption of a novel 0.1% saccharin during a 15-min session compared with water baseline intakes ($F_{(3, 36)} = 1.8$, $p > 0.05$). The means (±SEM) were 14.4 ± 1.2 mL, 12.1 ± 0.85 mL, 13.6 ± 0.90 mL, and 14.3 ± 1.1 mL for each of the Veh, Scop, AP5, and NBQX groups, respectively. However, as can be seen in Figure 2A, on the second taste presentation (LTM test) the control group as well as the AP5- and NBQX-treated groups showed similar reductions on saccharin intake, indicating comparable levels of learned taste aversion, unlike the Scop group, which drank substantially more saccharin solution than the other groups ($F_{(3, 36)} = 4.7$, $p < 0.01$).

Because scopolamine impaired CTA when injected before taste presentation, we decided to analyze its effects on taste–malaise association phase. Therefore, this drug was microinjected immediately after taste but before malaise induction. As can be seen in Figure 2B, the differences between vehicle and scopolamine groups were not statistically different on the acquisition day ($F_{(1, 13)} = 0.80$, $p > 0.05$). On the test trial, the means (±SEM) were 13.8 ± 0.80 mL for the Veh-A and 13.3 ± 1.2 mL for the Scop-A group; there were no significant differences between groups ($F_{(1, 13)} = 0.05$, $p > 0.05$). These results indicate that scopolamine's impairment observed on CTA is limited to an early taste processing phase.

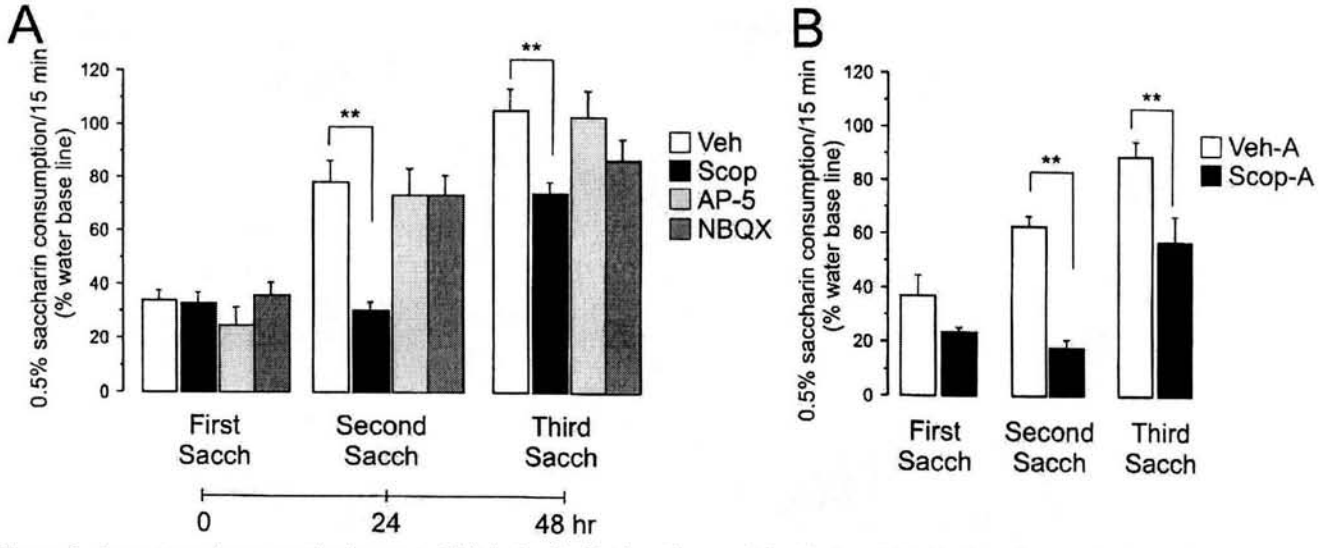

Figure 1 Percentage of consumption (mean ± SEM) during the first (novel), second (familiar), and third 0.5% sodium saccharin (sacch) presentation compared with water baseline; every saccharin presentation was given at 24-h intervals. Drugs were injected before (*A*) or immediately after (*B*) taste presentation.

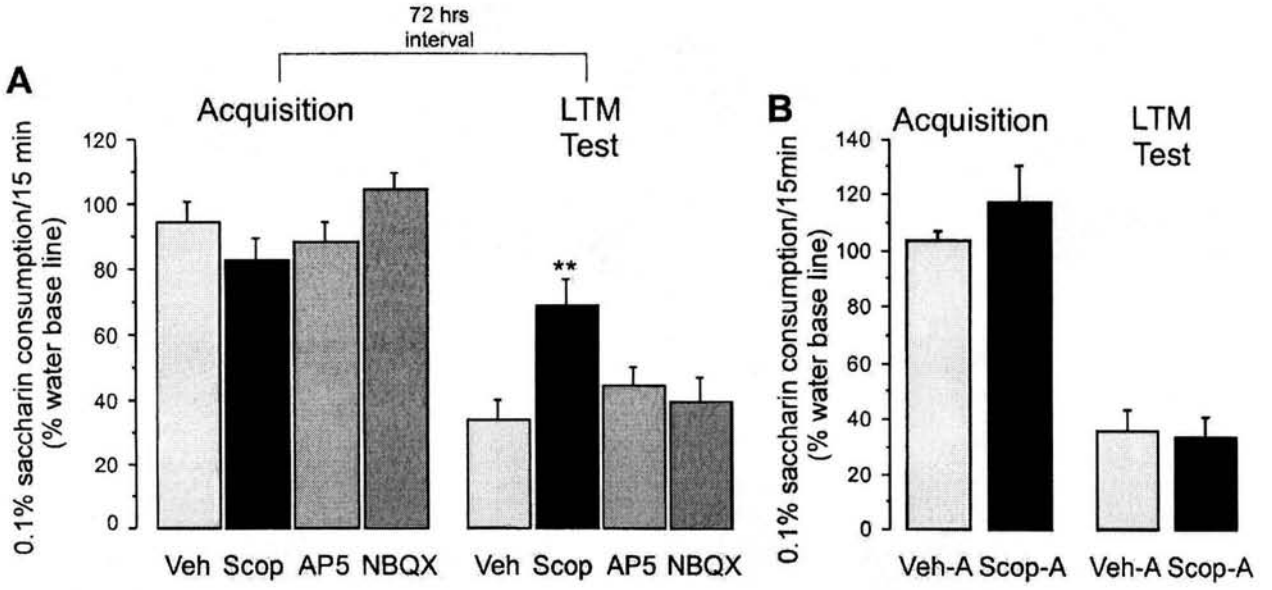

Figure 2 Effect of muscarinic receptor antagonist, scopolamine (Scop), NMDA receptor antagonist, (AP-5), AMPA receptor antagonist (NBQX), and vehicle (Veh) microinjected bilaterally into the perirhinal cortex upon conditioned taste aversion (CTA). (*A*) The microinjection was given 20 min before taste presentation on the acquisition day (saccharin followed by malaise-inducing agent injection; LiCl). (*B*) The microinjection was given immediately after taste exposure but before malaise induction on the acquisition day. In both *A* and *B* the long-term memory test (LTM; saccharin) was given 72 h after the acquisition trial. (**) $p < 0.01$ significantly different among groups.

Short-Term Memory of Learned Taste Aversion

The observed effects by scopolamine on long-term memory could be due to disruption of the TMT. Because it has been reported that muscarinic receptor blockade of the insular cortex impairs both short- and long-term memory of CTA (Ferreira et al. 2002), we decided to analyze its participation on the latter kind of memory. Therefore, scopolamine was microinjected in the perirhinal cortex 20 min before novel taste presentation, and these animals received a 15-min short-term memory test 4.5 h after acquisition trial (as described in Materials and Methods), as well as the long-term memory test.

On the acquisition trial, both vehicle and scopolamine groups drank the complete 10-mL saccharin solution ration used. As can be seen in Figure 3, during the short-term memory test there were differences between groups ($F_{(1,\ 17)} = 8.1$, $p < 0.05$). The scopolamine-treated group drank more saccharin solution than the control group, drinking 6.9 ± 1.2 mL (56%) of saccharin solution compared with 2.7 ± 0.73 mL (21%) for the control group (Fig. 3). These results indicate that, unlike vehicle, scopolamine treatment impairs short-term memory of CTA. Remarkably, neither water intake ($F_{(1,\ 17)} = 3.9$, $p > 0.05$; Veh, 6.6 ± 0.46; Scop, 4.4 ± 1.2) nor total fluid consumption (saccharin plus water) ($F_{(1,\ 17)} = 1.8$, $p > 0.05$; Veh, 9.3 ± 0.70; Scop, 11.3 ± 1.2) was significantly different between the groups.

The scopolamine group also drank more saccharin solution than the vehicle group in the long-term memory test ($F_{(1,\ 17)} = 23.9$, $p < 0.0001$). These results confirm the participation of perirhinal cortical muscarinic receptors on long-term memory of CTA (Fig. 2). Nevertheless, it was observed that the scopolamine-treated group drank more saccharin in the long-term memory test in this experiment, when a STM test was given, than they drank in previous experiments when the STM test was not performed (cf. the Scop group in Fig. 2A vs. Fig. 3). A comparison was made between the effect of scopolamine in both experiments. A two-way ANOVA (treatment [Veh vs. Scop] × experiment [with STM vs. without STM test]) revealed significant effect of treatment ($F_{(1,\ 34)} = 31.4$; $p < 0.0001$) and experiment ($F_{(1,\ 34)} = 18.7$, $p < 0.0001$), but not for treatment × experiment interaction ($F_{(1,\ 34)} = 3$, $p > 0.05$), meaning that in both experiments scopolamine impaired the long-term memory test. However, saccharin intake was different between experiments as indicated by the ANOVA analysis. A subsequent one-way ANOVA for these groups ($F_{(3,\ 34)} = 18.6$, $p < 0.0001$) and a post hoc test demonstrated that both scopolamine-treated groups are different ($p < 0.0001$) but vehicle groups are not ($p > 0.05$). This distinction might be caused by an accelerated extinction process triggered during the STM test on the scopolamine-infused group, reflecting the impairment induced by this treatment.

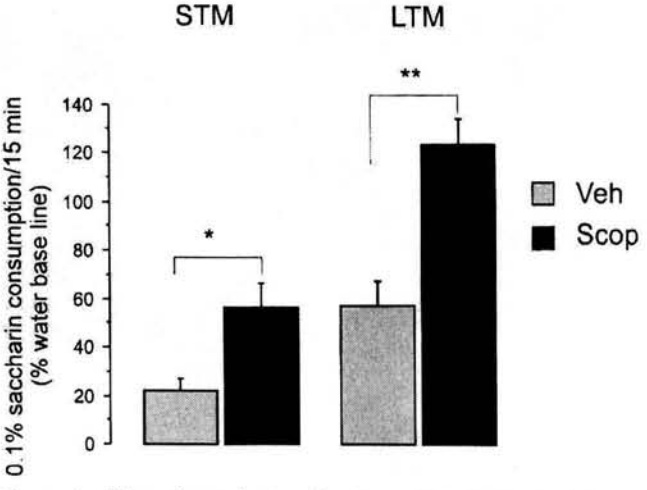

Figure 3 Effect of scopolamine (Scop) or vehicle (Veh) microinjected into perirhinal cortex 20 min before taste presentation on the acquisition day upon short-term memory (STM) and long-term memory (LTM) of conditioned taste aversion (CTA). To measure STM, both groups were presented with saccharin solution for the second time, 4.5 h after the acquisition trial. Three days after that, the LTM test was performed; that is, rats drank 0.1% saccharin solution for the third time during 15 min. (*) $p < 0.05$; (**) $p < 0.0001$ for difference between groups with respect to the control group.

Histology

According to Burwell (2001), perirhinal cortex comprises two narrow strips of cortex, areas 35 and 36, that are located along the rhinal sulcus. Figure 4 shows two representative sites of microinjection. In the first subject (Fig. 4A), the injector tip was localized just above area 36, whereas for the second subject (Fig. 4B), it was in area 35 (ectorhinal and perirhinal cortex, respectively, according to Paxinos and Watson 1998).

Although the rostral limit of perirhinal cortex is still controversial, we adopted the criteria used by Burwell (2001). Thus, we took the rostral perirhinal cortex as arising at the caudal limit of the claustrum, which equals −2.56 mm from Bregma (Paxinos and Watson 1998), whereas the caudal limit was considered as −6.72 mm from Bregma. Figure 4 shows the most rostral and caudal injection sites. All the microinjections were given in the anterior portion of the perirhinal cortex approximately from −3 mm to −3.6 mm relative to Bregma. One animal was excluded because of misplaced cannulae.

DISCUSSION

In the present report we studied the participation of perirhinal muscarinic, NMDA, and AMPA receptors in two different gustatory learned behaviors: conditioned taste aversion and attenuation of neophobia. Our results indicate that blockade of cholinergic muscarinic receptors but not NMDA or AMPA receptors in the perirhinal cortex impaired taste recognition memory as well as conditioned taste aversion. It is well known that the amygdala is important for learned taste aversion, neophobia, and attenuation of neophobia (Ellis and Kesner 1981; Yasoshima et al. 2000). Because the amygdala is anatomically close to perirhinal cortex (see Fig. 4), it might be possible that the effects observed are attributable to leakage of the drugs into this structure instead of blockade of the perirhinal cortex. However, several facts support the argument against this possibility. First, a small volume of 0.5 µL was injected per hemisphere (Myers 1966). Second, it has been reported that infusion of AP-5 or NBQX in the basolateral amygdala impairs CTA learning to saccharin (Yasoshima et al. 2000), whereas we did not find any significant effect on CTA with the same drugs. Fitzgerald and Burton (1983) reported that electrolytic amygdala lesions or severing the connection between the amygdala and the temporal cortex (perirhinal connections included) produce similar deficits on neophobia. However, in the present report neither cholinergic nor glutamatergic receptor antagonists disrupted neophobia, which not only shows that the effects observed were caused by blockade of perirhinal cholinergic activity but also ruled out the idea that the effects of scopolamine were due to impaired taste perception.

As proposed previously (Domjan 1976), novelty seems to enhance the aversive qualities of a highly concentrated saccharin solution (Bartoshuk 1979; Smith 2000). However, animals overcome this neophobia, displaying higher saccharin consumption on subsequent presentations, which reflects a learning process (Gutierrez et al. 2003b). Therefore, we used the neophobic response as a novelty detection index and the subsequent attenuation of neophobia (AN) as a recognition memory index, based on the supposition that to display AN rats must recognize the taste as familiar and recall that the solution is safe to drink (Gutierrez et al. 2003b).

The behavioral results obtained on taste recognition memory revealed that attenuation of neophobia was prevented by scopolamine, which indicates that the transition from novel to familiar was not accomplished or that the categorization as a safe edible was prevented, and hence the animals still showed neophobia on the second taste presentation as proposed elsewhere (Gutierrez et al. 2003a,b). It should be noted that scopolamine prevented attenuation of neophobia even when it was administered after taste stimulation, which provides support to the idea that activation of cortical muscarinic receptors is necessary to consolidate this memory. Importantly, the same treatment has no noticeable effect on consolidation of CTA, which indicates that attenuation of neophobia and conditioned taste aversion seems to rely on different cortical processes once the rat has sampled the taste and formed a representation of it (Gutierrez et al. 2003b).

The impairment of taste recognition memory induced by alteration in perirhinal cholinergic cortical activity accords with the impairment found in previous studies using microinjections of scopolamine into monkey perirhinal cortex on a visual recognition memory task (Tang et al. 1997) and those reported in rats (Warburton et al. 2003) in which scopolamine treatment administered intraperitoneally or directly into the perirhinal cortex disrupted acquisition and/or consolidation of recognition memory on a preferential exploration task. This task uses the innate preference of rats to explore novel over familiar objects. Hence, the difference in time that a rat spends in contact with a novel object minus the time it spends with the familiar object is used as a spontaneous recognition memory index; scopolamine-treated animals spend similar times with both novel and "familiar" objects, indicating that the "familiar" object did not lose its "novelty qualities." These results indicate that the ability of scopolamine to prevent the transition from novel to familiar is not limited to taste stimuli.

Recently a link was demonstrated between the cholinergic system, long lasting depression (LLD), and recognition memory (Warburton et al. 2003). First, it has been reported that in the perirhinal and surrounding cortices, some neurons decrease their activity after repetition of a novel vi-

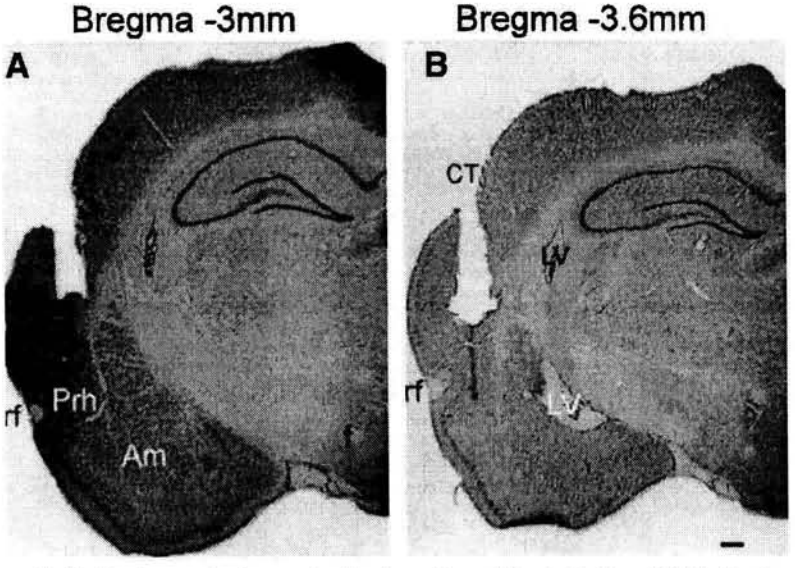

Figure 4 Photomicrograph of coronal rat brain sections, stained with Cresyl Violet, showing the cannula tract (CT) and the scar left by the injector and injection tip in the perirhinal cortex on two different rats (*A,B*). See text for explanation. (rf) Rhinal sulcus; (Prh) perirhinal cortex; (Amy) amygdala; (LV) lateral ventricle; and (f) fornix. Bar scale, 0.5 mm.

sual stimulus (Zhu et al. 1995). These results support the view that recognition memory could be mediated by neuronal depression, probably in the perirhinal cortex (Brown and Xiang 1998). Second, it has been proposed that cholinergic muscarinic receptor activity might be responsible for the depression presumably induced by recognition memory. In this regard, Massey et al. (2001) reported that activation of muscarinic receptors induces a long-lasting depression in perirhinal cortex slices; this synaptic depression is blocked by scopolamine but not by the NMDA receptor antagonist AP-5. Importantly, attenuation of neophobia was affected exclusively by blockade of muscarinic but not NMDA or AMPA receptors. In additional support of this idea, Warburton et al. (2003) reported that cholinergic antagonism induced by scopolamine selectively impaired LLD with no apparent effect on long-term potentiation (LTP) in perirhinal cortex slices. All in all, because there is converging evidence that muscarinic receptors participate in recognition memory, the activation of these receptors seems to induce a long-lasting depression, and their blockade affects specifically LLD and not LTP, these results indicate that cholinergic neurotransmission of perirhinal cortex is essential for recognition memory (Warburton et al. 2003). Our results are very consistent with this evidence and provide additional support of the view that cortical cholinergic muscarinic receptor activity is involved in different forms of recognition memory and in particular the recognition involved in taste information. However, it is still not clear if drinking a novel taste stimulus triggers synaptic depression in the perirhinal or insular cortex and if scopolamine could prevent these events. Further electrophysiological and biochemical studies are necessary to answer these questions.

The present results also indicate that blockade of perirhinal muscarinic but not NMDA or AMPA receptors impair the short- and long-term memory of CTA. These results provide additional support to the idea that the perirhinal cortex participates in CTA, as previously suggested (Fitzgerald and Burton 1983; Tassoni et al. 2000). Furthermore, the current data also provide neurochemical support of the idea that perirhinal cortex participation in taste aversion learning could be different from that of the insular cortex (gustatory part). Although scopolamine produces CTA impairment when injected in both cortices, NMDA and AMPA receptor blockers impaired CTA when they were microinjected into the insular cortex only (Berman et al. 2000; Ferreira et al. 2002; Gutierrez et al. 2003a). These results indicate that the perirhinal cortex participates in taste aversion learning, probably in an NMDA and AMPA receptor activity-independent manner. In addition, these results indicate that muscarinic receptor activity might be used in both perirhinal and insular cortices in the formation of the taste memory trace. Nevertheless, when scopolamine was injected immediately after taste intake but before the induction of malaise, no disruption was observed in CTA. This indicates that other memory components susceptible to be associated with malaise are in other brain structures like the insular cortex or amygdala (Gutierrez et al. 1999, 2003b). Similar results were found in the insular cortex (Naor and Dudai 1996), which also provides support of the idea that scopolamine does not interfere with the association between the taste memory trace and the malaise. These results are consistent with a previous report showing that perirhinal cortex might be relevant only in the early stages of the conditioned taste aversion learning (Tassoni et al. 2000).

In a previous study we demonstrated that scopolamine injected in the insular cortex impairs short-term memory of CTA only when rats drank saccharin after scopolamine treatment and not when scopolamine was administrated immediately after taste experience or before the short-term memory test (Ferreira et al. 2002). Although here scopolamine was administered only before taste presentation, it is reasonable to suggest that the observed short-term memory disruption was due to an indirect effect of the impairment induced on the taste memory trace rather than a direct effect on the association or consolidation of taste aversion learning.

The blockade of cholinergic muscarinic receptors of insular or perirhinal cortex seems to impair CTA in a similar manner. This indicates not only that the cholinergic mechanism is being shared by both insular and perirhinal cortex to form the taste memory trace but also that this cortical muscarinic-dependent process is not cortically redundant because perirhinal muscarinic-dependent activity could not be taken over by insular cortex receptors or vice versa. From this it can be proposed that the formation of the taste memory trace may occur in parallel and in distributed assemblies of neurons throughout the brain.

In addition, it has been demonstrated using a familiar saccharin solution that rats with ablations of the perirhinal cortex acquired spatial contextual control over liquid intake (saccharin or water). These animals show reduced liquid intake (familiar saccharin or water) in a place that has been paired with malaise (LiCl i.p. injection) compared with the consumption displayed in a place paired with saline injection (Howse et al. 2003), disclosing that rats lacking perirhinal cortex can acquire taste aversion learning to familiar taste stimuli. Although the Howse report seems to conflict with the present results and to those published by Tassoni et al. (2000), this is not necessarily the case. Importantly, compensatory redundant pathways could emerge after a chronic lesion (Gutierrez et al. 1999a). Also, the effect of this lesion has not been analyzed using a novel taste stimulus instead of a familiar one. Different anatomical and neurochemical substrates might be recruited by CTA learning depending on whether or not the rat had previous experience with the taste stimulus (Berman and Dudai 2001). For example, rats with ablations of the insular cortex displayed a deficiency in CTA only when they were trained with a novel taste (Kiefer and Braun 1977).

In summary, the present results indicate that perirhinal cortex participates in CTA, mainly during the formation of the taste memory trace. Furthermore, our results support a role of perirhinal cholinergic muscarinic receptors on taste recognition memory, extending previous results that support the participation of these receptors on recognition memory (Warburton et al. 2003) and also demonstrating that perirhinal cortex is concerned in the transition from novel to familiar safe taste.

MATERIALS AND METHODS

Subjects

Male Wistar rats (260–300 g) were used. They were housed in individual cages, with 12 h light/dark cycle and maintained at 22°C. All behavioral manipulations were performed inside the vivarium during the light phase. Rats received ad libitum lab chow throughout the experiment. Experiments were performed in accordance with Rules in Health Matters (Ministry of Health, Mexico) and with approval of the local Animal Care Committee.

Reagents

The muscarinic receptor antagonist scopolamine hydrobromide (Scop; SIGMA), the NMDA receptor antagonist, D,L-2-amino-5-phosphonovaleric acid (AP-5; RBI), and the AMPA receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium (NBQX; Tocris Cookson) were dissolved in Ringer solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 19 mM $NaHCO_3$, and 3.3 mM $C_6H_{12}O_6$; J.T. Baker), which, in turn, was used as vehicle. The concentrations used were 60 μg/μL scopolamine, 10 μg/μL AP-5, and 5 μg/μL NBQX, according to previous reports showing

CTA learning impairment when these specific concentrations were applied to the gustatory cortex (Insular Cortex; Gutierrez et al. 1999b, 2003a; Berman et al. 2000; Ferreira et al. 2002). All other chemicals were of analytical grade or the highest grade available.

Surgery and Microinjection

Animals were anesthetized with sodium pentobarbital (65 mg/kg) and mounted in the stereotaxic apparatus. A midline incision was made, and the lateral muscle was slightly detached, exposing the lateral border of the skull. Two holes were made at the following coordinates: anterior–posterior, −3 mm; lateral, ±6.5 mm relative to Bregma (Paxinos and Watson 1998). The tips of two stainless steel guide cannulae aimed at the perirhinal cortex were inserted bilaterally 5 mm below Bregma. Two screws were implanted and served as anchors in the skull bone. The whole assembly was attached to the skull with acrylic dental cement. A stylus was inserted into the guide cannula to prevent clogging. Rats were allowed to recover from surgery for at least 5 d before the beginning of behavioral training.

Microinjections were given bilaterally via a 30-gauge stainless steel injector 2.2 mm larger than the tips of the guide cannulae, connected via Teflon tubing to a 10-μL glass microsyringe attached to a microinfusion pump (Carnegie Medicin). Infusions of 0.5 μL were given per hemisphere over 1 min. The injector was left in the guide cannula one additional minute to allow complete diffusion. Intracortical infusions were given to hand-restrained conscious animals.

Behavioral Procedures and Experimental Design

Neophobia and Attenuation of Neophobia (AN)

We used the same protocol as in our previous study (Gutierrez et al. 2003a) with a highly concentrated saccharin solution, which enhances the robustness of neophobia (Domjan 1976). Briefly, as with CTA, rats were water-deprived for 24 h, and then they were given daily access to tapwater for 15 min during 3 d in their home cages. Volume consumption was registered with 0.5-mL accuracy. On day 4, animals were counterbalanced by weight and sorted into their corresponding groups, as described below, and the neophobic response was tested by presentation of 0.5% (w/v) sodium saccharin solution (SIGMA) for 15 min. To ensure that all animals consumed their daily fluid requirements regardless of their consumption of saccharin, an additional 15 min of water access was given. The same saccharin procedure was repeated on two consecutive days as an attenuation of neophobia test (AN). Thus, the neophobic response was analyzed in terms of the reduction in the intake of a novel taste solution relative to water baseline intake, and AN was indicated by the increased consumption of saccharin in the following presentations.

Four groups (Veh, $n = 16$; Scop, $n = 19$; AP5, $n = 8$ or NBQX, $n = 8$) received a single bilateral microinjection in the perirhinal cortex 20 min prior to the novel 0.5% saccharin solution on day 4 (Fig. 1A). Two additional groups (Veh-A, $n = 18$; Scop-A, $n = 19$) were microinjected immediately after taste presentation on day 4 (Fig. 1B). Two independent experiments were performed for the groups that received either scopolamine or vehicle. The results were very similar so the groups were pooled together. The results of these two subgroups were very similar, and because the differences were insignificant, they were pooled together.

Conditioned Taste Aversion (CTA): Long-Term Memory Test

Rats were water-deprived for 24 h and then were given 15 min of tapwater access in their home cage every day for four consecutive days. On the following day, the acquisition trial was performed by presentation of 0.1% (w/v) saccharin (in distilled water) for 15 min; 15 min later, after saccharin exposure was accomplished, a malaise-inducing drug (lithium chloride, 0.4 M LiCl; 2.94 mEq/kg; Baker) was injected intraperitoneally. Two subsequent drinking sessions were performed with water only, and 72 h after the acquisition trial, the long-term memory test trial was conducted. The test trial consisted in the presentation of 0.1% saccharin solution for a second time, and the decrease in consumption compared with water baseline was used as a measure of the strength of aversion.

Four groups received a single bilateral microinjection of vehicle (Veh, $n = 10$), scopolamine (Scop, $n = 9$), a NMDA receptor antagonist (AP5, $n = 13$), or an AMPA receptor antagonist (NBQX, $n = 8$) in the perirhinal cortex 20 min prior to the novel taste presentation on the acquisition trial (Fig. 2A). Two additional groups received a microinfusion of either vehicle (Veh-A, $n = 7$) or scopolamine (Scop-A, $n = 8$) immediately after taste presentation on the acquisition trial (Fig. 2B).

Conditioned Taste Aversion: Short-Term Memory Test

We used the same protocol as in our previous study (Ferreira et al. 2002). Briefly, from day 1 to day 4, two groups of rats were trained to drink water twice a day in their home cages. Each morning subjects had access to 10 mL of tapwater during a 15-min period; then, 4.5 h later, they had ad libitum access to water for another 15 min, and the average of this second consumption was taken as a baseline measure (Fig. 3). On day 5, 20 min before the beginning of the acquisition trial, one group received a single microinjection of vehicle (Veh, $n = 9$), whereas the other received scopolamine (Scop, $n = 10$). In the acquisition trial, rats were allowed to drink 10 mL of novel 0.1% (w/v) saccharin in distilled water, and 15 min later, a malaise-inducing drug (0.4 M LiCl; 2.94 mEq/kg) was injected intraperitoneally. Then, 4.5 h after first taste presentation, rats had ad libitum access to saccharin solution for 15 min as the short-term memory test. After saccharin exposure, 15 min of water access was given to avoid dehydration and to discard unspecific general fluid intake reduction. A 4.5-h interval was used to measure short-term memory to allow recovery from the poison-like effects induced by LiCl administration. Previous reports showed that lying on the belly (LOB), a behavioral index of malaise, dissipates within 2 h (Lamprecht et al. 1997), and our previous observation indicates that after 4.5 h, rats ingest 60% of the liquid during a 15-min session, compared with water baseline intake (Ferreira et al. 2002).

During the next two days, baseline water intake was re-established. Rats were exposed to saccharin solution 72 h after the acquisition trial for the third time to assess long-term memory of CTA.

Histology

At the end of the experiments, all rats were killed by an overdose of pentobarbital and perfused with saline followed by 0.4% paraformaldehyde. The brains were removed and placed in 30% sucrose/PB 0.1 M solution. Brains were sectioned and stained with Cresyl Violet to establish the place of microinjection.

Statistical Analysis

A one- or two-way ANOVA was used to compare means (±SEM) of milliliters of consumed water or saccharin among groups, presented as a percentage of the water consumed during baseline. The Fisher pairwise test was used for post hoc analysis with $p < 0.05$ considered significant.

ACKNOWLEDGMENTS

This work was supported by CONACYT-Mexico grants MRI 35806-N and DGAPA-IN215001. We acknowledge the technical assistance of Federico Jandete, Oreste Carbajal, Francisco Pérez Eugenio, and Yolanda Díaz de Castro and give thanks to Enrique Espinosa and Paul Afolabi Remi for English revision of the final text.

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REFERENCES

Aigner, T. and Mishkin, M. 1986. The effects of physostigmine and scopolamine on recognition memory in monkeys. *Behav. Neural Biol.* **45:** 81–87.

Bartoshuk, L.M. 1979. Bitter taste of saccharin related to the genetic ability to taste the bitter substance 6-*n*-propylthiouracil. *Science* **205:** 934–935.

Berman, D.E. and Dudai, Y. 2001. Memory extinction, learning anew, and learning the new: Dissociations in the molecular machinery of learning in cortex. *Science* **291:** 2417–2419.

Berman, D.E., Hazvi, S., Neduva, V., and Dudai, Y. 2000. The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste: Activation of ERK1-2 and formation of a memory trace. *J. Neurosci.* **20:** 7017–7023.

Bermudez-Rattoni, F. and Yamamoto, T. 1998. Neuroanatomy of CTA: Lesion studies. In *Conditioned taste aversion. Memory of a special kind* (eds. J. Bures et al.), pp. 28–44. Oxford University Press, New York.

Best, M.R., Domjan, M., and Haskins, W.L. 1978. Long-term retention of flavor familiarization: Effects of number and amount of prior exposures. *Behav. Biol.* **23:** 95–99.

Brown, M.W. and Xiang, J.-Z. 1998. Recognition memory: Neural substrates of the judgment of prior occurrence. *Prog. Neurobiol.* **55:** 149–189.

Buffalo, E.A., Ramus, S.J., Clark, R.E., Teng, E., Squire, L.R., and Zola, S.M. 1999. Dissociation between the effects of damage to perirhinal cortex and area TE. *Learn. Mem.* **6:** 572–599.

Buresova, O. and Bures, J. 1980. Post-ingestion interference with brain function prevents attenuation of neophobia in rats. *Behav. Brain Res.* **1:** 299–312.

Burwell, R.D. 2001. Borders and cytoarchitecture of the perirhinal and postrhinal cortices in the rat. *J. Comp. Neurol.* **437:** 17–41.

Burwell, R.D. and Amaral, D.G. 1995. Cortical afferents to the perirhinal, post-rhinal and entorhinal cortices of the rat. *Hippocampus* **5:** 390–408.

———1998. Perirhinal and postrhinal cortices of the rat: Interconnectivity and connections with the enthorinal cortex. *J. Comp. Neurol.* **391:** 293–321.

Deacon, T.W., Eichenbaum, H., Rosenberg, P., and Eckmann, K.W. 1983. Afferent connections of the perirhinal cortex in the rat. *J. Comp. Neurol.* **220:** 168–190.

Domjan, M. 1976. Determinants of the enhancement of flavored-water intake by prior exposure. *J. Exp. Psychol., Anim. Behav. Proces.* **2:** 17–27.

Ellis, M.E. and Kesner, R.P. 1981. Physostigmine and norepinephrine: Effects of injection into the amygdala on taste associations. *Physiol. Behav.* **27:** 203–209.

Ferreira, G., Gutierrez, R., De, L., and Bermudez, R. 2002. Differential involvement of cortical muscarinic and NMDA receptors in short- and long-term taste aversion memory. *Eur. J. Neurosci.* **16:** 1139–1145.

Fitzgerald, R.E. and Burton, M.J. 1983. Neophobia and conditioned taste aversion deficits in the rat produced by undercutting temporal cortex. *Physiol. Behav.* **30:** 203–206.

Goulet, S. and Murray, E.A. 2001. Neural substrates of crossmodal association memory in monkeys: The amygdala versus the anterior rhinal cortex. *Behav. Neurosci.* **115:** 271–284.

Gutierrez, H., Gutierrez, R., Ramirez, T., Silva, G., Ormsby, C.E., Miranda, M.I., and Bermudez, R. 1999a. Redundant basal forebrain modulation in taste aversion memory formation. *J. Neurosci.* **19:** 7661–7669.

Gutierrez, H., Hernandez-Echeagaray, E., Ramirez-Amaya, V., and Bermudez-Rattoni, F. 1999b. Blockade of *N*-methyl-D-aspartate receptors in the insular cortex disrupts taste aversion and spatial memory formation. *Neuroscience* **89:** 751–758.

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

Gutierrez, R., Rodriguez-Ortiz, C.J., De La Cruz, V., Núñez-Jaramillo, L., and Bermudez-Rattoni, F. 2003b. Cholinergic dependence of taste memory formation: Evidence of two distinct processes. *Neurobiol. Learn. Mem.* **80:** 323–331.

Howse, D.J., Squires, A.S., Martin, G.M., and Skinner, D.M. 2003. Perirhinal cortex lesions impair context aversion learning. *Learn. Mem.* **10:** 161–167.

Kiefer, S.W. and Braun, J.J. 1977. Absence of differential associative responses to novel and familiar taste stimuli in rats lacking gustatory neocortex. *J. Comp. Physiol. Psychol.* **91:** 498–507.

Kowalska, D.M., Kusmierek, P., Kosmal, A., and Mishkin, M. 2001. Neither perirhinal/entorhinal nor hippocampal lesions impair short-term auditory recognition memory in dogs. *Neuroscience* **104:** 965–978.

Lamprecht, R. and Dudai, Y. 1996. Transient expression of c-Fos in rat amygdala during training is required for encoding conditioned taste aversion memory. *Learn. Mem.* **3:** 31–41.

Lamprecht, R., Hazvi, S., and Dudai, Y. 1997. cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. *J. Neurosci.* **17:** 8443–8450.

Malkova, L., Bachevalier, J., Mishkin, M., and Saunders, R.C. 2001. Neurotoxic lesions of perirhinal cortex impair visual recognition memory in rhesus monkeys. *Neuroreport* **12:** 1913–1917.

Mandler, G. 1980. Recognizing: The judgment of previous occurrence. *Psychol. Rev.* **87:** 252–271.

Martin, S.J. and Morris, R.G.M. 2002. New life in an old idea: The synaptic plasticity and memory hypothesis revisited. *Hippocampus* **12:** 609–636.

Massey, P.V., Bhabra, G., Cho, K., Brown, M.W., and Bashir, Z.I. 2001. Activation of muscarinic receptors induces protein synthesis-dependent long-lasting depression in the perirhinal cortex. *Eur. J. Neurosci.* **14:** 145–152.

McDonald, A.J. and Jackson, T.R. 1987. Amygdaloid connections with posterior insular and temporal cortical areas in the rat. *J. Comp. Neurol.* **262:** 59–77.

Meunier, M., Bachevalier, J., Mishkin, M., and Murray, E.A. 1993. Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J. Neurosci.* **13:** 5418–5432.

Mumby, D.G. and Pinel, J.P.J. 1994. Rhinal cortex lesions and object recognition in rats. *Behav. Neurosci.* **108:** 11–18.

Murray, E.A. and Richmond, B.J. 2001. Role of perirhinal cortex in object perception, memory and associations. *Curr. Opin. Neurobiol.* **11:** 188–193.

Myers, R. 1966. Injection of solutions into cerebral tissue: Relation between volume and diffusion. *Physiol. Behav.* **1:** 171–174.

Nachman, M. and Jones, D.R. 1974. Learned taste aversions over long delays in rats: The role of learned safety. *J. Comp. Physiol. Psychol.* **86:** 949–956.

Naor, C. and Dudai, Y. 1996. Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. *Behav. Brain Res.* **79:** 61–67.

Otto, T. and Eichenbaum, H. 1992. Complementary roles of the orbital prefrontal cortex and the perirhinal–entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. *Behav. Neurosci.* **106:** 762–775.

Parker, A. and Gaffan, D. 1998. Lesions of the primate rhinal cortex cause deficits in flavour–visual associative memory. *Behav. Brain Res.* **93:** 99–105.

Paxinos, G. and Watson, C. 1998. *The rat brain in stereotaxic coordinates.* 4th edition, Academic Press, New York.

Sipos, M.L., Burchnell, V., and Galbicka, G. 1999. Dose-response curves and time-course effects of selected anticholinergics on locomotor activity in rats. *Psychopharmacology (Berl)* **147:** 250–256.

Smith, J.C. 2000. Microstructure of the rat's intake of food, sucrose and saccharin in 24-hour test. *Neurosci. Biobehav. Rev.* **24:** 199–212.

Tang, Y., Mishkin, M., and Aigner, T.G. 1997. Effects of muscarinic blockade in perirhinal cortex during visual recognition. *Proc. Natl. Acad. Sci.* **94:** 12667–12669.

Tassoni, G., Lorenzini, C.A., Baldi, E., Sacchetti, B., and Bucherelli, C. 2000. Role of the perirhinal cortex in rats' conditioned taste aversion response memorization. *Behav. Neurosci.* **114:** 875–881.

Warburton, E.C., Koder, T., Cho, K., Massey, P.V., Duguid, G., Bashir, Z.I., and Brown, M.W. 2003. Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. *Neuron* **38:** 987–996.

Yasoshima, Y. and Yamamoto, T. 1997. Rat gustatory memory requires protein kinase C activity in the amygdala and cortical gustatory area. *Neuroreport* **8:** 1363–1367.

Yasoshima, Y., Morimoto, T., and Yamamoto, T. 2000. Different disruptive effects on the acquisition and expression of conditioned taste aversion by blockades of amygdalar ionotropic and metabotropic glutamatergic receptor subtypes in rats. *Brain Res.* **869:** 15–24.

Zhu, X.O., Brown, M.W., and Aggleton, J.P. 1995. Neuronal signalling of information important to visual recognition memory in rat rhinal and neighbouring cortices. *Eur. J. Neurosci.* **7:** 753–765.

Received August 28, 2003; accepted in revised form November 21, 2003.

Artículo 3: *Perirhinal Cortex Muscarinic Receptor Blockade Impairs Taste Recognition Memory Formation*

Introducción

Actualmente es reconocida la existencia de distintos tipos de memoria una de ellas es la memoria declarativa y la otra es la memoria implícita, la primera requiere el recuerdo consciente de la información y la segunda no. Sin duda alguna uno de los tipos de memoria más elemental es la memoria de reconocimiento la cual subyace en la habilidad de los organismos para identificar y recordar que algún objeto o evento ha sido experimentado en el pasado (Brown y Xiang, 1998). Estudios pioneros han demostrado que la ablación de la corteza perirrinal en ratas o en monos, causa un deterioro severo en memoria de reconocimiento, al menos cuando se utiliza información visual (Meunier et al., 1993;Malkova et al., 2001), sin embargo, debido a que la corteza perirrinal es un área de asociación polimodal que recibe información de cortezas de asociación visual, olfativa, gustativa y somatosensorial, así como de otras áreas polimodales de asociación incluyendo la corteza prefrontal y entorhinal (Suzuki.W.A., 1996;Brown y Xiang, 1998), se ha propuesto que puede desempeñar un papel fundamental en hacer que un objeto nuevo sea percibido como familiar sin importar el tipo de información sensorial(Murray y Richmond, 2001), en apoyo a esta idea recientemente se ha encontrado que su ablación en monos deteriora la memoria de reconocimiento basada en tacto(Buffalo et al., 1999), mientras que en trabajos con ratas se ha encontrado un deterioro similar con estímulos olfativos(Otto y Eichenbaum, 1992). No obstante aún se desconoce su participación en memoria de reconocimiento gustativo.

Estudios electrofisiológicos de corteza perirrinal y cortezas aledañas reportaron un grupo de neuronas sensibles a la repetición, las cuales disminuían su frecuencia de disparo cuando un estímulo se convertía en familiar(Zhu X.O. et al., 1995), estos resultados llevaron a proponer a la disminución en actividad neuronal como mecanismo utilizado por la corteza perirrinal para mediar la memoria de reconocimiento de objetos(Brown y Xiang, 1998).

Planteamiento del problema

Recientemente, se demostró que la estimulación con carbacol, un agonista de receptores muscarínicos, en rebanadas de corteza perirrinal induce una depresión a semi-largo plazo (Long Lasting Depresión, LLD) dependiente de síntesis de proteínas, la cual no es bloqueada por antagonistas de receptores NMDA pero sí por la aplicación de escopolamina (Massey et al., 2001). Estos autores concluyen que probablemente el mecanismo que subyace a el LLD inducido por activación de receptores muscarínicos pueda ser el mismo o similar al mecanismo utilizado por la corteza perirrinal durante la codificación de la memoria de reconocimiento visual. No obstante a que aún queda por determinarse si la depresión en la actividad neuronal es el verdadero mecanismo de la memoria de reconocimiento, el mecanismo propuesto por Massey concuerda con los efectos conductuales inducidos con escopolamina, por ejemplo la aplicación sistémica de escopolamina así como su aplicación directamente en corteza perirrinal interfiere con la memoria de reconocimiento visual en monos (Aigner et al., 1991;Tang et al., 1997) y también con los resultados encontrados por (Duguid et al., 1998) quien reportó que el bloqueo de los receptores muscarínicos evita el decremento en la actividad neuronal medida por c-Fos asociado con la repetición de estímulos visuales(Duguid et al., 1998).

Pero aún más importante, todos estos resultados concuerda con los resultados que encontramos en los dos artículos anteriores en donde demostramos que la AN es detenida por escopolamina pero no por AP-5, antagonista de receptores tipo NMDA, así también la AN en cierta forma debe de subyacer en procesos de memoria de reconocimiento porque como se ha mostrado ésta solo ocurre cuando el sabor es familiar seguro. Por todo lo anterior, decidimos investigar los efectos de antagonistas de receptores metabotrópicos colinérgicos y receptores ionotrópicos a glutamato en corteza perirrinal sobre AN.

Adicionalmente, ya que recientemente se reportó que la corteza perirrinal esta involucrada en la adquisición del CAS(Tassoni et al., 2000), decidimos investigar la participación de estos receptores también en el CAS.

Objetivo

1. Determinar la participación de receptores metabotrópicos de acetilcolina y de los receptores ionotrópicos para glutamato en corteza perirrinal en AN y CAS

Resultados

(Ver *artículo 3*: *Perirhinal Cortex Muscarinic Receptor Blockade Impairs Taste Recognition Memory Formation)*

Discusión y Conclusiones

Los estudios incluidos en los tres artículos adjuntos se enfocaron en caracterizar la formación del TMS así como de su posterior etiquetamiento tratando de relacionar dos regiones corticales y sus sustratos neuroquímicos con estos eventos. Nuestros resultados indican que la AN es un aprendizaje que es "detenido" por escopolamina pero no por AP-5, sugiriendo que la AN es un aprendizaje dependiente de receptores muscarínicos pero no de receptores tipo NMDA en la corteza insular y la perirrinal.

En el primer artículo caracterizamos el efecto de la escopolamina en corteza insular sobre AN, en donde, se trató de descartar varias explicaciones alternativas, principalmente, si la escopolamina interfiere con la percepción del sabor, si induce aprendizaje estado-dependiente o si induce aversión *per se*. Los resultados sugieren que ninguna de estas tres hipótesis pueden explicar por completo el por qué los animales muestran un consumo similar de sacarina 0.5% al del día previo, lo que nos llevó a postular que la escopolamina detiene el proceso de AN, hasta que los animales prueben el sabor en ausencia de escopolamina.

Los datos indican que los animales tratados con escopolamina no recordaron haber bebido este sabor un día antes (a pesar de haberlo percibido porque mostraron neofobia) o que no recordaron qué consecuencias gástricas

conllevó su consumo y por tal lo trataron nuevamente con cautela como si fuera nuevo otra vez. Cualquiera de estas dos hipótesis pueden tener algo de verdad e incluso podrían ser complementarias. La primera alternativa predice que en la segunda presentación del sabor todos los procesos desencadenados por un sabor nuevo deben de estar presentes, mientras que la segunda alternativa predice que el sabor en su segunda presentación no es que sea "nuevo otra vez" sino que no tiene ningún significado (seguro) y por eso continúa induciendo neofobia. Estudios con marcadores celulares de novedad (proteínas que se expresen solamente cuando el sabor es nuevo) podrían ayudar a resolver esta interrogante i.e. en el caso de que la primer hipótesis sea la correcta la aplicación de escopolamina debería de recorrer la expresión de todos los marcadores de novedad a la par con la neofobia, de lo contrario la segunda alternativa podría ser la más adecuada. Al mismo tiempo, este primer trabajo sirvió para retomar a la AN como un modelo para estudiar la neurobiología del aprendizaje y la memoria desencadenada por el sistema gustativo en ausencia de malestar.

Dos resultados fueron desconcertantes con respecto a lo que se conocía del CAS. El primero es que la escopolamina detiene la AN aún cuando ésta es microinyectada inmediatamente o hasta dos horas después de la presentación del sabor novedoso, es decir en la fase de consolidación. mientras que en el CAS, la aplicación de escopolamina inmediatamente después del sabor no tiene ningún efecto conductual. La posibilidad de que la AN y el CAS sean mediados por diferentes mecanismos neuronales ya había sido postulado previamente por varios estudios conductuales(Braveman y Jarvis, 1978;Miller y Holzman, 1981b;Stewart y Reidinger, 1984), de estimulación eléctrica (Kesner y Berman, 1977) y farmacológicos (Buresova y Bures, 1980;Aguado et al., 1994;Ellis y Kesner, 1981). Por ejemplo Buresova y Bures 1980 demostraron que ratas anestesiadas con pentobarbital poco después de la presentación de sacarina nueva y antes de la inducción de malestar gástrico son capaces de aprender aversión al sabor, dejando claro que la asociación entre el sabor y el malestar puede llevarse a cabo bajo la influencia de anestesia. Sin embargo, la

atenuación de la neofobia a jugo de manzana es bloqueada por la administración de anestesia inmediatamente o hasta 2 horas después de la presentación del sabor. Otros tratamientos también afectan diferencialmente la AN y el CAS entre ellos hipotermia, choques electroconvulsivos y decorticación funcional. En el segundo artículo adjunto abordamos esta pregunta con mayor rigor científico, nuestros resultados confirman que la AN y el CAS pueden estar mediados por mecanismos independientes incluso dentro de una misma región cortical y cuando se utiliza la misma concentración de sacarina para inducir ambos aprendizajes, i.e. una vez que el TMS ha sido formado la AN continua siendo una memoria dependiente de receptores muscarínicos mientras que el CAS parece no requerir más de estos receptores. Al hacer una revisión de los efectos de la escopolamina en otros paradigmas conductuales se puede observar que no es extraño que la escopolamina interfiera en procesos de consolidación (ver tabla 1) de hecho recientemente se propuso que el sistema colinérgico tiene la capacidad de modular procesos de consolidación(Power et al., 2003).

Tabla 1 Efecto de la escopolamina en diversas tareas conductuales antes y después de la fase de adquisición.

Referencia	Lugar de la inyección	Tarea Conductual	Inyección escopolamina **Antes** (adquisición) **después**	
Resultados de esta tesis(Naor y Dudai, 1996a)	Corteza Insular y Perirrinal	CAS	Interfiere	No efecto
Resultados de esta tesis	Corteza Insular y Perirrinal	AN	Interfiere	Interfiere
(Barros et al., 2002)	Amígdala	Prevención pasiva	Interfiere	Interfiere
(Whitehouse, 1964)	Sistémica	Aprendizaje Discrimintivo	Interfiere	¿?
(Aigner et al.,	Sistémica	Memoria de reconocimiento	Interfiere	¿Probablemente?

1991;Duguid et al., 1998)				
(Tang et al., 1997)	Corteza Perirrinal Monos	Memoria de reconocimiento visual	Interfiere	Interfiere
(Warburton et al., 2003)	Perirrinal, vía i.p.	Familarización con objetos nuevos en un campo abierto	Interfiere	¿Probablemente?
(Riekkinen et al., 1995)	Corteza cingulada posterior	Laberinto de agua de Morris	Interfiere	No efecto
(Rudy, 1996)	sistémica	Temor condicionado al contexto y a pista auditiva	Interfiere	Interfiere
(McIntyre et al., 1998)	Amígdala	Laberinto radial	No efecto	¿?
(Schroeder y Packard, 2002)	Amígdala	Preferencia condicionada al lugar inducida por anfetamina o por comida	¿?	Interfiere

¿?=No explorado
¿Probablemente?= es difícil distinguir su efecto debido a las características del paradigma conductual

El segundo resultado fue que la AN es insensible a antagonistas de receptores tipo NMDA y AMPA en corteza insular e incluso en corteza perirrinal. No obstante, este resultado fue consistente con la falta de efecto inducido por MK-801 (un antagonista no competitivo de receptores NMDA) aplicado de manera sistémica, el cual interfiere con la adquisición del CAS y la inhibición latente pero no con la AN(Aguado et al., 1994). Los receptores tipo NMDA han sido ampliamente involucrados en procesos de aprendizaje y memoria así como en la potenciación a largo plazo (LTP)(Tang et al., 1999). La inducción de LTP en la proyección amígdala basolateral-corteza insular antes de la adquisición del CAS incrementa su retención(Escobar y Bermudez, 2000). Aún más importante tanto la LTP como la adquisición del CAS son deteriorados por la aplicación de antagonistas de receptores tipo NMDA en corteza insular (Escobar et al.,

1998;Escobar et al., 2002). Ya que la AN es insensible al bloqueo de receptores NMDA se sugiere que este aprendizaje podría ser mediado por un mecanismo diferente a la LTP, probablemente por medio de una depresión a largo plazo (LTD) tal vez similar al mecanismo propuesto por Brown 1998 que estaría mediando la memoria de reconocimiento visual.

En este sentido en el tercer artículo, abordamos a la AN como un modelo para estudiar memoria de reconocimiento gustativo, esto se debió básicamente por las similitudes entre nuestros resultados y aquellos encontrados por el equipo de Brown, básicamente que la escopolamina interfiere con la capacidad de recordar que un estímulo visual o un objeto ha ocurrido en el pasado (en nuestro caso un sabor) a la par con la depresión en la actividad celular medida por c-Fos de un estímulo visual familiar y el descubrimiento de una LTD inducida por activación de receptores muscarínicos en corteza perirrinal que es sensible a escopolamina pero no a AP-5. Nuestros datos concuerdan con estos resultados, es decir escopolamina pero no AP-5 microinyectados en corteza perirrinal, interfieren con la AN aunque aún queda por ser determinado si beber un sabor por primera vez induce LTD en corteza perirrinal o insular.

Muy poca información señalaba la participación de la corteza perirrinal en aprendizaje gustativo, pero un número creciente de evidencias la relacionan en memoria de reconocimiento visual, somatosensorial y olfativo pero aún no se ha explorado su participación utilizando estímulos gustativos, No obstante, esta corteza recibe información gustativa proveniente de la amígdala y de corteza insular(Saper, 1982). Sewards y Sewards (2001) basándose en evidencias anatómicas y haciendo analogías con el sistema visual: propusieron que además de la corteza gustativa primaria, localizada en la corteza insular granular de la rata, existen tres áreas gustativas de asociación. La secundaria localizada en la corteza insular disgranular, la terciaria corteza insular agranular y la cuaternaria en la corteza perirrinal. Además de esto, al cortar las proyecciones de la amígdala y corteza perirrinal se interfiere con la retención del CAS(Yamamoto et al., 1984) y disminuye la expresión de neofobia(Fitzgerald y Burton, 1983). Recientemente, se descubrió que la inhibición de potenciales de acción (con

tetrodotoxina) en esta corteza afecta únicamente la formación del trazo de memoria del sabor porque la tetrodotoxina solo interfirió la adquisición del CAS cuando se inyectó antes pero no inmediatamente después de la presentación del sabor ó 6-24 hr después de la inducción de malestar o antes de la prueba. Sin embargo, no se conocía qué sistema de neurotransmisión podría estar mediando este efecto. Nuestros resultados no solo reafirmaron la participación de la corteza perirrinal en el CAS sino que también la relacionaron por primera vez con la AN y además señalaron que la corteza perirrinal participa en la formación del trazo de memoria del sabor probablemente por medio de la activación de receptores muscarínicos, en conjunto con la actividad de estos receptores de corteza insular. El bloqueo de estos receptores también interfirió con la memoria a corto plazo medida 4 horas después de la adquisición del CAS, resultados que fueron muy similares a los que previamente habíamos encontrado en corteza insular (Ferreira et al., 2002), lo que sugiere que la asociación TMS-malestar se deterioró desde la formación de la memoria a corto plazo probablemente porque la escopolamina redujo o degradó la formación del TMS, el cual solo puede formar una asociación con malestar muy débil.

El bloqueo de los receptores ionotrópicos a ácido glutámico en corteza perirrinal no interfirió de manera estadísticamente significativa en la adquisición del CAS, estos resultados fueron contrastantes con los encontrados en corteza insular. En corteza insular el bloqueo de estos receptores no interfiere con la memoria de corto pero si con la de largo plazo del CAS cuando se inyectó AP-5 antes de la presentación del sabor. Este mismo fármaco no tiene ningún efecto cuando se aplica inmediatamente después del sabor (Ferreira et al., 2002;Gutierrez et al., 2003b). lo cual sugiere que estos receptores en corteza insular pueden estar participando en la consolidación del significado aversivo del sabor. Sin embargo, ya que en corteza perirrinal el bloqueo de estos receptores no tuvo ningún efecto en la adquisición del CAS, esta corteza probablemente solo está involucrada en la formación del TMS (Tassoni et al., 2000) y en la consolidación del significado seguro del sabor y estos dos procesos por

supuesto podrían estar siendo mediados por la activación de receptores muscarínicos.

Cual es papel de Corteza insular en aprendizaje gustativo

El hecho de que la corteza insular no participe en la percepción del sabor pero esté involucrada en el proceso de extinción (Braun, 1995;Berman y Dudai, 2001), inhibición latente(Naor y Dudai, 1996b), aversión aprendida al sabor y ahora propuesto en esta tesis también en AN, y en que todos estos procesos dependen de la modificación del significado del sabor, probablemente la función de la CI es consolidar o almacenar la memoria del valor actual del sabor (su etiquetamiento actual) corroborando la hipótesis de Braun de que el papel de la corteza insular es la de formar la memoria del "significado aprendido del sabor" cual sea este.

En resumen, cuando se bebe un sabor por primera vez, más allá de los reflejos de rechazo o aceptación que pudiera desencadenar de manera innata (neofobia, rechazo o aceptación), éste no tiene ningún otro significado para el organismo que lo ingiere. Así este evento debe de desencadenar por primera vez la formación del trazo de memoria del sabor probablemente vía activación de receptores muscarínicos en corteza insular y perirrinal que de acuerdo a las consecuencias gástricas post-ingestionales el TMS adquirirá un significado (aversión, preferencia) que será almacenado como seguro o peligroso para ingerirlo y precisamente es esta "etiqueta" (significado) que el animal evocará cuando lo consuma por segunda ocasión el ahora sabor familiar.

En esta tesis tratamos de demostrar que este primer etiquetamiento debe de ser un proceso que probablemente ocurre en paralelo y de manera distribuida a nivel cortical pero probablemente también en estructuras subcorticales (Buresova y Bures, 1973;Kesner y Berman, 1977;Ellis y Kesner, 1981) y que la corteza insular por medio de receptores muscarínicos y tipo NMDA entre otros participa diferencialmente en la adquisición del primer significado del TMS. Desafortunadamente, las investigaciones presentadas en esta tesis se limitaron a describir los sustratos neuroquímicos cuando el TMS adquiere su primer significado, sin embargo es bien conocido que este primer etiquetamiento no es

inmutable y rígido ya que se puede modificar básicamente en cada subsecuente encuentro con el sabor en donde se reafirmará el etiquetamiento previo o este cambiará gradualmente, experimentos futuros deben de estar encaminados en el entendimiento de los sustratos neuroquímicos inducidos por el TMS cuando éste ha adquirido más de un significado a la vez. Es bien sabido que el TMS puede tener varios significados al mismo tiempo, por ejemplo, la aplicación de un inhibidor de síntesis de proteínas en corteza insular no interfiere la evocación de un CAS pero detiene el proceso de extinción, lo que demuestra que la extinción de la aversión al sabor es un aprendizaje nuevo e independiente al de la aversión aprendida (Berman y Dudai, 2001). En la figura 9 se muestran los diversos significados que un sabor puede tener para el animal a lo largo de su vida. Probablemente estos compiten por ser evocados (Eisenberg et al., 2003), sin embargo esta competencia debe de cumplir el orden y la dirección propuesta en la figura 9.

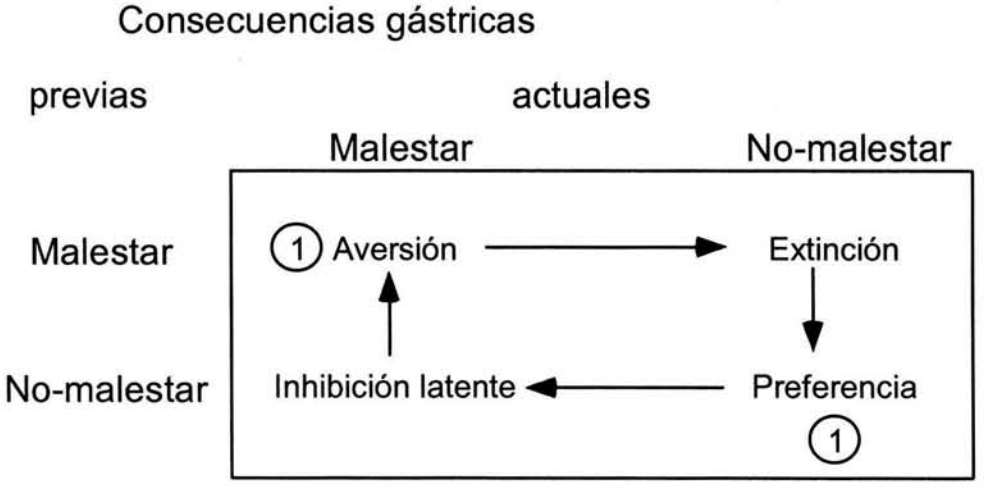

Figura 9 muestra los posibles significados que un animal puede aprender de un sabor con base en sus consecuencias gástricas previas y las que presente en el último consumo del sabor dentro de la caja se muestra el significado que será evocado en el siguiente encuentro con el sabor, en el primer encuentro los animales pueden aprender aversión o preferencia ver figura 1 del segundo articulo adjunto a este tesis.

En este modelo tanto la extinción y la inhibición latente son procesos de transición, fases intermedias entre la aversión y preferencia, aunque tentadora esta idea esto no implica que la inhibición latente y extinción subyazcan en procesos celulares similares entre ellos o intermedios entre aquellos que soportan a la aversión o a la preferencia, sino que probablemente existan algunos procesos moleculares adicionales aún no identificados responsables de estas memorias (inhibición latente y extinción). Estudios futuros de este modelo nos ayudarán a entender las interacciones y la complejidad de todos los procesos de aprendizaje y memoria basados en información gustativa.

Bibliografía

1. Aggleton JP, Petrides M, Iversen DS (1981) Differential effects of amygdaloid lesions on conditioned taste aversion by rats. Physiology & Behavior 27: 397-400.

2. Aguado L, San Antonio A, Perez L, del Valle R, Gomez J (1994) Effects of the NMDA receptor antagonist ketamine on flavor memory: conditioned aversion, latent inhibition, and habituation of neophobia. Behav Neural Biol 61: 271-281.

3. Aigner TG, Walker DL, Mishkin M (1991) Comparison of the effects of scopolamine administered before and after acquisition in a test of visual recognition memory in monkeys. Behavioral and Neural Biology 55: 61-67.

4. Arwas S, Rolnick A, Lubow RE (1989) Conditioned taste aversion in humans using motion-induced sickness as the US. Behav Res Ther 27: 295-301.

5. Barnett SA (1958) Experiments in "neophobia" in wild and laboratory rats. British Journal of Psychology 49: 195-201.

6. Barros DM, Pereira P, Medina JH, Izquierdo I (2002) Modulation of working memory and of long-but not short-term memory by cholinergic mechanisms in the basolateral amygdala. Behavioural Pharmacology 13: 163-167.

7. Berman DE, Dudai Y (2001) Memory extinction, learning anew, and learning the new: dissociations in the molecular machinery of learning in cortex. Science 291: 2417-2419.

8. Berman DE, Hazvi S, Neduva V, Dudai Y (2000) The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste: activation of ERK1-2 and formation of a memory trace. J Neurosci 20: 7017-7023.

9. Bermudez R, McGaugh JL (1991b) Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion. Brain Res 549: 165-170.

10. Bermudez R, McGaugh JL (1991a) Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion. Brain Res 549: 165-170.

11. Best MR, Domjan M, Haskins WL (1978) Long-term retention of flavor familiarization: effects of number and amount of prior exposures. Behav Biol 23: 95-99.

12. Bower B (1997) Forbidden Flavors scientists consider how distiguishting tastes can linger surreptitiously in memory. Science News 151: 151.

13. Bradley RM, Mistretta GM (1971) Intravascular taste in rats as demostrated by conditioned aversion to sodium saccharin. Journal of Comparative and Physiological Psychology 75: 186-189.

14. Braun JJ (1995) Gustatory Cortex: Definition and Function. In: The cerebral cortex of the rat (Kolb, Tees, eds), pp 407-430. Massachusetts: The MIT Press, Cambridg.

15. Braun JJ, Slick TB, Lorden JF (1972) Involvement of gustatory neocortex in the learning of taste aversions. Physiol Behav 9: 637-641.

16. Braveman NS, Jarvis PS (1978) Independence of neophobia and taste aversion learning. Animal Learning & Behavior 6: 406-412.

17. Brouwer JN, Hellekant G, Kasahara Y, van d, Zotterman Y (1973) Electrophysiological study of the gustatory effects of the sweet proteins monellin and thaumatin in monkey, guinea pig and rat. Acta Physiol Scand 89: 550-557.

18. Brown MW, Xiang J-Z (1998) Recognition memory: neural substrates of the judment of prior occurrence. Progress in Neurobiology 55: 149-189.

19. Buffalo, E. A., Ramus, S. J., Clark, R. E., Teng, E., Squire, L. R., and Zola, S. M.(1999) Dissociation Between the effects of damage to perirrinal cortex and area TE. Learning and Memory 6, 572-599. 1999.

20. Bures J (1998) Ethology, physiological psychology, and neurobiology of CTA. In: Conditioned Taste Aversion: memory of a special kind (Bures J, Bermudez-Rattoni F, Yamamoto T, eds), pp 1-10. New York: Oxford University Press.

21. Bures J, Bermudez-Rattoni F, Yamamoto T (1998) Conditioned Taste Aversion. Memory of a special kind. New York: Oxford science publications.

22. Bures J, Buresova O (1989) Conditioned taste aversion to injected flavor: differential effect of anesthesia on the formation of the gustatory trace and on its association with poisoning in rats. Neurosci Lett 98: 305-309.

23. Buresova O, Bures J (1973) Cortical and subcortical components of the conditioned saccharin aversion. Physiol Behav 11: 435-439.

24. Buresova O, Bures J (1980) Post-ingestion interference with brain function prevents attenuation of neophobia in rats. Behav Brain Res 1: 299-312.

25. Burghardt GM, Hess EH (1966) Food imprinting in the snapping turtle. Science 151: 108-109.

26. Burritt EA, Provenza FD (1989) Food aversion learning: ability of lambs to distinguish safe from harmful foods. J Anim Sci 67: 1732-1739.

27. Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ (2000) T2Rs function as bitter taste receptors. Cell 100: 703-711.

28. Chaudhari N, Yang H, Lamp C, Delay E, Cartford C, Than T, Roper S (1996) The taste of monosodium glutamate: membrane receptors in taste buds. J Neurosci 16: 3817-3826.

29. Cornwell J, Kovanic K (1981) Testosterone reduces olfactory neophobia in male golden hamsters. Physiol Behav 26: 973-977.

30. Cubero I, Thiele TE, Bernstein IL (1999) Insular cortex lesions and taste aversion learning: effects of conditioning method and timing of lesion. Brain Res 839: 323-330.

31. Deutsch JA, Molina F, Puerto A (1976) Conditioned taste aversion caused by palatable nontoxic nutrients. Behav Biol 16: 161-174.

32. Dogterom GJ, van H (1988) Attenuation of neophobia and conditioned taste aversion in the rabbit. Behav Brain Res 28: 253-257.

33. Domjan M (1977) Attenuation and enhancement of neophobia for edibles substance. In: Learning Mechanisms in Food Selection (Barker LM, Best MA, Domjan M, eds), pp 151-179. TX: Baylor University Press.

34. Domjan M (1976) Determinants of the enhancement of flavored-water intake by prior exposure. J Exp Psychol Anim Behav Process 2: 17-27.

35. Domjan M (1985) Cue-consequence specificity and long-delay learning revisited. Ann N Y Acad Sci 443: 54-66.

36. Duguid, G., Wan, H., Dix, S., Bashir, Z. I., Aggleton, J. P., and Brown, M. W. (1998) Effects of lesions and scopolamine on the differential expresion of c-fos produced by novel and familiar stimuli in rat perirhinal cortex. Soc.Neurosci. 24[757.9].

37. Eisenberg M, Kobilo T, Berman DE, Dudai Y (2003) Stability of retrieved memory: inverse correlation with trace dominance. Science 301: 1102-1104.

38. Ellis ME, Kesner RP (1981) Physostigmine and Norepinephrine: effects of injection into the amygdala on taste associations. Physiol Behav 27: 203-209.

39. Escobar ML, Alcocer I, Bermudez R (2002) In vivo effects of intracortical administration of NMDA and metabotropic glutamate receptors antagonists on neocortical long-term potentiation and conditioned taste aversion. Behav Brain Res 129: 101-106.

40. Escobar ML, Bermudez R (2000) Long-term potentiation in the insular cortex enhances conditioned taste aversion retention. Brain Res 852: 208-212.

41. Escobar ML, Chao V, Bermudez R (1998) In vivo long-term potentiation in the insular cortex: NMDA receptor dependence. Brain Res 779: 314-319.

42. Falciglia GA, Couch SC, Gribble LS, Pabst SM, Frank R (2000) Food neophobia in childhood affects dietary variety. J Am Diet Assoc 100: 1474-1481.

43. Ferreira G, Gutierrez R, De L, Bermudez R (2002) Differential involvement of cortical muscarinic and NMDA receptors in short- and long-term taste aversion memory. Eur J Neurosci 16: 1139-1145.

44. Fitzgerald RE, Burton MJ (1983) Neophobia and conditioned taste aversion deficits in the rat produced by undercutting temporal cortex. Physiol Behav 30: 203-206.

45. Franchina JJ, Slank KL (1989) Role of telereceptive and interoceptive (taste) cues in ingestional neophobia in chicks (Gallus domesticus). Behav Neural Biol 52: 116-122.

46. Frank ME (1985) Sensory physiology of taste and smell discriminations using conditioned food aversion methodology. Ann N Y Acad Sci 443: 89-99.

47. Galef BGJ (1977) Mechanisms for the social transmision of acquired food preference from adult to weanling rats. In: Learning Mechanisms in Food Selection (Barker LM, Best MA, Domjan M, eds), pp 99-122. TX: Baylor University Press.

48. Gallo M, Roldan G, Bures J (1992) 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.

49. Gamzu E (1977) The multifaceted nature of taste-aversion-inducing agents: is there a single common factor? In: Learning Mechanisms in Food Selection (Barker LM, Best MA, Domjan M, eds), pp 477-510. TX: Baylor University Press.

50. Garb J, Stunkard A (1974) Taste aversion in man. American Journal of Psychiatry 131: 1204-1207.

51. Garcia J, Ervin FR, Koelling RA (1966) Learning with prolonged delay of reinforcement. Psychonomic Science 5: 121-122.

52. Garcia J, Hankins WG, Rusiniak KW (1974) Behavioral regulation of the milieu interne in man and rat. Science 185: 824-831.

53. Garcia J, Kimeldorf DJ, Koelling RA (1955) Conditioned aversion to saccharin resulting for exposure to gamma radiation. Science 122: 157-158.

54. Garcia J, Koelling RA (1966) Relation of cue to consequence in avoidance learning. Psychonomic Science 4: 123-124.

55. Garcia J, Lasiter PS, Bermudez R, Deems DA (1985) A general theory of aversion learning. Ann N Y Acad Sci 443: 8-21.

56. Gilbertson TA (2003) The lure of dietary fat: are taste buds partly to blame? Chemo Sense 5: 1-6.

57. Gilbertson TA, Damak S, Margolskee RF (2000) The molecular physiology of taste transduction. Curr Opin Neurobiol 10: 519-527.

58. Gilley DW, Franchina JJ (1985) Effects of preexposure flavor concentration on conditioned aversion and neophobia. Behav Neural Biol 44: 503-508.

59. Grill HJ, Norgren R (1978) Chronically decerebrate rats demonstrate satiation but not bait shyness. Science 201: 267-269.

60. Gustavson CR (1977) Comparative and field aspects of learned food aversions. In: Learning Mechanisms in Food Selection (Barker LM, Best MA, Domjan M, eds), pp 23-43. TX: Baylor University Press.

61. Gutierrez H, Gutierrez R, Ramirez T, Silva G, Ormsby CE, Miranda MI, Bermudez R (1999a) Redundant basal forebrain modulation in taste aversion memory formation. J Neurosci 19: 7661-7669.

62. Gutierrez H, Hernandez-Echeagaray E, Ramirez-Amaya V, Bermudez-Rattoni F (1999b) Blockade of N-methyl-D-aspartate receptors in the insular cortex disrupts taste aversion and spatial memory formation. Neuroscience 89: 751-758.

63. Gutierrez R, Rodriguez O, De L, Nunez J, Bermudez R (2003a) Cholinergic dependence of taste memory formation: Evidence of two distinct processes. Neurobiol Learn Mem 80: 323-331.

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

65. Hayne H, Rovee C, Collier G, Tudor L, Morgan CA (1996) Learning and retention of conditioned aversions by freely feeding chicks. Dev Psychobiol 29: 417-431.

66. Hellekant G, Glaser D, Brouwer JN, van d (1976) Gustatory effects of miraculin, monellin and thaumatin in the Saguinus midas tamarin monkey studied with electrophysiological and behavioural techniques. Acta Physiol Scand 97: 241-250.

67. Herness SM (1999) Cellular mechanisims of taste transduction. Annu Rev Physiol 61: 873-900.

68. Johnson C, Beaton R, Hall K (1975) Poison-based avoidance learning in nonhuman primates: use of visual cues. Physiol Behav 14: 403-407.

69. Johnson C, Mason A, Beaton R (1979) Long-term retention of a visually cued poison-based aversion in monkeys. Percept Mot Skills 49: 721-722.

70. Johnson E (2000) Food-neophobia in semi-free ranging rhesus macaques: effects of food limitation and food source. Am J Primatol 50: 25-35.

71. Kalat JW (1974) Taste salience depends on novelty, not concentration, in taste-aversion learning in the rat. Journal of Comparative and Physiological Psychology 86: 47-50.

72. Kesner RP, Berman RF (1977) Effects of midbrain reticular formation, hippocampal, and lateral hypothalamic stimulation upon recovery from neophobia and taste aversion learning. Physiol Behav 18: 763-768.

73. Kiefer SW, Leach LR, Braun JJ (1984) Taste agnosia following gustatory neocortex ablation: dissociation from odor and generality across taste qualities. Behav Neurosci 98: 590-608.

74. Koivisto UK, Sjoden PO (1996) Reasons for rejection of food items in Swedish families with children aged 2-17. Appetite 26: 89-103.

75. Lamprecht R, Dudai Y Transient expression of c-Fos in rat amygdala during training is required for encoding conditioned taste aversion memory. Learn Mem 3: 31-41.

76. Lamprecht R, Dudai Y (1995) Differential modulation of brain immediate early genes by intraperitoneal LiCl. Neuroreport 7: 289-293.

77. Lamprecht R, Hazvi S, Dudai Y (1997) cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. J Neurosci 17: 8443-8450.

78. Lasiter PS (1983) Gastrointestinal reactivity in rats lacking anterior insular neocortex. Behav Neural Biol 39: 149-154.

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

80. Lasiter PS, Deems DA, Oetting RL, Garcia J (1985b) Taste discriminations in rats lacking anterior insular gustatory neocortex. Physiol Behav 35: 277-285.

81. Lopez G, Fernandez R, Escobar ML, Bermudez R, Tapia R (1993) Effects of excitotoxic lesions of the nucleus basalis magnocellularis on conditioned taste aversion and inhibitory avoidance in the rat. Pharmacol Biochem Behav 45: 147-152.

82. Lorden JF (1976) Effects of lesions of the gustatory neocortex on taste aversion learning in the rat. J Comp Physiol Psychol 90: 665-679.

83. Mackay B (1974) Conditioned food aversions produced by toxicosis in Atlantic cod. Behav Biol 12: 347-355.

84. Malkova L, Bachevalier J, Mishkin M, Saunders RC (2001) Neurotoxic lesions of perirhinal cortex impair visual recognition memory in rhesus monkeys. Neuroreport 12: 1913-1917.

85. Marcontell DK, Laster AE, Johnson J (2002) Cognitive-behavioral treatment of food neophobia in adults. Journal of anxiety disorders 16: 341-349.

86. Margolskee RF (1995) Receptor mechanisms in gustation. In: Handbook of olfaction and gustation (Richard LD, ed), pp 575-595. New York: Marcel Dekker, Inc.

87. Maslow AH (1932) The "emotion" of disgust in dogs. Journal of Comparative Psychology 14: 401-407.

88. Massey PV, Bhabra G, Cho K, Brown MW, Bashir ZI (2001) Activation of muscarinic receptors induces protein synthesis-dependent long-lasting depression in the perirhinal cortex. European Journal of Neuroscience 14: 145-152.

89. McIntyre CK, Ragozzino ME, Gold PE (1998) Intra-amygdala infusions of scopolamine impair performance on a conditioned place preference task but not a spatial radial maze task. Behav Brain Res 95: 219-226.

90. Meunier M, Bachevalier J, Mishkin M, Murray EA (1993) Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. J Neurosci 13: 5418-5432.

91. Mierson S (1995) Transduction of taste stimuli by receptor cells in the gustatory system. In: Handbook of olfaction and gustation (Richard LD, ed), pp 597-610. New York: Marcel Dekker, Inc.

92. Miller IJ (1995) Anatomy of the peripheral taste system. In: Handbook of olfaction and gustation (Richard LD, ed), pp 521-547. New York: New York.

93. Miller RR, Holzman AD (1981a) Neophobia: generality and function. Behav Neural Biol 33: 17-44.

94. Miller RR, Holzman AD (1981b) Neophobias and conditioned taste aversions in rats following exposure to novel flavors.

95. Mitchell D (1976) Experiments on neophobia in wild and laboratory rats: a reevaluation. J Comp Physiol Psychol 90: 190-197.

96. Murray, E. A. and Richmond, B. J. (2001) Role of perirhinal cortex in object perception, memory and associations. Curr Opin Neurobiol 11, 188-193.

97. Nachman M (1963) Learned aversion to the taste of lithium chloride and generalization to other salts. Journal of Comparative and Physiological Psychology 56: 343-349.

98. Nachman M (1970) Learned taste and temperature aversions due to lithium chloride after temporal delays. Journal of Comparative and Physiological Psychology 73: 20-30.

99. Nachman M, Hartley PL (1975) Role of illness in producing learned taste aversions in rats: a comparison of several rodenticides. J Comp Physiol Psychol 89: 1010-1018.

100. Nachman M, Jones DR (1974) Learned taste aversions over long delays in rats: The role of learned safety. J Comp Physiol Psychol 86: 949-956.

101. Naor C, Dudai Y (1996a) Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. Behav Brain Res 79: 61-67.

102. Naor C, Dudai Y (1996b) Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. Behav Brain Res 79: 61-67.

103. Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS (2002) An amino-acid taste receptor. Nature 416: 199-202.

104. Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS (2001) Mammalian sweet taste receptors. Cell 106: 381-390.

105. Otto, T. and Eichenbaum, H. (1992) Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. Behav Neurosci. 106, 762-775.

106. Pliner P, Lahteenmaki L, Tuorila H (1998) Correlates of human food neophobia. Appetite 30: 93.

107. Pliner P, Pelchat M, Grabski M (1993) Reduction of neophobia in humans by exposure to novel foods. Appetite 20: 111-123.

108. Power AE, Vazdarjanova A, McGaugh JL (2003) Muscarinic cholinergic influences in memory consolidation. Neurobiol Learn Mem 80: 178-193.

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

110. Reilly S, Trifunovic R (2001) Lateral parabrachial nucleus lesions in the rat: neophobia and conditioned taste aversion. Brain Res Bull 55: 359-366.

111. Reutter K, Witt M (1993) Morphology of vertebrate taste organs and their nerve supply. In: Mechanisms of taste transduction (Sidney A.Simon, Roper DS, eds), pp 29-82. Boca Raton, Florida: CRC Press, Inc.

112. Revusky SH, Bedarf EW (1967) Association of illness with prior ingestion of novel foods. Science 155: 219-220.

113. Riekkinen P, Kuitunen J, Riekkinen M (1995) Effects of scopolamine infusions into the anterior and posterior cingulate on passive avoidance and water maze navigation. Brain Res 685: 46-54.

114. Rosenblum K, Meiri N, Dudai Y (1993) Taste memory: the role of protein synthesis in gustatory cortex. Behav Neural Biol 59: 49-56.

115. Rozin P (1977) The significance of learning mechanisms in food selection: somebiology, psychology and sociology of science. In: Learning Mechanisms in Food Selection (Barker LM, Best MA, Domjan M, eds), pp 557-589. TX: Baylor University Press.

116. Rudy JW (1996) Scopolamine administered before and after training impairs both contextual and auditory-cue fear conditioning. Neurobiol Learn Mem 65: 73-81.

117. Sakai N, Yamamoto T (1998) Role of the medial and lateral parabrachial nucleus in acquisition and retention of conditioned taste aversion in rats. Behav Brain Res 93: 63-70.

118. Sako N, Okamoto K, Mori T, Yamamoto T (2002) The hardness of food plays an important role in food selection behavior in rats. Behav Brain Res 133: 377-382.

119. Saper CB (1982) Convergence of autonomic and limbic connections in the insular cortex of the rat. J Comp Neurol 210: 163-173.

120. Schafe GE, Sollar SI, Bernstein IL (1995) The CS-US Interval and taste aversion learning: a brief look. Behavioral Neuroscience 109: 799-802.

121. Schafe GE, Bernstein IL (1998) Forebrain contribution to the induction of a brainstem correlate of conditioned taste aversion. II. Insular (gustatory) cortex. Brain Res 800: 40-47.

122. Schroeder JP, Packard MG (2002) Posttraining Intra-basolateral amygdala scopolamine impairs food-and amphetamine-induced conditioned place preference. Behav Neurosci 116: 922-927.

123. Scott TR, Giza BK (1996) Issues of gustatory neural coding: where they stand today. Physiol Behav 69: 65-76.

124. Scott TR, Mark GP (1987) The taste system encodes stimulus toxicity. Brain Res 414: 197-203.

125. Sewards TV, Sewards MA (2001) Cortical association areas in the gustatory system. Neuroscience and Biobehavioral Reviews 25: 395-407.

126. Siegel S (1974) Flavor preexposure and "learned safety". Journal of Comparative and Physiological Psychology 86: 674-678.

127. Smith DV, Theodore RM (1984) Conditioned taste aversions: generalization to taste mixtures. Physiol Behav 32: 983-989.

128. Smith JC, Roll DL (1967) Trace conditioning with x-rays as an aversive stimulus. Psychonomic Science 9: 11-12.

129. Spector AC, Breslin P, Grill HJ (1988) Taste reactivity as a dependent measure of the rapid formation of conditioned taste aversion: a tool for the neural analysis of taste-visceral associations. Behav Neurosci 102: 942-952.

130. Spector AC, Norgren R, Grill HJ (1992) Parabrachial gustatory lesions impair taste aversion learning in rats. Behav Neurosci 106: 147-161.

131. Stewart CN, Reidinger RF (1984) Disparity between formation of conditioned flavor aversions and neophobia during grooming in rats and mice. Physiol Behav 32: 955-959.

132. Suzuki.W.A. (1996) The anatomy, physiology and functions of the perirhinal cortex. Curr Opin Neurobiol 6, 179-186.

133. Swank MW (1999) Coordinate regulation of Fos and Jun proteins in mouse brain by LiCl. Neuroreport 10: 3685-3689.

134. Swank MW (2000) Phosphorylation of MAP kinase and CREB in mouse cortex and amygdala during taste aversion learning. Neuroreport 11: 1625-1630.

135. Swank MW, Schafe GE, Bernstein IL (1995) c-Fos induction in response to taste stimuli previously paired with amphetamine or LiCl during taste aversion learning. Brain Res 673: 251-261.

136. Tang Y, Mishkin M, Aigner TG (1997) Effects of muscarinic blockade in perirhinal cortex during visual recognition. Proc Natl Acad Sci U S A 94: 12667-12669.

137. Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GAZM, Liu G, Tsien JZ (1999) Genetic enhancement of learning and memory in mice. Nature 401: 63-69.

138. Tassoni G, Lorenzini CA, Baldi E, Sacchetti B, Bucherelli C (2000) Role of the perirhinal cortex in rats' conditioned taste aversion response memorization. Behav Neurosci 114: 875-881.

139. Travers JB, Dinardo LA, Karimnamazi H (1997) Motor and premotor mechanisms of licking. Neuroscience Bio Behav 21: 631-664.

140. Travers SP, Norgren R (1991) Coding the sweet taste in the nucleus of the solitary tract: differential roles for anterior tongue and nasoincisor duct gustatory receptors in the rat. J Neurophysiol 65: 1372-1380.

141. Warburton EC, Koder T, Cho K, Massey PV, Duguid G, Bashir ZI, Brown M.W. (2003) Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. Neuron 38: 987-996.

142. Weiner I, I, Tarrasch RR, Hasson OO, Forian RR, Smith AA, Rawlins JJ, Feldon JJ (1994) The effects of chronic administration of ceronapril on the partial reinforcement extinction effect and latent inhibition in rats. Behav Pharmacol 5: 306-314.

143. Welzl H, D'Adamo P, Lipp HP (2001) Conditioned taste aversion as a learning and memory paradigm. Behav Brain Res 125: 205-213.

144. Whitehouse JM (1964) Effects of atropine on discrimination learning in the rat. J Comp Physiol Psychol 57: 13-15.

145. Wong GT, Gannon KS, Margolskee RF (1996) Transduction of bitter and sweet taste by gustducin. Nature 381: 796-800.

146. Wong R, McBride CB (1993) Flavour neophobia in gerbils (Meriones unguiculatus) and hamsters (Mesocricetus auratus). Q J Exp Psychol B 46: 129-143.

147. Yamamoto T (1993) Neural mechanisms of taste aversion learning. Neurosci Res 16: 181-185.

148. Yamamoto T, Azuma S, Kawamura Y (1984) Functional relations between the cortical gustatory area and the amygdala: electrophysiological and behavioral studies in rats. Exp Brain Res 56: 23-31.

149. Yamamoto T, Matsuo R, Kiyomitsu Y, Kitamura R (1989) Taste responses of cortical neurons in freely ingesting rats. J Neurophysiol 61: 1244-1258.

150. Yamamoto T, Shimura T, Sako N, Azuma S, Bai WZ, Wakisaka S (1992) C-fos expression in the rat brain after intraperitoneal injection of lithium chloride. Neuroreport 3: 1049-1052.

151. Yamamoto T, Shimura T, Sako N, Yasoshima Y, Sakai N (1994) Neural substrates for conditioned taste aversion in the rat. Behav Brain Res 65: 123-137.

152. Yasoshima Y, Morimoto T, Yamamoto T (2000) Different disruptive effects on the acquisition and expression of conditioned taste aversion by blockades of amygdalar ionotropic and metabotropic glutamatergic receptor subtypes in rats. Brain Res 869: 15-24.

153. Yasoshima Y, Yamamoto T (1997) Rat gustatory memory requires protein kinase C activity in the amygdala and cortical gustatory area. Neuroreport 8: 1363-1367.

154. Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112: 293-301.

155. Zhu X.O., Brown M.W., Aggleton JP (1995) neuronal Signalling of information important to visual recognition memory in rat rhinal and neighbouring cortices. European Journal of Neuroscience 7: 753-765.

Abreviaturas

Atenuación de la neofobia (AN)

Condicionamiento aversivo a los sabores (CAS)

Corteza insular (CI)

Formación reticular (FR)

Inhibición latente (IL)

N-Metil-d-Aspartato (NMDA)

Núcleo del tracto del solitario (NTS)

Núcleo parabraquial (PBN)

Tálamo ventroposteromedial parte parvocelular (VPMpc)

Trazo de memoria del sabor (TMS)