UNIVERSIDAD DE OVIEDO
DEPARTAMENTO DE PSICOLOGÍA

Universidad de Oviedo

TESIS DOCTORAL

Patricia Gasalla Canto

APRENDIZAJE DE AVERSIÓN AL SABOR Y RESPUESTAS CONDICIONADAS DE NÁUSEA

TASTE AVERSION LEARNING AND CONDITIONED DISGUST REACTIONS

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TASTE AVERSION LEARNING AND CONDITIONED DISGUST REACTIONS

DIRECTOR:

Dr. Matías López Ramírez

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RESUMEN DEL CONTENIDO DE TESIS DOCTORAL

1.- Título de la Tesis	
Español/Otro Idioma: Aprendizaje de aversión al sabor y respuestas condicionadas de náusea	Inglés: Taste aversion learning and conditioned disgust reactions

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RESUMEN (en español)

En esta tesis doctoral se examina el efecto de la preexposición a los estímulos en el aprendizaje de la aversión al sabor en ratas. Más específicamente, se analiza el efecto de la exposición repetida a los estímulos condicionado (EC) e incondicionado (EI) sobre la adquisición de respuestas de náusea. Tradicionalmente, estos efectos se han evaluado midiendo los cambios en el consumo voluntario de la solución gustativa tras el condicionamiento. En los experimentos aquí realizados se utilizaron las técnicas de reactividad al sabor y de análisis del patrón de ingesta para evaluar el cambio en la palatabilidad de los sabores derivado de su asociación con una droga emética.

En la Parte I de la tesis se expone el marco teórico de la investigación, los objetivos específicos de la misma y la metodología empleada. En la Parte II se exponen los estudios realizados agrupados por temáticas: preexposición al EI, preexposición al EC y bases neurales.

En cuanto al efecto de preexposición al EI, los experimentos presentados en el capítulo 5 muestran que el preentrenamiento con cloruro de litio (LiCl) interfiere con la adquisición de las respuestas condicionadas de náusea a una solución de sacarina. Los resultados se explican en términos de bloqueo por el contexto, es decir, las respuestas de nausea elicitadas por el contexto pueden interferir con la adquisición posterior de las respuestas de disgusto a un sabor emparejado con el LiCl. En apoyo de esta hipótesis, los datos muestran que el efecto de bloqueo se atenúa si las ratas

reciben exposiciones no reforzadas al contexto (extinción del condicionamiento contextual) antes de asociar el sabor al LiCl.

El capítulo 6 examina el papel que juegan las claves relacionadas con la infusión intraoral del fluido sobre el control del efecto de preexposición al EI. Se encontró que la preexposición al LiCl no atenúa la expresión de respuestas condicionadas de disgusto a la sacarina (como ocurre normalmente en el aprendizaje de aversión al sabor) debido a que las claves derivadas de la infusión se asocian al malestar provocado por el litio durante las sesiones de preexposición. Por lo tanto, las claves relacionadas con la infusión pueden provocar reacciones de nausea condicionada e interferir con el condicionamiento posterior de respuestas aversivas (disgusto condicionado) a una solución de sacarina.

En cuanto al efecto de preexposición al EC, los resultados de esta tesis doctoral muestran que la exposición no reforzada a un sabor atenúa el efecto de la aversión al sabor medida a través del test de reactividad al sabor y del análisis del patrón de ingesta. En concreto, como se concluye en el capítulo 7, la exposición repetida a un sabor antes de su condicionamiento con LiCl atenúa tanto el descenso en el consumo voluntario de la solución como la frecuencia de la respuesta de lameteo como índice del valor hedónico de la solución. Además, se examinó el patrón de extinción del condicionamiento aversivo con ambas medidas. Los resultados obtenidos indican que el descenso en el consumo voluntario de la solución es más resistente a la extinción que los cambios en la palatabilidad con independencia de la preexposición previa al fluido, lo que sugiere que el la experiencia previa con el sabor influye en la magnitud del aprendizaje aversivo gustativo y no tanto en su naturaleza.

Por último, se exploraron las regiones cerebrales implicadas en el condicionamiento de respuestas de nausea en el aprendizaje de aversión al sabor. En el capítulo 8, se analizaron las redes neuronales implicadas en la aversión al sabor midiendo el metabolismo oxidativo de las regiones cerebrales asociadas a la inhibición latente de repuestas condicionadas de náusea. Se apreció un descenso en los niveles del metabolismo oxidativo en el núcleo parabraquial (PBN), el área tegmental ventral (VTA), el núcleo del lecho de la estría terminal (NET), el núcleo

de la amígdala basolateral (BLA) y el prefrontal (mPFC) como resultado de la asociación del sabor con los efectos eméticos del cloruro de litio. Por otro lado, se describieron nuevas redes neuronales como resultado del procesamiento de sabores familiares o novedosos.

Finalmente, en el capítulo 9, se utilizó la expresión de la proteína c-Fos como marcador de la actividad neuronal para explorar el papel que desempeñan el núcleo accumbens (NAcb) y la corteza insular (IC) en la adquisición de respuestas condicionadas de náusea. Los resultados obtenidos sugieren una reducción de la actividad c-Fos en el NAcb (corteza y núcleo) cuando se infunde a los animales con un sabor que ha sido preexpuesto antes del condicionamiento. En cambio, se produce una reducción de la actividad neuronal en la corteza insular cuando el sabor es familiar.

Destacar, por último, que la investigación de las respuestas condicionadas de nausea presentada en esta tesis doctoral presenta una implicación práctica potencial en el diseño de técnicas de intervención clínica encaminadas a reducir el impacto de las náuseas derivadas de los tratamientos de quimioterapia en algunos tipos de cáncer.

RESUMEN (en Inglés)

In this thesis, preexposure effects in taste aversion learning in rats are examined. More specifically, the effect of repeated exposure to the conditioned stimulus (CS) and unconditioned stimulus (US) on the acquisition of nausea responses are analyzed. Traditionally, these effects were evaluated by measuring changes in the voluntary intake of the taste solution after conditioning. In the experiments carried out here, the taste reactivity test and analysis of licking behavior were used in order to assess changes in the palatability of flavor solutions paired with lithium chloride.

The theoretical framework, the methodology and the specific aims of this thesis are presented in Part I. In Part II of this thesis experiments are grouped by the

following topics: US-preexposure, CS-preexposure and the neural bases of CTA.

In relation to the US-preexposure effect, the experiments reported in Chapter 5 showed that pretraining with lithium chloride (LiCl) interfered with the production of conditioned disgust reactions to a saccharin solution. These findings are explained in terms of blocking by context, that is, contextually elicited conditioned nausea can block the subsequent development of conditioned disgust reactions to a LiCl-paired flavor. Supporting this hypothesis, results showed that the blocking effect was abolished when rats were given non-reinforced exposure to the previously LiCl-paired context before aversive conditioning of the saccharin in compound with the context.

Chapter 6 examined the role played by the cues generated by intraoral infusions of water in producing the US-preexposure effect. It was found that LiCl preexposure did not attenuate the production of conditioned disgust reactions to saccharin (typically produced by taste aversion learning) due to stimulation arising from oral infusions that became associated with LiCl during preexposure. Therefore, infusion-related cues can evoke a conditioned state of nausea and interfere with the subsequent conditioning of disgust reactions.

In terms of the CS-preexposure effect, the results suggest that non-reinforced exposure to a flavor attenuates the effect of taste aversion on taste palatability as assayed by taste reactivity and the analysis of licking behavior. Specifically, in Chapter 7, preexposure to a flavor prior to its pairing with lithium-induced nausea attenuated both the reduction in consumption and taste palatability. In addition, the patterns of extinction were evaluated, and it was found that suppressed consumption appears to be more resistant to extinction than changes in palatability, in spite of preexposure to the flavor cue, suggesting that latent inhibition influences the quantity but not the quality of a conditioned taste aversion.

Additionally, this thesis analyzed brain regions involved in conditioned disgust reactions to flavors. In Chapter 8, the neuronal networks involved in taste aversion were examined by measuring brain oxidative metabolism following latent inhibition of LiCl-induced conditioned disgust. Taste aversion learning was

associated with decreased levels of oxidative metabolism in the parabrachial nucleus, tegmental ventral area, bed nucleus of stria terminalis, basolateral amygdala and prefrontal cortex. Further, novel patters of brain networks involved in the processing of the hedonic value of familiar/novel flavors were identified and described..

Finally, in Chapter 9, c-Fos expression as a marker of neural activation was used to explore the involvement of the insular cortex and accumbens in the expression of lithium-induced conditioned disgust. The findings provide evidence of reduced neural activity in NAcb (core and shell) in response to taste palatability after flavor preexposure in taste aversion learning, whilst reduced activity in IC was found in response to infusions of familiar tastes.

The investigation of nausea conditioned responses presented in this thesis could also be of translational value. In particular, knowledge of the mechanisms involved in conditioned nausea could provide a useful tool for the design of clinical intervention techniques for reducing the impact of nausea derived from chemotherapy treatments in cancer.

SR. DIRECTOR DE DEPARTAMENTO DE PSICOLOGÍA
SR. PRESIDENTE DE LA COMISIÓN ACADÉMICA DEL PROGRAMA DE DOCTORADO EN PSICOLOGÍA

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"Now let us fight to fulfill that promise! Let us fight to free the world - to do away with national barriers - to do away with greed, with hate and intolerance. Let us fight for a world of reason, a world where science and progress will lead to all men's happiness".

Charles Chaplin, 'The Great Dictator', 1940

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ÍNDICE

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Abreviaturas

Adenosin-trifosfato (ATP)

Amygdala (AMY)

Aprendizaje aversivo gustativo (AAG)

Cannabidiol (CBC)

Cloruro de litio (LiCl)

Complejo dorso-vagal (CDV)

Corteza prefrontal medial (mPFC)

Estímulo condicionado (EC)

Estímulo incondicionado (EI)

Inhibición latente (IL)

Núcleo del tracto solitario (NET)

Núcleo del lecho la estría terminal (NET)

Núcleo parabraquial (NPB)

Receptor cannabinoide$_1$ (CB_1)

Receptor cannabinoide$_2$ (CB_2)

Respuesta condicionada (RC)

Respuesta incondicionada (RI)

Serotonina (5-HT)

Δ^9-tetrahidrocannabinol (Δ^9-THC)

Abbreviations

Acetylcholine (ACh)

Amygdala, basolateral nucleus (BLA)

Amygdala, central nucleus (CeA)

Bed nucleus of stria terminalis (BNST)

Cingulate cortex (Cg)

Conditioned stimulus (CS)

Conditioned taste aversion (CTA)

Cytochrome c oxidase (CO)

Dopamine (DA)

Infralimbic cortex (ILc)

Insular cortex (IC)

Intraoral (IO)

Latent inhibition (LI)

Lithium Chloride (LiCl)

Magnetic resonance imaging (MRI)

Medial prefrontal cortex (mPFC)

Nucleus accumbens (NAcb)

Parabrachial nucleus (PBN)

Prelimbic cortex (PrL)

Sodium Chloride (NaCl)

Taste reactivity (TR) Test

Unconditioned stimulus (US)

Ventral posterolateral thalamic nucleus (VPL)

Ventral posteromedial thalamic nucleus (VPM)

Ventral tegmental area (VTA)

DECLARACIÓN

- Los experimentos recogidos en el capítulo 7 de esta tesis están publicados como:

  Dwyer, D. M., Gasalla, P., López, M. (2013). Nonreinforced flavor exposure attenuates the effects of conditioned taste aversion on both flavor consumption and cue palatability. *Learning & Behavior*, 41, 390-401. dx.doi.org/10.3758/s13420-013-014-x

- Los experimentos recogidos en el capítulo 5 de esta tesis han sido redactados para su publicación y están sometidos a revisión:

  Gasalla, P., Soto, A., López, M. (2014). Blocking of acquisition of lithium-induced conditioned disgust reactions by contextual cues. *Physiology & Behavior.*

- Los experimentos recogidos en el capítulo 6 de esta tesis han sido redactados para su publicación y están bajo revisión en:

  Gasalla, P., Soto, A., López, M. (2014). Role of intraoral infusion cues in producing the unconditioned stimulus (US) pre-exposure effect in taste aversion learning. *Behavioural Processes.*

- Los experimentos recogidos en el capítulo 8 de esta tesis han sido redactados para su publicación y están bajo revisión como:

  Gasalla, P., Begega, A., Soto, A., López, M. (2014). Functional brain networks underlying nausea-induced conditioned disgust in rats. *Brain Research.*

- Los experimentos recogidos en el capítulo 9 de esta tesis han sido redactados para su publicación y están sometidos a revisión en:

  Gasalla, P., Begega, A., Soto, A., López, M. (2014). Differential involvement of the nucleus accumbens and the insular cortex in the hedonic reactivity to taste stimuli in taste aversion learning. *Brain Research Bulletin*.

Resumen

En esta tesis doctoral se examina el efecto de la preexposición a los estímulos en el aprendizaje de la aversión al sabor en ratas. Más específicamente, se analiza el efecto de la exposición repetida a los estímulos condicionado (EC) e incondicionado (EI) sobre la adquisición de respuestas de náusea. Tradicionalmente, estos efectos se han evaluado midiendo los cambios en el consumo voluntario de la solución gustativa tras el condicionamiento. En los experimentos aquí realizados se utilizaron las técnicas de reactividad al sabor y de análisis del patrón de ingesta para evaluar el cambio en la palatabilidad de los sabores derivado de su asociación con una droga emética.

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El capítulo 6 examina el papel que juegan las claves relacionadas con la infusión intraoral del fluido sobre el control del efecto de preexposición al EI. Se encontró que la preexposición al LiCl no atenúa la expresión de respuestas condicionadas de disgusto a la sacarina (como ocurre normalmente en el aprendizaje de aversión al sabor) debido a que

las claves derivadas de la infusión se asocian al malestar provocado por el litio durante las sesiones de preexposición. Por lo tanto, las claves relacionadas con la infusión pueden provocar reacciones de nausea condicionada e interferir con el condicionamiento posterior de respuestas aversivas (disgusto condicionado) a una solución de sacarina.

En cuanto al efecto de preexposición al EC, los resultados de esta tesis doctoral muestran que la exposición no reforzada a un sabor atenúa el efecto de la aversión al sabor medida a través del test de reactividad al sabor y del análisis del patrón de ingesta. En concreto, como se concluye en el capítulo 7, la exposición repetida a un sabor antes de su condicionamiento con LiCl atenúa tanto el descenso en el consumo voluntario de la solución como la frecuencia de la respuesta de lameteo como índice del valor hedónico de la solución. Además, se examinó el patrón de extinción del condicionamiento aversivo con ambas medidas. Los resultados obtenidos indican que el descenso en el consumo voluntario de la solución es más resistente a la extinción que los cambios en la palatabilidad con independencia de la preexposición previa al fluido, lo que sugiere que el la experiencia previa con el sabor influye en la magnitud del aprendizaje aversivo gustativo y no tanto en su naturaleza.

Por último, se exploraron las regiones cerebrales implicadas en el condicionamiento de respuestas de nausea en el aprendizaje de aversión al sabor. En el capítulo 8, se analizaron las redes neuronales implicadas en la aversión al sabor midiendo el metabolismo oxidativo de las regiones cerebrales asociadas a la inhibición latente de repuestas condicionadas de náusea. Se apreció un descenso en los niveles del metabolismo oxidativo en el núcleo parabraquial (PBN), el área tegmental ventral (VTA), el núcleo del lecho de la estría terminal (NET), el núcleo de la amígdala basolateral (BLA) y el prefrontal (mPFC) como resultado de la asociación del sabor con los efectos eméticos del

cloruro de litio. Por otro lado, se describieron nuevas redes neuronales como resultado del procesamiento de sabores familiares o novedosos.

Finalmente, en el capítulo 9, se utilizó la expresión de la proteína c-Fos como marcador de la actividad neuronal para explorar el papel que desempeñan el núcleo accumbens (NAcb) y la corteza insular (IC) en la adquisición de respuestas condicionadas de náusea. Los resultados obtenidos sugieren una reducción de la actividad c-Fos en el NAcb (corteza y núcleo) cuando se infunde a los animales con un sabor que ha sido preexpuesto antes del condicionamiento. En cambio, se produce una reducción de la actividad neuronal en la corteza insular cuando el sabor es familiar.

Destacar, por último, que la investigación de las respuestas condicionadas de nausea presentada en esta tesis doctoral presenta una implicación práctica potencial en el diseño de técnicas de intervención clínica encaminadas a reducir el impacto de las náuseas derivadas de los tratamientos de quimioterapia en algunos tipos de cáncer.

Parte I

Marco teórico de la investigación

Capítulo 1

El aprendizaje aversivo gustativo

CAPITULO I

El aprendizaje aversivo gustativo

1.1. Aproximación histórica

Un mecanismo altamente especializado de adaptación al entorno, resultado de la evolución, es la capacidad de las especies animales de seleccionar su dieta (nutrientes, fluidos) para preservar y mantener su equilibrio homeostático. Los animales seleccionan su dieta de manera innata, sin embargo, es necesario aprender a seleccionar aquellos alimentos que garanticen su supervivencia en situaciones en las que sus hábitos alimenticios se vean alterados por cambios en la naturaleza (Domjan, 1998). Ante una situación de necesidad de energía, el animal debe ser capaz de identificar alimentos que regulen el estado interno del organismo. Si el consumo de esos alimentos resulta en la reducción de las necesidades biológicas del animal, las conductas encaminadas a la obtención del alimento se reproducirán en el futuro. Pero si el consumo de esos alimentos resulta en envenenamiento, las propiedades de la comida como el olor o el sabor se asociarán con el malestar gástrico y será evitado en sucesivas ocasiones. Este aprendizaje asociativo de especial relevancia biológica se denomina aprendizaje aversivo gustativo (AAG) (Garcia, Kimmeldorf y Koelling, 1955; Garcia y Koelling, 1966; ver Reilly y Schachtman, 2009 para una revisión reciente). En el AAG el animal aprende a asociar una sustancia novedosa con las consecuencias que produce su ingesta, aun cuando los efectos negativos sean moderadamente aversivos.

Este tipo de aprendizaje se ha identificado en una variedad de especies, desde invertebrados como la babosa de jardín (Limax maximus) (Sahley, Rudy y Gelperin,

1981) hasta en humanos (Bernstein, 1978; Logue, 1985). Sin embargo la especie que más se ha empleado para el estudio del AAG es la rata. Además de por razones prácticas y de carácter ético, el principal motivo es que la rata carece del reflejo del vómito, lo cual la convierte en un buen candidato para evaluar el efecto de sustancias tóxicas en la selección de alimentos.

Las primeras investigaciones sobre el fenómeno de AAG surgen en el contexto de la Segunda Guerra Mundial, y deriva de investigaciones de carácter militar que pretendían dar solución a dos problemas relacionados con la nueva era atómica: el control de la plaga de roedores en las trincheras y la necesidad de estudiar los efectos de la radiación en los seres vivos (ver Freeman y Riley, 2009, para una revisión histórica). En este momento histórico, surge todo un cuerpo de investigaciones encaminadas a desarrollar nuevas tecnologías de eliminación de plagas, entre ellas la elaboración de nuevos venenos y cebos. Sin embargo, durante los primeros ensayos prácticos se encontraron con un problema metodológico: la neofobia. En este sentido, las ratas sin experiencia previa con la comida envenenada probaban cantidades no letales de ésta, y tras recuperarse del malestar provocado por el veneno aprendían a evitar esa comida en sucesivas ocasiones (Elton, 1954). Para solucionar este inconveniente, uno de los investigadores de la Oficina de Población Animal, Julian Rzóska, llevó a cabo una serie de experimentos en el laboratorio con ratas en situación de privación a las que se les administraba comida envenenada (Rzóska, 1954). Las principales conclusiones de estos trabajos fueron que éstas ratas asociaban el malestar provocado por el veneno con las cualidades sensoriales de la comida, y de esta forma, aprendían a evitarla en sucesivas ocasiones. Además, si se les administraba un veneno con el que ya tenían experiencia (o novedoso) mezclado en un alimento nuevo, las ratas no lo rechazaban. Parecía por tanto que la clave estaba en la base de comida a la que tenían acceso, y si ésta estaba

catalogada como peligrosa o segura. A partir de estas conclusiones, la aversión a la comida fue definida como un fenómeno asociativo en el que las cualidades sensoriales de un alimento se asocian con las consecuencias tóxicas derivadas de su consumo.

El segundo problema que debían afrontar los investigadores eran los efectos de la radiación en los seres vivos. Sin duda, uno de los pioneros que guiaron la investigación a este respecto fue John Garcia (ver por ejemplo Garcia, 1980; Garcia et al., 1955). Sus investigaciones estaban orientadas a evaluar los efectos de la radiación en varios aspectos fisiológicos y conductuales, así como a desarrollar métodos de protección. En el trascurso de sus investigaciones, observó un patrón llamativo de consumo de agua y comida en las ratas que habían sido irradiadas. Garcia observó que el consumo de agua o comida era menor en la cámara experimental que en las jaulas hogar. Dado que las botellas de plástico que contenían el agua en la cámara experimental y en las jaulas hogar variaban, comprobó que las botellas de la cámara experimental dejaban un sabor en el agua que posteriormente era asociado al malestar provocado por la radiación (Garcia et al., 1955). En palabras de Garcia, "el progresivo cambio en la conducta consumatoria durante las exposiciones repetidas (a la radiación) puede ser, en parte, una respuesta condicionada en la cual la evitación del agua y la comida está fortalecida por el aprendizaje derivado del emparejamiento repetido con la situación de radiación" (Garcia et al., 1955, p. 157).

Garcia y sus colaboradores demostraron que el consumo de una solución de sacarina se ve reducido (en relación a su consumo inicial) por el emparejamiento de la solución con el malestar provocado por las exposiciones a la radiación. Demostraron además que estos resultados no eran fruto de la mera habituación o del cambio en la preferencia por la sacarina. De esta forma, el AAG fue introducido como una forma de condicionamiento clásico en la que el sabor funciona como un estímulo condicionado

(EC) y las consecuencias de la administración de la radiación como un estímulo incondicionado (EI).

Si el AAG era ciertamente una forma de condicionamiento clásico debería seguir las mismas reglas que otros modelos de condicionamiento establecidos. Garcia y sus colaboradores llevaron a cabo un análisis temporal en el que variaban el orden de la presentación de la sacarina y la radiación utilizando procedimientos simultáneos, de huella o hacia atrás. El condicionamiento hacia atrás fue el único procedimiento que resultó inefectivo, así que aparentemente el AAG parecía ajustarse a los mismos parámetros que el aprendizaje "pavloviano" (Pavlov, 1927). Sin embargo la capacidad de la radiación de funcionar como un EI fue ampliamente discutida, dado que ésta era "imperceptible a los sentidos" (Garcia, 1980) y por lo tanto carecía de propiedades estimulares. Garcia demostró que bajas dosis de presentaciones cortas de radiación en la cabeza servía como EC que predecía la aparición de un shock (Garcia y Buchwald, 1963).

1.2. El renglón torcido del aprendizaje asociativo

Con los avances en su investigación, Garcia y sus colaboradores observaron que en realidad el AAG era un tipo muy particular de condicionamiento que no seguía las reglas de asociación propias del condicionamiento clásico (Seligman, 1970). En realidad poseía parámetros de adquisición particulares que llevaron a suscitar el interés por su estudio en los investigadores del aprendizaje. Hay tres características que convierten al AAG en un aprendizaje asociativo diferente a los demás: su adquisición en un único ensayo, que se produce incluso con largas demoras entre el EC y el EI y la especificidad de la relación clave-consecuencia.

La primera característica hace referencia a la posibilidad de desarrollar una fuerte aversión a un sabor en un solo ensayo de adquisición (Garcia et al., 1955). Evolutivamente, el organismo está preparado para aprender de forma rápida la relación entre estados internos del organismo y claves sensoriales externas (sabor y olor), y de esta forma garantizar la supervivencia. Por lo tanto, adaptativamente, es importante para el animal aprender de manera rápida la relación que existe entre el consumo de sustancias y las consecuencias derivadas de ese consumo. El aprendizaje en un solo ensayo también se ha observado en otro tipo de condicionamientos, como es el caso del condicionamiento del miedo, y ocasionalmente en el salival.

La segunda característica hace referencia a la larga demora que es capaz de soportar el AAG entre la presentación del EC y del EI. Clásicamente, las reglas del aprendizaje asociativo definen que las presentaciones del EC y el EI deben ser contigüas (variando el intervalo óptimo en función de la respuesta que se estudia, aproximadamente entre 0,5 y 2 segundos). Excepcionalmente, el AAG es capaz de desarrollarse con periodos de demora que van desde minutos a horas o incluso de forma solapada (Garcia y Koelling, 1966). Una de las explicaciones que se propuso fue que la demora de la asociación EC-EI estaba mediada por los post-efectos sensoriales, esto es, la regurgitación de la ingesta podría provocar indirectamente una contigüidad temporal entre los estímulos. Sin embargo, esta explicación se abandonó rápidamente debido a que las ratas carecen del sistema reflejo del vómito, y metabolizan sustancias como la sacarina rápidamente. Posteriormente se propusieron otras explicaciones (para una revisión, ver Domjan, 1985) entre ellas, se sugería que el aspecto crítico era que el AAG implicaba el condicionamiento de una respuesta afectiva y no tanto de una respuesta instrumental. Otra sugería que se establecía una especial relación clave-respuesta-consecuencia o teorías que sugerían que la larga demora se produciría siempre y cuando

no haya otro estimulo que provoque interferencia durante ese periodo. La explicación más aceptada a este fenómeno fue propuesta por Bures y cols. (Bures, Bermudez-Rattoni y Yamamoto, 1998). Estos autores proponen que la presentación de un EC forma una memoria a corto plazo gustativa que se asocia a los efectos del EI, por lo que la asociación EC-EI no se formaría por la interacción directa de sus circuitos sensoriales. El hecho de que el condicionamiento simultáneo provoque una aversión débil en comparación con el demorado, puede ser el resultado de la lenta formación de la memoria a corto plazo gustativa. Durante los tres primeros minutos tras la presentación del EC, la memoria gustativa se está fromando lo que debilita su asociabilidad. Esta capacidad de asociación se hace máxima entre los 10 y los 60 minutos disminuyendo paulatinamente hasta las 8-12 horas posteriores. Independientemente de la explicación, el hecho es que la larga demora que es capaz de soportar el AAG posee sentido en el contexto ecológico. Los efectos nocivos de una sustancia peligrosa para el organismo no suelen presentarse hasta que ha sido digerida y absorbida por la sangre, si los animales no fueran capaces de asociar esos efectos a las cualidades de un alimento no serían capaces de aprender a evitar ese alimento.

La tercera característica hace referencia a la especificidad clave-consecuencia. Existe una predisposición a asociar estímulos de determinadas modalidades sensoriales (Domjan, 1985). Según Seligman (1970) los organismos están preparados biológicamente para asociar ciertos estímulos condicionados con consecuencias particulares. El aprendizaje de selección de alimentos pertenecería a la categoría de comportamiento "preparado" (predisposición biológica para aprender), formado por la asociación de unas claves específicas (sabor, olor, apariencia del alimento) con los cambios que provocan en el estado interno del organismo y no entre esas claves y otro tipo de señales exteroceptivas (una descarga eléctrica por ejemplo). Parecería por tanto

que los seres vivos hubiéramos desarrollado dos sistemas defensivos, uno que relacionaría estímulos exteroceptivos con ofensas periféricas, y otro que relacionaría cambios gastrointestinales con las cualidades sensoriales de los alimentos que se consumen (Garcia y Koelling, 1966; Garcia, Lasiter, Bermudez-Rattoni y Deems, 1985). Una explicación alternativa a la predisposición asociativa sería la hipótesis de la similitud estimular, en la que se sugiere que tanto el malestar provocado por una sustancia tóxica como la digestión de un alimento tendrían una temporalidad parecida, mientras que una luz y un shock son eventos con un procesamiento rápido. Esto implicaría que fueran más fácilmente asociables entre si debido a su similitud estimular. Sin embargo esta hipótesis no es capaz de dar explicación a que estímulos visuales o táctiles de un alimento sean capaz de asociarse en un solo ensayo con una sustancia tóxica incluso con 30 minutos de demora en su presentación (Domjan, 1985).

1.3. La metodología del aprendizaje aversivo gustativo

El experimento de AAG estándar implica dos fases principales. El primer paso consiste en adaptar a las ratas a un programa de privación de agua (alrededor de 23 horas) y habituar a los animales a beber en tubos calibrados (pipetas o botellas experimentales) diariamente. En la fase de adquisición, los animales son expuestos a un sabor novedoso y posteriormente a un agente aversivo que provoca malestar gástrico. Durante la fase de prueba se mide (tradicionalmente) el descenso en el consumo del fluido que ha sido asociado con el malestar gástrico o su ingesta relativa (en una prueba de elección con dos botellas).

Para medir la magnitud de la aversión condicionada suelen dos tipos de pruebas (Bures et al., 1998):

- Procedimiento de una sola botella: en esta prueba está disponible una botella con el sabor emparejado al malestar, cuyo consumo se compara con el de un grupo de control que no ha recibido (o al menos de manera contingente) el agente aversivo. Otra opción alternativa sería comparar el consumo del EC con el de agua durante los días de habituación. La principal limitación de este procedimiento es que, cuando la aversión es débil, el programa de privación al que está sometido la rata puede hacer que beba el fluido a pesar de la aversión, y que cuando es fuerte, elimine totalmente el consumo quedando las diferencias en la intensidad del AAG oscurecidas por el efecto suelo. Una posible solución a este problema sería analizar la resistencia a la extinción o utilizar el procedimiento de elección de dos botellas.
- Procedimiento de dos o más botellas: el animal tiene la oportunidad de escoger entre agua destilada y el fluido asociado al agente aversivo. Durante la fase de adquisición ambas botellas contendrían el fluido que se pretende condicionar. Sin embargo esta prueba también tiene alguna limitación. Puede darse el caso en el que los animales escojan una botella al azar y continúen bebiendo de esa botella sin probar la alternativa. Una solución al problema pasaría por colocar de manera igualmente accesible botellas intercaladas con los fluidos, limitadas en cantidad, asegurándose de que tengan que beber de varias pipetas para saciarse. Este método es más sensible a aversiones no muy intensas.

De manera habitual se suele utilizar un sabor dulce (sacarina o sacarosa) como *estímulo condicionado* en el AAG. Para la percepción de este sabor es necesario procesar la información sensorial procedente de la estimulación de los receptores gustativos, olfativos y del nervio trigémino. Esto proporciona un "input" que regula la conducta de ingesta y por lo tanto el estado interno del organismo. Cada sabor es asociado con una valoración hedónica, es decir, puede resultar agradable o

desagradable. Si el sabor es valorado hedónicamente como positivo, las respuestas conductuales serán de aceptación (apetitivas), mientras que si la valoración hedónica es negativa, las respuestas serán de rechazo (aversivas). Los animales se aproximarán a sabores salados o dulces (agradables) y evitará aquellos ácidos o amargos (desagradables) como la quinina o el limón (Grill y Norgren, 1978; Spector, Breslin y Grill, 1988).

Por otro lado, el *estímulo incondicionado* que de forma habitual se utiliza en el AAG es el cloruro de litio (LiCl). El LiCl es una dróga emética que provoca náusea en la rata (especie incapaz de vomitar). La topografía de la náusea en la rata y de la arcada en la musaraña (*Suncus murinus*), especie capaz de vomitar, es muy similar. Los músculos que se ponen en marcha en ambas respuestas son los mismos. Es razonable pensar que la respuesta de náusea en la rata representa un vestigio del precursor del vómito en este animal, que no emite la respuesta completa de vómito. Durante el AAG el animal aprende a asociar las cualidades del sabor consumido (EC) con las consecuencias derivadas de la administración del LiCl, siendo la nausea un factor crítico para el desarrollo de la aversión al sabor. Cabe destacar que la magnitud de la aversión depende de la dosis de LiCl administrado y no tanto de la concentración o el volumen de la solución inyectada. Adicionalmente, el litio es capaz de producir malestar y náusea con independencia del método de administración, ya sea este inyectado intraperitoneal, subcutáneo o a través de una cánula directamente en el estómago (Nachman y Ashe, 1973). El litio también ha sido utilizado para el tratamiento clínico de trastornos bipolares y depresión por sus efectos en la actividad exploratoria, agresión, hiperlocomoción, regulación del apetito, patrones de sueño, conducta sexual, respuesta a las hormonas, aprendizaje, cognición, etc. (O´Donell y Gould, 2007).

Aunque el LiCl es un EI eficaz, otro tipo de sustancias han sido empleadas como EI en el AAG, por ejemplo, el fluoroacetato de sodio que también produce fuertes aversiones al sabor (Riley y Tuck, 1985 para una revisión de las sustancias que inducen AAG).

Capítulo 2

Aversión y evitación: dos procesos diferentes.

CAPITULO 2

Aversión y Evitación: dos procesos diferentes

Se presentan ciertas dificultades a la hora de definir el EI en el paradigma de AAG. Se utilizan términos como el malestar provocado por sustancias tóxicas, émesis o náusea. Pero no todos los agentes que inducen AAG son toxinas, ni tampoco sería adecuado hablar de émesis dado que la rata es incapaz de vomitar. Además no es necesaria la náusea para que se desarrolle AAG, de hecho hay muchas drogas reforzantes capaces de inducir evitación (Parker, 1995). La evitación de un fluido también es producido por drogas que el animal se auto-administra o por drogas que establecen una preferencia por un contexto determinado. De hecho, la magnitud de la aversión al sabor está relacionada con la cantidad de droga consumida durante las sesiones de auto-administración. Al principio se defendió que las drogas a dosis reforzantes producían aversión al sabor debido a que la náusea era uno de los efectos secundarios de su consumo, la cual quedaba asociada al fluido novedoso. Sin embargo, si esto fuera cierto, y la evitación al sabor producido por dosis reforzantes de drogas está mediada por la náusea, también deberían desarrollarse respuestas condicionadas de disgusto cuando al animal se le administra el fluido condicionado. Sin embargo existe considerable evidencia de que esto no es cierto (Parker, 1982, 1995, 2003), al contrario que las drogas eméticas, diferentes dosis de drogas reforzantes (p. ej., cocaína, anfetaminas, morfina, nicotina, etc.) no producen respuestas de rechazo en la rata

(Parker, Limebeer y Rana, 2009; ver Lin, Arthurs y Reilly, 2014, para una revisión reciente).

Parker (2003) propuso una distinción entre "evitación" de un fluido y "aversión" al sabor propiamente dicha, para referirse a dos aspectos diferenciables en el condicionamiento de una aversión gustativa. Las ratas (especie animal carente del sistema reflejo de vómito) evitan consumir un fluido (*evitación del sabor*) asociado con un cambio en su estado fisiológico, ya sea inducido por náusea, debido a la administración contingente con el sabor de una droga emética como LiCl, o con drogas con propiedades reforzadoras como las anfetaminas o la morfina. Sin embargo, las ratas muestran reacciones de rechazo (*aversión al sabor*) sólo al administrarles un sabor previamente emparejado con drogas con propiedades eméticas. La evitación del sabor estaría relacionada con la anticipación de las consecuencias negativas del consumo de una sustancia, es decir, una respuesta de miedo condicionada que proporciona una oportunidad de supervivencia, dado que la rata no es capaz de expulsar a través del vómito sustancias que suponen un peligro para su organismo. Por otro lado, la aversión al sabor estaría mediada por el condicionamiento de la náusea, e implicaría un cambio en la palatabilidad del sabor, mensurable a través de la expresión de reacciones de rechazo cuando se le administra el sabor condicionado (ver Parker, 2003, 2014 para revisión). Una de las primeras evidencias para esta distinción entre evitación y aversión fue presentada por Pelchat, Grill, Rozin y Jacobs (1983). Estos autores analizaron las reacciones de rechazo que producían los animales cuando bebían una solución de sacarina que había sido previamente asociada con una baja dosis de litio o con la administración de un shock. Los dos grupos bebieron menos sacarina que los animales control (salino), pero solamente el grupo que había recibido la sacarina asociada con el litio mostró reacciones de rechazo al fluido. Estos autores concluyeron que la sacarina

era evitada por las ratas por distintos motivos. En el caso de las ratas que habían recibido la sacarina asociada con el malestar producido por la droga emética, evitaban el fluido porque les resulta desagradale desde el punto de vista hedónico. En el caso de la sacarina emparejada con descargas eléctricas, los animales la evitaban porque se había convertido en una señal de peligro, un proceso de señalización más propio del condicionamiento clásico.

Clásicamente se ha utilizado pruebas de consumo para evaluar la evitación al sabor. Esta prueba requiere dos tipos de conductas, "preparatorias" de aproximación a la botella y "consumatorias" de ingesta del fluido (Konorski, 1967). Utilizando este procedimiento la rata aprende la contingencia entre el sabor (EC) y el malestar (EI) y/o la contingencia instrumental entre aproximarse a la botella (respuesta instrumental) y el malestar (reforzador) en presencia del sabor (estímulo discriminativo). Por lo tanto la prueba de consumo podría ser el reflejo del aprendizaje instrumental de aproximación a la botella, o de la respuesta condicionada de disgusto.

Una manera más adecuada de medir la aversión al sabor sería utilizar una prueba que evalúe específicamente respuestas consumatorias del fluido. El test de reactividad al sabor introducido por Grill y Norgren (1978), es una técnica alternativa que mide cambios en la palatabilidad asociados con las consecuencias de la ingesta de un fluido. En esta prueba se mide las reacciones oro-faciales que produce una solución cuando se infunde directamente en la cavidad bucal a través de una cánula que ha sido previamente implantada a la rata (Parker, 1980). Cuando una solución agradable como la sacarosa es infundida directamente en la boca, las ratas muestran reacciones de ingesta (Berridge, 2000; Berridge y Grill, 1983, 1984). Cuando la solución infundida ha sido emparejada con un estado de náusea, las ratas muestran reacciones de rechazo, una medida directa del condicionamiento de la aversión al sabor. Estas reacciones de

disgusto son similares a las producidas por sustancias desagradables como la quinina (Grill, 1985).

2.1. Medidas del cambio en la palatabilidad: test de reactividad al sabor y análisis del patrón de ingesta

Las respuestas conductuales que reflejan el condicionamiento de las reacciones de disgusto (aversivas) medidas a través de la técnica de reactividad facial están encaminadas a expulsar la solución de la cavidad bucal. Las respuestas aversivas más representativas son la respuesta de arcada, frotamientos de la barbilla contra el suelo y movimientos sincrónicos hacia atrás y hacia adelante de las patas delanteras. Las respuestas de ingesta (apetitivas) más representativas son los movimientos rítmicos de la boca y movimientos (protusiones) de la lengua que facilitan el consumo de la solución. La Tabla 1 presenta las respuestas características evaluadas en el test de reactividad al sabor. Entre las reacciones orofaciales de rechazo al sabor elicitadas por un sabor previamente emparejado con un tratamiento emético, la arcada (*gape*, en inglés) es la más representativa.

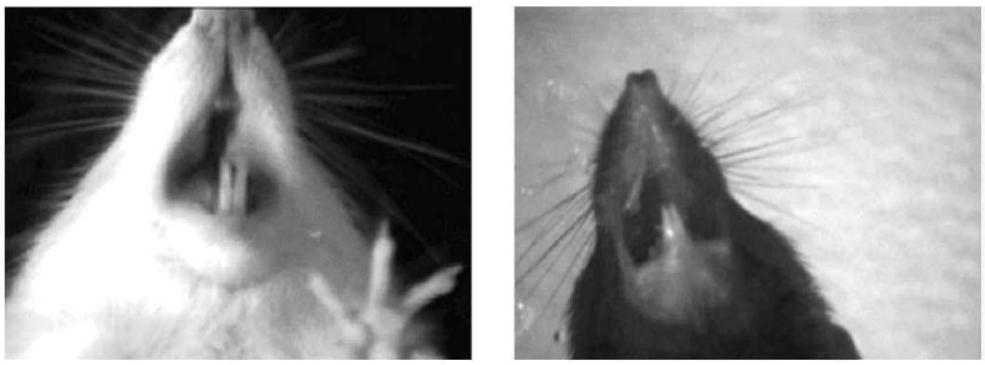


Figura 1*:* Reacción orofacial característica de la respuesta de náusea en la rata y en la musaraña (Parker, 2003).

Tabla 1. Respuestas características evaluadas en el test de reactividad facial (adaptado de Bures et al.,1998).

Respuestas de ingesta (ingesta del fluido continua)
Movimientos rítmicos de la boca (mouth movements) bajada simétrica de la mandíbula a una tasa de 6 Hz
Movimientos de la lengua (tongue protrusions) extensiones centrales de la lengua sobre el labio superior
Movimientos laterales de la lengua (lateral tongue protrusions) extensión lateral de la lengua combinada con la retracción unilateral del labio superior
Lameteo de las patas anteriores (paw licking) extensiones centrales de la lengua directamente sobre las patas anteriores
Respuestas aversivas (rechazo del fluido infundido)
Arcadas (gaping) bajada de la mandíbula que lleva a la exposición de los incisivos inferiores, retracción de la comisura de la boca y expulsión del fluido.
Frotamiento de la barbilla (chin rubbing) frotar la barbilla contra la superficie del suelo y expulsar el fluido
Movimiento de las patas delanteras (paw treading) avance y retroceso de las patas anteriores en alternancia sincronizada
Movimiento de cabeza (head shakes) movimientos rápidos de lado a lado de la cabeza con la boca abierta permitiendo la expulsión del fluido con la boca.
Limpieza con las patas (paw wipes) usar las patas anteriores para eliminar el fluido de la boca
Movimientos bruscos de las extremidades anteriores (forelimb flails) movimientos rápidos horizontales con las extremidades anteriores eliminando el fluido del pelaje.
Goteo pasivo (passive drip) en ausencia de respuestas de ingesta, el fluido acumulado en la boca gotea contra el suelo

En uno de los estudios pioneros sobre cambios en la palatabilidad, Pelchat et al. (1983) analizaron como variaban los patrones de reactividad facial tras emparejar una solución de sacarosa con litio, un shock en las patas o con lactosa. La primera vez que la sacrosa era infundida intraoralmente, las ratas mostraron conductas de ingesta de la solución, principalmente movimientos de la boca y de la lengua. Tras la infusión, a las ratas se les administró uno de los tres estímulos. Tras este único ensayo de

condicionamiento, las ratas a las que se les administró una descarga eléctrica o la lactosa continuaron mostrando reacciones apetitivas cuando la sacarosa era infundida en la cavidad oral. Sin embargo se produjo un cambio en el patrón de reactividad de las ratas que habían recibido litio: disminuyeron las reacciones apetitivas y se produjeron respuestas de disgusto a la solución infunida (principalmente arcadas, frotamientos de barbilla y sacudidas de la cabeza). El test de reactividad al sabor permitió observar los cambios en la palatabilidad de la sacarosa como consecuencia de su asociación con el estado de náusea inducido por el litio.

El test de reactividad al sabor mide, por lo tanto, cambios en la palatabilidad positivos y negativos. Pero a la vez que surgía esta alternativa de medida de la aversión, una tradición separada fue desarrollando un método que estaba centrado en el uso de equipos automáticos que analizaban las respuestas de lameteo (licking) en roedores y el análisis del patrón de ingesta (Davis, 1989; Dwyer, 2012). Las ratas ingieren los fluidos con un ritmo de lameteo o frecuencia de lametones determinada. Esas respuestas de lameteo tienden a producirse en agrupaciones (ó *clusters*) que están separados unos de otros en función de pausas de tiempo variable. El número de lameteos por cluster (tamaño del cluster) depende de la naturaleza de la solución consumida. Está positiva y monotónicamente relacionada con la concentración de una solución gustativa agradable (por ejemplo la sacarina), así como decrece con el consumo una sustancia aversiva (como por ejemplo la quinina) (Davis y Smith, 1992). Es importante resaltar que la conducta de lameteo (o licking) no es sustitutiva del consumo. Mientras que el mayor consumo de una solución dulce se produce a concentraciones moderadas de la solución, el tamaño del cluster aumenta cuanto mayor es la concentración de la solución dulce. Este microanálisis de las respuestas de licking es una medida alternativa al test de reactividad de la valoración hedónica de las soluciones que se consumen (Dwyer, 2012).

Uno de los hechos que respaldan que el tamaño del cluster es un reflejo de la palatabilidad de los sabores es que le afectan la misma variedad de manipulaciones que influyen en la TR. Por ejemplo emparejar una sustancia agradable como la sacarina con el malestar inducido por la administración de litio, provoca el mismo efecto tanto si se mide a través de la técnica de TR como a través del análisis de la conducta de ingesta. Además esta técnica de análisis es igual de sensible para diferenciar la evitación de un sabor de la aversión propiamente dicha (Dwyer, Boakes y Hayward, 2008). Cuando un sabor dulce se asocia con los efectos negativos de la administración de una droga emética, el tamaño del cluster y el consumo en una prueba posterior disminuyen (en comparación con un grupo de control no emparejado). Sin embargo, cuando un sabor dulce se asocia con los efectos de la administración de una droga como las anfetaminas (a dosis reforzantes), el consumo desciende pero el tamaño del cluster permanece inalterado. Al igual que en la TR las drogas a dosis reforzantes no provocarían aversión al sabor pero sí evitación al sabor. Una de las posibles explicaciones alternativas a este fenómeno podría ser que en realidad, las técnicas no son lo suficientemente sensibles para detectar estos cambios cuando se trata de las anfetaminas. Para una verdadera demostración de que la técnica del análisis del patrón de ingesta mide cambios en la palatabilidad y que adicionalmente la evitación y la aversión son dos procesos diferentes, sería necesario el uso de alguna manipulación que fuera capaz de alterar el tamaño del cluster sin alterar el consumo de una solución. Las investigaciones en otro paradigma de aprendizaje, la preferencia condicionada al sabor, constituyen esa demostración.

La asociación de una sustancia neutra desde el punto de vista de su valor hedónico, con una solución agradable como el azúcar, tiene como resultado un aumento en la preferencia de ese sabor cuando posteriormente se presenta solo. Dwyer (2008)

realizó una serie experimental utilizando ese paradigma para demostrar cómo podía variar el tamaño del cluster sin alterar el consumo. Para ello emparejó un sabor novedoso con una solución de maltodextrina al 16% (CS+), y otro sabor alternativo con una solución al 2% (CS-). Posteriormente en la prueba, presentó los dos sabores emparejados con 16% o con 2% de maltodextrina (un polisacárido sin dulzor y con propiedades nutricionales) y se analizaron tanto el consumo de las soluciones como el tamaño del cluster. Los resultados obtenidos mostraron que el consumo del CS+ fue mayor cuando los sabores se presentaban con una concentración de 2% de maltodextrina, pero no hubo diferencias en el consumo cuando ambos eran presentados con el 16% de maltodextrina. Sin embargo, en cuanto al tamaño del cluster, fue mayor para el CS+ con independencia de la concentración, es decir tanto al 2% como al 16%. Estos resultados pueden explicarse en términos de un aumento en la percepción de la concentración de maltodextrina. Es importante tener en cuenta que el consumo de una sustancia dulce o agradable depende de la concentración de la misma. En este sentido, el consumo de una solución dulce se produce a concentraciones moderadas de la solución, mientras que cuanto mayor es la concentración de una solución, mayor es el tamaño del cluster. Combinar una clave condicionada con maltodextrina con la presentación de la maltodextrina por sí misma, tuvo efectos análogos a la percepción incrementada de la concentración. Afectó al consumo a bajas dosis, pero no a concentraciones más altas, mientras que el patrón de ingesta se vio afectado a cualquier concentración. Estos resultados sugieren que la preferencia por el sabor produce un incremento de la valoración hedónica del CS+, que es exactamente lo contrario a lo que se produce en el AAG, donde la palatabilidad produce un decremento de la valoración del sabor condicionado. Por lo tanto el test de reactividad facial y el análisis del patrón de la conducta de ingesta son dos pruebas que evalúan cambios en la valoración hedónica de

sustancias agradables y desagradables, y pruebas más adecuadas para medir la aversión condicionada a sabores. Constituyen además una prueba de que la aversión y la evitación son dos procesos que cabe diferenciar en el AAG.

2.2. Efecto de los tratamientos antieméticos en el condicionamiento de la aversión al sabor

Otro argumento a favor de que la evitación de un fluido y la aversión al sabor constituyen dos procesos diferentes es la disociación que se produce tras la administración de fármacos antieméticos. En este sentido, cuando se administra un fármaco antiemético antes del condicionamiento se impide el establecimiento y la expresión de la aversión al sabor, pero no interfiere con la evitación del fluido (Limebeer y Parker, 2000; Parker, 2003). Los agentes antieméticos bloquean los receptores de la serotonina (5-HT), cuya activación median la respuesta de émesis durante tratamientos de quimioterapia. Si las reacciones de disgusto condicionadas están producidas por el efecto de las drogas eméticas, entonces es factible pensar que tratamientos antieméticos, como el uso de ondasetrón (un antagonista de los receptores 5-HT_3), podrían atenuar las reacciones condicionadas de disgusto en la rata. Limebeer y Parker (2000) demostraron que pre-tratamientos con ondasetrón interfieren con el establecimiento y la expresión de respuestas condicionadas de disgusto. Estos resultados sugieren que la aversión y no la evitación, es el reflejo de la náusea condicionada que puede ser atenuada por tratamientos que disminuyan la liberación de serotonina.

No solo los antagonistas de los receptores de 5-HT_3 son capaces de atenuar las náuseas condicionadas, los derivados del cannabis como el Δ^9-tetrahidrocannabinol (Δ^9-THC), principal componente psicotrópico del cannabis, disminuye el malestar provocado por las drogas eméticas (Parker, Rock y Limebeer, 2011; Rock, Limebeer,

Mechoulam, Piomelli y Parker, 2008). Esta acción antiemética parece estar mediada por la acción de sus principales receptores: el Cannabinoide$_1$ (CB_1) y el Cannabinoide$_2$ (CB_2). El receptor CB_1 se ha localizado en el tracto gastrointestinal y en el sistema nervioso, principalmente en el complejo dorso-vagal (CDV). Este complejo está formado por el área postrema, núcleo del tracto solitario y el núcleo dorsal del vago, además está implicado en las reacciones de vómito y náusea inducidas tanto por la acción de drogas eméticas como por activación vagal gastrointestinal. Cuando las náuseas están inducidas por agentes tóxicos, el Δ^9-THC activa los receptores CB_1 en el núcleo del tracto solitario con el objetivo de disminuir el malestar. Pero el CDV no solamente tiene receptores CB_1, sino que también contiene receptores 5-HT_3, convirtiéndolo en el sitio potencial del efecto antiemético (Parker y Limebeer, 2006). De hecho, el sistema serotoninérgico interactúa con el cannabinoide en el control de la émesis. Se sabe que la combinación de bajas dosis de ondasetrón y Δ^9-THC puede ser una alternativa terapéutica para el tratamiento de los vómitos inducidos por quimioterapia (Parker et al., 2009; Sharkey, Darmani y Parker, 2014 para revisión). Estos vómitos inducidos por el tratamientos de algunos tipos de cáncer, es uno de los efectos secundarios más aversivos que pone en peligro la adhesión de los enfermos a los tratamientos. Aunque los fármacos antieméticos han mejorado extensiblemente estos efectos, los vómitos ocurren aproximadamente en un 40% de los pacientes y la náusea en un 75%. Además algunos pacientes muestran esta respuesta de manera anticipatoria, es decir antes de la siguiente sesión de tratamiento. El desarrollo de nauseas anticipatorias pueden ocurrir después del primer ciclo de tratamiento y son especialmente refractarias a los tratamientos farmacológicos antieméticos, desarrollándose aproximadamente en el 30% de los pacientes tras el cuarto o quinto ciclo (Limbeer, Hall y Parker, 2006).

2.3. Un modelo de náusea anticipatoria

Se ha discutido que las náuseas y los vómitos anticipatorios que muestran los pacientes que reciben tratamiento quimioterapéuticos son consecuencia de un condicionamiento pavloviano (Stockhorst, Steingrueber, Enck y Klosterhalfen, 2006; Symonds y Hall, 2000). Se ha propuesto que los estímulos del ambiente clínico (olores, aparato de infusión, la habitación, el contexto, el personal de hospital, etc.) se convierten en un EC asociado al tratamiento (EI) que evoca la respuesta condicionada de náusea y los vómitos. Después de una o varias presentaciones contingentes del EC y el EI, los pacientes desarrollan respuestas condicionadas (RC) de náuseas y vómitos al volver a entrar en el contexto hospitalario. Además, estas respuestas condicionadas correlacionan con las propiedades aversivas de los fármacos administrados en quimioterapia y con el número de episodios de nausea y vómitos tras el tratamiento. El desarrollo de nausea anticipatorias dependería de la capacidad que tiene el EC (contexto hospitalario) para predecir las consecuencias derivadas de la administración de los fármacos. Dado que el desarrollo de las náuseas anticipatorias tiene efectos aversivos en los pacientes enfermos de cáncer y puede ser explicado en términos de condicionamiento clásico, es interesante el uso de un modelo animal que permita estudiar los mecanismos explicativos de su adquisición y expresión.

Si bien uno de estos modelos podría ser la evitación del sabor condicionada, sería más adecuado el análisis de las respuestas condicionadas de nauseas a través de la técnica de reactividad facial. La desventaja que supone el uso de técnicas clásicas como el descenso en el consumo de una solución que ha sido emparejada con una droga emética (Symonds y Hall, 1997) es que no es una medida necesariamente selectiva de la náusea, sino el reflejo de la anticipación de las consecuencias aversivas del consumo de la solución.

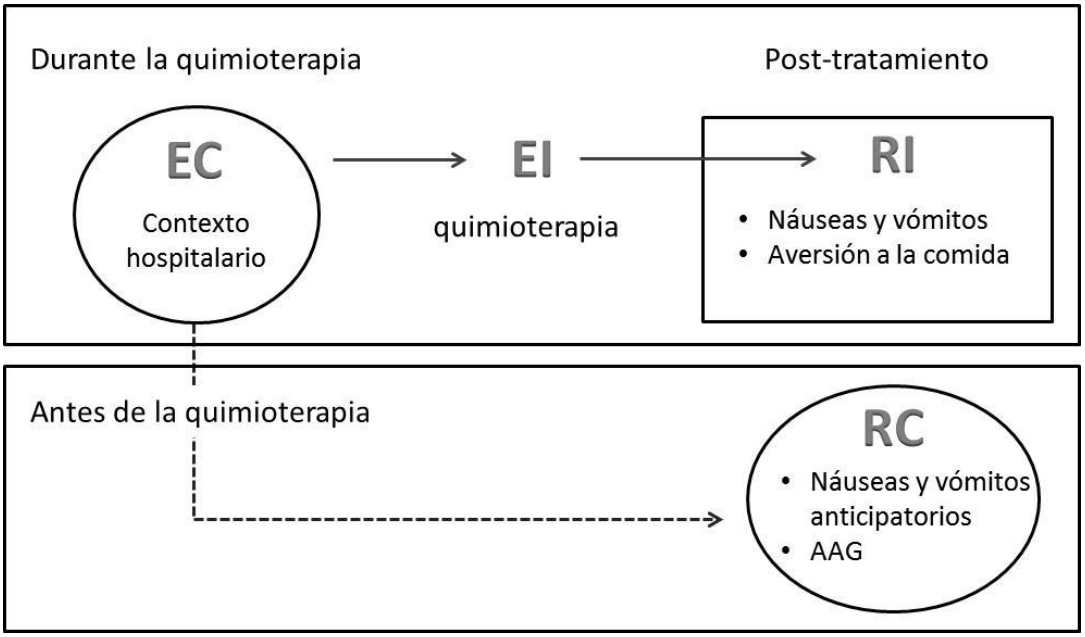


Figura 2: Modelo de condicionamiento Pavloviano en tratamientos de quimioterapia (adaptado de Stockorst et al., 2006).

El uso del análisis de las reacciones condicionadas de rechazo sería una herramienta preclínica que podría utilizarse para el estudio del desarrollo de las náuseas anticipatorias, así como para probar la eficacia de nuevos tratamientos farmacológicos.

La náusea es por tanto, un poderoso reforzador que resulta altamente aversivo para los pacientes que reciben tratamiento con quimioterapia. Además, sus efectos no disminuyen con el curso del tratamiento, de hecho se vuelven más desagradables a medida que avanzan los ciclos. Sin embargo, sí se ha evaluado el descenso en la efectividad de la náusea como reforzador en el paradigma de la evitación al sabor.

Capítulo 3

Efectos de preexposición a los estímulos en el aprendizaje aversivo gustativo.

CAPITULO 3

Efectos de preexposición en el aprendizaje aversivo gustativo

3.1. Preexposición al EI

La efectividad de un EI como reforzador puede verse reducida por su exposición repetida antes del condicionamiento. En este sentido, la administración repetida de LiCl, antes de su asociación con un sabor novedoso, atenúa el efecto de evitación condicionada. Este efecto se denomina preexposición al EI (Hall, 2009; Randich y LoLordo, 1979; Riley y Simpson, 2001). Se han propuesto dos mecanismos explicativos para este fenómeno en el AAG: la habituación y el bloqueo por el contexto. Desde un punto de vista no asociativo, se ha sugerido que la efectividad de un EI puede disminuir por la mera presentación repetida del estímulo. Desde un punto de vista asociativo, la presentación repetida de un EI en un contexto determinado sin la presencia de un EC explícito, hace que las claves contextuales distintivas del ambiente sean asociadas al malestar provocado por la administración del EI aversivo. Estas claves contextuales funcionarían como un EC capaz de bloquear (Kamin, 1969) el condicionamiento de un sabor novedoso. Cabe resaltar que estas dos interpretaciones no son mutuamente excluyentes, las dos pueden estar mediando el efecto de preexposición al EI en algunos casos, mientras que en otros puede ser el resultado de una de ellas. Es necesario evaluar a qué mecanismo responde el efecto de preexposición al EI en cada situación de aprendizaje.

3.1.1 Habituación al EI

A nivel conductual, el fenómeno de habituación se refiere a la disminución progresiva en la magnitud de la respuesta elicitada siguiendo la exposición repetida a un estímulo del entorno. Más específicamente, la habitaución al EI se refiere a la disminución de las propiedades motivacionales del estímulo como resultados de su presentación repetida. Pero para demostrar que el responsable del efecto de preexposición al EI es la habituación, es necesario comprobar que el proceso de aprendizaje que subyace a la pérdida de la RI es también el responsable de reducir la capacidad del EI (en este caso la náusea) para actuar como reforzador. Batson (1983) observó que las respuestas conductuales que producían las ratas tras una inyección de litio (bajada de temperatura corporal y disminución en la actividad general) no variaban a lo largo de ocho ensayos. Las ratas no mostraron signos de habituación a los efectos del litio, sin embargo los animales que habían recibido estas sesiones, mostraron un condicionamiento más débil cuando posteriormente se utilizó el litio como EI en un paradigma de AAG. Este autor defiende que el efecto de preexposición al EI se explicaría por la hipótesis asociativa y no por la tolerancia al litio. Sin embargo esta conclusión dependería de la droga que se utilice como EI. En el caso del litio no se produciría tolerancia, sin embargo, si se utilizase morfina como EI sí dependería de un proceso de habituación y no de una interferencia asociativa (Dacanay y Riley, 1982). Si se utilizan drogas adictivas (en vez de eméticas), el efecto que se produce podría explicarse en términos de habituación a la droga debido a la rápida tolerancia que producen este tipo de sustancias. Además su efecto no atiende a manipulaciones contextuales, producen débiles AAG, no dependen de la dosis que se administra y aumentar el número de ensayos de condicionamiento no disminuye el efecto de

preexposición al EI (al contrario que el litio). Por otro lado, el litio tiene muy baja tolerancia y provoca fuertes AAG que son dependiente de dosis.

Uno de los efectos inmediatos de las inyecciones de litio es que las ratas tienden a rechazar el consumo de sustancias palatables (por ejemplo sacarina) mientras están bajos los efectos del litio. De Brugada, González y Cándido (2003) llevaron a cabo un experimento que también pone en tela de juicio la habituación como responsable del efecto de preexposición al EI en el AAG inducido por litio. Estos autores compararon el consumo de sacarina tras una inyección de litio en tres grupos de ratas. Uno de los grupos recibió exposiciones repetidas al EI, mientras que a los otros dos se les administró salino. El día del condicionamiento el grupo con experiencia repetida al litio y uno sin experiencia previa, recibieron una inyección de litio antes de beber sacarina. El tercer grupo sirvió como control salino del condicionamiento y la preexposición. Los resultados mostraron que los dos grupos que recibieron litio bebieron menos sacarina el día del condicionamiento que el grupo salino, y además no hubo diferencias entre ellos. Sin embargo el día de la prueba, si se produjo un retraso en el condicionamiento de la sacarina en el grupo con experiencia repetida al litio en comparación con el grupo que sólo se le administró litio el día del condicionamiento. Estos resultados muestran que las inyecciones repetidas de litio no parecen habituarse, pero sin embargo sí se obtiene el efecto de preexposición al EI. Otro tipo de proceso puede estar mediando los resultados obtenidos en este estudio, por lo tanto es necesario volver a retomar la hipótesis de la interferencia asociativa.

3.1.2. Bloqueo por el contexto

El efecto de preexposición al EI podría explicarse en términos de interferencia asociativa. De acuerdo con esta hipótesis la exposición repetida al EI sin un EC explícito, dará lugar a la formación de una asociación entre las claves contextuales del

ambiente y los efectos aversivos del EI, y esta asociación podría interferir con la adquisición de la asociación sabor-EI si ésta se lleva a cabo en el mismo contexto. Los principales modelos explicativos del efecto de preexposición al EI en el AAG se pueden dividir principalmente en aquellos que explican el fenómeno en términos de un déficit de adquisición o de un déficit de recuperación de la información.

- Los modelos de la *recuperación* asumen que la asociación contexto-EI no interfiere en la adquisición de la asociación posterior sabor-EI en el condicionamiento, sino que la interferencia se produce en su manifestación, cuando la prueba se realiza en el mismo contexto previamente emparejado con el malestar (Bouton, 1993; Miller y Matzel, 1988).
- Los modelos basados en un déficit de *adquisición* predicen que se producirá el efecto de preexposición al EI cuando todas las fases experimentales se llevan a cabo en el mismo contexto, y por lo tanto las claves contextuales serán mejores predictoras del malestar que el sabor (Mackintosh, 1975; Pearce y Hall, 1980; Rescorla y Wagner, 1972; Wagner, 1981). Por ejemplo, los modelos atencionales explican el efecto de preexposición al EI en términos de cambios en la atención que se le presta a los estímulos que dependen de la experiencia previa que se tenga con ellos.

Si la hipótesis de la interferencia asociativa es correcta, habría que poner a prueba a las claves contextuales en dos aspectos principales: su capacidad para funcionar como EC y su vulnerabilidad a sufrir las mismas manipulaciones que otros ECs en el paradigma del bloqueo.

1.- Si las claves contextuales se presentan de manera contingente con el malestar provocado por el EI, y adquieren fuerza asociativa capaz de bloquear el condicionamiento posterior del sabor, sería necesario demostrar la capacidad que tienen

para actuar como EC. De hecho, hay autores (Garcia, 1989) que defienden que la náusea activa una "defensa intestinal" que permitiría aprender de claves como el sabor, pero no de claves exteroceptivas, como las contextuales. Sin embargo habría una excepción, la presencia de un sabor podría fomentar el aprendizaje de otras claves (como las contextuales) cuando se presentan conjuntamente, fenómeno conocido como *potenciación* del condicionamiento. Según Garcia, la presencia de claves gustativas favorecería que las claves contextuales se integrasen en el sistema de defensa digestivo. Por lo tanto, estrictamente, estos autores defienden que el condicionamiento contextual sólo puede ocurrir cuando se consuma una solución en ese contexto. ¿Es verdaderamente necesaria la mediación de las claves gustativas?.

Hay resultados experimentales donde se demuestra que el condicionamiento contextual ocurre más rápidamente si se les permite a las ratas beber una solución novedosa durante el condicionamiento, incluso con agua (Mitchell y Heyes, 1996). En este sentido Symonds et al. (1998) demostraron que el consumo de agua en un contexto distintivo antes de su asociación con litio facilitó el condicionamiento de las claves contextuales (pero ver Hall, Symonds y Rodriguez, 2009). El nivel de condicionamiento fue medido a través del descenso en el consumo de una solución familiar. Los animales que no recibieron agua durante el condicionamiento no desarrollaron aversión al contexto. Sin embargo esta reducción en el consumo puede ser el reflejo de una generalización (a la solución familiar) de la aversión adquirida al agua durante el condicionamiento, que además sólo se reflejaría en presencia de la claves contextuales debido a que la aversión es dependiente de contexto. Una alternativa para evaluar si lo que se ha producido es generalización o condicionamiento contextual es el uso de un procedimiento de bloqueo. Si realmente las claves contextuales se han condicionado deberían bloquear el condicionamiento posterior de un sabor novedoso. Estos autores

demostraron que el contexto que había sido previamente emparejado con el litio, era capaz de bloquear el condicionamiento de una sustancia novedosa con independencia de si habían recibido agua o no en las sesiones de condicionamiento. Una posible interpretación de estos datos es que asociar un contexto con náusea puede producir condicionamiento contextual incluso en ausencia de claves gustativas durante el condicionamiento (ver también Symonds y Hall, 1997), lo que va en contra de la propuesta inicial de Garcia.

2.- Si las claves contextuales son capaces de bloquear el condicionamiento posterior de un sabor, entonces deberían resultar igualmente sensibles a las manipulaciones del paradigma del bloqueo. Una de estas manipulaciones es el cambio de contexto entre las fases de preexposición y condicionamiento, que atenúa el efecto de preexposición al EI (Batson y Best, 1979; Dacanay y Riley, 1982). Otras manipulaciones estarían encaminadas a reducir la fuerza asociativa entre el contexto y el EI. Por ejemplo, añadir una fase de extinción del condicionamiento contextual antes de la fase de condicionamiento con el sabor, afecta a la fuerza de la asociación sabor-EI (Batson y Best, 1979). Introducir un mayor intervalo temporal (intervalo de retención) entre la fase de exposición al EI y la fase de condicionamiento también atenúa el efecto de preexposición al EI (Aguado, De Brugada y Hall, 1997). Otra de las manipulaciones posibles sería utilizar un contexto familiar durante la preexposición. La utilización de un contexto familiar atenúa el efecto de preexposición al EI debido a que ese contexto sufriría una suerte de inhibición latente. La asociación contexto-EI sería más débil en comparación con un contexto que sea novedoso para los animales, o en comparación con un contexto familiar al que se le añade una clave novedosa (por ejemplo un olor) (Cole, VanTilburg, Burch-Vernon y Riccio, 1996). Estos autores demostraron que no se produce efecto de preexposición al EI cuando todas las fases experimentales se llevan a

cabo en las jaulas hogar, sin embargo otros autores (por ejemplo De Brugada et al., 2003) demostraron que el efecto de preexposición se producía a pesar de que se llevara a cabo en un contexto familiar. Nótese que cuando todas las fases experimentales ocurren en el mismo contexto, necesariamente se prueban las claves bloqueadas en presencia de las claves que bloquean el condicionamiento, lo que aumentaría la RC. En el procedimiento estándar de bloqueo, el fallo de la clave bloqueada para producir RC ocurre cuando se prueba en ausencia de la clave bloqueante. Presentar las dos en conjunto produciría una fuerte RC. Una explicación alternativa para los resultados de estos autores es que las claves contextuales incluyen más estímulos que aquellos relacionados con el ambiente general. Las claves relacionadas con la manipulación del animal y con la administración de las inyecciones son también contingentes al estado de náusea y suponen una novedad en comparación con las claves ambientales de las jaulas hogar. Por lo tanto estas claves son también un buen candidato para asociarse con el estado de náusea, capaces de bloquear el condicionamiento posterior del sabor (en ausencia de claves contextuales novedosas) (De Brugada y Aguado, 2000; De Brugada et al., 2003; De Brugada, Hall y Symods, 2004).

Finalmente en cuanto a la hipótesis de bloqueo por el contexto, se debería destacar que los efectos producidos por la administración del litio pueden producir condicionamiento no solo de sabores sino de un conjunto de claves exteroceptivas que pueden adquirir fuerza asociativa y bloquear el consiguiente condicionamiento de un sabor, resultando en un efecto de preexposición al EI. Cuando el contexto es nuevo, las claves relacionadas con el ambiente contribuyen al bloqueo, cuando el contexto es familiar las claves manipulativas y relacionadas con la inyección tienen el rol dominante.

Sin embargo, hay que tener en cuenta que todos estos estudios que medían el condicionamiento contextual utilizaron pruebas de consumo para su análisis. Sin embargo, quizás esta no sea la mejor manera de evaluar el condicionamiento contextual, dado que el estado de náusea no solamente provoca un cambio en la palatabilidad sino también un cambio en el estado fisiológico del organismo que lleva al animal a evitar la solución en sucesivas ocasiones. Esto podría estar oscureciendo los resultados de los estudios, dado que el aprendizaje de las claves contextuales podría estar señalando un peligro potencial, más que evocar una respuesta condicionada de náusea. La mejor manera de evaluar si el contexto es capaz de bloquear el condicionamiento posterior de una solución novedosa es utilizando la técnica de la reactividad facial. Limebeer, Hall y Parker (2006) utilizaron esta técnica para evaluar el condicionamiento contextual (ver también Limebeer et al., 2008) en cuatro ensayos de condicionamiento en los cuales a las ratas se les administraba una inyección de litio en un contexto novedoso. El día de la prueba se les infundió una solución novedosa en el contexto condicionado. Las ratas mostraron reacciones condicionadas de rechazo a la solución novedosa, confirmando que le contexto es capaz de asociarse con los efectos aversivos de la administración del EI y modular respuestas aversivas a un sabor novedoso.

Por lo tanto, el EI es capaz de asociarse a ECs, pero también las claves contextuales podrían funcionar como EC y evocar RCs. De hecho el contexto puede adquirir capacidad asociativa sin necesidad de que medie el EI. Un claro ejemplo de esto es el paradigma de inhibición latente, donde no es necesario que medie el EI para que se adquieran asociaciones con el contexto.

3.2. Preexposición al EC

La presentación en solitario de un estímulo da lugar al retraso en la adquisición de una asociación posterior en la que el estímulo preexpuesto predice la parición de una consecuencia. Este fenómeno se conoce con el nombre de Inhibición Latente ó preexposición al EC. En el AAG, el consumo repetido de un sabor antes de su asociación con el malestar gástrico producido por la administración de una droga emética, produce un retraso en el condicionamiento de ese sabor (Lubow, 1989; 2009). Constituye un mecanismo de aprendizaje altamente adaptativo desde el punto de vista biológico al evitar la adquisición de una aversión a sabores o alimentos con los que está familiarizado el individuo.

En un procedimiento estándar de IL un grupo (preexpuesto) recibe exposiciones no reforzadas a un sabor mientras que otro grupo (no preexpuesto) recibe agua. En una segunda fase de condicionamiento ambos grupos reciben el sabor seguido de la administración de una droga. Finalmente en la fase de prueba, se mide el descenso en el consumo de la solución asociada al malestar. En el grupo que ha recibido exposiciones no reforzadas se produce un retraso en la adquisición del AAG en comparación con el grupo sin experiencia previa con el sabor.

Las explicaciones de este fenómeno, al igual que en el efecto de preexposición al EI, se dividen principalmente en dos categorías: un fallo en la adquisición (por ejemplo, Lubow 1989; Lubow, Weiner y Schnur, 1981; Mackintosh, 1975; Pearce y Hall, 1980; Wagner, 1981) o en la recuperación (Bouton, 1993; Miller y Matzel, 1988). Las teorías atencionales, por ejemplo, asumen que se producen cambios en la atención que reciben los estímulos en función de su capacidad para predecir sus consecuencias. Solamente aquellos estímulos que resultan inesperados son atendidos y tienen la capacidad de asociarse con un EI y por tanto, ser buenos predictores de ese estímulo. De hecho se

presta menos atención a los estímulos que son capaces de predecir exactamente sus consecuencias (en caso de la inhibición latente, la no ocurrencia de consecuencias). Más específicamente, Lubow (1989) en su *teoría de la atención condicionada*, argumenta que la atención a ciertos estímulos aumenta cuando un estímulo predice una consecuencia importante, pero disminuye si carece de valor informativo. Además, la respuesta de desatención puede condicionarse al contexto, y por tanto provocar una reacción de desatención al EC condicionada. Como los sujetos no prestan atención al EC, no consiguen aprender la asociación EC-EI. El modelo SOP de Wagner (1981) es el que mejor explica y predice el efecto de IL, donde otorga un papel fundamental a las claves contextuales. Durante la preexposición, el EC queda asociado al contexto, lo que disminuye su asociabilidad. Esta disminución hace que el EC no sea procesado activamente durante el condicionamiento puesto que ya existe una representación del EC en la memoria que se encuentra activada asociativamente por la presencia del contexto, resultando en un retraso en la asociación EC-EI dado que estos dos estímulos se procesarían de manera independiente.

Las explicaciones que se centran en el fallo en la recuperación asumen que durante la fase de prueba se produce una competición entre la asociación adquirida durante la preexposición (EC-sin consecuencias) y el condicionamiento (EC-EI).En virtud del proceso de interferencia proactiva, las primeras asociaciones dificultarían la recuperación de la asociación EC-EI produciendo ese aparente retraso (Bouton, 1993). Específicamente, Miller y Matzel (1988) defendían que la fuerza de la RC en la fase de prueba estaba determinada por tres tipos distintos de asociaciones: la que se establecía entre el EC y el contexto, la que se establecía entre el EC y el EI en la fase de condicionamiento y finalmente la que se establecía entre el contexto y el EI. En la fase de prueba, la representación del EI sería activada directamente por el EC e

indirectamente por el contexto (el estímulo comparador). La fuerza de la RC vendría determinada por la diferencia entre la activación directa e indirecta del EI, evocando respuesta o no respectivamente. En el caso de la IL, la activación indirecta del EI sería fuerte y evocaría una débil RC.

3.2.1. Variables moduladoras de la Inhibición Latente

La expresión de la IL en el AAG es susceptible de ser modulada por una gran variedad de factores (para revisión Lubow, 2009), entre los que destacan:

- Tipos de estímulos: la IL en el AAG puede producirse con estímulos gustativos agradables (sacarina, azúcar, maltodextrina, leche) pero también con estímulos que carecen de valoración hedónica positiva (vinagre, sal, limón), con olores e incluso con objetos que están relacionados con la comida (por ejemplo las cualidades de los dispensadores de comida). Sin embargo hay excepciones en las que utilizando un sabor novedoso, un polisacárido altamente palatable (Policosa, polímero de glucosa con alta capacidad calórica), tanto el grupo preexpuesto como el no preexpuesto reconocen el sabor como familiar y no se produce diferencias entre ellos (Koh y Bernstein, 2005; Barot y Bernstein, 2005). Entre los argumentos explicativos, Barot y Bernstein (2005) defienden que los efectos derivados de la ingesta de la Policosa son altamente reforzantes y por tanto, sólo produce una aversión condicionada moderada. Sin embargo, eso no explicaría por qué no hay diferencias entre el grupo que ha tenido experiencia previa con el fluido y el grupo que no la ha tenido. Estos autores también descartan un efecto de la concentración, de la falta de perceptibilidad del sabor o una generalización del sabor a otros incluidos en su dieta diaria. Finalmente proponen que este sabor podría constituir el sexto sabor básico para las ratas, lo cual lo convertiría en un sabor único.

- Métodos de presentación del fluido: cambiar el método de presentación del fluido, de activo (botella) a pasivo (infusión intraoral) o viceversa, puede modular el efecto de IL en la misma medida que un cambio de contexto. Por ejemplo, cuando las ratas son preexpuestas de manera activa y la prueba también es de consumo activo, las ratas tienen un consumo mayor con independencia del método de condicionamiento en comparación a un grupo que ha sido preexpuesto de manera pasiva (Fouquet, Oberling y Sadner, 2001). Otro estudio de Yamamoto, Fresquet y Sadner (2002) mostró resultados similares usando el método activo y la auto-activación de las infusiones intraorales. Otra vez, el grupo que había sido preexpuesto de manera intraoral, pero condicionada con el procedimiento estándar, consumió menos que los otros grupos.
- Intensidad y especificidad de los estímulos preexpuestos: el efecto de IL incrementa en función de la concentración del sabor preexpuesto (Rodriguez y Alonso, 2002). Además, realizar pequeñas modificaciones de las propiedades del estímulo preexpuesto entre fases, produce una generalización del efecto de IL, mientras que grandes modificaciones en el tipo de estímulo utilizado entre fases provoca una atenuación del efecto de IL (Lubow, 1989).
- Número y duración de las fases experimentales: el efecto de IL aumenta con el incremento de la duración y el número de las preexposiciones (De la Casa y Lubow, 1995; Lubow, 1989). Igualmente, el consumo de la solución durante la fase de preexposición aumenta con el incremento de las horas de privación de agua de las ratas durante esta fase. El efecto de IL puede producirse con un número limitado de preexposiciones, sin embargo, si la cantidad total de fluido es muy limitada, entonces en vez de IL se producirá una facilitación del AAG. Por otro lado, un aumento en el número de ensayos de condicionamiento disminuye las diferencias

entre el grupo preexpuesto y no preexpuesto en la magnitud del AAG. En los ensayos de prueba (presentar el EC en ausencia de EI, es decir, ensayos de extinción), si el condicionamiento es fuerte, el grupo preexpuesto recupera los niveles iniciales de consumo del fluido más rápidamente que el grupo no preexpuesto. También, si el número de ensayos de extinción aumenta, ambos grupos alcanzan niveles asintóticos similares (Lubow, 2009).

- Duración del intervalo de retención: la IL puede ser modulada por un cambio en el intervalo de retención en tres momentos diferentes (para revisión, ver Lubow y De la Casa, 2005): (1) *Intervalo preexposición-condicionamiento*: el efecto de IL disminuye en función de la duración de este intervalo (1-2 días Vs. 10-100 días; sobre 20 días normalmente). Sin embargo, hay que tener en cuenta que el intervalo de retención en este momento de la fase experimental suele pasarse en el mismo contexto que la fase de preexposición y condicionamiento. Cuando el contexto del intervalo de retención es distinto al de las fases de preexposición y condicionamiento, no hay atenuación de la IL con el aumento del intervalo. (2) *Intervalo preexposición-prueba*: el efecto de IL también disminuye con el aumento del intervalo (3 días Vs. 12-21 días). (3) *Intervalo condicionamiento-prueba*: la atenuación de la IL con el aumento en este intervalo ha sido un argumento utilizado para defender las teorías explicativas de la IL centradas en el fallo de la recuperación. Pero al igual que en otras fases, esta atenuación solo ocurre cuando el contexto del intervalo de retención es el mismo que en las otras fases experimentales. Pero si el contexto durante el intervalo de retención es distinto, entonces se produce una potenciación del efecto de IL (super-IL) (Lubow y De la Casa, 2002, 2005; De la Casa, Díaz y Lubow, 2003; De la Casa y Lubow, 2005).

- Papel del contexto: los fenómenos de preexposición son específicos de contexto, esto es, son más robustos cuando todas las fases experimentales suceden en el mismo contexto. Sin embargo hay estudios que se contradicen en los resultados (ver Lubow, 2009, Tabla 3.1, p. 44) cuando se cambia el contexto global entre fases. Algunos experimentos sugieren que los cambios contextuales reducen el efecto de IL (ver por ejemplo Hall y Chanell, 1986; Manrique et al., 2004) mientras que otros sugieren que el cambio contextual no afecta a la IL (por ejemplo Best y Meachum, 1986; Chamizo, 1996). Sin embargo, un cambio en las claves locales (como es el sonido que se produce al beber de los tubos calibrados) sí produce un efecto de atenuación de la IL, mientras que la IL podría parecer más resistente a cambios de contexto globales. Pero, ¿Hasta qué punto es esto correcto? A pesar de esta inconsistencia, sí hay evidencia de que los aprendizajes adquiridos en un contexto determinado se transfieren difícilmente cuando se miden en otro contexto alternativo. En un estudio reciente de Quintero et al. (2011) se analiza el rol de las claves contextuales en la IL. Los autores proponen que los resultados contradictorios relativos al cambio de contexto son el resultado de utilizar las jaulas hogar (contexto altamente familiar) como uno de los contextos. Estos autores concluyen que un cambio de contexto entre la preexposición y el condicionamiento/test (pero no entre la preexposición/condicionamiento y el test) reduce el efecto de la inhibición latente cuando ambos contextos son novedosos. Pero si uno de los contextos es la jaula hogar, hay un aumento del efecto de IL cuando el cambio se produce entre el condicionamiento y el test. Por lo tanto, parecería que los resultados del cambio de contexto están mediados tanto por la fase en la que se produce el cambio, como por la familiaridad con el contexto. En este sentido, un cambio de un contexto familiar a uno novedoso reduce el efecto de

IL, pero un cambio de uno novedoso a uno familiar (cuando el cambio se produce después del condicionamiento) potencia el efecto de IL.

Pero no solamente el cambio de contexto es capaz de modular este efecto, el contexto siempre ha jugado un papel clave en el AAG. De una manera más general Lubow (2009) resume los hallazgos más significativos a este respecto en 4 puntos: la preexposición al contexto de adquisición antes de la asociación EC-EI facilita el AAG; la exposición no reforzada (extinción) del contexto del condicionamiento después de la asociación EC-EI debilita el AAG; la re-exposición a ese mismo contexto después de la extinción aumenta el AAG (efecto de renovación) y finalmente, el contexto del intervalo de retención afecta al efecto de IL, como se ha comentado en el punto anterior.

3.2.2. Algunas consideraciones sobre la Inhibición Latente

Existen ciertos problemas derivados de este paradigma que deben tenerse en consideración en el AAG. Por ejemplo, sería necesario tener en cuenta que el efecto de inhibición latente puede ser el resultado de un descenso de la atención que se presta al EC en el grupo preexpuesto, como a que el EC puede ser más saliente para el grupo no preexpuesto, y por tanto presentar una ventaja para adquirir nuevas asociaciones. En este sentido, la IL sería el resultado de lo que ocurre en el grupo preexpuesto y también de lo que ocurre en el no preexpuesto (Lubow, 2009).

Otro posible problema es que las exposiciones no reforzadas al fluido pueden producir habituación a la neofobia (Lin, Amodeo, Arthurs y Reilly, 2012; Neath, Limebeer, Reilly y Parker, 2010; Reilly y Bornovalova, 2005). Cuando una rata prueba una solución novedosa no puede predecir las consecuencias del consumo de la solución, así que consume menos cantidad del fluido hasta estar seguro de que no supone un

peligro. Poco a poco, con los ensayos, el consumo aumenta hasta alcanzar una asíntota. Si el grupo preexpuesto muestra un patrón mayor de consumo durante todo el procedimiento experimental en comparación con un grupo para el cual el sabor es novedoso, puede ser debido a la habituación de la neofobia y no a un efecto de IL.

Otro de los errores en los que se suele recaer usando este paradigma es confundir el cambio de contexto con la familiaridad del contexto. En este sentido, muchas veces cuando se cambian los contextos entre fases se suele utilizar un contexto novedoso y las jaulas hogar, que son altamente familiares. Una de las variables que habría que controlar sería la familiaridad con los contextos. Se deberían utilizar dos contextos con el que se tenga la misma experiencia y puedan ser contrabalanceados en el diseño experimental (ver por ejemplo Hall y Channell, 1986).

Finalmente, se deben tener en cuenta el efecto suelo y el efecto techo son problemas que deben tenerse en consideración a la hora de analizar resultados de la IL. Un solo ensayo de condicionamiento puede provocar un fuerte AAG, haciendo que el grupo no preexpuesto apenas consuma la solución el día de la prueba. Por el contrario, los ensayos de preexposición pueden hacer que el grupo preexpuesto consuma el máximo posible de la solución. Además los ensayos de preexposición suelen ocurren en situación de privación de agua. El EC en el grupo preexpuesto podría adquirir propiedades reforzantes que llevan a la disminución de la pulsión, que podrían alterar el tamaño del efecto de IL aunque no explicar el fenómeno (ver por ejemplo Domjam, 1972).

Capítulo 4

Bases neurales de la aversión al sabor

CAPITULO 4

Bases neuronales de la aversión al sabor

Toda manifestación conductual tiene su base en procesos moleculares que ocurren en el cerebro, de hecho, los aprendizajes alteran su estructura. Dejando a un lado el reduccionismo de lo psicológico a lo neural (considerar el cerebro como causa), no deja de ser interesante complementar el estudio conductual con el estudio fisiológico, alejarse de las causas únicas para ofrecer un estudio más amplio, dado que toda ciencia acaba relacionándose con las que están por encima y por debajo de su nivel de análisis. Por tanto, estudiar las bases neuronales de la aversión al sabor aportaría información adicional sobre este aprendizaje. Una de las formas de poder conocer los sustratos neuronales de una conducta, es a través de la medición del incremento de la actividad en la región que está implicada en la conducta a estudiar. De hecho, las regiones cerebrales relacionadas en el procesamiento del sabor y las señales de cambio visceral se han estudiado usando técnicas anatómicas, electrofisiológicas, de neuroimagen, aunque mayoritariamente se han utilizado lesiones de núcleos particulares y el análisis de genes de expresión temprana c-Fos (para revisiones, ver Bermudez-Rattoni, 2004; Berstein, Wilkins y Barot, 2009; Núñez-Jaramillo, Ramírez-Lugo, Herrera-Morales y Miranda, 2010; Reilly, 2009). Estas técnicas nos pueden ofrecer información de cómo se trasmite y procesa la información gustativa.

El gusto es el único sistema sensorial que además de reconocer las cualidades del fluido (en este caso) está innatamente asociado con los aspectos hedónicos del reforzamiento y la aversión. La información sobre el sabor es trasmitida de las vías

centrales a las zonas corticales donde se procesan los aspectos cualitativos y cuantitativos del sabor (Yamamoto 2006). Hay una aceptación generalizada de que un área cerebral a la que llega información tanto gustativa como visceral (en términos de cambio en el estado fisiológico) está implicada en la valoración hedónica del sabor. Sin embargo, si a esa región llega información sobre las cualidades gustativas en exclusiva, (o información visceral), entonces estaría relacionado con el procesamiento de aspecto puramente sensoriales del sabor consumido (Sewards, 2004). Así, existirían representaciones independientes para los aspectos sensoriales y aquellos relacionados con la valoración hedónica de un sabor, que además, puede variar con el aprendizaje como es el caso del AAG. En oposición, las cualidades químicas relacionadas con las cualidades gustativas permanecen inalteradas.

Entre las regiones cerebrales de las que se conoce con mayor precisión su función en la aversión al sabor destacan: la *corteza insular* (IC), *núcleo del tracto solitario* (NTS), *núcleo parabraquial* (PBN) y la *amígdala* (AMY). Otras áreas que se están relacionadas con el AAG son el *núcleo accumbens* (NAcb), *corteza prefrontal medial* (PFC), *tálamo posteromedial* (VPM), *núcleo de la estría terminal* (NET) y el *área tegmentoventral* (VTA).

Algunas de estas regiones participan en el procesamiento de las cualidades sensoriales mientras que otras se encargarían de los aspectos hedónicos. Existirían por lo tanto dos vías separadas que se encargan de aspectos distinto en el procesamiento de sabores (ver Figura 2). El uso de técnicas inmunoquímicas ha permitido la distinción de las bases neuroanatómicas para las dos vías. Por ejemplo, se sabe que pequeñas sub-poblaciones neuronales en el PBN responden de manera diferente a los sabores básicos (Yamamoto, 2006). El sistema del sabor está compuesto por el núcleo del tracto solitario, el núcleo parabraquial, el tálamo posteromedial y la corteza insular (aunque

esta región quizás estuviera mejor ubicada como estructura de relevo. El sistema relacionado con la recompensa está compuesto por el área tegmental ventral y el núcleo accumbens principalmente. Las regiones que funcionan como intermediarios entre los dos sistemas son la amígdala y el prefrontal. Más específicamente, la información sensorial del sabor) que llega de las ramificaciones nerviosas de la cara (facial, glosofaríngeo y vagal) es enviada a la primera estación de relevo, el núcleo del tracto solitario. La segunda estación hasta la que asciende la información sensorial del sabor es el núcleo parabraquial, que envía la información al tálamo posteromedial (implicado en la discriminación de las cualidades gustativas).

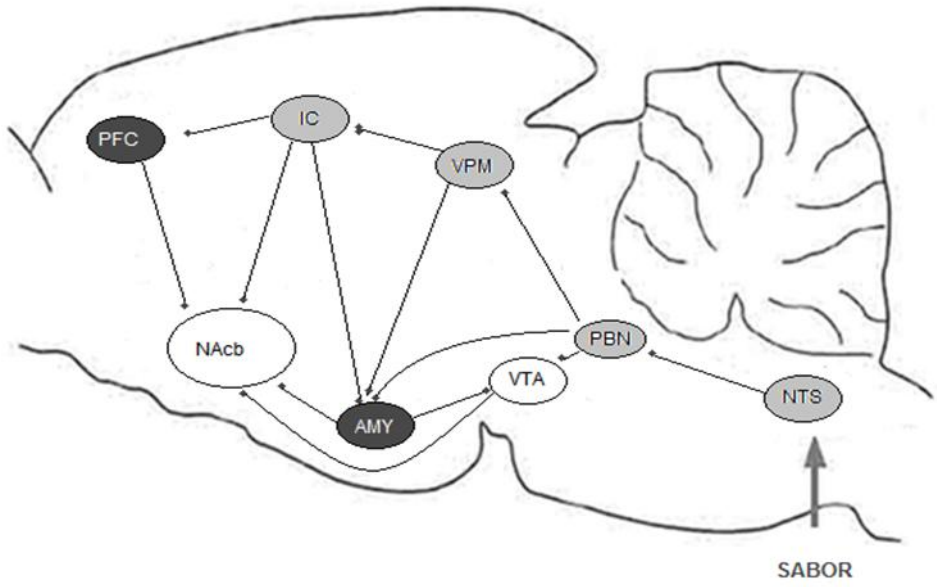


Figura 3.- Esquema simplificado de las regiones cerebrales implicadas en el procesamiento del sabor (Adaptado de Yamamoto, 2006). Las regiones en gris representan la vía sensorial, las regiones en blanco representan la vía de la recompensa, y en negro las estrucutras que funcionarían de intermediarias entre las dos vias.

El tálamo actúa como filtro y envía la información a las zonas corticales relacionadas con el sabor. La corteza insular se encarga de procesar el sabor y la información visceral. Esta información sigue un sistema paralelo al descrito. Aunque no se sabe con certeza cómo funcionan las regiones que median entre el sistema del reforzamiento y el de reconocimiento del sabor, se sabe que la corteza insular (dorsomedial) envía axones a la corteza prefrontal, la cual está conectada con el sistema límbico, siendo de interés las aferencias con la amígdala. A esta región también llega información procedente del núcleo accumbens, relacionado con el reforzamiento, que a su vez recibe aferencias del área tegmental ventral, implicada en la valoración hedónica y conectada con el parabraquial (Yamamoto, 2006, Yamamoto y Ueji, 2011).

4.1. Regiones cerebrales más relevantes en el AAG

Núcleo del tracto solitario

Es la primera estación de relevo de la información gustativa ascendente, recibe proyecciones de los nervios facial, glosofaríngeo y vago. Además recibe proyecciones de la amígdala y envía información al parabraquial. Sus lesiones bilaterales no previene el AAG (para revisión Reilly y Schatman, 2009). El NTS posee neuronas gustativas localizadas en su parte más rostral, y muestran respuestas electrofisiológicas tanto a estímulos gustativos positivos como a negativos, por lo que se considera que esta región está relacionada con el procesamiento de aspectos puramente sensoriales. En este sentido, infusiones IO de sacarina, sacarosa o quinina aumenta la activación c-Fos en esta subregión (Yamamoto y Saka, 2000). Sin embargo en su parte caudal e intermedia se han encontrado evidencias de confluencia de información gustativa sensorial y visceral, dado que tanto información de infusiones IO como intragástricas aumentan la actividad c-Fos en la parte caudal y medial del núcleo. Estos hallazgos pueden considerarse como una evidencia a favor de que estas subregiones procesan aspectos

hedónicos. Más específicamente, se han localizado una serie de neuronas en la porción parvocelular de la región intermedia que responden a EC aversivos (infundidos IO, no bebidos) pero no a estímulos no condicionados con independencia de su valor hedónico inicial (Swank, Schafe y Bernstein, 1995). Estos hallazgos relacionan directamente al núcleo del tracto solitario con el AAG.

Núcleo Parabraquial

Es el centro de relevo de segundo orden, recibe aferencias del NTS que proyectan a la subdivisión medial del PBN. En este núcleo confluye información sensorial, visceral y en ciertas subregiones ambas informaciones (para revisión Bures et al., 1998; de la Torre-Vacas y Agüero-Zapata, 2006; Reilly, 1999, 2009). El parabraquial se divide principalmente en dos subregiones: medial y lateral. A su vez la medial puede dividirse en externa y central, y la lateral en externa, central, ventral, dorsal, extrema, superior e interna. Las dos subregiones del medial y las tres primeras del lateral han sido relacionadas con la representación gustativa. Por otro lado las subregiones medial externa, lateral externa y la zona de la cintura parabraquial, reciben información tanto sensorial gustativa como visceral, mientras que la medial central y la lateral ventral recibe información únicamente sensorial. En resumen, esta estructura es la principal región donde converge la información gustativa-visceral, básica para el AAG. Lesiones del PBN (antes de la adquisición) interrumpe el AAG, pero no impide el procesamiento aislado del sabor y el malestar. Por lo tanto, este núcleo juega un papel fundamental en la adquisición y consolidación del AAG, mientras que no es crítico para la recuperación de esta memoria (Nuñez-Jaramillo et al., 2010).

Amígdala

Al igual que en el núcleo parabraquial, en la amígdala también confluye la información visceral y gustativa. Está implicada en el procesamiento emocional y juega un papel determinante en la formación de la memoria aversiva. La amígdala suele

dividirse genéricamente en dos subnúcleos: amígdala central, que recibe aferencias que proviene del tronco, y la amígdala basolateral, que recibe aferencias de tálamo y la corteza. Sin embargo en general la amígdala recibe inputs del hipotálamo, el tálamo, de todas las modalidades sensoriales y de otras partes del sistema límbico. Este núcleo evalúa la información afectiva procedente de los sentidos (para revisión Bures et al., 1998; Reilly y Bornovalova, 2005), aunque está más relacionada con el procesamiento visceral, dado que su inactivación antes de la inyección de litio interrumpe el AAG (Roldan y Bures, 1994), y la activación c-fos depende de la dosis de litio que se administre (Ferreira, Ferry, Meurisse y Lévy, 2006). La amígdala basolateral juega un papel crucial en el AAG. De hecho, las lesiones producidas en la amígdala basolateral pero no en la central retrasan el AAG (Yamamoto, 1995). La información sobre el EC llega a la amígdala basolateral desde la corteza insular y la información del EI desde el tálamo y de la corteza insular. Esta información debe contrastarse con la información hedónica que ya se posee, y modificarse si esto fuera preciso. La amígdala central integraría la información gustativa de la amígdala basolateral, del parabraquial y la procedente del hipotálamo (que aporta información sobre el estado interno del organismo para regular la conducta de ingesta) para proporcionar valor emocional a la nueva información gustativa (Schafe y Berstein, 1996). La amígdala por tanto, se ha relacionado con algunos aspectos de regulación de la ingesta, de hecho, ratas con lesiones en la amígdala basolateral no muestran efecto de neofobia (Bures et al., 1998), lo que la implica directamente (en conjunto con el córtex insular) en el reconocimiento de la familiaridad de un EC.

Corteza insular

La corteza insular recibe aferencias gustativas y viscerales que provienen del tronco, de la amígdala y el tálamo. Es por tanto otro de los principales centros de

integración visceral-sensorial. En la corteza insular hay neuronas sensoriales que responden a sabores básicos con independencia de su valoración afectiva, y neuronas "hedónicas" que responden de manera distinta a estímulos aversivos y apetitivos (Sewards, 2004). Además se han observado cambios moleculares a corto plazo que podrían estar relacionados con el procesamiento sensorial y otros a largo plazo, que podrían estar implicados en la formación de la memoria gustativa (Yasoshima y Yamamoto, 1998). El IC está conectado al parabraquial y al núcleo del tracto solitario y ejerce un efecto modulador en estos núcleos similar al que ejerce la amígdala (una de las razones por las que se considera que podría ser también una región que funciona de interface entre el sistema del sabor y el sistema del refuerzo). A pesar de esto, mucha literatura aporta pruebas de que su lesión no tiene influencia en la adquisición del AAG. Una de los motivos que pueden explicar esta contradicción es que la mayoría de los estudios se lleva a cabo exponiendo repetidamente el sabor, por lo que los resultados podrían verse oscurecidos por el efecto de IL. Algunos autores han defendido que la función que cumple en el AAG es la de procesar la saliencia (asociativa) del EC, esto es, ratas con lesiones en la corteza insular son incapaces de adquirir aversión condicionada a un sabor palatable (sacarina) pero la lesión no tendría influencia en el desarrollo de una aversión condicionada a un sabor desagradable (quinina) (véase la revisión de Reilly, 2009). Esta implicación de la corteza insular ha suscitado muchas críticas y se han planteado interpretaciones alternativas sobre su papel en la familiaridad del sabor. Estas interpretaciones sugieren que las ratas lesionadas tratan sabores novedosos como sabores familiares (Roman y Reilly, 2007) lo que se traduce en una atenuación de la AAG. Más recientemente se ha analizado el papel de la corteza insular en la familiaridad con sabores (Moraga-Amaro, Cortés-Rojas, Simon y Stehberg, 2014). Estos autores proponen que la corteza insular no está implicada en la adquisición o

retención de la familiaridad de sabores. Además, otros mecanismos compensatorios podrían permitir a animales con lesiones en la corteza insular aprender acerca de la familiaridad de los sabores. Si bien estos autores proponen que la corteza insular es necesaria para la adquisición del AAG, sugieren que no lo es para la adquisición o el almacenamiento de la información sobre la familiaridad con los sabores, lo que implica una clara disociación del papel que juega en el AAG y la familiaridad con el sabor. Estos autores explican la discrepancia entre sus resultados y aquellos que hablan de la implicación de la corteza insular con la familiaridad del sabor, debido a que lesiones en esta región disminuyen el efecto de neofobia. Proponen que el papel del IC es modular conductas asociadas con respuestas de rechazo de soluciones (neofobia y AAG) pero que no tiene un papel fundamental en la familiaridad o este es asumido por otras áreas.

En contraposición con esta hipótesis, existen estudios de los mecanismos moleculares relacionados con el cambio de un sabor novedoso a familiar que sugieren una participación de la corteza insular en este aprendizaje (ver Bermudez-Rattoni, 2014 para revisión). Específicamente, la primera presentación de un sabor induce un incremento en la liberación de acetilcolina en la corteza insular y tras varias presentaciones del sabor, la liberación va disminuyendo. Además, es posible convertir, en un paradigma de IL, un sabor familiar en "novedoso" a través de inyecciones de agonistas colinérgicos que incrementan su saliencia que finalmente se traduce en un fuerte AAG. Este autor concluye que la corteza insular es necesaria para la conversión de un estímulo de novedosos a familiar, así como la amígdala es necesaria para convertir un sabor novedoso en un sabor aversivo familiar. Esto convertiría a esta estructura en el lugar donde se forma la memoria de la aversión al sabor.

4.2. Otras regiones involucradas en el AAG

Si bien las estructuras anteriormente mencionadas son las principales en el AAG, hay otras estructuras que también participan en esta variedad de aprendizaje. Una de ellas en el área tegmental ventral que está relacionada con las consecuencias derivadas del consumo del fluido (motivación del incentivo). Las lesiones de esta región cerebral suprime el consumo de una solución preferida sin influir en el consumo de agua u otros sabores (Yamamoto, 2006). Además, la administración de morfina incrementa el consumo de sacarosa sin afectar al de la quinina, mientras que en animales con lesiones en el VTA no se experimenta este incremento. La otra estructura principal del sistema de la recompensa es el núcleo accumbens, implicado en la conducta de ingesta de estímulos altamente palatables. La administración de inyecciones de morfina en el núcleo accubmens aumenta la palatabilidad de los sabores en un test de reactividad facial (Pecina y Berridge, 2000). El NAcb proyecta fibras principalmente al hipotálamo lateral, región que está implicada en la conducta de ingesta y en la regulación del apetito.

Otra estructura cerebral que podría estar implicda en el AAG es el núcleo del lecho de la estría terminal. Se ha demostrado que inyecciones intraperitoneales de LiCl aumenta la actividad c-fos en esta región (St. Andre, Albanos y Reilly, 2007). Además recibe inputs de información gustativa del PBN. Sin embargo, se ha visto que lesiones en esta región no tienen influencia directa sobre el AAG (Reilly, 2009). Otra región cerebral que también recibe aferencias del PBN es el tálamo posteromedial, y a su vez éste proyecta a la corteza insular. Se han encontrado resultados contradictorios en cuanto a si lesiones en el tálamo posteromedial atenúan o no el AAG (ver Bures et al., 1998), sin embargo cabe la posibilidad de que estas diferencias se deban a que lesionan diferentes subnúcleos del tálamo o las lesiones incluyen varias zonas adyacentes

(dorsomedial, centromedial, parafascicular) que destruyen fibras que unen el PBN y la corteza insular (Yamamoto, 1995).

Finalmente cabría mencionar la corteza prefrontal medial. Esta corteza puede subdividirse en varias regiones que tienen relacionadas distintas funciones. Por ejemplo la corteza infralímbica está implicada en la actividad visceral (recibe aferencias de la corteza insular) y está relacionada con el sistema límbico, jugando un papel significativo en procesos de memoria, cognitivos y emocionales (Núñez-Jaramillo et al., 2010). Por otro lado la corteza prelímbica se ha relacionado con procesos atencionales, en memoria de trabajo y en toma de decisiones (procesos cognitivos en general). Estas dos regiones junto con otras zonas del prefrontal se encargan de la supervisión de funciones atencionales y el aprendizaje de contingencias, así como de la flexibilidad conductual. La corteza cingulada, situada en la parte más dorsal del prefrontal medial, está implicada en la selección de respuestas motoras, memoria motora general y procesamiento temporal de la información (integración de la información actual y pasada, decisivo en el paradigma de inhibición latente) incluyendo cualidades afectivas. Todas estas regiones cumplen alguna función moduladora dentro del AAG, si bien no hay numerosos estudios que clarifiquen su papel, es importante tenerlas en cuenta cuando se realicen estudios de las regiones cerebrales implicadas en el AAG.

4.3. Métodos de estudio

Una de las técnicas ampliamente utilizadas para identificar las bases neurales del aprendizaje, específicamente del AAG, es la medición de genes de expresión temprana, como por ejemplo c-fos (Sheng y Greenberg, 1990). La técnica inmunohistoquímica c-fos nos permite medir actividad neuronal a corto plazo y es un marcador potencial de cambios en la actividad cerebral como resultado de un aprendizaje concreto (ver

Bernstein et al., 2009 para una revisión). Esta técnica ofrece una serie de ventajas que la ha convertido en una de las más utilizadas para el estudio de las bases neuronales del aprendizaje. Es capaz de marcar poblaciones de neuronas que están activadas por estimulación muy específica, su expresión basal es relativamente baja (lo que facilita eliminar el "ruido") y además se puede combinar con otros métodos de tinción. Sin embargo su principal limitación es la temporal, es decir, se limita a medir lo que ha ocurrido en un momento temporal específico, lo que puede suponer una desventaja en algunos estudios. Además las neuronas difieren en su capacidad y en la latencia para expresar c-fos. Por ende, se requiere una fuerte actividad neural para que la c-fos sea expresada y detectada, lo que implica que la ausencia de expresión c-fos no tiene porqué significar necesariamente que la neurona no tenga un aumento en la actividad neuronal.

El aumento de la actividad neuronal requiere mayor demanda energética, lo que implica un aumento del flujo de sangre y mayor nivel de glucosa. Esta demanda energética conlleva un incremento del consumo de oxígeno y de la respiración celular. En las neuronas, las demandas energéticas hacen funcionar la bomba Na^+/K^+, que pone en marcha la generación de señales eléctricas para la trasmisión neuronal. Este proceso requiere de un incremento de la síntesis de adenosin-trifosfato (ATP) que es proporcionada por el metabolismo oxidativo y glicolítico de los análogos de la glucosa. Dada la estrecha relación existente entre la actividad eléctrica de las neuronas y el metabolismo energético oxidativo, otra forma de estudiar la actividad es mediante técnicas histoquímicas como la enzima mitocondrial c oxidasa (CO). La técnica inmunohistoquímica c-fos mide cambios a corto plazo (evocados por la estimulación durante un periodo de prueba bien establecido), mientas que la técnica CO permite identificar estructuras adicionales implicadas en cambios metabólicos a largo plazo tales como incrementos en los niveles de enzima o de neurotransmisores, síntesis de

proteínas que formarán la membrana o cambios morfológicos (mayor densidad en la hendidura postsinápica), así como incrementos en la actividad eléctrica neuronal, reflejando otros procesos que también requieres de ATP en la célula (Wong-Riley, 1989). Los cambios en CO revelados por histoquímica reflejarían variaciones importantes en la capacidad metabólica endógena a largo plazo, se usa para evaluar cambios enzimáticos que ocurren durante todo el periodo de experimentación (en ocasiones periodos de varias semanas). Las ventajas que ofrece la técnica histoquímica de la C oxidasa frente a otras técnicas es su elevada resolución espacial tanto en microscopía óptica como electrónica, lo que permite una delimitación precisa de las regiones cerebrales de interés. Esta técnica también tiene la capacidad para marcar de manera diferencial las neuronas frente a las células gliales. Las neuronas obtienen su energía por metabolismo oxidativo mientras que las células gliales lo hacen a través de la glicolisis anaeróbica; por tanto, la mayoría de los procesos gliales no contienen ninguna o pocas mitocondrias reactivas claras para la CO, sólo la glía limitante y unos pocos astrocitos tienen mitocondrias reactivas oscuras pero en menor número que las neuronas. Por tanto ambas técnicas, la inmunohistoquímica c-fos y la histoquímica CO, nos pueden proporcionar información relevante en el estudio de las bases neurales de la aversión al sabor.

Debemos tener en cuenta que el aprendizaje de la aversión al sabor depende en gran medida de la familiaridad que se tiene con ese sabor (como ya se ha explicado con anterioridad en esta introducción). La familiaridad con un sabor atenúa de manera crítica el AAG. Variar la familiaridad con los sabores nos proporciona una herramienta útil para medir la mediación molecular en el aprendizaje, dado que la expresión génica y la síntesis de proteínas está fuertemente influida por cómo es de novedoso un sabor, y el análisis de esa mediación nos proporciona información sobre donde se localizan las

regiones que están implicadas en el AAG. Específicamente, que un sabor sea novedoso o no provoca respuestas neurales diferentes, que pueden o no ser susceptibles de ser asociadas con aquellas señales derivadas de la administración del EI. Por esto, el paradigma de Inhibición latente es muy útil para conocer las bases neurales del AAG, y es el paradigma que va a utilizarse en esta tesis para el análisis de las regiones cerebrales de la AAG.

Parte II

Estudio Experimental

OBJETIVOS

Esta tesis doctoral tiene como objetivo general estudiar los cambios hedónicos producidos durante el aprendizaje de la aversión al sabor a través del análisis de las respuestas condicionadas de náusea. Para ello, con la técnica de reactividad al sabor, se examina si la exposición a los estímulos (condicionado e incondicionado) antes de la adquisición de la aversión atenúa las respuestas condicionadas de náusea. Paralelamente se examinan mediante técnicas inmunohistoquímicas (proteína c-fos) e histoquímicas (CO) las estructuras cerebrales que subyacen al cambio en la palatabilidad del sabor producido durante el condicionamiento de la aversión. Estos objetivos generales se concretan en los siguientes objetivos específicos:

1. Con la técnica de reactividad al sabor se evalúa si la experiencia con el agente inductor de malestar gástrico (cloruro de litio; LiCl) antes del condicionamiento de la aversión atenúa la adquisición de las respuestas condicionadas de náusea.
2. Se examina la capacidad que tienen las claves contextuales presentes durante el condicionamiento para interferir con la adquisición de una aversión gustativa y más concretamente de las respuestas condicionadas de náusea.
3. Se examina el efecto que tiene la extinción de la asociación contexto-náusea en la adquisición de una aversión a un sabor presentado en el contexto previamente asociado al litio.

4. Estudiar el papel que juegan las claves relacionadas con la infusión intraoral de los fluidos en el desarrollo de una aversión gustativa y específicamente su capacidad para interferir con el condicionamiento de las respuestas de náusea.
5. Examinar si la exposición no reforzada a un fluido atenúa la adquisición de la aversión al sabor a través del análisis del patrón de ingesta (microestructura de la conducta consumatoria) y la cantidad de fluido consumido.
6. Investigar las bases neuronales implicadas en el condicionamiento de respuestas de náusea en el paradigma de aversión al sabor:
    a) analizar la actividad metabólica cerebral producida en las distintas estructuras relacionadas con el aprendizaje de aversión al sabor. Para ello, se cuantificará la actividad citocromo c oxidasa (CO) mediante densitometría como índice del metabolismo oxidativo neural.
    b) analizar la actividad c-fos de las estructuras cerebrales que median los cambios en la palatabilidad del fluido, en concreto corteza insular (CI) y el núcelo accumbens (NAcb).

Capítulo 5

PREEXPOSICIÓN AL EI: EL PAPEL DE LAS CLAVES CONTEXTUALES

The US-preexposure effect: the role of contextual cues

5.1. Introduction

It is well established in rats that pairing contextual cues with the effects of a lithium chloride (LiCl) injection will endow the context with conditioned aversive properties. Different test procedures have been employed to assess context aversion learning, including the amount consumed of a palatable fluid in the presence of the contextual cues (e.g., Best, Brown, & Sowell, 1984; Boakes, Westbrook, & Barnes, 1992), the amount of time spent in an environment previously paired with LiCl on a place-preference test (e.g., Tenk, Kavaliers, & Ossenkopp, 2005; White & Carr, 1985), and blocking of a taste aversion by prior nausea-based context conditioning (e.g., Batson & Best, 1979; Willner, 1978). By means of the blocking procedure, Batson and Best (1979), for example, reported that rats previously given pairings of a black chamber with a LiCl injection showed an attenuated aversion to a saccharin solution presented in compound with the pretrained context and followed by LiCl administration.

More recently, the blocking procedure has been also used by Symonds and Hall (1997; see also Symonds et al., 1998) to demonstrate that pre-trained contextual stimuli can interfere with the acquisition of an aversion to a novel flavor. In this study, rats received initial exposure to two distinctive contexts, one of which was associated with an injection of LiCl whereas the other was not. In the conditioning phase, the rats received a novel flavor in the home cage before being placed either in the context in which they had experienced the lithium injection (the blocking group) or in the no-injection context (the control group) prior to a further injection of LiCl. In a subsequent test phase in which the flavor was presented in the home cage, it was found that rats in the blocking group consumed more of the flavor than rats in the control group. It was concluded that the context-LiCl association had blocked the acquisition of an aversion

to the novel flavor. Similarly, Rodríguez, López and Symonds (2000) also reported that environmental cues paired with lithium-induced nausea could block subsequent conditioning of a taste aversion. In this study (Experiment 2), the conditioning of an aversion to sucrose was attenuated when this flavor was associated with an injection of LiCl in the same context in which the lithium was previously administered. In a more recent example, Kwok and Boakes (2012) have also reported using a discrimination procedure that a context previously paired with LiCl injections blocks the acquisition of a taste aversion. An important feature of the blocking test procedure employed in these experiments is that the critical test takes place in the absence of the pre-trained contextual cues, providing, therefore, an unambiguous demonstration that the attenuated flavor aversion occurs because the context has itself acquired aversive properties (see Hall, 2009, for an analysis of this method).

Nevertheless, as recently argued by Parker (Parker, 2003; Parker et al., 2009), the reduction in consumption of a previously LiCl-paired flavor may not reflect the establishment of a conditioned taste aversion. According to the Parker´s proposal, an injection of lithium not only induces a state of nausea, but it also produces a novel change in physiological state that signals danger to the rat. The association between the flavor and the dangerous change of physiological state might be responsible for the suppression of intake (taste avoidance) observed in a standard consumption test for flavor aversion learning. Similarly, suppressed consumption of a fluid when presented in a context previously paired with LiCl might not reflect a state of conditioned nausea. Therefore, it has been proposed that nausea-induced taste aversions can be more directly assessed by means of the taste reactivity (TR) test introduced by Grill & Norgren (1978). In this test rats are implanted with intraoral cannulas and the orofacial reactions accompanying an intraoral infusion of the flavor previously paired with LiCl are

recorded. Rats display conditioned disgust reactions (i.e., rejection reactions), such as gaping, chin rubbing, and paw treading, when infused with a previously LiCl-paired flavor. TR analysis has shown than an animal may avoid a flavor but show no aversive reactions to that flavor. For example, Parker (1982; 1995) has reported different hedonic responses to flavors previously paired with LiCl and amphetamine. While rats avoided consumption of a flavor that has been paired amphetamine (a drug that has rewarding rather than emetic properties), only LiCl-paired flavors elicited conditioned disgust reactions. Furthermore, treatments that alleviate nausea (e.g., ondansetron) interfere with the acquisition and expression of aversive reactions but not conditioned suppression of consumption (Limebeer & Parker, 2000), indicating that taste aversion, reflected as conditioned disgust reactions, is motivated by conditioned nausea (see Parker, 2014, for a recent review).

There is recent evidence showing that rats not only display disgust reactions to a LiCl-paired flavor, but they also display conditioned disgust reactions to a contextual cue previously paired with lithium. For example, Limebeer et al. (2006) reported that when rats were repeatedly infused with saccharin in a context previously paired with LiCl, they not only displayed conditioned disgust reactions during the flavor infusions but also during the inter-infusion intervals, indicating that contextual cues can acquire the ability to elicit conditioned nausea in the same way a flavor previously paired with the nausea-inducing effects of LiCl. Similarly, Limebeer et al. (2008) also reported that, even in the absence of a flavored solution, rats display conditioned disgust reactions during reexposure to a distinctive context previously paired with lithium. Interestingly, pretreatment with the antiemetic agent, Δ^9-tetrahydrocannabinol (Δ^9-THC), reduces contextually elicited disgust reactions in rats (Limebeer et al., 2006). As well, the non-psychoactive cannabinoid cannabidiol (CBD) prevents the expression of conditioned

disgust elicited by a LiCl-paired context (e.g., Rock et al., 2008; Rock et al., 2014). These results support strongly the view that lithium-paired cues other than a flavor cue appear to be capable of eliciting conditioned nausea in rats. Therefore, conditioned disgust might be a more selective marker of a LiCl-induced context aversion than is flavor avoidance.

With this in mind, the present study tested the idea that a context previously paired with lithium-induced nausea would block the subsequent establishment of conditioned disgust reactions to a LiCl-paired saccharin solution. Whereas it has long been known that nausea-based context conditioning can interfere with the acquisition of a taste aversion as revealed by fluid intake, its effectiveness to interfere with the acquisition of conditioned disgust reactions has not been yet examined. Experiment 1 investigated whether LiCl exposure prior to conditioning of a saccharin solution presented in compound with the context would attenuate flavor aversion learning as measured by both conditioned suppression of consumption and the number of disgust reactions elicited by the intraoral infusion of the saccharin solution in the taste reactivity test. Experiment 2 tested the blocking by context account of conditioned disgust reactions by examining the level of aversive taste reactivity responses elicited by the contextual cues independently of conditioning to the saccharin solution. Finally, Experiment 3 looked at whether blocking of acquisition of conditioned disgust was obtained when rats are given non-reinforced exposure to the context previously paired with the lithium injection (i.e., context extinction) before the saccharin conditioning in compound with the context was established.

5.2. Experiment 1

The specific aim of this experiment was to test whether a context previously paired with lithium injections interferes with the acquisition of conditioned disgust reactions to a LiCl-paired flavor as assessed by the taste reactivity test. As mentioned above, rats not only display conditioned disgust reactions to a nausea-paired flavor, but they also display aversive reactions to a context previously paired with LiCl. Therefore, it is plausible that contextually elicited conditioned nausea would interfere with the development of conditioned disgust reactions to a flavor cue presented in compound with the context and paired with lithium.

Table 2: Design of Experiment 1

Group	Preexposure	Conditioning	TR test	Consumption
Pre	4 x Li	Sac (IO) → Li	Sac (IO)	2 x Sac
Non	4 x Sal	Sac (IO) → Li	Sac (IO)	2 x Sac

Note. Pre and Con refer to preexposed and non-preexposed groups; Sac = saccharin; Li = injection of lithium chloride; Sal = injection of physiological saline; IO = intraoral infusion; Pre-exposure, conditioning and taste reactivity test took place in the conditioning chamber; Consumption test took place in the home cages

The design of this experiment is summarized in Table 2. On each of 4 preexposure trials, rats in Group Pre (preexposed) were injected with LiCl and rats in Group Non (non-preexposed) were injected with physiological saline before being placed in a distinctive context (the conditioning chamber) for 60 min. All rats received then an intraoral infusion of saccharin while in the conditioning chamber immediately followed by an injection of LiCl. During the taste reactivity test, the rats were intraorally infused with the saccharin solution and their orofacial reactions examined. Finally, a consumption test was conducted in which the rats were given saccharin in a

graduated tube, and their consumption was measured. It was expected that the contextual cues should associate with the LiCl-induced nausea during the preexposure phase and this would be expected to block the subsequent development of conditioned disgust reactions to the lithium-paired flavor.

5.2.1. Materials and methods

5.2.1.1. Subjects

Twenty male Wistar rats, approximately 90 days of age and with a mean free-feeding weight of 286 g (range, 251-318 g) at the start of the experiment, were used. Upon arrival, they were housed individually in standard plastic cages in a colony room maintained on a 12-h light/dark cycle (lights on at 08:00 h) and at an ambient temperature of 23º C. All experimental manipulations took place during the light phase. Throughout the experiment, rats were maintained on a water deprivation-schedule as described below. Food was always available in the home cages. All behavioral procedures were conducted in accordance with guidelines of the European Council Directive (86/609/EEC) and Spanish regulation RD-1201/2005 regarding the care and use of laboratory animals.

5.2.1.2. Fluids and apparatus

The fluids used were solutions of lithium chloride (0.15 M LiCl), isotonic saline (0.9% NaCl solution), and saccharin (0.1% w/v). LiCl and NaCl were administered intraperitoneally (i.p.) at a volume of 10 ml/kg of body weight. The saccharin solution was infused directly into the mouth of the subject through an oral cannula implanted prior to the experiment, the details of which are described below.

The behavioral procedure took place in a conditioning chamber located in a dark room. The chamber was made of clear Plexiglas sides (26 cm x 23 cm x 14 cm) with a

dark lid, and was placed on a table with a clear Plexiglas top. Two 50-Watt white lights on each side of the table provided a light illumination. A mirror beneath the chamber on a 45º angle facilitated viewing of the ventral surface of the rat during the intraoral infusion. Fluids were administered to the animals through an infusion pump (KD Scientific) connected to the implanted cannula. While the rats were infused with the fluids, their orofacial responses were videotaped using a videocamara (Sony Optical 20 X) with a telephoto lens. The videocamara was connected to a computer to record the orofacial reactions of the rats during the infusion. The videotapes were scored using the Observer XT 9.0 (Noldus Information Technology, Sterling, VA) event recording program. The videotapes were scored by two raters blind to the experimental groups.

The behaviors scored included the frequency of the disgust reactions of gaping (rapid, large-amplitude opening of the mandible with retraction of the corners of the mouth), chin rubbing (mouth or chin in direct contact with the floor or wall of the chamber and body projected forward), and paw treading (forward and backward movement of the forepaws in synchronous alternation). These scores were summated to provide a total disgust reaction score. The inter-rater reliability (r´s > 0.91) for each behavior scored was highly significant.

5.2.1.3. Cannulation surgery

The rats were surgically implanted with an intraoral cannula using a very similar method to that described in Parker (1995). The surgical anesthesia preparation included administration of an intraperitoneal (i.p.) injection of ketamine (50 mg/kg) combined with medetomidina (0.15 mg/kg), a drug with analgesic properties. Following surgery, the rats were administered ketofreno (1.5 mg/kg, s.c.), an anti-inflammatory drug, and the antibiotic enrofloxacino (0.3 mg/kg, s.c.). A thin-walled 15-gauge stainless steel needle was inserted at the back of the neck, directly subcutaneously around the ear and

brought out behind the first molar inside mouth. A length of intramedic polyethylene tubing with an inner diameter of 0.86 mm and an outer diameter of 1.27 mm was then run through the needle after which the needle was removed. Two square elastic discs were placed over the tubing and drawn to the exposed skin at the back of the neck for the purpose of stabilizing the cannula. The tubing was held secure in the oral cavity by an O-ring, which was sealed behind the tubing prior to cannulation surgery. Following surgery, rats were monitored for three days and had their cannula flushed daily with chlorhexidine to prevent infection. For the purpose of fluids infusion, the cannula was connected to the infusion pump by slipping the tubing of the cannula inside a second polyethylene tubing (inner diameter 1.19 mm; outer diameter 1.70 mm) attached to the infusion pump.

5.2.1.4. Behavioral procedure

During recovery from surgery, 2 rats lost their cannula and were removed from the experiment. The remaining rats were randomly assigned to either the preexposed (Pre; n= 9) group or the non-preexposed (Non; n=9) group. Three days after the surgery, the rats were placed on a water deprivation-schedule, comprising 1-h access to water each day, approximately 2 h after the experimental sessions. Throughout the experiment, this water deprivation regime was maintained.

On each of 4 preexposure sessions (see Table 1), rats were placed in the conditioning chamber, where they spent 5 min before being injected with lithium (Group Pre) or physiological saline (Group Non). Rats spent 60 min after the injection in the conditioning chamber before being returned to the home cage. After the second and fourth pre-exposure sessions, the animals were given water for 24 h in their home cage. On the next day, the rats were habituated to the infusion procedure. They were placed in the conditioning chamber (the taste reactivity apparatus) with their cannula

attached to the infusion pump for fluid delivery. After a period of 5 min, water was infused into their intraoral cannula for 1 min at the rate of 1 ml/min in order to habituate them to this fluid delivery method. The day following the habituation session, the conditioning trial was administered. In this session, all rats were placed in the conditioning chamber and intraorally infused with 0.1% saccharin for 5 min at a rate of 1 ml/min while their orofacial responses were video-recorded. Immediately following the saccharin infusion, the rats were all injected with LiCl. The rats spent one hour after the injection in the conditioning chamber before being returned to the colony room. The next day was a recovery day on which the rats were given water for 24 h in their home cages.

On the next day the taste reactivity (TR) test was administered. On this session, the rats were intraorally infused with the saccharin solution for 5 min at a rate of 1 ml/min and the orofacial responses videotaped. Finally, a consumption test was administered on each of the following two days. On each of these sessions, the rats were given access to the saccharin solution in a graduated tube for 15 min in their home cages, and their consumption was measured. Rats were given supplementary water for 60 min at the usual time on each day.

5.2.1.5. Data analysis

For statistical analysis, differences between groups in taste reactivity during conditioning and testing were evaluated using independent *t*-tests. The behavioral data entered into analysis were the total number of disgust reactions (gapes, chin rubs, and paw treads summated). Fluid intake during the consumption test was analyzed by an analysis of variance (ANOVA) for repeated measures, with group and trial as factors, and post hoc comparisons by independent *t*-tests. All tests reported here used a criterion for significance of $p = 0.05$. Data was expressed as mean $\pm$ SEM.

5.2.2. Results and discussion

Rats in Group Pre and Group Non did not significant differ in their mean number of aversive reactions during the infusion of novel saccharin in the conditioning session with LiCl, $t(16) = 1.83$; $p = 0.08$. The mean numbers of aversive responses for the two groups were: Group Pre: 3.3 (± 1.16); Group Non: 1.1 (± 0.51).

Figure 4 (panel A) presents the mean number of conditioned disgust reactions displayed by rats during the 5-min intraoral infusion of saccharin in the taste reactivity test. It may be seen that Group Pre displayed significantly less disgust reactions than did Group Non on this session, $t(16) = -2.14$; $p = 0.04$, suggesting that pretreatment with LiCl may have interfered with the acquisition of conditioned disgust reactions to the saccharin solution. The mean number of conditioned disgust reactions for each group during this session was: Group Pre: 9.55 (± 2.80); Group Non: 19.01 (± 3.39).

The panel B of Figure 4 presents the mean amount of saccharin consumed by the Groups Pre and Non on each of the 2 consumption tests. A 2 x 2 repeated measures ANOVA of consumption data with group and trial as factors revealed significant main effects of trial, $F(1,16 = 29.49$; $p < 0.001$, and group, $F(1,16 = 9.39$; $p = 0.007$. The interaction between these two factors was no significant ($F < 1$). As assessed by subsequent independent *t* tests, Group Pre drank significantly more of the saccharin solution than did Group Non on trial 1, $t(16) = 2.69$; $p = 0.01$, and trial 2, $t(16) = 2.72$; $p = 0.01$.

The present experiment shows that a context previously paired with lithium injections can attenuate subsequent conditioning of an aversion to saccharin as revealed by a reduction in flavor avoidance. This finding confirms previous reports of blocking of a taste aversion by environmental cues previously paired with lithium-induced nausea (e.g., Batson & Best, 1979; Rodríguez et al., 2000; Symonds & Hall, 1997).

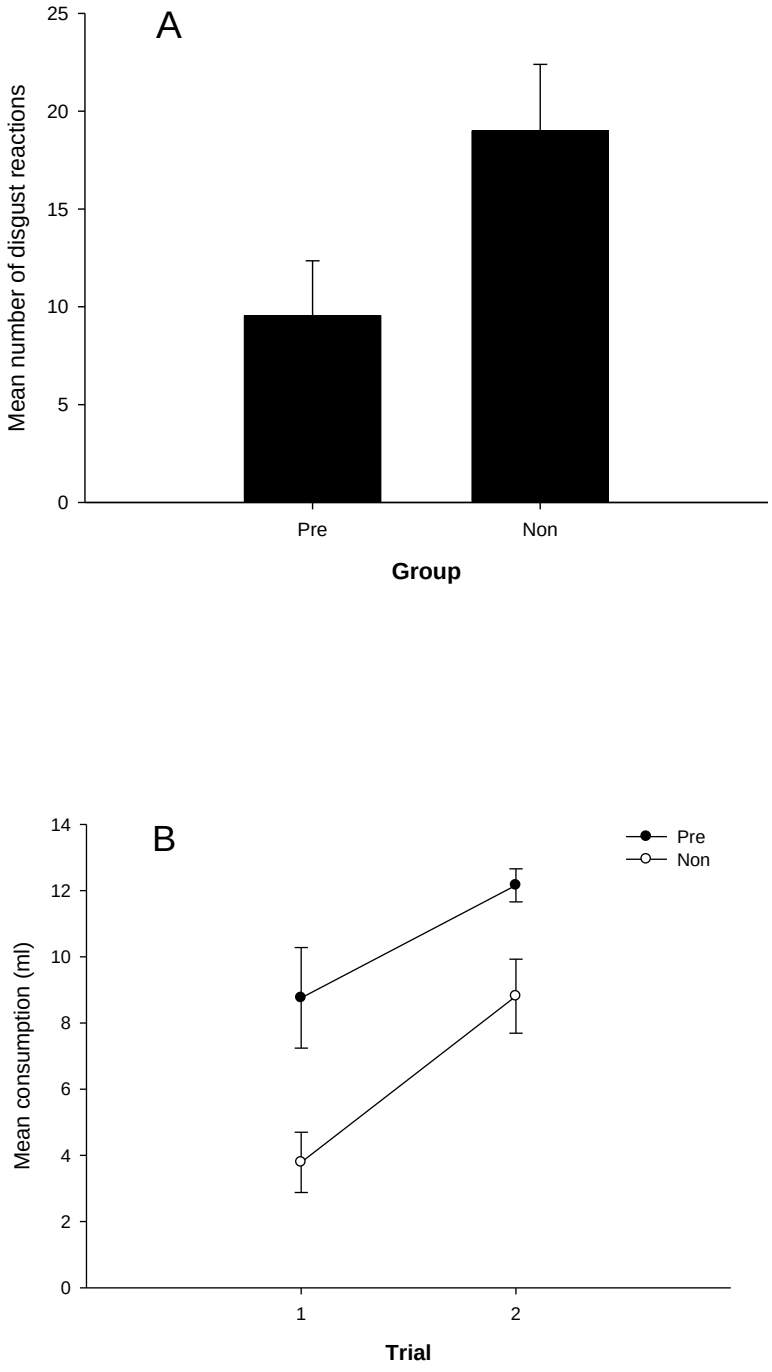


Figure 4: Experiment 1: Mean number of conditioned disgust reactions elicited by the infusion of saccharin during the TR test (A) and mean amount of solution consumed on each of the consumption tests (B) for both the Group Pre and the Group Non. Error bars indicate the standard error of mean (*SEM*s).

The new finding was that pretraining with LiCl also interfered with the establishment of conditioned disgust reactions to the saccharin solution, suggesting that the effects of taste aversion on hedonic reactions had also been attenuated. The results are consistent with the hypothesis that contextually elicited conditioned nausea can block the subsequent establishment of conditioned disgust reactions to a lithium-paired flavor. There is, however, a major potential problem with this explanation. The problem, obviously, is that in our test procedure the saccharin (the blocked cue) is presented in compound with the context (the blocking cue) and, as a consequence, we do not know the level of conditioning to the saccharin independently of conditioning to the context. It should be noted, however, that in the bottle test the Group Pre had a weaker aversion to saccharin than Group Non, a result consistent with the claim that contextual cues are associated with LiCl and then block conditioning to the saccharin. Moreover, the most likely effect of a compound test would be to artificially increase the apparent disgust reactions to saccharin in Group Pre via the summation of any context-based responses with those elicited by saccharin. Thus the compound test would work against seeing the blocking effect that we have reported here. Regardless, one possible strategy for testing the blocking by context account of the present results could be to compare taste reactivity to the flavor cue with that to contextual cues alone – this was done in Experiment 2.

5.3. Experiment 2

This experiment evaluate the potential of the contextual cues alone (i.e., in the absence of the flavor) to display conditioned disgust reactions, in order to confirm that the attenuated aversion to saccharin observed in Experiment 1 was due to the operation of contextually elicited conditioned nausea. A comparison between the levels of aversive responses elicited by the saccharin infusion and by the context alone directly

addresses this idea. Experiment 2 (see Table 3) also includes saline-paired controls to provide a baseline measure of responding without conditioned taste aversion treatment to saccharin. Two groups of rats (Groups Pre-Li and Pre-Sal) were pretrained with LiCl whereas other two groups (Groups Non-Li and Non-Sal) received saline injections during pretraining. In the conditioning sessions, subjects in Groups Pre-Li and Non-Li were injected with LiCl after an intraoral infusion of saccharin whereas those in Groups Pre-Sal and Non-Sal were given saline injections. In the TR test, each subject was tested with the context alone, an intraoral infusion of saccharin, and finally, with an infusion of water, while their orofacial reactions were recorded. It was expected that rats pretrained with LiCl before conditioning of saccharin in compound with the context (Group Pre-Li) would display conditioned disgust reactions when exposed to the context alone, and that they would display reduced disgust reactions when infused with the saccharin solution during testing.

Table 3: Design of Experiment 2

Group	Preexposure	Conditioning	TR Test	Consumption
Pre-Li	4 x Li	2 x Sac (IO) → Li	Context/Sac/Water	3 x Sac
Non-Li	4 x Sal	2 x Sac (IO) → Li	Context/Sac/Water	3 x Sac
Pre-Sal	4 x Li	2 x Sac (IO) → Sal	Context/Sac/Water	3 x Sac
Non-Sal	4 x Sal	2 x Sac (IO) → Sal	Context/Sac/Water	3 x Sac

Note. The first term in each group´s label indicates that the subjects received either LiCl (Pre; preexposed) or saline (Non; non-preexposed) during pretraining; Groups Pre-Li and Non-Li received injections of LiCl during conditioning while the Groups Pre-Sal and Non-Sal received saline; Preexposure, conditioning, and taste reactivity test were conducted in the conditioning chamber; Consumption test was conducted in the home cages; Li = LiCl; Sal = saline; Sac = Sacharin; IO: intraoral infusion.

5.3.1. Method

5.3.1.1. Subjects, fluids, and apparatus

Thirty two male Wistar rats, approximately 90 days old and with a mean free-feeding weight of 331 g (range, 220-393 g) at the start of the experiment, were utilized for the present study. The animals were housed and maintained as in Experiment 1. The solutions and apparatus were identical to those used in Experiment 1. The rats were implanted with intraoral cannula as in the previous experiment. Three rats lost their cannula throughout the experiment and were removed from the sample. The remaining animals were randomly assigned in four groups as followed: Group Pre-Li (n=7); Group Non-Li (n=8); Group Pre-Sal (n=7); and Group Non-Sal (n=7).

5.3.1.2. Procedure

The procedures were identical to those described for Experiment 1, with two exceptions. First, the rats received two conditioning trials with lithium during the conditioning phase, and second, in the TR test the level of conditioning to the context was evaluated independently of conditioning to the flavor cue. Three days after the surgery, the rats were placed on a water deprivation-schedule during which they could consume water for 1 h in their home cages at the same time each day. During the pre-exposure phase, subjects in groups Pre-Li and Pre-Sal received four daily pretraining sessions consisting of an i.p. injection of LiCl (0.15 M; 10 ml/kg) 5 min after to placement in the conditioning chamber. Rats in groups Non-Li and Non-Sal were injected with isotonic saline (10 ml/kg) on these sessions. The animals were kept in the conditioning chamber for 60 min after the injection. They were given a water recovery session after the second and fourth conditioning trials. After completion of this phase, the rats were habituated to the taste reactivity procedure by infusion with water for a period of 1 min at the rate of 1 ml/min. The next four days constituted the conditioning

phase. The rats received two conditioning trials separated by a recovery day during which they were given water in the home cage. On each of the two conditioning trials, the animals were placed in the conditioning chamber and intra-orally infused with 0.1% saccharin for 5 min at a rate of 1 ml/min whereas their orofacial responses were videotaped. Immediately following the fluid infusion, the rats in Groups Pre-Li and Non-Li were injected (i.p.) with LiCl whereas those in Groups Pre-Sal and Non-Sal received an injection of physiological saline. After the injections, the animals were kept 60 min in the conditioning chamber before being returned to the home cage.

The TR test occurred the next day. This test was divided into three 2.5 min-periods. During this session, each rat was placed in the conditioning chamber for 2.5 min while their orofacial responses were recorded; they were then intra-orally infused with the saccharin solution (0.1%) for 2.5 min at a rate of 1 ml/min, and finally, with water (1 ml/min) for another 2.5 min. Half of the animals in each group received first the IO infusion of saccharin and then that of water; for the remaining animals, this arrangement was reversed. During the fluid infusion the rats´ orofacial responses were recorded. On the next three days, the consumption test was administered. On each of these sessions, the rats were given access to a drinking tube containing the saccharin solution for 15 min in their home cages, and the amounts consumed were measured. Rats were given supplementary water for 60 min in the home cage at the end of each of these tests.

5.3.1.3. Data analysis

Data was expressed as mean $\pm$ SEM. Taste reactivity scores during the conditioning sessions were analyzed by a 2 x 2 x 2 repeated measures analysis of variance (ANOVA), with two between-subjects factors (preexposure and drug) and one within-subjects factor (trial). Significant main effects and/or interactions were further

analyzed by means of ANOVAs for each trial and post-hoc analyses (Student-Newman-Keuls tests). Intake scores during the consumption tests were analyzed by a similar analysis of variance (ANOVA) for repeated measures. The data for each behavior scored during the TR test was entered into a 2 (preexposure) x 2 (drug) ANOVA. All tests reported here used a criterion for significance of $p = 0.05$. The inter-rater reliability (r´s > 0.87) for each behavior scored was highly significant.

5.3.2. Results and discussion

The Figure 5 (panel A) shows the mean number of disgust reactions elicited by the infusion of saccharin during the conditioning phase. On the first conditioning trial, the groups did not differ in their mean rate of aversive reactions during the saccharin infusion. However, on trial 2, rats in Group Pre-Li displayed more disgust reactions than the rats from the other three groups. The number of disgust reactions across the two days of the conditioning phase was analyzed by a 2 x 2 x 2 repeated measures ANOVA, with two between-subjects factors (preexposure and drug) and one within-subjects factor (trial). This analysis revealed significant main effects of trial, pre-exposure, and drug [$Fs(1,25) > 5.93$; $ps < 0.02$]. The interactions involving these factors (preexposure x trial, drug x trial, and preexposure x drug x trial) were all significant [$Fs(1,25) > 14.75$; $ps < 0.001$]. Subsequent ANOVAs for each trial revealed only an effect of group in trial 2, $F(1,25) = 34.33$; $p < 0.001$, indicating that Group Non-Li had a significantly higher mean rate of aversive reactions than the remaining groups. The post hoc analyses (Student-Newman-Keuls test) confirmed that Group Non-Li displayed significantly more disgust reactions than the other three groups ($p < 0.05$), which did not significantly differ from each other.

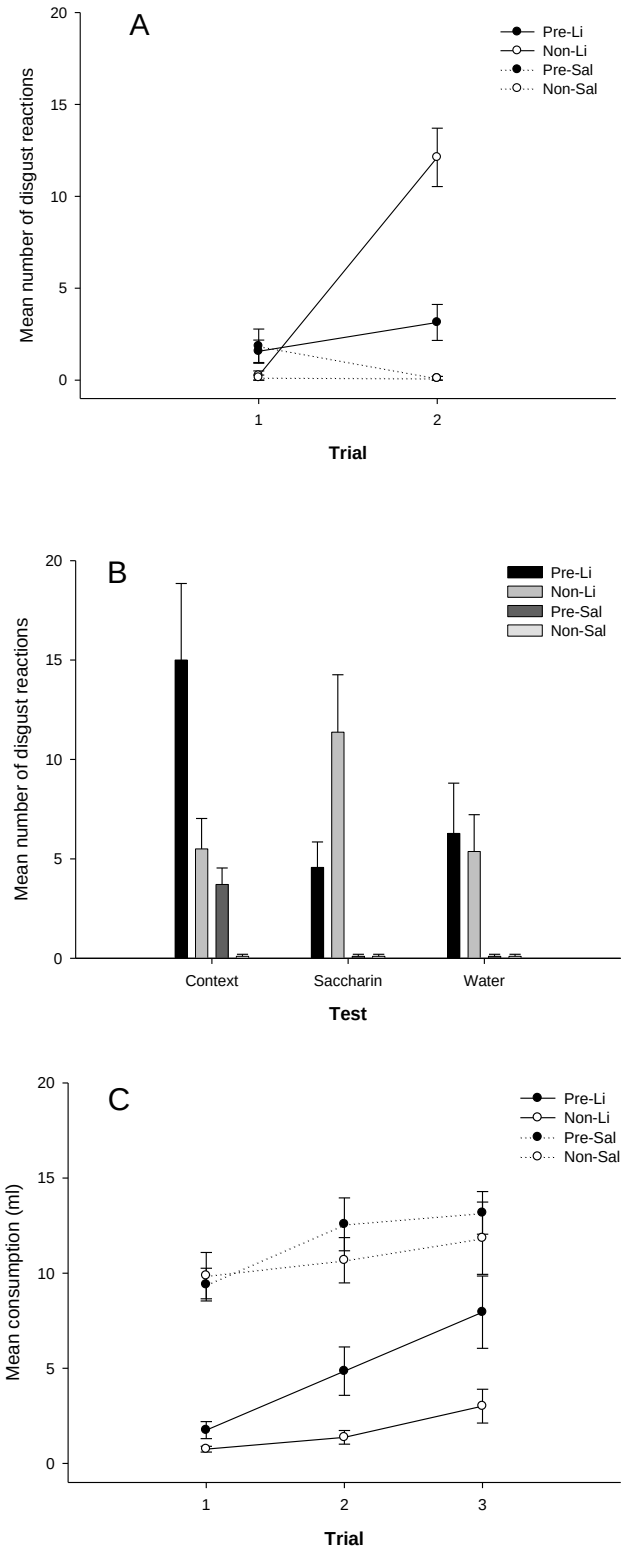


Figure 5: Experiment 2**:** Mean number of conditioned disgust reactions displayed by the different groups during pre-training (A), during the TR test with the context alone and the IO infusions of saccharin and water (B), and mean intake of saccharin during the consumption test trials (C). Error bars represent the standard error of mean (*SEM*s).

Panel B of Figure 5 shows the mean number of conditioned disgust reactions (gaping, chin rubbing, and paw treading summated) displayed by the various groups during the TR test. Separate 2 (preexposure) x 2 (drug) between-groups analyses of variance (ANOVAs) were conducted for the number of aversive reactions during the exposure to the conditioning chamber (i.e., the contextual cues), during the intraoral infusion of saccharin, and during the water infusion. Analysis of the rates of aversive reactions elicited by the context alone revealed significant main effects of preexposure, $F(1,25) = 18.32$; $p < 0.001$, and drug, $F(1,25) = 27.77$; $p < 0.001$, and a significant interaction between these two factors, $F(1,25 = 4.94$; $p = 0.036$. To determine the locus of this interaction, a one-way ANOVA was performed with group as the only between-groups variable, revealing a significant effect of this factor, $F(3,25) = 16.49$; $p < 0.001$. Post hoc Student-Newman-Keuls comparisons ($p < 0.05$) found that Group Pre-Li displayed significantly more conditioned disgust reactions to the contextual cues than the remaining groups, which did not differ one from another.

Analysis of the number of conditioned disgust reactions displayed by the rats during the intraoral infusion of saccharin in the TR test revealed significant main effects of pretraining, $F(1,25) = 5.31$; $p < 0.03$, and drug, $F(1,25) = 20.22$; $p < 0.001$, as well as a significant pretraining by drug interaction, $F(1,25) = 5.31$; $p < 0.03$. The one-way ANOVA with group as the between-subject variable revealed a significant effect of group, $F(3,25) = 10.88$; $p < 0.001$. According to the post-hoc analyses, rats in Group Non-Li displayed more disgust reactions than the other three groups during the IO infusion of saccharin. Finally, analysis of the number of disgust reactions during the water infusion revealed a main effect of drug condition, $F(1,25) = 12.27$; $p = 0.02$, but not an effect of pretraining nor an interactions between these two factors ($Fs < 1$). Subsequent one-way ANOVA revealed a significant effect of group, $F(3,25) = 4.11$; $p =$

0.017. Post-hoc Student-Newman-Keuls comparisons showed that rats injected with LiCl during the conditioning trials (Groups Pre-Li and Non-Li) displayed significantly more disgust reactions than those injected with saline (Groups Pre-Sal and Non-Sal).

Panel C of Figure 5 shows the mean amount of saccharin solution consumed by the groups on each of the subsequent consumption tests. Fluid intake by groups Pre-Li and Non-Li increased over the trials, whereas consumption intake by groups Pre-Sal and Non-Sal remained high across the sessions. A three-way (pretraining x drug x trial) ANOVA of consumption scores revealed significant main effects of trial, $F(2,50) = 17.39$; $p < 0.001$, pretraining, $F(1,25) = 4.81$; $p = 0.03$, and drug, $F(1,25) = 74.48$; $p < 0.001$, and a significant pretraining x trial interaction, $F(2,50) = 3.26$; $p = 0.04$. There was neither a significant drug x trial interaction, however, nor a significant interaction of the three factors ($Fs < 1$). The pretraining x drug interaction also was not significant $F(1,25) = 1.46$; $p = 0.23$. Subsequent ANOVAs for each trial revealed a significant effect of group on each trial [$Fs(3,25) > 9.74$; $ps < 0.001$]. The post-hoc Newman-Keuls comparisons ($p < 0.05$) showed that subjects in groups Pre-Sal and Non-Sal drank the saccharin solution at a higher rate than did the groups Pre-Li and Non-Li in trials 1 and 2. In trial 3, however, the Group Non-Li drank significantly less of the saccharin solution than did the other three groups, which did not differ from each other.

As already mentioned, we interpreted the results obtained in Experiment 1 as evidence that contextual cues associated with the lithium-induced nausea interfere with the subsequent development of conditioned disgust reactions to the saccharin solution. However, because in the TR test the animals were intra-orally infused with the flavor while in the conditioning chamber (i.e., in the presence of the contextual cues), it was not possible to determine the level of conditioning to the flavor cue independently of conditioning to the context. The current experiment was an attempt to eliminate this

problem by testing the rats with the context alone, and then with intraoral infusions of saccharin and water. A comparison between levels of responding to the saccharin infusion and to the context alone could provide a confirmation of the blocking by context explanation. When rats (Group Pre-EI) were tested in the absence of the flavor cue, they displayed a high rate of disgust reactions; when infused with saccharin, however, they displayed reduced disgust reactions, indicating that prior context conditioning attenuated the subsequent development of aversive reactions to the saccharin. Supporting this analysis, the consumption test showed that rats in Group Pre-Li, given prior pairings of the context and illness, increased their saccharin intake across the consumption test trials as compared with the Group Non-Li, a result that is consistent with the observation that pretrained contextual cues blocked the acquisition of the aversion to the flavor cue.[1]

5.4. Experiment 3

In this experiment, we examined whether the blocking effect (i.e., attenuation of conditioned disgust reactions) observed in the previous experiments can be abolished when the context-lithium association has been extinguished by exposing the rats to the context before the saccharin and the context are conditioned as a compound. It is known that extinction reduces the strength of the context-lithium association and therefore the ability of the context to block a subsequent taste aversion as assessed by a consumption test (e.g., Batson & Best, 1979; Boakes et al., 1997; Iguchi, Fukumoto, Sawa, & Ishii, 2014). Based on this finding, it would be expected that extinction would weaken the

[1] A 2-way ANOVA with just Pre-Li vs Non-Li as a between-subjects factor and test trial as a within-subject factor revealed significant main effects of group, $F(1,13) = 7.43$; $p = 0.01$, and trial, $F(2,26) = 19.55$; $p < 0.001$, as well as a significant trial by group interaction, $F(2,26) = 4.30$; $p = 0.02$. Subsequent *t*-tests showed that Group Pre-Li drank significantly more saccharin than did Group Non-Li on trials 1-3, $ts(13) > 2.21$; $ps < 0.04$.

association between the context and nausea, allowing for the establishment of conditioned disgust reactions to the saccharin solution when presented in compound with the context and paired with LiCl administration.

The design of this experiment is summarized in Table 4. Briefly, two groups of rats, Pre and Pre-Ext, were administered LiCl injections in a distinctive context (the conditioning apparatus), after which rats in Group Pre-Ext were given saline injections in the context in order to extinguish its ability to elicit conditioned nausea, whereas rats in Group Pre received the saline in the home cages (a third group of rats, Group Non, was given saline injections during pre-exposure and extinction sessions). Following this, all rats received two conditioning trials in which an infusion of saccharin in the presence of the contextual cues was paired with lithium. The ability of context alone and saccharin to elicit disgust reactions was then examined in the TR test.

Table 4: Design of Experiment 3

Group	Preexposure	Extinction	Conditioning	TR test
Pre	Ch: 4 x Li	Hc: 4 x Sal	2 x Sac (IO) → Li	Context/Sac/Water
Non	Ch: 4 x Sal	Ch: 4 x Sal	2 x Sac (IO) → Li	Context/Sac/Water
Pre-Ext	Ch: 4 x Li	Ch: 4 x Sal	2 x Sac (IO) → Li	Context/Sac/Water

Note. Groups Pre (preexposed) and Pre-Ext (preexposed-extinguished) were given LiCl injections during preexposure phase, and Group Non (non-preexposed) received saline injections; during extinction, Group Pre received saline injections in the home cage (Hc), and Groups Non and Pre-ext were injected with saline in the conditioning chamber (Ch); Li = LiCl; NaCl = saline; Sac = saccharin; IO = intraoral infusion.; Sac: saccharin; Li: lithium chloride; Sal: saline.

5.4.1. Method

5.4.1.1. Subjects, fluids, and apparatus

Thirty male Wistar rats, approximately 90 days old, weighing from 273 to 379 g at the start of the experiment served as subjects. Except otherwise stated, deprivation conditions, apparatus, and other procedural details were the same as in Experiment 2. Each subject was implanted with an oral cannula using the procedure described in Experiment 1. The flavor used during the experiment was a 0.1 % (w/v) saccharin solution. The rats were injected with either 10 ml/kg of .15 M LiCl or isotonic saline (10 ml/kg). Two rats lost their cannula throughout the experiment, so that the number of subjects in each group was as follows: Group Pre (n=8); Group Non (n=10); and Group Pre-Ext (n=10).

5.4.1.2. Procedure

The pretraining phase was similar to that of Experiment 2 (see Table 4). In each of 4 daily trials, the rats were placed in the conditioning chamber for 2.5 min while their orofacial responses were video-recorded. Immediately after, the subjects were injected with LiCl (Group Pre and Group Pre-Ext) or saline (Group Non). The rats were kept in the conditioning chamber for 60 min after the injection. They received a water recovery day after the second and fourth pretraining trials. The next 4 sessions constituted the extinction phase. During these sessions, the rats in Group Non and Group Pre-Ext were placed in the conditioning chamber for 2.5 min and their orofacial responses recorded before being injected with saline (10 ml/kg; 0.9 % NaCl solution). They were kept in the conditioning apparatus for 60 min after the injection. The animals in Group Pre received a saline injection in each session before being returned to their home cage. After completion of this phase, the rats were habituated to the taste reactivity procedure by infusion with water for a period of 1 min at the rate of 1 ml/min.

On each of the following two sessions, the rats received the conditioning trials. On these sessions, the rats were placed in the conditioning chamber and intraorally infused with 0.1 % saccharin for 5 min while their reactions were video-recorded. Immediately following the saccharin infusion, the rats were all injected with LiCl. The rats were kept 60 min in the conditioning chamber before being returned to their home cages. After a recovery day with water in the home cages, the TR test was administered. As in Experiment 2, each rat was placed in the conditioning chamber for 2.5 min while their orofacial responses were recorded; they were then intraorally infused with saccharin (0.1%) for 2.5 min at a rate of 1 ml/min, and finally, with water (1 ml/min) for another 2.5 min. The sequence of infusions (saccharin and water) was counterbalanced as in Experiment 2. No consumption test was conducted in this experiment.

5.4.1.3. Data analysis

The behaviors scored during the pre-exposure phase were analyzed by means of a 3 (group) x 4 (trial) mixed factor analysis of variance (ANOVA) and during the extinction phase by a similar ANOVA for repeated measures. The data for each behavior scored during the conditioning trials was analyzed by means of a 3 (group) x 2 (trial) ANOVA. Significant main effects and/or interactions were further analyzed by means of follow-up ANOVAs and post-hoc analyses (Student-Newman-Keuls tests). The taste reactivity scores during testing were analyzed by one-way ANOVAs with group as between-group factor. All tests reported here used a criterion for significance of $p = 0.05$. The inter-rater reliability (r´s > 0.93) for each behavior scored was highly significant.

5.4.2. Results and discussion

The panel A of Figure 5 (left-hand side) shows the mean number of conditioned disgust reactions displayed by the rats to contextual cues on each of the 4 pretraining sessions. The number of contextually elicited disgust reactions increased over the trials to the same extent in Groups Pre and Pre-Ext. The 3 x 4 ANOVA conducted on these data revealed significant main effects of trial and group [$F(3,75) = 37.68$; $p < 0.001$ and $F(2,25) = 16.76$; $p < 0.001$, respectively] and a significant interaction between trial and group, $F(6,75) = 10.01$; $p < 0.001$. To further analyze this interaction, separate one-way ANOVAs were performed with group as the only between-subject factor, with the disgust scores collected each day. These ANOVAs revealed a significant effect of group in trials 3 and 4 [*Fs* $(2,25) > 3.82$; *ps* < 0.035], but not in trials 1 and 2 (*Fs* < 1). Subsequent Newman-Keuls post hoc analysis showed that rats in Groups Pre and Pre-Ext expressed significantly more conditioned disgust reactions in trials 3 and 4 than rats in Group Non, which did not change over trials.

Figure 6A (right-hand side) presents the mean number of disgust reactions expressed by rats in Groups Pre-Ext and Non during the extinction phase. The 2 (group) x 4 (trial) ANOVA of the number of disgust reactions during these sessions revealed significant main effects of group, $F(1,18) = 24,76$; $p < 0.001$, and trial, $F(3,54) = 11.31$; $p < 0.001$, and a significant group x trial interaction, $F(3,54)= 11.72$; $p < 0.001$. Simple main effects analysis of the interaction revealed that the number of contextually elicited disgust reactions declined over trials for Group Pre-Ext, $F(3,27) = 11,55$; $p< 0.001$), and that Group Non did not change over the trials, not displaying disgust reactions ($F < 1$). Analysis of group differences for each trial revealed that during extinction trials 1 and 2, Group Pre-Ext displayed significantly more disgust reactions than Group Non (*ps* $<$ 0.05) but that the groups did not differ on extinction trials 3 and 4 (*ps* > 0.05).

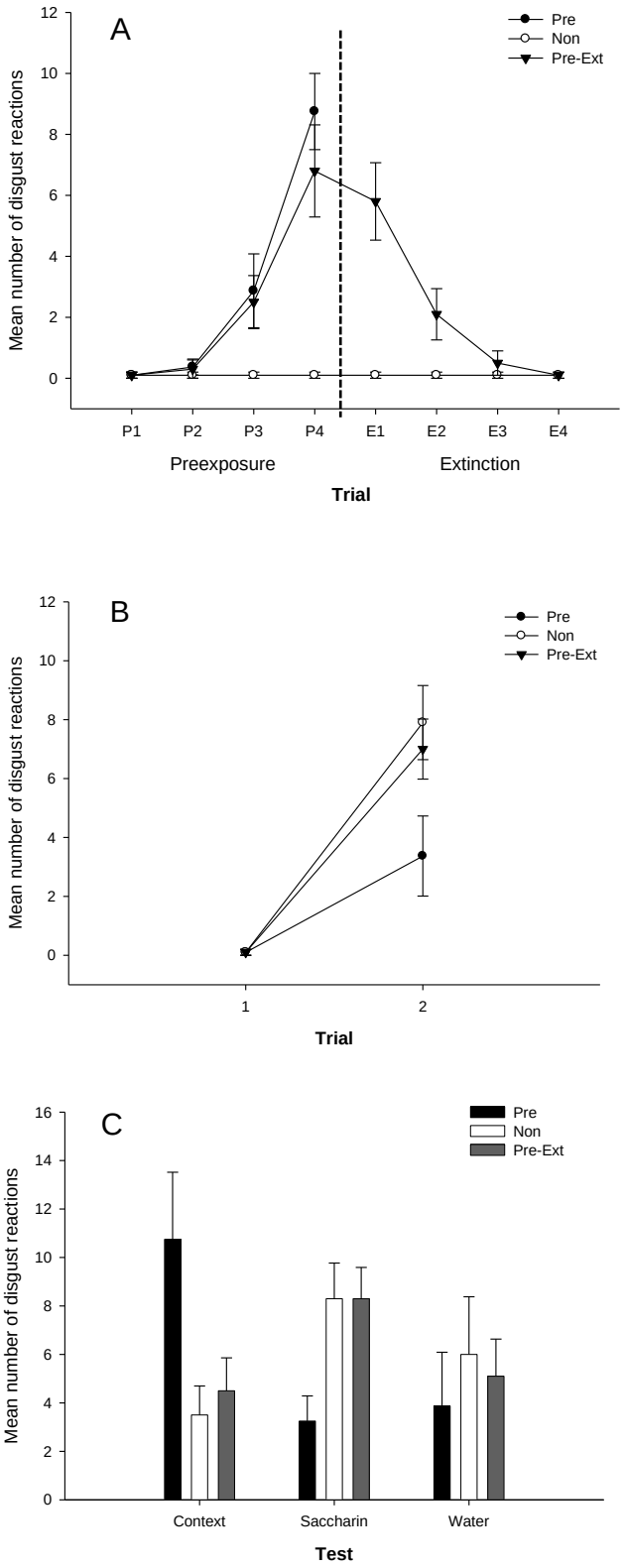


Figure 6: Experiment 3: Mean number of disgust reactions elicited by contextual cues during the preexposure and extinction phases (A), during the conditioning trials (B), and during testing with the context alone and the infusions of saccharin and water (C), for the different groups. Error bars represent the standard error of mean (*SEM*s).

During the conditioning phase Groups Non and Pre-Ext displayed significantly more disgust reactions than Group Pre as conditioning proceeded. Figure 6 (panel B) presents the mean number of conditioned disgust reactions elicited by the infusion of saccharin during the two conditioning trials. The 3 x 2 ANOVA, with group and trial as factors, conducted with the data from these sessions revealed significant main effects of group, $F(2,25) = 3.65$; $p = 0.041$, and trial, $F(1,25) = 73.93$; $p < 0.001$, and a significant interaction between these factors, $F(2,25) = 3.54$; $p = 0.04$. Subsequent one-way ANOVAs for each trial revealed only a significant effect of group in trial 2, $F(2,25) = 3.60$; $p = 0.042$. The post hoc analyses (Student-Newman-Keuls) confirmed that rats in Group Non and Pre-Ext expressed significantly more conditioned disgust reactions than rats in Group Pre ($p < 0.05$) on conditioning trial 2.

Panel C of Figure 6 presents the mean number of disgust reactions for the different groups during the TR tests. The one-way ANOVA conducted with the aversive reactions elicited by the context alone revealed a significant effect of group, $F(2,25) = 4.54$; $p = 0.021$. According to the post-hoc analyses, Group Pre had a significantly higher rate of disgust reactions than the other groups ($p < 0.05$), which did not differ from each other, indicating that the LiCl-associated context acquired the ability to elicit conditioned nausea, and that non-reinforced exposures to the context after lithium-context pairings resulted in attenuated conditioned disgust. Analysis of the number of aversive reactions during the intraoral infusion of saccharin revealed a significant effect of group, $F(2,25) = 4,61$; $p = 0.019$. As shown in panel C of Figure 5, the rats in Group Pre displayed significantly less disgust reactions than did Groups Non and Pre-Ext, which did not differ from each other ($p < 0.05$). These results suggest that contextually elicited conditioned nausea blocked the acquisition of conditioned disgust reactions to the saccharin solution. Finally, ANOVA also revealed that there was no significant

effect of group on the number of disgust reactions displayed by the rats during the water infusion ($F < 1$). The post-hoc analyses confirmed that the groups did not differ in the number of disgust reactions elicited by the intraoral infusion of water in the TR test (*ps* > 0.05).

The results of this experiment confirm those obtained in Experiment 2, suggesting a role for nausea-based context conditioning in the production of conditioned disgust reactions to a novel taste paired with lithium in the presence of the context. More specifically, our data show that the animals that received training designed to extinguish the association between the contextual cues and the LiCl-induced nausea developed more disgust reactions to the saccharin when this flavor was presented later in compound with the context and paired with LiCl. Thus, these results support the hypothesis that blocking by contextual cues can be responsible for the attenuation of conditioned disgust elicited by saccharin following aversive conditioning in compound with the context.

5.5. General discussion

This study explored the possibility that a context previously associated with lithium could interfere with the establishment of conditioned disgust reactions to a flavor presented in compound with the context and paired with lithium. Although it is known that exposure to a previously lithium-paired context elicits conditioned nausea in rats (Limebeer et al., 2006, 2008), its effects on the subsequent acquisition of disgust reactions by a LiCl-paired flavor have not been previously examined. Two main findings were obtained in the present study: firstly, pre-trained contextual cues blocked the subsequent acquisition of an aversion to saccharin as revealed by an attenuated avoidance of the solution in a standard consumption test; secondly, and most novel, prior context-lithium pairings also interfered with the establishment of conditioned

disgust reactions to the saccharin solution when examined with the taste reactivity test. Thus, the findings of our study indicate that blocking of a taste aversion by pre-trained contextual cues is not limited to fluid consumption. Specifically, when rats were given a LiCl injection during the pretraining sessions, such pretraining not only attenuated the reduction in consumption typically produced by taste aversion learning, but also reduced the rate of disgust reactions elicited by the saccharin when infused to the rats in the TR test (Experiment 1). Experiment 2 confirmed that the attenuation of lithium-induced disgust reactions observed in Experiment 1 is primarily a consequence of blocking by context, as revealed by the effectiveness of the contextual cues alone, in the absence of the flavor cue, to elicit conditioned disgust reactions. Finally, Experiment 3 showed that the extinction of the context-nausea association by exposure to the context before saccharin conditioning with LiCl abolished the blocking effect; that is, following context extinction, the rats displayed disgust reactions when infused with the saccharin in the TR test but they did not display aversive reactions when exposed to context alone. Together, these results support the proposal that contextually elicited conditioned nausea may be responsible for the subsequent retardation in the acquisition of conditioned disgust reactions to a flavor infused in the presence of the context and paired with lithium.

The above conclusion is consistent with the results of previous studies showing that exposure to a context previously associated with nausea elicits conditioned disgust reactions in rats (Limebeer et al., 2006; 2008). As mentioned in the introduction, in these studies rats injected with LiCl before being placed in a distinctive context showed aversive taste reactivity responses when infused with a novel taste in that context; more importantly, the rats also displayed disgust reactions when exposed to the context in the absence of the fluid, suggesting that animals had associated the context with the LiCl-

induced nausea. Accordingly, the study reported here shows for first time that contextually elicited conditioned nausea can block the subsequent establishment of conditioned disgust reactions to a flavor paired with nausea. Our results add to other studies using different measures to demonstrate that contextual cues can elicit conditioned nausea and then block a taste aversion, as for example, avoidance of palatable solutions in the conditioned context (Best et al., 1984; Boakes et al., 1992), decrease of general activity in the presence of environmental cues paired with LiCl (Meachum & Bernstein, 1992) and, finally, by the blocking test procedure (Symonds & Hall, 1979; Rodríguez et al., 2000). As well, environmental cues can modulate conditioned nausea as recently demonstrated by Brown, Penney, Skinner and Martin (2011) using a context discrimination task. In this study, rats received a saccharin solution paired with lithium injections in one context, alternating with presentations of the saccharin followed by saline in another context. After discrimination training, the rats consumed more the saccharin solution in the non-poisoned context than in the lithium-paired context, revealing that contextual cues gained control over fluid consumption. The new finding was that the rats also showed more aversive responses (and less appetitive ones) to the saccharin when consumed in the danger context, indicating that aversive taste reactivity responses were strongly correlated with fluid consumption.

Finally, the results of this study can be compared to those from other studies of our laboratory examining the effects of flavor preexposure on taste aversion learning by both consumption and taste palatability tests. We found that non-reinforced exposure to a flavor prior to conditioning with LiCl not only disrupts suppressed consumption of the LiCl-paired flavor, but also attenuates the development of conditioned disgust reactions to the flavor (López et al., 2010), indicating that the attenuating effects of flavor

preexposure in taste aversion learning (i.e., latent inhibition) can be assessed using both voluntary fluid intake and the display of disgust reactions in the taste reactivity test.

In conclusion, the study reported here shows that a context-LiCl association can block subsequent conditioning of a lithium-induced flavor aversion as reflected by both an attenuation of suppressed consumption and attenuated conditioned disgust reactions in the taste reactivity test. The present results extend our understanding of blocking in taste aversion learning as a consequence of prior LiCl-induced context conditioning by showing that blocking of a taste aversion is not limited to the fluid avoidance response. Our results indicate that the association between contextual cues and nausea also interferes with the establishment of disgust reactions to a lithium-paired flavor.

Capítulo 6

PREEXPOSICIÓN AL EI: EL PAPEL DE LAS CLAVES RELACIONADAS CON LA INFUSIÓN

The US-preexposure effect: the role of infusion related cues

6.1. Introduction

As pointed in the preceding chapter, repeated exposure to LiCl injections in a novel context attenuates the subsequent acquisition of conditioned disgust reactions to a saccharin solution – the US preexposure effect. In addition, attenuation of conditioned disgust is abolished when animals receive non-reinforced exposures to the context (i.e, context extinction) before taste aversion conditioning. These results support the suggestion that contextual cues can operate as a CS and therefore evoke conditioned nausea.

It is not only environmental cues that can acquire associative strength in procedures of this sort. In addition, cues related to handling or associated with the intraperitoneal administration of LiCl can block the acquisition of a taste aversion (e.g., de Brugada & Aguado, 2000; de Brugada et al., 2003). This proposal receives support from observations that procedures designed to restrict the aversive properties of injection cues, by intermixed saline injections during preexposure (Willner, 1978) or by administering the LiCl orally during conditioning (de Brugada et al., 2004) can attenuate the US-preexposure effect. Further support for the proposal of blocking by non-flavor cues comes from experiments showing that the latent inhibition (LI) effect depends on a common method of fluid delivery during preexposure and conditioning (López et al., 2010). In this regard, the effect of preexposure to a taste on consumption and disgust reactions differed as a function of the method of fluid exposure. If rats were IO infused, saccharin preexposure resulted in attenuated disgust reactions in the TR test but did not attenuate the voluntary flavor consumption; in contrast, when the solution was delivered by bottle during preexposure, the LI effect was only evident in the flavor consumption test, whilst having no effect on conditioned disgust reactions in the TR test. These results suggest that IO fluid delivery would act as a specific context that may

well modulate the acquisition of a taste aversion. However the ability of cues arising from the intraoral infusions of fluids to act as external contextual cues remains unclear.

The present study therefore tests the hypothesis that an association between cues related to the intraoral infusion and the lithium could interfere with the subsequent acquisition of a LiCl-induced taste aversion. If infusion-related cues are associated with the state of nausea produced by LiCl injections, rats would display conditioned disgust reactions when subsequently infused with a novel palatable flavor. Experiment 4 tested this hypothesis. Experiment 5 examined the level of aversive taste reactivity responses elicited directly by the IO cues (in the absence of the flavor) and the environmental context.

6.2. Experiment 4

The aim of this experiment was to analyze the effect of intraoral (IO) infusions of water prior to the LiCl injections during preexposure trials in the establishment of conditioned disgust reactions to a taste solution. It is well-established that repeated exposure to LiCl attenuates the subsequent LiCl-induced taste aversion measured by suppression of consumption. As we noted above, previous studies from our laboratory reported an attenuation of conditioned disgust reactions to a saccharin solution as a result of LiCl preexposure. Contextually elicited conditioned nausea would interfere with the development of conditioned disgust reactions to a flavor cue presented in compound with the context and paired with the lithium. In order to test the role of the stimulation arising from oral infusions in taste aversion learning, two groups of rats received intraoral infusions of water before being injected with LiCl (Group Pre) or Saline (Group Non). During the conditioning phase, all rats were infused with saccharin prior to being injected with LiCl. If general contextual cues are associated with the state of nausea, LiCl preexposure should retard the establishment of conditioned disgust

reactions as a result of the context-nausea association. But, if an association between the infusion cues and the illness-inducing effect of lithium was effectively formed, rats would display conditioned disgust reactions to a novel flavor delivered intraorally and we should therefore observe an attenuation of the US-Preexposure effect. The design of Experiment 4 is summarized in Table 5.

Table 5: Design of Experiment 4

Group	Preexposure	Conditioning	TR test
Pre	4 x water (IO) → Li	Sac (IO) → Li	Sac (IO)
Non	4 x water (IO) → Sal	Sac (IO) → Li	Sac (IO)

Note. The first term in each group´s label indicates that the rats received either LiCl (Pre; preexposed) or saline (Non; nonpreexposed) injections during the preexposure phase immediately after the IO infusion of water. Preexposure and conditioning were conducted in the taste reactivity apparatus. IO: introral infusion; Sac: saccharin; Li: lithium chloride; Sal: saline.

6.2.1. Material and methods

6.2.1.1. Subjects, apparatus and cannulation surgery

The subjects were 16 male Wistar rats from Oviedo vivarium that weighed 247-342 g at the start of the experiment. Upon arrival, they were individually housed in opaque plastic cages in a room maintained at 21° C with a 12/12h light-dark cycle. All experimental manipulations were performed during the light portion of the cycle. Throughout the experiment, food was always available in the home cages. All experimental procedures were in accordance with guidelines for the care and use of laboratory animal in the Spanish regulation (RD 12301/2005) and European (86/609/EEC) concerning animal experimentation.

Training and testing were carried out in a taste reactivity apparatus in a room without natural light. Except otherwise stated, deprivation conditions, apparatus, cannulation surgery and other procedural detail were the same as in Chapter 5.

6.2.1.2. Measurement of conditioning

Orofacial reactions made by the animal during the infusion of fluids were recorded and subsequently quantified with the software the Observer XT 9.0´ (Noldus Information Technology) designed for recording and analyzing behavioral activity of animals. Orofacial reactions quantified were: gaping (rapid, large-amplitude opening of the mandible with retraction of the corners of the mouth), chin rubbing (mouth or chin in direct contact with the floor or the wall of the chamber with the body projecting forward) and paw treading (forward and backward movement of the forepaws in synchronous alternation).

6.2.1.3. Procedure

During the course of the experiment, one rat lost their cannula and was removed from the study. The remaining rats were randomly assigned to groups as follows: Group Pre (n=8) and Group Non (n=7). After their recovery from surgery, rats were placed on a 24-h water deprivation-schedule with access to water in their home cages for 1 hour daily. During the four pre-exposure sessions, all animals were placed in the conditioning chamber with their cannula attached to the infusion pump and intraorally infused with water for 5 min at the rate of 1ml/min. Immediately following the water infusion, the rats in Group Pre were injected with LiCl (10ml/kg of .15M LiCl) whereas those in the Group Non were injected with saline. All the animals spent one hour after the injection in the conditioning chamber before being returned to the colony room. A day of free water consumption recovery was given after the second and fourth pre-exposure sessions. The following day of the last recovery session, the conditioning trial

was carried out. In this session all animals received an intraoral infusion of 0.1% saccharin for 5 min (rate 1ml/min) and immediately after they were injected with LiCl (10 ml/Kg of .15M). Rats spent one hour after the injection in the experimental room before being returned to the colony. Following a recovery day on which rats were given water for 24 h, the taste reactivity test was administered. On this session, the rats were intraorally infused with the saccharin solution for 5 min at a rate of 1 ml/min and the orofacial responses videotaped.

6.2.2. Results and discussion.

During the preexposure sessions, rats were infused with water prior being injected with LiCl or saline. Rats that received LiCl injections (Group Pre) showed an increase of the number of disgust reactions (gaping, chin rubbing and paw treading) across the sessions, relative to the rats injected with saline (Group Non). A 2 X 4 repeated measures ANOVA, with group and session as the factors, was conducted on the data from this phase. This analysis revealed significant main effects of session (here, and throughout, a significance level of p <.05 was adopted), [$F(3,39) = 3.446$; $p = .026$], group [F (1,13) = 21.013; p <.001], and a significant group X session interaction [$F(3,39) = 3.033$; $p = .041$]. Panel A of Figure 7 shows the mean number of disgust reactions displayed by the animals during the IO infusion of water in these sessions.

During the IO infusion of saccharin in the conditioning session with LiCl, rats in Group Pre displayed more disgust reactions than did Group Non. A t-test conducted with the data from this session revealed that the groups differed in their number of disgust reactions displayed to saccharin [$t(13) = 2.461$; $p = .029$]. Panel B of Figure 7 shows the mean number of conditioned disgust reactions during the conditioning session. However, no differences between groups for conditioned disgust reactions on

the TR test were found [$t(13) = -1.006$; $p = .333$]. Groups Pre and Non displayed similar level of conditioned disgust reactions while they were IO infused with saccharin (see Panel C).

These data revealed no attenuation effect of LiCl preexposure on the establishment of conditioned disgust reactions to saccharin, in spite of the fact that during the preexposure phase, repeated water infusions followed by LiCl injections elicited the development of conditioned nausea that increased throughout the sessions. Further, during conditioning, Group Pre displayed more conditioned disgust reactions to saccharin than Group Non, suggesting that contextual cues have acquired aversive properties and that they should have served to block subsequent taste conditioning.

One possible explanation is that the infusion of water just before the injections of LiCl promotes the formation of an association between the IO infusion cues and its effects, and therefore, may evoke conditioned nausea independently of the solution infused. Water administration could potentiate the intraoral cues-lithium association. Indeed, there is some evidence that context conditioning occurs more readily when rats are permitted to drink a novel flavor during conditioning, or even when animals are given unflavored water during conditioning (see Boakes et al., 1997; Symonds et al., 1998). For example, in the study by Symonds et al. (1998), rats experienced two distinctive contexts, one that was followed by a LiCl injection and the one that was not. During training, the half of the rats had access to water in the LiCl-paired context, whereas the other half did not receive exposure to water. A context aversion was then assessed by a consumption test in which the rats were offered a sucrose solution in each of the contexts. It was found that the rats that received water during training showed a suppression of sucrose consumption in the LiCl-paired context, whilst the effect was absent in subjects not given access to water during conditioning.

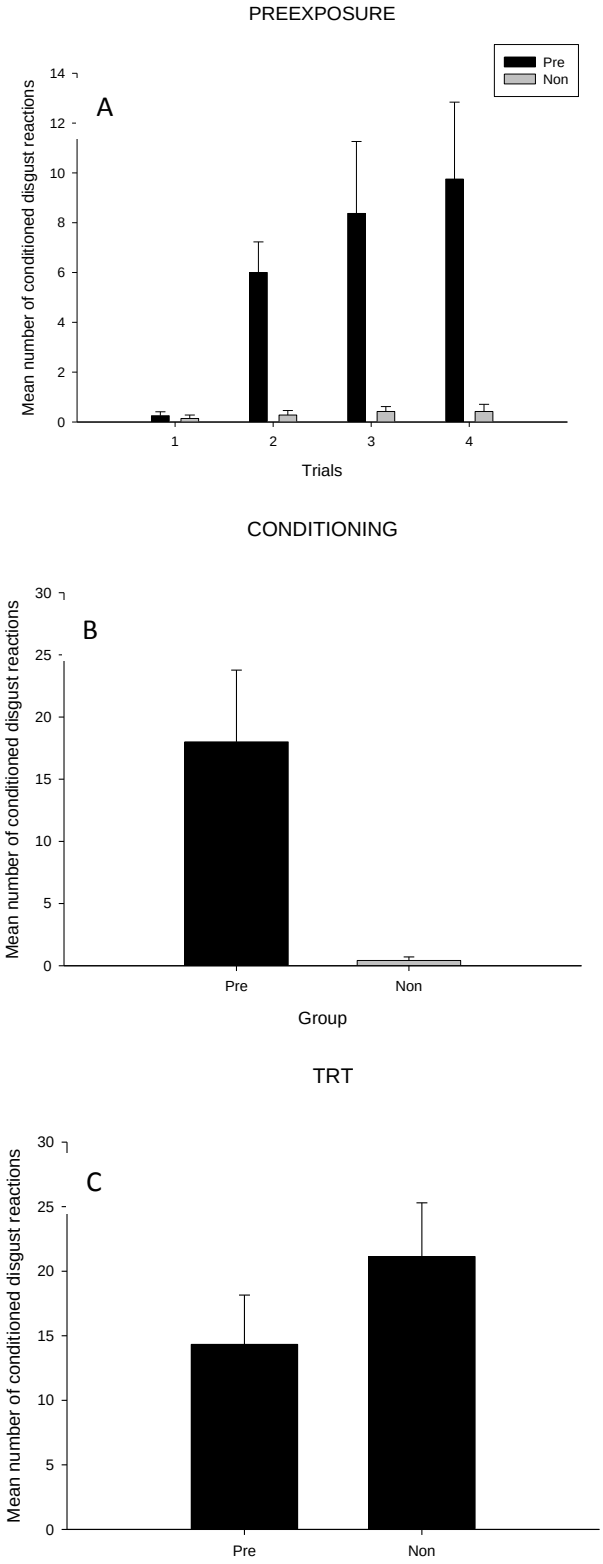


Figure 7: Experiment 4: Mean number of conditioned disgust reactions (gaping, chin rubbing, and paw treading) displayed by the rats during the infusion of water in the preexposure phase (A), and during the infusion of saccharin in the conditioning session (B). Panel C presents the mean number of conditioned disgust reactions elicited by the infusion of saccharin on the taste reactivity test. Error bars represent the standard error of the mean.

Although the results of the present experiment suggest that the saccharin aversion has been blocked by the IO cues present during the infusion, there remains a major potential problem with this explanation. It is clear that in our test procedure, the saccharin (the blocked cue) is presented in compound with the cues arising from the IO infusion (the blocking cue) and, as a consequence, we do not know the level of conditioning to the saccharin independently of conditioning to the intraoral cues. One possible strategy for addressing this issue is to compare taste reactivity to the flavor cue with that to intraoral cues in the absence of the saccharin. This is the aim of the next experiment.

6.3. Experiment 5

Stimulation arising from the oral infusion of water during LiCl preexposure involves a range of proximal cues that might well contribute to the observed effect in the previous experiment, that is, an attenuation of the delay of conditioned disgust reactions after LiCl preexposure assessed by the TR test. As mentioned above, one strategy for testing the role of IO cues could be to compare taste reactivity to the flavor cue with that evoked by IO cues alone (water IO infusions). For this purpose, four groups of rats were given IO water-infusions followed by either LiCl (Groups Pre-Li and Pre-Sal) or saline (Groups Non-Li and Non-Sal) injections immediately before being placed in the conditioning chamber for an hour. During the conditioning sessions, all rats were intraorally infused with saccharin followed by an injection of LiCl (Pre-Li and Non-Li) or Saline (Pre-Sal and Non-Sal). On the TR test, subjects were tested with the context alone, an intraoral infusion of saccharin, and with an infusion of water whereupon the conditioned reactions were recorded.

6.3.1. Material and methods

6.3.1.1. Subjects and apparatus.

The subjects were 36 male Wistar rats weighing 194-373 at the start of the experiment. Housing and maintenance conditions were the same as those described for the previous experiment. Similarly, the apparatus and solutions were the same as those employed in the previous experiment. All rats were implanted with an intraoral cannula following the procedure described for the previous experiment. Six rats lost their cannula during the experimental procedure and were removed from the study. The remaining rats were randomly assigned into four groups as follows: Group Pre-Li (n = 7); Group Non-Li (n = 7); Group Pre-Sal (n = 7); and Group Non-Sal (n = 9).

6.3.1.2. Procedure.

Table 6: Design of Experiment 5

Group	Preexposure	Conditioning	TR Test
Pre-Li	4 x w (IO) → Li	2 x Sac (IO) → Li	Context/Sac/Water
Non-Li	4 x w (IO) → Sal	2 x Sac (IO) → Li	Context/Sac/Water
Pre-Sal	4 x w (IO) → Li	2 x Sac (IO) → Sal	Context/Sac/Water
Non-Sal	4 x w (IO) → Sal	2 x Sac (IO) → Sal	Context/Sac/Water

Note. The first term in each group´s label indicates that the rats received either LiCl (Pre-Li and Pre-Sal; preexposed) or saline (Non-Li and Non-Sal; non-preexposed) injections during the preexposure phase immediately after IO water infusions. Both Li groups (Pre-Li and Non-Li) received LiCl injections during the conditioning phase, but Sal groups (Pre-Sal and Non-Sal) received saline injections. Preexposure and conditioning were conducted in the taste reactivity apparatus. IO: intraoral infusion; Sac: saccharin; Li: lithium chloride; Sal: saline.

The design of this experiment (see Table 6) is identical to Experiment 4 with some exceptions. The rats received two conditioning trials during the conditioning phase, and on the TR session, the level of conditioned responses to IO cues (as well as contextual cues) were evaluated independently of the flavor cue. During the four preexposure trials, all rats were intraorally infused with water for 5 minutes at a rate of 1ml/min while orofacial responses were video-recorded. Immediately after the IO water infusion, rats in Group Pre-Li and Pre-Sal were injected with LiCl whereas those in Group Non-Li and Non-Sal were given an injection of saline. Following this phase, all rats were placed in the conditioning chamber for 60 min and then returned to their home cages. A day of recovery with water in the home cages was given after the second and fourth sessions in this phase. On the day after the second recovery session a conditioning trial was carried out. In this session, rats were placed in the conditioning chamber and intraorally infused with saccharin for 5 min at a rate of 1ml/min prior being injected with LiCl (Groups Pre-Li and Non-Li) or saline (Groups Pre-Sal and Non-Sal). After an additional water recovery day, the second conditioning trial was performed. The TR test was then administered. First, all rats were placed in the conditioning chamber for 2.5 minutes and their orofacial responses to general contextual cues were videotaped. They were then intraorally infused with water for 2.5 min (1ml/min) and finally with saccharin (0,1%) for another 2.5 min (1ml/min). Fluids were counterbalanced and the orofacial reactions during infusions were recorded.

6.3.2. Result and discussion

During the preexposure sessions, animals in the groups injected with LiCl progressively acquired an increased level of conditioned rejection reactions throughout preexposure trials. A 4 x 4 repeated measures ANOVA conducted on the data revealed a

main effect of trial [F (3,78) = 23.20; p > .001], group [F(1,26) = 13.29; p < .001] and trial-group interaction [F(9,78) = 8.28; p <.001]. To further analyze this interaction, separate one-way ANOVAs were performed and revealed a main effect of group on trials 2, 3 and 4 [Fs (3,26) > 6.94; ps < .001] but not on trial 1 [F(3,26) = 1.15; p = .346]. Student-Newman-Keuls post-hoc analysis (p<.05) confirmed that groups injected with LiCl during preexposure (Pre-Li and Pre-Sal Groups) displayed more conditioned disgust reactions on trials 2, 3 and 4. Panel A of Figure 8 shows the results for the preexposure phase.

The number of disgust reactions elicited by the IO infusion of saccharin during the conditioning trials was analyzed by a 2 x 2 x 2 repeated measures ANOVA, with two between-group factors (preexposure and drugs administered during conditioning) and one within-subjects factor (trials of conditioning). The analysis revealed significant main effects of trial, preexposure, and drugs [Fs(1,26) > 10.87; ps<.003]. The interactions (preexposure x trial, drug x trial) were both significant [Fs(1,26) > 12.92; ps <.001] whereas preexposure x drug x trial interaction was not (F < 1). In addition, one-way ANOVAs were performed with both conditioning sessions. On the first trial of conditioning, Groups Pre-Li and Pre-Sal displayed more disgust reactions to saccharin infusions than Non-Li and Non-Sal Groups [F(3,26) = 29.63; p < .001]. A Student-Newman-Keuls post-hoc analysis confirmed these results. In addition, a one-way ANOVA conducted with data from the second trial (see panel B of Figure 7) revealed an effect of group [F(3,26) = 16.77; p < .001). Post hoc Student-Newman-Keuls analysis revealed that Groups Pre-Li and Non-Li displayed more conditioned disgust reactions than Groups Pre-Sal and Non-Sal.

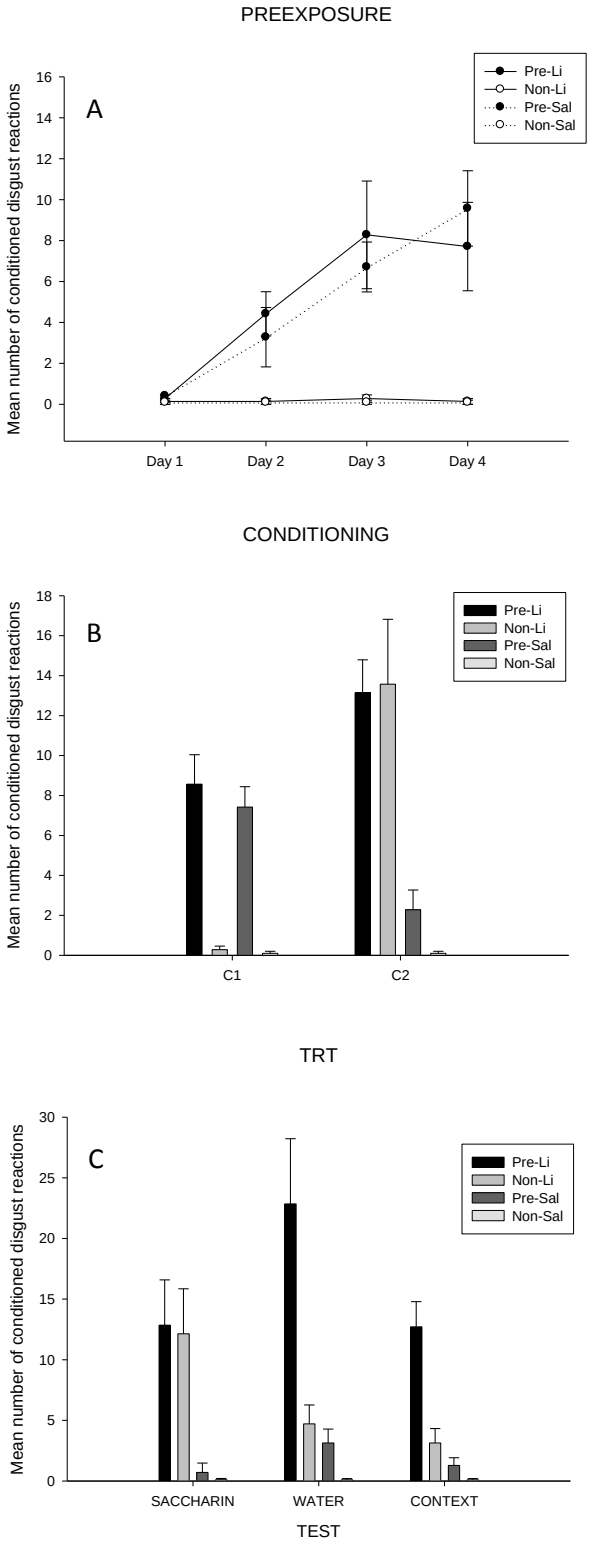


Figure 8: Experiment 5: Panel A shows the mean number of conditioned disgust reactions elicited to water infusions by rats during the four trials of preexposure phase. Panel B shows the mean number of conditioned disgust reactions to a saccharin infusion on both conditioning trials. Panel C represents the mean number of conditioned disgust reactions in response to water infusions, saccharin infusion and context cues on the TR test. Error bars represent the standard error of the mean.

A 2 (preexposure) x 2 (drug) ANOVA was carried out with the number of conditioned disgust reactions displayed by the rats during the IO infusion of saccharin on the TR test. The analysis revealed a significant main effect of drug [$F(1,26) = 22.46$; $p < .001$] but neither effects of preexposure or a drug x preexposure interaction ($F<1$). Subjects that received saccharin infusions followed by a LiCl injection during conditioning sessions displayed more conditioned disgust reactions to saccharin infusions on the TR test independently of their experience during the preexposure phase. Student-Newman-Keuls post–hoc analysis revealed that both Pre-Li and Non-Li displayed more conditioned reject reactions to saccharin infusions than Pre-Sal and Non-Sal. The results of the TR test can be seen in Panel C in Figure 8.

Analysis of the disgust reactions during water infusion revealed significant main effects of preexposure [$F(1,26) = 15.85$; $p < .001$] and drug [$F(1,26) = 20.87$; $p < .001$], and a significant interaction between these two factors [$F(1,26) = 7.87$; $p = .009$]. A subsequent one-way ANOVA with group as a between-subjects variable revealed a main effect of group [$F(3,26) = 14.73$; $p < .001$].

Student-Newman-Kleus comparisons showed that Pre-Li Group displayed more conditioned disgust reactions to water infusions than the remaining groups, which did not differ one from another. This result suggests that IO cues have acquired aversive properties throughout the preexposure phase and therefore, the conditioned disgust reactions displayed by Group PE to the saccharin infusion might be reflecting conditioned disgust reactions to IO cues instead of to the saccharin solution (see panel C of Figure 8). Finally, analysis of aversive reactions elicited by the context alone reveled a main effect of preexposure [$F(1,26) = 21.86$; $p < .001$], drug [$F(1,26) = 39.38$; $p < .001$] and preexposure x drug interaction [$F(1,26) = 12.73$; $p < .001$]. A one-way ANOVA conducted on the data from the TR test to contextual cues revealed an effect of

group [F (3,26) = 24.43; $p < .001$]. Post-hoc analysis found that Group Pre-Li displayed more conditioned disgust reactions to general contextual cues than the remaining groups, which did not differ one from another. These results suggest that not only the IO cues (cues related to infusion) acquired aversive properties, but also the general contextual cues that are present at the time of experiencing illness during training.

In summary, this experiment provides some evidence that infusion-related cues associated with the LiCl are responsible for the expression of the disgust reactions displayed by the animals when infused with saccharin during conditioning and testing. Animals injected with LiCl following a water IO infusion displayed an increased level of conditioned disgust reactions across preexposure trials. These conditioned reactions might be reflecting the association between the infusion-related cues and the state of nausea. Results from the second conditioning trial and the TR test with the saccharin revealed an attenuation of the US-preexposure effect in Group Pre-Li. As shown, Pre-Li and Non-Li groups displayed a similar level of rejection reactions when infused with saccharin. These results would reflect conditioned nausea produced by the infusion-related cues rather than by the saccharin in Group Pre-Li. Results from the infusion with water support this account. As can be seen, rats in Group Pre-Li displayed more disgust reactions during this test than the remaining groups. These results suggest a role for the cues provided by the IO infusion of water during preexposure in the control of conditioned disgust reactions.

6.4. General discussion

The aim of the current work was to evaluate whether an association between IO infusion-related cues and the state of nausea could interfere with the acquisition of a subsequent LiCl-induced taste aversion. Experiment 4 revealed no attenuation effect of LiCl preexposure on the establishment of conditioned disgust reactions to the saccharin.

Experiment 5 showed that the rate of disgust reactions during the infusion of saccharin during testing was indeed a consequence of blocking by the IO cues present during the saccharin infusion as revealed by the infusion of water during testing. Since it is not possible to estimate saccharin palatability in the absence of the IO cues, we adopted the strategy of examining disgust reactions elicited by the infusion of water (i.e., in the absence of the flavor cue). As already mentioned, a decrease in voluntary consumption is not a reliable way to evaluate palatability shifts. The main finding obtained in this study suggests that IO cues may play a similar role to that of the physical external context (e.g., Limebeer et al., 2006, 2008) or the injection-related cues (De Brugada et al., 2004) in the attenuation of the establishment of conditioned disgust reactions.

The present results are consistent with those obtained from several other studies examining the role of injection-related cues. For example, Willner (1978) examined the role of these cues by intermixed saline injections with LiCl injections during the preexposure phase, finding an attenuation of the US-preexposure effect. This procedure presumably extinguished the association between the injection-related cues and illness, and therefore its ability to block a subsequent taste aversion. More recently, De Brugada et al. (2004; see also De Brugada et al., 2003) have provided evidence for this hypothesis using a conditioning procedure in which the US was orally consumed, a method that did not involve cues related to injections. With this procedure, rats refused to consume a salty solution, sodium chloride (NaCl), after the oral administration of LiCl. In this study, the authors found that prior exposure to LiCl injections attenuates the subsequent taste aversion learning when the US is delivered by injections, but not when it is induced by oral administration. These results suggested that injection-related cues could be associated with nausea induced by LiCl and that this association may be critical in attenuating subsequent conditioning to a novel taste.

We propose that the cues provided by the IO of water during the preexposure phase can play a similar role to that provided by injection-related cues. In accord with this proposal, rats infused with water during the LiCl-preexposure sessions displayed aversive reactions to saccharin on the conditioning day, as well as during the preexposure phase. Therefore, IO cues associated with LiCl injections can evoke a conditioned state of nausea and block the development of a subsequent conditioned taste aversion.

Although it is well established that conditioned nausea is elicited by contextual cues previously paired with LiCl (e.g., Limebeer et al., 2006, 2008), the results in the present experiments cannot be explained in terms of blocking by external contextual cues. Results from experiments described in chapter 5 have shown that LiCl preexposure attenuates the establishment of conditioned disgust reactions to a flavor paired with the drug. This finding confirms that a context previously paired with lithium acquired the ability to evoke conditioned nausea, and therefore, interfere with the subsequent establishment of conditioned disgust reactions to a flavor. Even though the context-illness association may contribute to the attenuating effects of LiCl-preexposure observed in other studies, it is important to note that in the current experiments, all subjects received IO infusions of water prior to the injections of LiCl during the preexposure phase, indicating that it is the infusion-related cues that appear to play a dominant role in the control of conditioned disgust. It can therefore be argued that IO infusion cues are more salient than the external context in which lithium preexposure is carried out, enabling the infusion-related cues to overshadow the acquisition of conditioned nausea by the external context. Conditioned disgust reactions elicited by the context alone during testing support this hypothesis.

The results obtained in the present chapter are in agreement with those of previous studies from our laboratory examining latent inhibition of conditioned disgust in taste aversion learning (López et al., 2010). It was found that non-reinforced exposure to a flavor prior its conditioning with LiCl results not only in suppressed consumption of the LiCl-paired flavor, but also in attenuated conditioned disgust reactions to the flavor. However, a LI effect on either consumption or disgust reactions depends on a common method of fluid delivery during preexposure and testing. This dissociation might be related to context specificity provided by the methods of delivery, in which a change between contexts may attenuate the LI effect.

In summary, the present study provides some evidence that the attenuating effects of US-preexposure in taste aversion conditioning can be assessed by the presence or absence of conditioned disgust reactions on the TR test. We also suggest that stimuli provided by the IO infusion of water in our procedure can modulate the US-preexposure effect in taste aversion learning in a similar way to that of environmental cues or injection-related cues. Subsequent research using alternative methods to examine taste palatability, such as the microstructural analysis of licking behavior (Dwyer, 2012) might provide useful information about the mechanisms involved in the acquisition and expression of nausea-induced taste aversions.

Capítulo 7

El efecto de la preexposición al EC sobre el consumo y la palatabilidad del sabor

The CS-preexposure effect as assessed by both flavor consumption and cue palatability

7.1. Introduction

It is well established in rats that pairing a novel taste with illness induced by the injection of an emetic drug (e.g., lithium chloride, LiCl) results in decreased consumption of the taste when it is subsequent contacted, a learning paradigm termed conditioned taste aversion (see Reilly & Schachtman, 2009, for a recent review on this phenomenon). Although taste aversions produced by different methods are often considered together, it has been argued by Parker and colleagues (see Parker, 2003; Parker et al., 2009) that a reduction in the consumption of a taste previously paired with aversive consequences may be motivated by two different processes; the association of the taste with the nausea, or by its association with a potential danger (e.g., that produced by a novel change in rat´s physiological state). This distinction is largely based on the presence or absence of aversive (rejection) reactions in the taste reactivity test introduced by Grill and Norgren (1978). In this test, rats are infused with a flavored solution via a cannula implanted in their oral cavity and the orofacial reactions elicited by the flavor are recorded. Rats usually display rejection reactions, such as gaping, chin rubbing, and paw treading, when infused with unpalatable solutions such as bitter tasting quinine. Critically, rats also display the same rejection reactions to otherwise palatable tastes (such as sweet sucrose) that have been previously paired with nausea produced by LiCl administration, reflecting a shift in hedonic value, or palatability, of the taste (e.g., Parker, 1982; Pelchat et al., 1983). In contrast when sucrose is paired with peripheral pain (electric shock), the consumption of that solution is reduced to a degree comparable to that induced by pairing the solution with LiCl, but does not produce a change in palatability of the taste stimulus as measured by the taste reactivity test (e.g. Pelchat et al., 1983). This was interpreted in terms of the solution becoming a danger signal without a change in its affective properties. Further evidence that taste

aversion learning is mediated both by internal nausea linked to disgust reactions as well as by other mechanisms includes the fact that that rats can suppress intake of flavors paired with rewarding drugs, such as cocaine or amphetamine, that do not result in the production of rejection reactions to the conditioned stimulus flavors (e.g., Parker, 1982; 1995), and that antiemetic drugs that can interfere with the establishment of disgust reactions to a LiCl-paired flavor without affecting the amount consumed of the flavored solution (e.g., Limebeer & Parker, 2000).

It is also well established that exposure to a stimulus prior to it being paired with some reinforcing event will attenuate (or even prevent) learning about the cue-event relationship. This phenomenon is referred latent inhibition (LI) and has been demonstrated with a wide variety of preparations including when the cue stimulus is a flavor, and the subsequent event is the administration of LiCl (for reviews, see Lubow, 1989, 2009). But while it has long been known that flavor pre-exposure reduces conditioned taste aversion as measured by voluntary fluid ingestion in simple consumption tests, its effects on taste palatability is not well known. In a recent study conducted to evaluate whether flavor pre-exposure concurrently attenuates the effects of taste aversion on both fluid consumption and conditioned disgust reactions as an index of palatability, we found that pre-conditioning flavor exposure not only disrupts suppressed consumption, but also attenuates the establishment of conditioned disgust reactions to flavor paired with LiCl (López et al., 2010). However, the effects of pre-conditioning exposure to saccharin on acquired consumption and disgust reactions differed as a function of the how the saccharin exposure was performed. That is, when rats were given intraoral infusions of saccharin prior to conditioning with LiCl, saccharin pre-exposure resulted in attenuated conditioned disgust reactions in the taste reactivity test, but did not attenuate the reduction in flavor ingestion during a voluntary

consumption test; in contrast, when pre-exposed to the solution by bottle, the taste aversion induced reduction in consumption of saccharin was attenuated, but there was no effect of exposure on the acquisition of conditioned disgust reactions to saccharin. In short, latent inhibition effects on either consumption or disgust reactions required a common method of fluid delivery during pre-exposure and testing.

This apparent dissociation in latent inhibition effects on consumption and taste reactivity measures might relate to the context specificity of latent inhibition whereby a change of context between exposure and test will attenuate or abolish the latent inhibition effect (e.g. Hall & Channell, 1986; Lovibond, Preston, & Mackintosh, 1984; Boakes, Westbrook, Elliot, & Swinbourne, 1997). In the experiments by López et al. (2010) taste reactivity analyses were performed during intraoral fluid delivery, while consumption was assessed by giving free access to the test solution in a bottle. On the grounds that the method by which fluid access was given would presumably be highly salient to the rats, López et al. (2010) suggested that it would act as a contextual cue, and so exposure to the flavor before training should only influence conditioned taste aversion when the exposure and test methods of fluid delivery matched (which is exactly the pattern of results that was observed). However, while context-based latent inhibition effects certainly offer an account of the apparent dissociation in the taste reactivity and consumption measures, this account cannot be tested by traditional taste reactivity methods because the reliance on intraoral infusion means that the fluid delivery context will be perfectly correlated with the type of response being assessed. Moreover, it is at least possible that consumption and taste reactivity reflect two different aspects of the conditioned response and that flavor exposure might influence them independently. In terms of Konorski's (1967) distinction between preparatory and consummatory conditioning, bottle-based consumption tests afford preparatory

responses (e.g. approach or withdrawal from the bottle) while intraoral infusion does not, but intraoral infusion does afford consummatory responses (including hedonic reactions). This division between consummatory and preparatory responses has previously been considered in light of the fact that the hedonic effects of conditioned taste aversion appear to extinguish faster than the effects on consumption (Cantora, López, Aguado, Rana, & Parker, 2006; Dwyer, 2009).

With these issues in mind, the goal of the present studies was to determine whether latent inhibition in taste aversion has concurrent effects on consumption of, and hedonic reactions to, the target taste when the possibility of context effects produce by fluid delivery methods is removed. This was achieved by the microstructural analysis of licking behaviour during voluntary consumption (for reviews of this methodology see, Davis, 1973, 1989; Dwyer, 2012). The ingestive behavior of rats consuming fluids consists of sustained runs of rapidly occurring rhythmic licks (referred to here as clusters) separated by pauses of varying lengths. It is consistently observed that palatable sugar solutions increase the quantity of fluid consumed, the number of licks, and the number of licks per cluster (e.g., Davis & Perez, 1993; Davis & Smith, 1992); in contrast, the aversive taste of quinine reduces the rate of licking and the size of licking clusters (e.g., Spector & St. John, 1998). Also, pairing an otherwise palatable taste with LiCl results in a reduction of the lick cluster size similar to that produced by quinine (Baird, John, & Nguyen, 2005; Dwyer, 2009). Moreover, amphetamine based aversions do not produce the same degree of a change in lick cluster size as do aversions produced by LiCl (Dwyer et al., 2008; but see also Lin et al., 2012; Arthurs, Lin, Amodeo, & Reilly, 2012). In addition, it has been demonstrated that the administration of benzodiazepine drugs, which modulate ingestion responses in the taste reactivity test and enhance hedonic reactions to food in humans, enhance lick cluster size (e.g.,

Cooper, 2005; Higgs & Cooper, 1998). All these findings indicate that the analysis of the microstructure of licking behavior can be taken as an effective indicator of rodents´ hedonic reactions. Moreover, the measurement of licking behavior does not affect the means of fluid delivery (it relies on simple electrical means to record the time of each lick to a freely available bottle) and so does not produce a context change similar to that created by the use of intraoral fluid delivery. Thus the current experiments, using the latent inhibition paradigm, examined both the amount of consumption and the microstructure of licking behavior in order make an unambiguous assessment of concurrent changes in consumption and taste palatability following flavor pre-exposure in taste aversion learning.

7.2. Experiment 6

The design of Experiment 6 is shown in Table 7. Half of the animals (Group LI) were exposed to 0.1% (w/w) saccharin without any experimentally defined consequences across four drinking sessions, while the remainder (Group Control) received water. Following this exposure phase all animals received two sessions in which saccharin was paired with intraperitoneal injections of lithium chloride (LiCl). The responses to saccharin were then examined across ten drinking sessions in extinction. Throughout the initial exposure, conditioning, and test phases, the timing of all licks was recorded to allow for the analysis of lick cluster sizes. On the basis of previous analyses of latent inhibition in conditioned taste aversion, rats in Group LI should consume more saccharin those in Group Control during the test phase (i.e., after saccharin was paired with LiCl). Furthermore, to the extent that LI also attenuates the degree to which conditioned taste aversion influences hedonic reactions then lick cluster sizes elicited by saccharin should be larger in Group LI than in Group Control.

Table 7: Design of Experiments 6

Group	Exposure	Conditioning	Test
LI	4 × saccharin	2 × saccharin →	10 × saccharin
Control	4 × Water	5ml/kg 0.15M LiCl	

Note: There was one 15 min drinking session per day (followed 1 hr later by 1 hr access to water in the home cage). In Experiment 6 saccharin was presented at 0.1% (w/w).

7.2.1. Method

7.2.1.1. Subjects.

Twenty-four male Lister hooded rats (*Rattus Norvegicus*) were obtained from Harlan, Bicester, UK for the purposes of the study. Their weights before the beginning of the study ranged from 289g to 361g, with a mean weight of 333g. The rats were housed in pairs in a room illuminated between the hours of 0800-2000, where they had ad-lib access to food and received 60 min access to water per day approximately, 1 hr after the experimental sessions.

7.2.1.2. Fluids and Apparatus

Rats were trained and tested in twelve custom-made drinking chambers (Med Associated Inc., St Albans, USA). These measured 32 × 15 × 12 cm (L × W × H), with steel mesh flooring and with white acrylic walls. The drinking chambers were located in a room separate from that containing the home cages. Fluids were made accessible through drinking spouts made of stainless steel, attached to 50ml cylinders. These could be inserted on the left or right hand side of the lid (made of wire mesh). The distance between the holes for the bottles was 8cm. Only the left hand side was used for the current studies. A contact sensitive lickometer registered the time of each lick to the nearest 0.01s. This was recorded by a computer using MED-PC software (Med

Associates Inc.). The amount of fluid consumed by each rat was measured by weighing the drinking bottle before and after each session. The stimuli were tap water or solutions of 0.1% (w/w) saccharin.

7.2.1.3. Procedure

All experimental drinking sessions were 15 min in duration and there was one session on each day. To acclimatize the rats to the experimental apparatus they were given two 15-min sessions with access to water. The following four sessions comprised the exposure phase: rats in Group LI received saccharin in each session, while those in Group Control received water (see Table 1). Following the exposure phase, all rats received a 2-day conditioning phase in which exposure to saccharin was followed by an intraperitoneal injection of LiCl (0.15M at 5ml/kg bodyweight) on both days. The test phase consisted of ten drinking sessions in which saccharin was presented without any experimental consequences.

7.2.1.4. Data Analysis.

In addition to the consumption data, the mean cluster size for each rat was extracted from the record of licks for analysis. A cluster was defined as a set of licks each separated by an inter-lick-interval of no more than 0.5 s. This criterion is used by Davis and his co-workers (e.g., Davis & Perez, 1993; Davis & Smith, 1992) and in the majority of our previous studies using lick analysis techniques (for a review see, Dwyer, 2012). Although other criteria have been used (e.g., Dwyer, Pincham, Thein, & Harris, 2009; Spector, Klumpp, & Kaplan, 1998), parametric analyses suggest that there is little practical difference between them as most pauses greater than 0.5 s are also greater than 1 s (e.g., Davis & Smith, 1992; Spector, et al., 1998). Mixed analyses of variance (ANOVA) were used to analyze the test data with factors of exposure condition (LI vs.

Control) and session (Conditioning 1 and 2, Tests 1-10). All tests reported here used a criterion for significance of $p = 0.05$.

On several occasions no licks were recorded for individual rats (test session 1 – three rats from group control, test session 2 – two rats from group control, test sessions 6, 8, and 9 – one rat from group control). Consumption was correspondingly very low at these times suggesting that these were genuine absences of licking, rather than a failure of the recording equipment. As lick cluster size measures are undefined in the absence of any recorded licks, these empty cells were replaced with the relevant group means for that session in the analyses reported below. A preliminary analysis using only the animals for which data was available for every test session revealed the same general pattern of effects suggesting that this treatment of the data did not generate spurious effects.

7.2.2. Results

Table 8 shows the data averaged across the exposure phase. Consumption of saccharin in Group LI was higher than consumption of water in Group Control, $t(22) = 5.63$, $p < .001$, SED = 0.55, but the mean lick cluster sizes did not differ between groups, $t < 1$.

Table 8: Exposure Phase data from Experiment 6

Group	Solution	Consumption (g)	Lick Cluster Size
LI	Saccharin	11.9 (0.5)	32.6 (2.4)
Control	Water	8.8 (0.2)	35.4 (2.4)

Note: Data is shown as mean (with SEM).

Figure 9 shows the data from the conditioning and tests sessions (consumption in Panel A and lick cluster size in Panel B). Inspection of Panel A suggests that consumption of saccharin was generally lower for the Control than the LI group, and that consumption in both groups dropped from the level seen on conditioning session 1, before partially recovering across testing in extinction. ANOVA conducted on the amount consumed revealed significant effects of exposure condition (LI vs. Control), $F(1, 22) = 9.90$, $p = .005$, MSE = 3.67), session, $F(11, 242) = 73.43$, $p < .001$, MSE = 2.93, but no interaction between these two factors, $F(11, 242) = 1.41$, $p = .167$, MSE = 2.93. Simple effects analyses revealed that the difference between the Groups LI and Control was significant on every session (lowest $F(1, 22) = 4.35$, $p = .049$, MSE = 8.79, for test session 10) except for test session 1 ($F(1, 22) = 2.08$, $p = .164$, MSE = 3.31). In addition, consumption was significantly lower than on conditioning session 1 in all subsequent sessions for both Group LI (lowest $F(1, 22) = 24.85$, $p < .001$, MSE = 0.82, for the comparison to test session 10) and Group Control (lowest $F(1, 22) = 31.08$, $p < .001$, MSE = 0.82, for the comparison to test session 10).

Inspection of Panel B suggests similar results for the analysis of mean lick cluster sizes. These were generally lower for the Control than the LI group, and mean lick cluster sizes in both groups dropped from the level seen on conditioning session 1. Unlike with consumption, this recovery did approach initial levels of lick cluster size by the end of extinction testing. ANOVA conducted on the lick cluster size data revealed significant effects of exposure condition (LI vs. Control), $F(1, 22) = 13.58$, $p = .001$, MSE = 62.13), session, $F(11, 242) = 20.76$, $p < .001$, MSE = 96.27, but no interaction between these two factors, $F < 1$. Simple effects analyses revealed that the difference between the Groups LI and Control was significant on conditioning session 2, and test sessions 3 – 9 (lowest $F(1, 22) = 5.33$, $p = .031$, MSE = 161.04, for test session 5), but

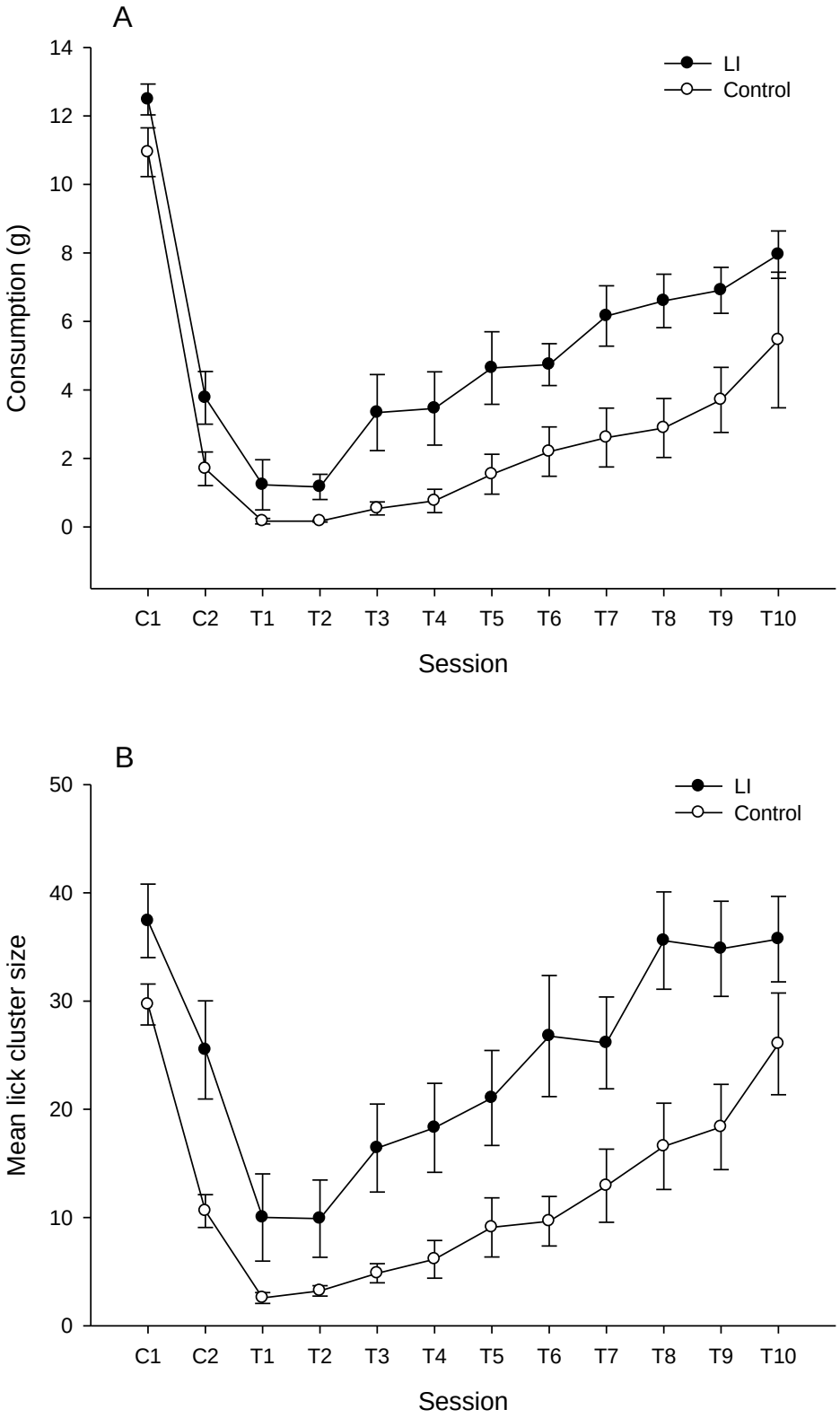


Figure 9: Experiment 6: Shows mean consumption (panel A) and lick cluster size (panel B) per session for both the LI and Control groups. C1 and C2 refer to conditioning sessions 1 and 2, while T1-T10 refer to extinction test sessions 1-10.

not on conditioning session 1, and test sessions 1, 2, and 10 (highest $F(1, 22) = 3.94$, $p = .060$, MSE = 90.55, for conditioning session 1). In addition, in Group LI lick cluster size was significantly lower than on conditioning session 1 in conditioning session 2, test sessions 1 – 5 and 7 (lowest $F(1, 22) = 5.29$, $p = .031$, MSE = 26.80, for the comparison to conditioning session 2), but was not significantly different from the initial level on test sessions 6 or 8 – 10 (highest $F(1, 22) = 3.44$, $p = .077$, MSE = 32.9, for the comparison to test session 6). In Group Control lick cluster size was significantly lower than on conditioning session 1 in conditioning session 2, test sessions 1 – 9 (lowest $F(1, 22) = 5.33$, $p = .031$, MSE = 17.46, for the comparison to test session 9), but was not significantly different from the initial level on test session 10 ($F < 1$).

In summary, exposure to saccharin prior to taste aversion conditioning with LiCl resulted in both higher levels of consumption, and higher lick cluster sizes compared to a non-exposed control. This is consistent with exposure producing a latent inhibition effect that was apparent in both consumption and lick microstructure measures. In addition, the effects of the taste aversion treatment on consumption were more resistant to extinction treatment than were the effects of taste aversion on lick cluster size. Finally, the fact that the effects produced by latent inhibition were not affected by the stage of conditioning or extinction test suggests that exposure may have attenuated a neophobic reaction to the saccharin solution and thus that, in the present circumstances, an attenuation of neophobia made at least some contribution to the latent inhibition effect observed[2].

[2] In the present case the difference in lick cluster sizes between the exposed and non-exposed groups during the initial conditioning session (i.e. when the control group first had access to saccharin) did not reach standard levels of statistical significance (albeit that at $p = .06$, it would have been significant on a 1-tailed test).

In this light, it is interesting that studies of neophobia reduction using taste reactivity (Neath et al., 2010) and lick microstructure methods (Lin, Amodeo, Arthurs, & Reilly, 2012) have produced inconsistent effects. But before considering the theoretical implications of the results of Experiment 6 in more detail, we sought to replicate and extend them to a within-subject design.

7.3. Experiment 7

In Experiment 6, the fact that exposure influenced consumption (and to an extent lick cluster size) at the start of the conditioning phase meant that it is hard to completely disentangle any effects of neophobia attenuation from latent inhibition more generally. Using a within-subject manipulation of the taste aversion manipulation means that we were able to compare both conditioned and unconditioned differences in the responses to the flavored solutions. The design of Experiment 7 is shown in Table 9. Half of the animals (Group LI) were exposed to two separate CS flavors (1% (w/w) NaCl, and 4% (w/w) maltodextrin (although presented without consequences in the exposure phase these were to be counterbalanced between the CS+ and CS-), while the remainder (Group Control) received water. Following this exposure phase all animals received two sessions a two-day conditioning phase where the CS+ flavor was paired with the IP injection of LiCl and the CS- flavor was paired with the IP injection of NaCl. The responses to the CS+ and CS- were then examined across 16 drinking sessions in extinction (these alternated between the CS+ and CS-). As in Experiment 6, both consumption and lick mean lick cluster size measures were taken throughout. If non-reinforced exposure does produce latent inhibition effects on both the consumption and palatability of the cue flavors, then animals in the LI group should show attenuated effects of taste aversion learning on the difference between the CS+ and CS- flavors in terms of both consumption and lick cluster size compared to the Control group. In

addition, if non-reinforced exposure does attenuate neophobic responses to the test flavors (as was suggested by the results of Experiment 6), then the responses across both the CS+ and CS- during the conditioning phase (i.e. before any taste aversion had been created) should also differ between the LI and Control groups.

Table 9: Design of Experiment 7

Group	Exposure	Conditioning	Test
LI	4 × CS+, 4 × CS-	CS+ → 5ml/kg 0.15M LiCl	8 × CS+
Control	8 × Water	CS- → 5ml/kg 0.9% NaCl	8 × CS-

Note: There was one 15 min drinking session per day (followed 1 hr later by 1 hr access to water in the home cage). In Experiment 2, CS+ and CS- were counterbalanced between 1% (w/w) NaCl, and 4% (w/w) maltodextrin. All injections (LiCl or NaCl) were given by the intraperitoneal route and occurred immediately after the end of the relevant drinking session.

7.3.1. Method

7.3.1.1. Subjects, Apparatus and Stimuli

Twenty-four male Lister hooded rats, obtained from the same source and maintained in the same fashion as in Experiment 1, were used. Their weights before the beginning of the study ranged from 354g to 441g, with a mean weight of 400g. The drinking chambers used were the same as described for Experiment 1. The stimuli were tap water or solutions of 1% (w/w) NaCl, or 4% (w/w) maltodextrin (C*Dry MD 01904, Cerestar-UK, Manchester, UK).

7.3.1.2. Procedure

All experimental drinking sessions were 15 min in duration and there was one session on each day. To acclimatize the rats to the experimental apparatus they were given one 15-min session with access to water. The following eight sessions comprised the exposure phase: rats in Group LI received alternating sessions with NaCl and maltodextrin, while those in Group Control received water (see Table 6). Following the exposure phase, all animals received a two-day conditioning phase. On the first conditioning day all rats received NaCl in the drinking session: for half of the rats in both groups LI and Control this was followed by an intraperitoneal injection of LiCl (0.15M at 5ml/kg bodyweight); the remainder of the rats received an intraperitoneal injection of NaCl (0.9% at 5ml/kg bodyweight). On the second conditioning day all rats received maltodextrin in the drinking session: rats that had received LiCl on the first conditioning session now received an injection of NaCl while the remainder received an injection of LiCl. Thus, for both the LI and Control groups the CS+ and CS- were counterbalanced between NaCl and maltodextrin. The test phase consisted of 16 drinking sessions alternating between the CS+ and the CS-.

7.3.1.3. Data Analysis

The data was prepared for analysis in the same general manner as in Experiment 6. In addition, as will be seen below, there were large unconditioned differences in the lick cluster sizes elicited by NaCl and maltodextrin during the exposure phase (these continued into the conditioning and test phases). Thus a factor of solution counterbalance (CS+ = NaCl vs CS+ = maltodextrin) was added to the analysis of the consumption and lick cluster size data from the conditioning and test phases.

7.3.2. Results

Table 10 shows the data averaged across the exposure phase. Taking first the LI group, while consumption of the solutions to become the CS+ and CS- was equivalent, there was a tendency for consumption of maltodextrin to be lower than that of salt. These trends were stronger in the lick cluster size data. An ANOVA was performed on the consumption data from the LI group with factors of whether that solution was to be paired with LiCl or not (CS+/CS-) and the nature of the solution (Salt/Maltodextrin).

Table 10: Exposure Phase data from Experiment 7

	Solution	Consumption (g)	Lick Cluster Size
LI	CS+ Salt	13.7 (0.9)	48.3 (3.6)
	CS+ Maltodextrin	12.4 (0.8)	36.8 (3.8)
	CS- Salt	13.4 (0.7)	51.1 (5.3)
	CS- Maltodextrin	12.3 (0.7)	33.9 (3.3)
Control	Water	8.8 (0.4)	36.3 (5.6)

Note: Data is shown as mean (with SEM). Data from the LI group is shown as a function of whether that solution was to be paired with LiCl or not (CS+/CS-) and as a function of the nature of the solution (Salt/Maltodextrin).

This revealed that there was no main effect of CS, $F < 1$, that the main effect of solution type approached standard levels of significance, $F(1,10) = 3.66$, $p = .085$, MSE = 2.32, and that there was no interaction between these factors, $F < 1$. A similar analysis of the lick cluster size data revealed no main effect of CS, $F < 1$, a significant effect of solution type, $F(1,10) = 24.88$, $p = .001$, MSE = 49.77, and no interaction between these factors, $F < 1$. In addition, consumption of the flavored solutions as a whole in Group LI was higher than consumption of water in Group Control, $t(22) =$

6.90, $p < .001$, SED = 0.61, but the mean lick cluster sizes did not differ between groups, $t(22) = 1.64$, $p = .115$, SED = 2.99.

Figure 10 shows the data from the conditioning and tests sessions (consumption in Panel A and lick cluster size in Panel B). Inspection of Panel A suggests that, in both the LI and Control groups, consumption of the CS+ dropped following the conditioning session before recovering across extinction testing – with the initial reduction being smaller in the LI than Control groups. That is, pre-exposure to the CS+ and CS- attenuated, but did not prevent, the formation of a conditioned taste aversion. The consumption data was subjected to a mixed ANOVA with within-subject factors of CS (CS+/CS-) and test session, plus between subject factors of exposure group (LI/Control) and stimulus assignment (CS+ = NaCl/CS+ = maltodextrin). The most theoretically relevant results from the analysis were as follows: There was a main effect of CS, $F(1,20) = 93.14$, $p < .001$, MSE = 25.60, a session by CS interaction, $F(8,160) = 34.18$, $p < .001$, MSE = 4.25, and an exposure by CS interaction, $F(1,20) = 5.77$, $p = .026$, MSE = 25.60. These revealed that consumption of the CS+ was greater in the LI than the Control group on test sessions 1-4 (lowest $F(1, 20) = 4.94$, $p = .038$, MSE = 9.63, for test session 2), but consumption of the CS+ did not differ between groups at any other time (highest $F(1, 20) = 1.45$, $p = .242$, MSE = 16.17, for test session 5).

Consumption of the CS- did not differ between the LI and Control groups on any session (highest $F(1, 20) = 2.24$, $p = .150$, MSE = 6.20, for test session 6). In addition, in group LI consumption of the CS+ was significantly reduced relative to the conditioning session baseline on test sessions 1-4 (lowest $F(1, 20) = 5.22$, $p = .033$, MSE = 1.01, for the comparison to test session 4), but was not significant different to the baseline on test sessions 5-8 (highest $F(1, 20) = 2.45$, $p = .133$, MSE = 1.22, for the comparison to test session 4).

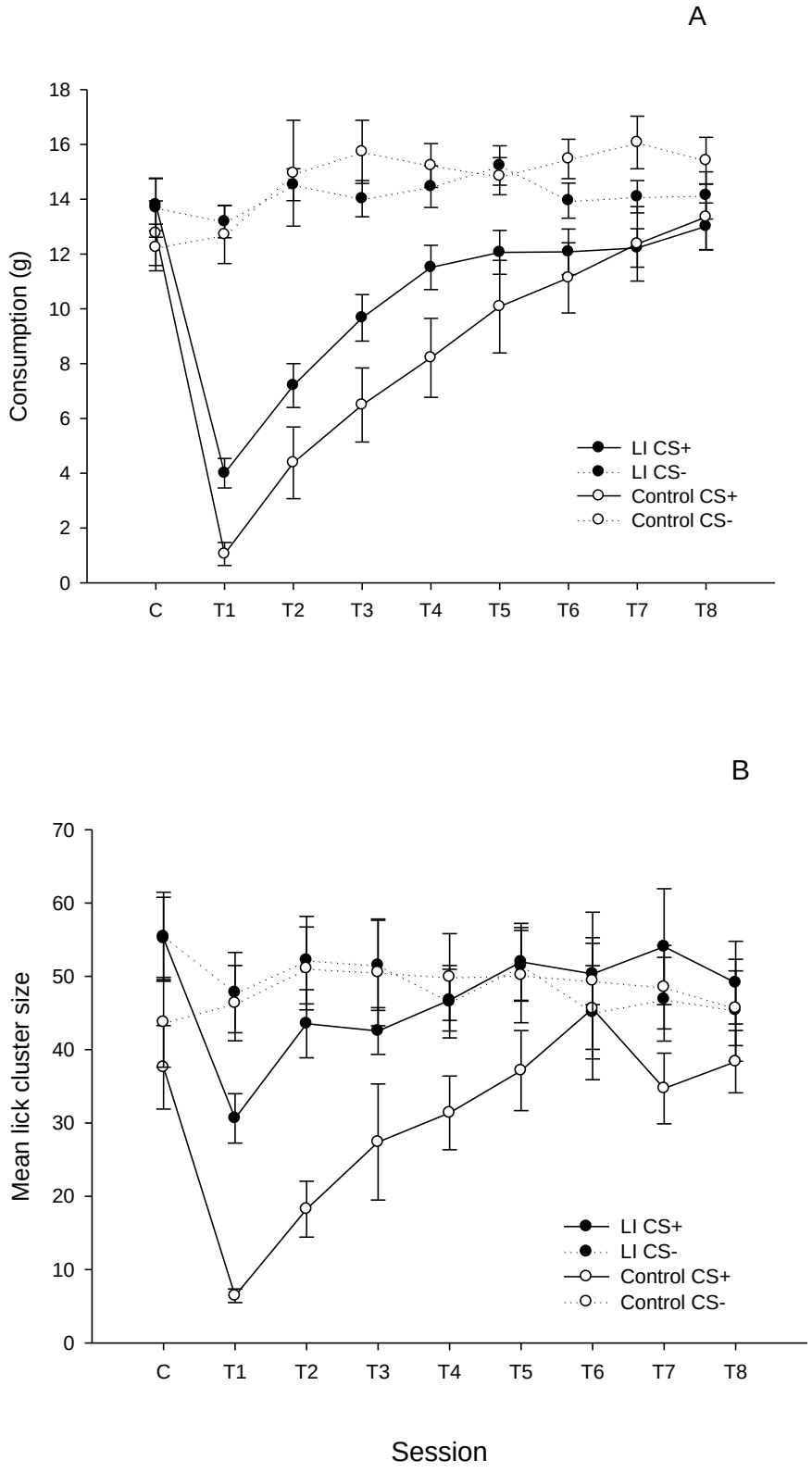


Figure 10: Experiment 7: Shows mean consumption (panel A) and lick cluster size (panel B) per session for both the CS+ and CS- flavors for the LI and Control groups. C refers to the conditioning session while T1-T8 refer to extinction test sessions 1-8.

In group LI consumption of the CS- did not differ from the conditioning session baseline during any subsequent test (highest $F(1, 20) = 2.94$, $p = .102$, MSE = 0.80, for the comparison to test session 5). In group Control consumption of the CS+ was significantly reduced relative to the conditioning session baseline on test sessions 1-5 (lowest $F(1, 20) = 5.33$, $p = .032$, MSE = 1.35, for the comparison to test session 5), but was not significant different to the baseline on test sessions 6-8 (highest $F(1, 20) = 2.19$, $p = .155$, MSE = 1.22, for the comparison to test session 6). In contrast to group LI, in group Control the consumption of the CS- did exceed that of the conditioning session baseline on test sessions 2-8 (lowest $F(1, 20) = 8.40$, $p = .009$, MSE = 0.80, for the comparison to test session 5), but was equivalent to baseline consumption on test session 1 ($F < 1$). Finally, with respect to the possibility of neophobia reduction, an analysis of consumption during the conditioning phase averaged across the CS+ and CS- revealed that there was no difference between the LI and Control groups, $F(1, 20) = 1.56$, $p = .222$, MSE = 5.91

Table 11 shows the aversion ratio for the CS+ (calculated as the consumption of the CS+ divided by consumption of both the CS+ and CS-). Taking such a ratio controls for differences in overall response levels (as was seen in the consumption data presented above) in the assessment of the relative response to the CS+ and CS-. Inspection of Table 10 suggests that the ratio dropped below 0.5 (indicating an aversion to the CS+) following conditioning in both the LI and Control groups, and that this decrease was larger in the Control group. This data was analysed with a mixed ANOVA with factors of exposure group (LI/Control) and session. This revealed main effects of exposure, $F(1,22) = 4.53$, $p = .045$, MSE = 0.009, and session, $F(8,176) = 63.39$, $p < .001$, MSE = 0.005, as well as an interaction between them $F(8,176) = 3.56$, $p = .001$, MSE = 0.005.

Table 11: Aversion ratio data from Experiment 7

Group	Session	Consumption	Lick Cluster Size
LI	Conditioning	.51 (.44 - .57)	.50 (.40 - .59)
	Test 1	.23 (.17 - .29)	.40 (.34 - .46)
	Test 2	.32 (.25 - .40)	.46 (.37 - .55)
	Test 3	.40 (.32 - .48)	.47 (.37 - .56)
	Test 4	.44 (.37 - .51)	.51 (.40 - .61)
	Test 5	.44 (.37 - .51)	.50 (.41 - .60)
	Test 6	.46 (.41 - .51)	.54 (.46 - .62)
	Test 7	.46 (.41 - .51)	.53 (.46 - .60)
	Test 8	.48 (.43 - .53)	.54 (.48 - .59)
Control	Conditioning	.51 (.45 - .57)	.47 (.38 - .57)
	Test 1	.08 (.02 - .14)	.14 (.07 - .20)
	Test 2	.20 (.12 - .27)	.27 (.18 - .36)
	Test 3	.28 (.19 - .36)	.34 (.25 - .44)
	Test 4	.32 (.25 - .40)	.40 (.29 - .50)
	Test 5	.37 (.30 - .44)	.43 (.40 - .56)
	Test 6	.41 (.35 - .46)	.48 (.35 - .49)
	Test 7	.42 (.37 - .48)	.42 (.41 - .52)
	Test 8	.46 (.41 - .50)	.47 (.41 - .52)

Note: Shows aversion ratios for both consumption and lick cluster measures (with 95% confidence intervals). The ratio data shown here was calculated by dividing the response to the CS+ (either in terms of consumption or mean lick cluster size) by the sum of the responses to both the CS+ and CS-.

A simple effect analysis of the interaction revealed that there was a significant difference between groups LI and Control on test sessions 1-4 (lowest $F(1, 22) = 4.46$, $p = .046$, MSE = 0.20, for test session 3) but not during the conditioning session, or test sessions 5-8 (highest $F(1, 22) = 2.20$, $p = .152$, MSE = 0.008, for test session 7). It is also worth noting that confidence intervals for the aversion ratio in group LI did not include 0.5 (i.e. indicating a rejection of the CS+ relative to the CS-) on test sessions 1-3, but did include 0.5 at all other times. In group Control the aversion ratio confidence intervals did not include 0.5 on test sessions 1-7, but did include 0.5 during conditioning and test session 8. In summary, the ratio data analyzed here reflect the consumption data described above – exposure to the CS solutions prior to conditioning attenuated, but did not prevent, the formation of a conditioned taste aversion.

Turning to the lick cluster size data in Panel B of Figure 10, for both the LI and Control groups, the mean lick cluster size elicited by the CS+ reduced following the conditioning session before recovering across extinction testing – with the initial reduction being smaller in the LI than Control groups. Thus, pre-exposure to the CS+ and CS- attenuated, but did not prevent, taste-aversion produced changes in affective responses to flavored solutions. In addition, the mean lick cluster sizes (across both the CS+ and CS-) appeared somewhat lower for the Control group than the LI group during the conditioning session – an effect consistent with a neophobic response. The lick cluster data was subjected to the same mixed ANOVA as the consumption data: Within-subject factors of CS (CS+/CS-) and test session, plus between subject factors of exposure group (LI/Control) and stimulus assignment (CS+ = NaCl/CS+ = maltodextrin). The most theoretically relevant results from the analysis were as follows: There was a main effect of CS, $F(1,20) = 2625$, $p < .001$, MSE = 395.80, a session by CS interaction, $F(8,160) = 8.36$, $p < .001$, MSE = 140.69, and an exposure by

CS interaction, $F(1,20) = 16.59$, $p = .001$, MSE = 395.80. Respectively, these confirmed that pairing the CS+ with LiCl reduced the lick cluster size for the CS+, this reduction decreased over extinction testing, and that the size of the CS+ vs CS- difference was attenuated by exposure to the CS solutions[3]. In order to further explore the effects of exposure on conditioning, simple effect tests were performed to compare the LI and Control groups for mean lick cluster sizes elicited by the CS+ and CS-. These revealed that lick cluster size for the CS+ was greater in the LI than the Control group during the conditioning session, as well as test sessions 1-5 (lowest $F(1, 20) = 4.56$, $p = .045$, MSE = 301.40, for test session 4), but lick cluster sizes for the CS+ did not differ between groups at any other time (highest $F(1, 20) = 4.08$, $p = .057$, MSE = 549.27, for test session 7). Lick cluster sizes for the CS- did not differ between the LI and Control groups on any session (highest $F(1, 20) = 2.98$, $p = .099$, MSE = 274.77, for the conditioning session). In addition, in group LI lick cluster sizes for the CS+ were significantly reduced relative to the conditioning session baseline on test sessions 1-3 (lowest $F(1, 20) = 7.75$, $p = .011$, MSE = 20.58, for the comparison to test session 3), but was not significant different to the baseline on test sessions 4-8 (highest $F(1, 20) = 2.98$, $p = .100$, MSE = 23.75, for the comparison to test session 4). In group LI lick cluster size for the CS- did not differ from the conditioning session baseline during any

[3] The remainder of the full 4-way ANOVA was as follows: There was a main effect of test session, $F(8,160) = 5.11$, $p < 0.001$, MSE = 212.07, but no interactions between session and exposure condition, $F(8,160) = 1.16$, $p = 0.325$, MSE = 212.07, or session and stimulus assignment, $F(8,160) = 1.15$, $p = 0.333$, MSE = 212.07. There was an interaction between CS and stimulus assignment, $F(1,20) = 43.71$, $p < .001$, MSE = 395.80, such that the CS+ vs CS- difference was only present when the maltodextrin was the CS+ solution (means not shown). There was an interaction between CS, exposure condition, and stimulus assignment, $F(1,20) = 6.74$, $p = .017$, MSE = 395.80, reflecting the fact that the CS by exposure condition interaction was carried by the conditions in which Maltodextrin was the CS+. Finally, there was no significant interaction between session, CS, and exposure condition, $F < 1$, a significant interaction between session, CS, and stimulus assignment, $F(8,160) = 4.59$, $p < .001$, MSE = 140.69, but no 4-way interaction between session, CS, exposure condition, and stimulus assignment, $F < 1$.

subsequent test (highest $F(1, 20) = 3.06$, $p = .096$, MSE = 32.94, for the comparison to test session 8). In group Control lick cluster size for the CS+ was significantly reduced relative to the conditioning session baseline on test sessions 1-3 (lowest $F(1, 20) = 5.03$, $p = .036$, MSE = 20.58, for the comparison to test session 3), but was not significant lower than the baseline on test sessions 4-8 (highest $F(1, 20) = 1.62$, $p = .218$, MSE = 23.75, for the comparison to test session 4). In group Control the lick cluster size for the CS- did not differ from that of the conditioning session baseline on any test session (highest $F(1, 20) = 1.61$, $p = .219$, MSE = 33.51, for the comparison to test session 2. Finally, with respect to the possibility of neophobia reduction, an analysis of lick cluster sizes during the conditioning phase averaged across the CS+ and CS- revealed that these were lower in group Control and in group LI, $F(1, 20) = 5.35$, $p = .031$, MSE = 240.03.

Table 10 shows the aversion ratio for the CS+ (calculated as for the consumption data). Taking such a ratio allows for differences in overall response levels (such as was seen in the lick cluster data presented above) to be controlled for in the assessment of the relative response to the CS+ and CS-. Inspection of Table 10 suggests that the ratio dropped below 0.5 (indicating a reduction in the lick cluster size for the CS+ relative to that for the CS-) following conditioning in both the LI and Control groups, and that this decrease was larger in the Control group. This data was analyzed with a mixed ANOVA with factors of exposure group (LI/Control) and session. This revealed main effects of exposure, $F(1,22) = 4.65$, $p = .017$, MSE = 0.012, and session, $F(8,176) = 16.09$, $p < .001$, MSE = 0.009, as well as an interaction between them $F(8,176) = 3.42$, $p = .001$, MSE = 0.009. A simple effect analysis of the interaction revealed that there was a significant difference between groups LI and Control on test sessions 1, 2, and 7 (lowest $F(1, 22) = 4.91$, $p = .037$, MSE = 0.013, for test session 7) but not during the conditioning session, or test sessions 3-6 and 8 (highest $F(1, 22) = 3.77$, $p = .065$, MSE

= 0.025, for test session 3). It is also worth noting that confidence intervals for the aversion ratio in group LI did not include 0.5 (i.e. indicating a lower lick cluster sizes for the CS+ relative to the CS-) on test session 1, but did include 0.5 at all other times. In group Control the aversion ratio confidence intervals did not include 0.5 on test sessions 1-3 and 7, but did include 0.5 during conditioning and test sessions 4-6 and 8. Thus, the ratio data analyzed here reflect the consumption data described above – exposure to the CS solutions prior to conditioning attenuated, but did not prevent, changes in the lick cluster size measure of affective responses produced by conditioned taste aversion.

In summary, exposure to the cue flavors prior to taste aversion conditioning with LiCl resulted in a reduction in the subsequent differences between the CS+ and CS- flavors for both consumption and lick cluster size measures relative to non-exposed controls. While there was also some evidence for exposure reducing neophobic responses (especially in terms of the differences between groups LI and Control for the lick cluster measure during the conditioning phase) the use of a within-subject manipulation of aversion conditioning meant that any neophobia reduction could be parceled out of the exposure effect on learning itself. In addition, the effects of taste aversion persisted for longer on consumption than they did on lick cluster size. The theoretical implications of these results will be considered in the General Discussion.

7.4. General Discussion

The main purpose of these experiments was to provide a demonstration of the attenuating effects of flavor pre-exposure (i.e., latent inhibition) on taste aversion learning as assessed by microstructural analysis of licking behavior as a means to ascertain whether latent inhibition has concurrent effects on consumption and hedonic responses. Although latent inhibition effects in taste aversion have been examined

extensively using consumption tests (i.e., prior exposure attenuates subsequent suppressed consumption of an illness-paired flavor), the effect of flavor pre-exposure on taste palatability is not well known. In Experiment 6, non-reinforced exposure to saccharin prior to aversive conditioning with LiCl resulted in attenuated conditioned taste aversion, as assessed by the amount consumed from a bottle containing the solution (i.e., the typical latent inhibition effect in taste aversion learning). More interestingly, the pre-exposure treatment also reduced the effects of taste aversion on the size of licking clusters as compared to a non-exposed control, indicating that the effects of taste aversion on hedonic reactions had also been attenuated. That is, latent inhibition attenuates the effects of taste aversion on both consumption and taste palatability. In addition, it was found in this experiment that conditioned changes in taste palatability extinguished more rapidly than did consumption. Experiment 7 used a within-subject design to preclude any interpretation of the above-described pattern of results in terms of attenuating neophobia to the cue flavor. As in Experiment 6, flavor pre-exposure attenuated the formation of a conditioned taste aversion as measured by consumption and lick cluster size. More specifically, the exposure to the cue flavors (CS+ and CS-) prior to aversive conditioning with LiCl resulted in a reduction in the subsequent differences between the CS+ and CS- flavors for both consumption and lick cluster size measures. Again, conditioned changes in taste palatability extinguished more rapidly than did consumption. Therefore, the concurrent effects of latent inhibition on lick cluster size and consumption indicate that pre-conditioning exposure to the CS flavors attenuates the changes in both consumption and taste palatability produced by conditioned taste aversion in a way that was independent of exposure effects on neophobia.

The current results are largely consistent with previous experiments (López et al., 2010) using the taste reactivity methodology to examine changes in cue palatability following flavor pre-exposure in the taste aversion learning paradigm. López et al. (2010) demonstrated for the first time that flavor pre-exposure not only disrupts suppressed consumption, but also attenuates the establishment of conditioned disgust reactions to a LiCl-paired taste. However, the attenuating effects of flavor pre-exposure on both consumption and taste reactivity appeared to depend on a common method of fluid delivery during pre-exposure and testing. As noted in the introduction, the methods of flavor presentation differentially affected the consumption of the flavor and the display of disgust reactions. When the rats were intraorally infused with the flavor during pre-exposure, they did not display rejection reactions but showed a reduction in flavor consumption; in contrast, when the solution was provided by bottle during the pre-exposure phase, the rats displayed disgust reactions, but they drank the solution in the consumption test. López et al. (2010) interpreted this pattern of results as consistent with the idea that the contextual cues provided by the fluid delivery method (especially the intraoral infusion) can modulate the expression of latent inhibition in taste aversion learning. There is already some evidence that changing the fluid delivery method between pre-exposure and conditioning attenuates the latent inhibition effect on consumption measures in taste aversion learning (e.g., Fouquet et al., 2001; Yamamoto et al., 2002), as the strength of the taste aversion is weakened by changing the method of fluid exposure between conditioning and testing (e.g., Limebeer & Parker, 2006). The current studies, which demonstrate concurrent effects of latent inhibition on consumption and palatability without the contextual confound of different fluid delivery methods, thus support the suggestion that the absence of concurrent latent inhibition effects on consumption and palatability observed in the previous study by López et al.

(2010) was due to a context effect produced by the oral taste infusion method required for taste reactivity analyses.

Considered in this way, latent inhibition appears to produce the same general pattern of effects on lick cluster and taste reactivity measure in the context of conditioned taste aversion. Thus, latent inhibition joins a number of other manipulations which have parallel effects on these two measures (for a review see Dwyer, 2012). Such results suggest that microstructural analysis of lick patterns and taste reactivity may be complementary measures which both assess taste palatability or hedonic responses. However, it should be noted that there are at least some places where taste reactivity and lick microstructure measures diverge. This is apparent in the current context when the effects of flavor exposure on neophobia are considered. As previously noted, a study by Neath et al. (2010) using the taste reactivity method found that repeated intraoral exposure to saccharin caused an increase in consumption in an intake test but not an increase in hedonic reactions to the fluid in the taste reactivity test. In contrast, a recent study by Lin, Amodeo, et al. (2012) found that repeated exposure to saccharin results in an attenuation of the neophobic response to this solution as revealed by an increase in consumption and, importantly, an increase in the size of lick clusters. Although not designed as an explicit test of the effects of flavor exposure on neophobia our own studies reflect this pattern of results: Both of Experiments 6 and 7 here provided at least some suggestion that lick cluster sizes were indeed larger following flavor exposure, while our previous study of latent inhibition in taste aversion (López et al., 2010) did not see any evidence of flavor novelty on unconditioned taste reactivity responses. Taken at face value, these results appear to represent a dissociation between taste reactivity and lick microstructure measures, with the former suggesting that the reduction in neophobia with exposure does not affect the palatability of a taste, while

the latter suggests that it does. While it is premature to offer a definitive interpretation here, it is worth noting that (broadly speaking) taste reactivity analyses are aimed at making a qualitative distinction as to whether a pattern of facial responses are appetitive or aversive while lick microstructure analyses are a more quantitative measure. It is thus possible that release from neophobia might not change a taste from being aversive to being appetitive (hence the lack of a taste reactivity change) but merely change the degree to which it is appetitive (or aversive).

Finally, the results of the present experiments may also provide some information about hedonic processes underlying extinction of conditioned taste aversions. Previous studies examining the microstructure of licking during extinction of a taste aversion have shown that reduction in lick cluster size associated with a learned change in palatability extinguishes more quickly than does the avoidance of the flavor previously paired with the lithium (Dwyer, 2009). That is, the suppressed consumption appears to be more resistant to extinction than learned changes in taste palatability as indicated by the lick cluster size. Similarly, taste reactivity experiments show that a conditioned palatability shift precedes extinction of suppressed consumption (Cantora et al., 2006). The pattern of results obtained in the current study is consistent with these results: In Experiment 6 consumption in the last extinction trial was significantly lower than on the first conditioning session for both Group LI and Group Control but lick cluster size did return to baseline levels for both groups; while in Experiment 7 the differences in consumption between the CS+ and CS- reduced more slowly than did the differences in lick cluster size (for both the LI and Control groups). We (Cantora et al., 2006; Dwyer, 2009) have previously suggested that the difference in extinction rates for hedonic and consumption measures might result from preparatory responses associated with approaching the drinking bottle being more resistant to extinction than are the

consummatory responses (including hedonic ones) directed to the taste itself (e.g., Konorski, 1967; Wagner & Brandon, 1989). The current data is entirely consistent with this general idea, and the fact that prior exposure to the conditioned flavors has little or no effect on the relative speed of extinction suggests that there is little reason to think that latent inhibition differentially influences preparatory and consummatory responses taste aversion.

To summarize, we found that latent inhibition attenuates the effects of taste aversion on both consumption and taste palatability as assayed by the size of licking clusters. That is, non-reinforced exposure to a flavor to-be associated with illness resulted in faster recovery of the size of licking clusters and consumption after taste aversion treatment. The fact that the lick cluster and consumption changes were seen concurrently, and that exposure did not materially affect the relative speed of extinction in consumption and lick cluster measures, suggests that latent inhibition influences taste aversion through a single mechanism rather than having separate effects on preparatory and consummatory process. That said, differences do remain between studies using taste reactivity and lick microstructure methods. While some of these differences might well be attributable to context effects based upon fluid delivery methods, further studies will be needed to determine conclusively how the type of measure (e.g. amount consumed, lick microstructure, taste reactivity) are related to the processes involved in taste aversion learning.

Capítulo 8

Metabolismo cerebral y respuestas condicionadas de náusea

Brain metabolism associated to conditioned disgust reactions

8.1. Introduction

As noted in previously chapters, in taste aversion learning, animals learn to avoid a novel taste associated with illness. In order to establish the conditioned aversion, the taste can be delivered to the rats by different methods, the standard procedure being to present a bottle containing the solution. Alternatively, the TR method measures changes in the initial hedonic value of the taste stimulus due to aversive visceral experience. In particular, rodents display conditioned gaping reactions (a selective measure of the nausea) during re-exposure to the conditioned flavor. Therefore, TR is perhaps a more suitable procedure for measuring conditioned taste aversion than the decrease in the voluntary intake of the flavor that would simply reflect taste avoidance of the solution (Parker, 1995, 2003, 2006, 2014). In spite of this, palatability changes in CTA have been scarcely studied. It is well established that non reinforced exposure to a flavor prior to being paired with malaise will attenuate subsequent taste aversion conditioning, a phenomenon referred to as latent inhibition (LI) (Lubow, 1989, 2009). Whilst the LI effect has been extensively evaluated using voluntary fluid ingestion, the effects of prior exposure on taste palatability remain unclear.

Investigation of nausea-induced conditioning that reflects changes in taste palatability, otherwise known as *conditioned disgust,* has interesting implications for the design of clinical intervention techniques to reduce the impact of nausea derived from chemotherapy treatments in cancer. Patients usually develop anticipatory nausea and vomiting to contextual cues related to the hospital environment, effects that can cause patients to discontinue treatment (Stockhorst et al., 2006; Scalera & Bavieri, 2009). It is therefore important to develop animal models to explain the acquisition and expression of conditioned nausea reactions that might be used as a preclinical tool to measure the effectiveness of antiemetic treatments (Limebeer et al, 2006; Rodriguez et al., 2000;

Symonds & Hall, 2000). Research on the neuropharmacological and physiological mechanisms of CTA can lead to a better understanding of the side-effects of chemotherapy treatments. Many studies have analyzed the neuronal underpinnings of CTA (Bernstein et al., 2009; Nuñez-Jaramillo et al., 2010; Yamamoto & Ueji, 2011).

One of the most widely-used methods is the immunocytochemistry of the c-fos (Bernstein et al., 2014 for a review in CTA). This is an immediate-early gene whose expression indicates a short-term change in neuronal activity as a result of a particular moment. This feature might be a disadvantage in some learning studies designed to assess changes throughout the learning process. In addition, using 2-DG (Sokoloff et al., 1997) or FDG (Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999) allows us to examine changes in the central nervous system. It is well known that, increased neuronal activity requires increased energy demand, which implies an elevation in blood flow and glucose levels. This leads to greater energy demand for oxygen consumption and cell respiration. Therefore, these techniques allow for the marking of glucose analogues to observe areas of higher energy consumption. Due to the close relationship between the electrical activity of neurons and oxidative energy metabolism, an alternative way to study brain activity is by using histochemical techniques such as the enzyme mitochondrial C oxidase (CO) (Wong-Riley, 1989; Gonzalez-Lima & Jones, 1994).

Cytochrome Oxidase is a mitochondrial enzyme implicated in the oxidative phosphorylation process in which ATP is generated. This histochemical technique identifies additional structures involved in long-term metabolic changes such as increased levels of enzymes or neurotransmitters, membrane protein synthesis, morphological changes (high postsynaptic density) and neuronal electrical increased activity, reflecting other process that also require ATP in the cell. CO histochemistry

has been used in studies of learning and memory in a variety of animal species (Arias, Morán, Conejo, & Arias, 2013; Bruchey & Gonzalez-Lima, 2008; Conejo, González-Pardo, Gonzalez-Lima, & Arias, 2010; Conejo, Cimadevilla, González-Pardo, Méndez-Couz, & Arias, 2013; Fidalgo, Conejo, Gonzalez-Pardo, Lazo, & Arias, 2012), but has not been used in taste aversion learning. The use of CO histochemistry in a LI paradigm provides a range of advantages over the use of early gene expression. In addition to methodological advantages, it allows for the assessment of cumulative changes throughout the learning process, not only in specific brain regions, but also in terms of their functional connectivity (Sakata, Coomber, Gonzalez-Lima, & Crews, 2009). In addition, the LI procedure provides advantages over other learning paradigms in CTA In particular, the memory of the taste has already been formed during preexposure, and this allows for a comparison of taste memory strength in groups having different levels of familiarity with the flavor. Therefore, the aim of the present study is to examine, using CO histochemistry, the brain regions involved in conditioned taste aversion. In particular, we aim to explore the neural networks related to latent inhibition of nausea-induced conditioned disgust reactions.

8.2. Experiment 8

8.2.1. Method

8.2.1.1. Subjects, apparatus and cannulation surgery

The subjects were 31 male Wistar rats weighting 190-339 g (258g mean weight) at the start of the experiment. Upon arrival, they were individually housed in opaque plastic cages in a room maintained at 21° C with a 12/12h light-dark cycle. All experimental manipulations were performed during the light portion of the cycle. Food and water were always available in the home cages. Except otherwise stated,

deprivation conditions apparatus, cannulation surgery and other procedural detail were the same as in the experiments described in chapter 5 and 6.

Orofacial reactions made by the animal during the infusion of fluids were recorded and subsequently quantified with the software 'The Observer XT 9.0′ (Noldus Information Technology) designed for recording and analyzing behavioral activity of animals. Two behavioral patterns were analyzed, appetitive reactions (tongue protrusions and mouth movement summated) and disgust reactions (gapes, chin-rubbing and paw-treading summated).

8.2.1.2. Behavioral procedure

After recovery from the surgery required in order to implant the cannula, the rats were randomly assigned to four groups: preexposed group (PE, n=8), non-preexposed group (NPE, n=8), preexposed control (PEC, n=7) and non-preexposed control (NPEC, n=8). During the course of the experiment, all rats had unlimited access to water and food and the experiments sessions were initiated at 10 a.m. Two days after the cannulation surgery the rats were habituated to the infusion procedure, receiving a 5ml water infusion (rate of infusion 1ml/min) in the conditioning apparatus and returned to home cages. On the following four days (see Table 12), the rats of groups PE and PEC received a daily session of 5 ml saccharin (0.1 %) intraorally (rate of infusion 1ml/1min) whereas group NPE and NPEC received water infusions. On the fifth day, the conditioning trial was carried out. This trial consisted of one single session of an intraoral saccharin infusion (0.1%) for 5 minutes. Immediately following the saccharin infusion, rats from groups PE and NPE were injected with LiCl (20 ml/Kg, .15 M). Rats in groups PEC and NPEC received the LiCl injection 24-h after the saccharin infusion. Subsequently, the rats were returned to their home cages in order to recover from the LiCl effects. The next day was a recovery day without any experimental procedures. On

the next day the TR test was carried out. This test consisted of an intraoral administration of 5 ml of saccharin (0.1%) at a rate of 1ml/min in the conditioning chamber while their orofacial reactions were videotaped.

TABLE 12. Design of the experiment 8

Group	Preexposure	Conditioning	TR test
PE	4 x Sac (IO)	Sac (IO) → LiCl	Sac (IO)
NPE	4 x Water (IO)		
PEC	4 x Sac (IO)	Sac (IO) / LiCl	
NPEC	4 x Water (IO)		

Note: PE and PEC refers to preexposed groups, and NPE and NPEC refer to non-preexposed. In addition, PE and NPE groups received LiCl injections during conditioning whereas PEC and NPEC received LiCl injections on the day following the conditioning trial. Sac = saccharin; IO = intraoral infusions; LiCl = lithium chloride injection.

8.2.1.3. Cytochrome oxidase histochemistry

The changes in metabolic activity were analyzed using Cox histochemistry. The procedure followed was described by Wong-Riley (1989) based on certain modifications proposed by Gonzalez-Lima and Jones (1994). The structures were selected for analysis using the atlas of Paxinos and Watson (2005). 90 minutes after the taste reactivity test, rats were sacrificed by decapitation. Their brains were rapidly removed and frozen by immersion in isopentane (Sigma-Aldrich, Spain) at -70º C for two minutes and stored at -40º C to prevent tissue damage with loss of enzyme activity.

Subsequently, the brains were sectioned coronally with a microtome cryostat (Microm International Gmblt, HM 505E, Heidelberg, Germany) in sections (30 µm thick) that were placed on slides previously been cleaned with alcohol at 100º. The sections were fixed with 0.5 % (v/v) glutaraldehyde and 10% (W/V) sucrose in 0.1 M

phosphate buffer, pH 7.6. After that, sections were rinsed in 0.1 M phosphate buffer with 10% (W/V) sucrose. Following this procedure, they were immersed in 0.05 M Tris buffered solution, pH 7.6 containing 275 mg/l cobalt chloride, 10% (w/v) sucrose and 0.5 % (v/v) dymethylsulfoxide. The sections were incubated in darkness for 2 h at 37º C in a staining bath containing 0,06 g cytochrome c, 0.016 g catalase, 40 g sucrose, 2 ml dimethylsulfoxide and 0.4 g diaminobenzidine tetrahydrocloride (Sigma-Aldrich, Spain) dissolved in 800 ml of phosphate buffer (pH 7.6; 0.1 M). Finally, the sections were fixed at 4% (v/v) buffered formalin with 10% (w/v) sucrose, dehydrated in alcohol chain and coverslipped with Entellan (Merck, Germany). A series of sections of rat liver cut at different thicknesses (10, 30, 50 y 70 µm) were included together with brain tissue in each bath. These sections were used as standards to control for staining variability across different incubation baths, and were obtained from rat brain homogenates. The tissue homogenates were obtained from the brains of 12 adult male Wistar rats killed by decapitation and homogenized on ice. To establish comparisons in the staining of sections from different staining baths, measurements were taken from Cox stained brain homogenate standards. These activity values allowed for the construction of a linear regression curve to convert measured values of optical density of the selected structures in cytochrome oxidase activity values, and were calculated for each incubation bath.

Using an image processing system (Leica Q550, Germany) with a light source (Leica DM-RHC) connected to a camera CCD (Lohu, Japan) and image analysis software (Leica Q-Win), relative optical density (OD) readings were obtained from the brain regions of interest: medial prefrontal cortex (mPFC) including cingulate (Cg), prelimbic (PrL) and infralimbic cortex (ILc); insular cortex (IC); bed nucleus of stria terminalis (BNST); central and basolateral amygdala (CeA and BLA); ventral tegmental

area (VTA); parabrachial nucleus (PBN); ventral posteromedial thalamic nucleus (VPM) and core and shell nucleus of accumbens (NAcb). A total of 25 measurements were taken per region. The measures were averaged to obtain one data point per region for each animal.

8.2.1.4. Statistical Analysis

The data were analyzed using SPSS version 19.0 (SPSS Inc., Chicago, USA). Data for behavior scored in taste reactivity test were analyzed by a 2 (preexposure) x 2 (drug) analysis of variance (ANOVA). Significant main effects or interactions were further analyzed by means of ANOVAs and post-hoc analyses (Student-Newman-Keuls test). Significance was accepted when $p < 0.05$ in all cases. Differences in CO staining of each brain region between the selected groups were analyzed by a similar analysis of variance (ANOVA). In order to examine functional connectivity between brain regions, pairwise correlations in CO activity were analyzed by calculating Pearson´s product-moment correlation coefficients ($p<.05$). A "Jackknife" procedure (Shao & Dongsheng, 1995) was used with CO activity normalized values (for each animal, CO activity of each region divided by mean activity in all regions). In this procedure, all possible pairwise correlations are calculated from removing one subject each time. Thus, only correlations present in all comparisons are maintained to avoid the effect of a single subject on the results.

8.2.3. Results

8.2.3.1. Taste reactivity test

Figure 11 (panel A) presents the mean number of conditioned disgust reactions displayed by rats during the 5-min intraoral infusion of saccharin on the TR test. It can

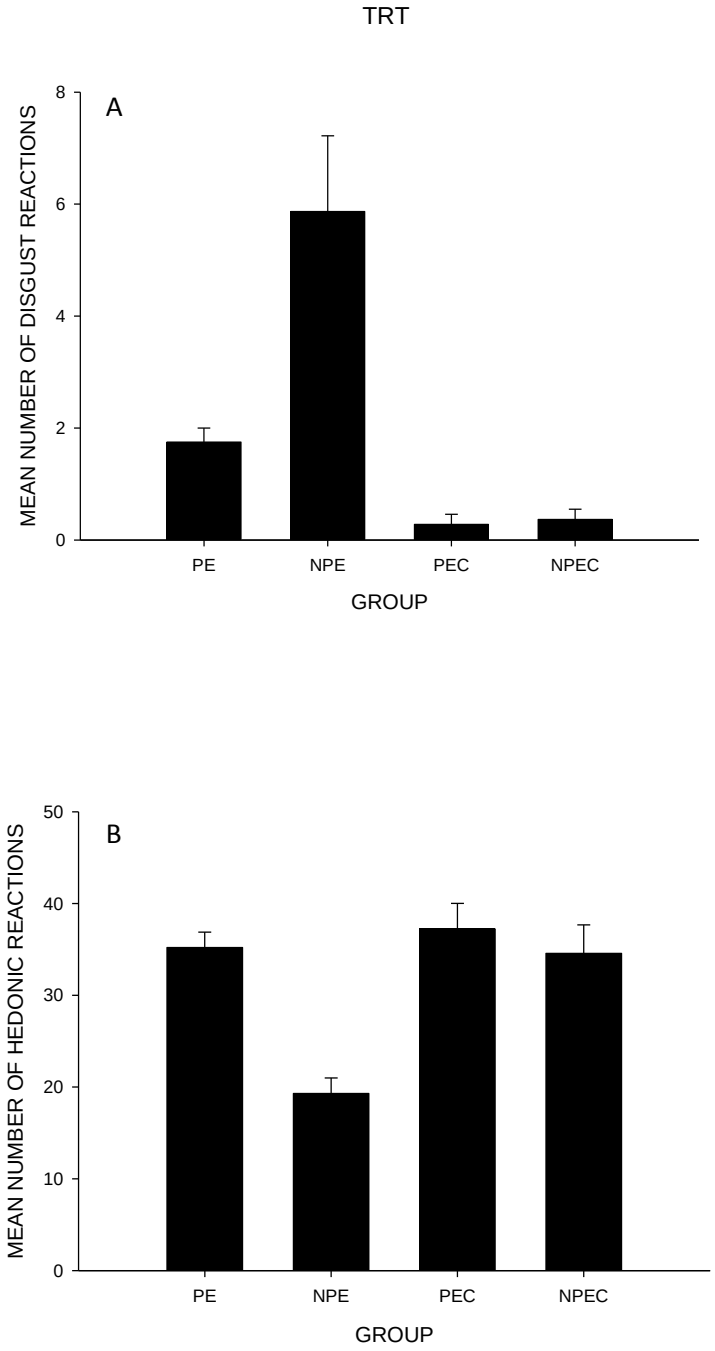


Figure 11: Experiment 8. Mean number of aversive and appetitive taste reactivity responses for the various groups: preexposed (PE), non-preexposed (NPE), preexposed control (PEC) and non preexposed control (NPEC).

be seen that rats in Group NPE showed a higher rate of aversive reactions than animals in the remaining groups, indicating that non-reinforced exposure to saccharin attenuated the establishment of LiCl-induced conditioned disgust reactions. The two-way ANOVA, with preexposure and drug as the between-subjects factors, conducted with these scores revealed significant main effects of preexposure [F (1,27) = 8.44; p = 0.007], conditioning [F (1,27) = 23.05; $p < 0.001$], and a significant preexposure x conditioning interaction [F (1,27) = 7.74; p = 0.010]. Subsequent comparisons using Student-Newman-Keuls tests showed that Group NPE displayed more aversive reactions to saccharin than the other groups, whilst the remaining groups did not differ from one another. Figure 11 (panel B) shows the mean number of appetitive reactions displayed by the rats during the TR test. The two-way ANOVA conducted with these data revealed a main effect of preexposure [F (1,27) = 15.326; $p < 0.001$] and conditioning [F (1,27) = 13.26; $p < 0.001$], and a significant preexposure x conditioning interaction [F (1,27) = 7.79; p = 0.010]. Post-hoc Student-Newman-Keuls comparisons showed that Group NPE displayed a lower rate of appetitive reactions to the saccharin infusion than the other groups ($p < 0.05$), which did not differ from one another.

8.2.3.2. CO activity

Table 13 shows mean CO activity measured in the regions of interest. The 2 (preexposure) x 2 (drug) ANOVA conducted on these data revealed a significant main effect of drug in mPFC subregions [cingulate, prelimbic and infralimbic; Fs (1,27) > 4.587; $ps < 0.041$], in bed nucleus of stria terminalis [F (1,27) = 12.55; $p < 0.001$], amygdala basolateral [F (1,27) = 4.28; p = 0.048], tegmental ventral area [F (1,27) = 11.064; p = 0.003], parabrachial [F (1,27) = 4.07; p =0.05] and thalamus [F (1,27) = 5.081; p = 0.033], but not a main effect of preexposure or preexposure x drug interaction ($Fs < 1$), with the exception of parabrachial [F (1,27) = 5.06; p = 0.033]. To

further analyze this interaction between conditioning and preexposure, separate one-way ANOVAs were performed with group as between group factor. These analyses revealed a significant effect of group [F (3,27) = 4.803; p = 0.008]. Subsequent Student-Newman-Keuls post hoc analysis showed that Groups PE and NPE expressed significantly lower CO activity in PBN than the remaining groups, which did not differ from one another.

TABLE 13. Cytochrome oxidase activity values of the measured regions

Regions	**PE**	**NPE**	**PEC**	**NPEC**
mPFC				
Cingulate	26.91 ± 1.28	27.51 ± 1.05	32.07 ± 0.80	33.13 ± 2.44
Prelimbic	27.76 ± 1.84	27.81± 0.96	30.96 ± 0.50	30.19 ± 0.96
Infralimbic	26.26 ± 1.68	26.72± 1.01	29.05 ± 0.63	28.99 ± 1.01
Accumbens				
Core	34.38 ± 1.70	36.97 ± 1.82	34.76 ± 0.97	36.07 ± 3.00
Shell	36.67 ± 1.71	37.36 ± 1.27	38.42 ± 1.08	41.52 ± 4.15
IC	24.62 ± 1.56	27.76 ± 1.72	27.89 ± 0.56	26.29 ± 1.01
BNST	25.75 ± 0.90	23.86 ± 1.00	28.26 ± 0.44	27.48 ± 0.89
AMY				
CeA	29.76 ± 1.50	28.81 ± 1.16	29.84 ± 0.54	29.98 ± 2.18
BLA	25.96 ± 2.41	27.57 ± 0.96	29.79 ± 0.57	31.13 ± 1.35
VTA	12.61 ± 1.00	15.40 ± 1.00	17.82 ± 1.04	17.51 ± 1.28
PBN	7.72 ± 0.79	9.16 ± 1.49	13.91 ± 1.63	11.82 ± 1.71
Thalamic n.				
VPM	18.75 ± 1.79	21.99 ± 0.95	24.32 ± 0.95	21.09 ± 2.02
VPL	28.71 ± 2.94	26.20 ± 2.83	21.10 ± 0.90	22.65 ± 2.34

Note: Data represent mean ± SEM values. mPFC = Prefrontal cortex; IC = Insular Cortex; BNST = bed nucleus of estria terminalis; AMY = amígdala (BLA = basolateral, CeA = central); VTA = ventral tegmental area; PBN= parabrachial nucleus; VPM = ventral posteromedial thalamic nucleus; VPL = ventral posterolateral thalamic nucleus. PE = preexposed group; NPE = non-preexposed group; PEC = preexposed control group; NPEC = non-preexposed control group.

Interregional correlations of CO activity were analyzed in order to establish different brain networks in each group. Figure 12 shows significant cross-correlations of CO activity for the groups. These correlations revealed two brain networks for Group

PEC. The first one included the parabrachial and tegmental ventral area with a positive correlation. The second network comprised a prefrontal cortical region, the cingulate cortices, which showed a positive correlation with the nucleus Accumbens core. In turn, NAcb shell had a positive correlation with CeA. This latter area also had a negative correlation with the ventral posterolateral thalamus, which showed a negative correlation with the prefrontal cortex and NAcb (shell).

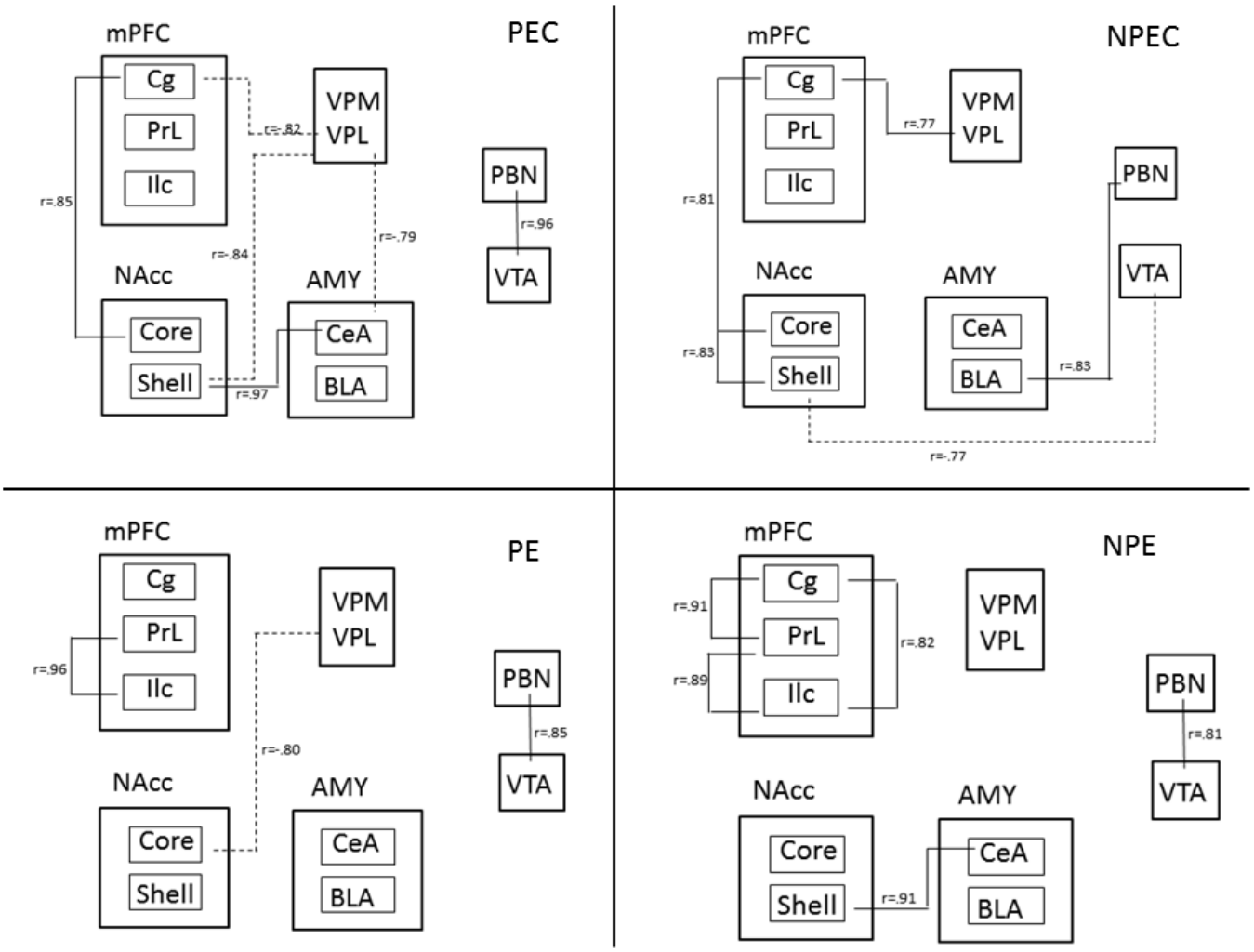


Figure 12: Schematic representations of interregional correlations of CO activity for the different groups. Solid lines represent high positive pairwise Pearson´s correlations (p<. 05), whereas negative correlations are represented by broken lines. Each quadrant represents a group correlation, namely, preexposed (PE), non-preexposed (NPE), preexposed control (PEC) and non preexposed control (NPEC).

For the NPEC group, correlations also revealed two brain networks. First, there was a positive correlation between the amygdala BSL and parabrachial. Subsequent correlations show a brain network that comprised cingulate cortices, which had a positive correlation with the thalamus and with the NAcb core and shell. Shell also showed a negative correlation with the tegmental ventral area.

PE and NPE groups showed few positive correlations between brain regions. In particular, the PE group showed positive correlations between prelimbic and infralimbic, NAcb core and thalamus, and finally between the parabrachial and tegmental areas. NPE group had positive correlations between the prefrontal areas (Cg, PrL and Ilc), Nacb shell and central amygdala, and between parabrachial and tegmental areas.

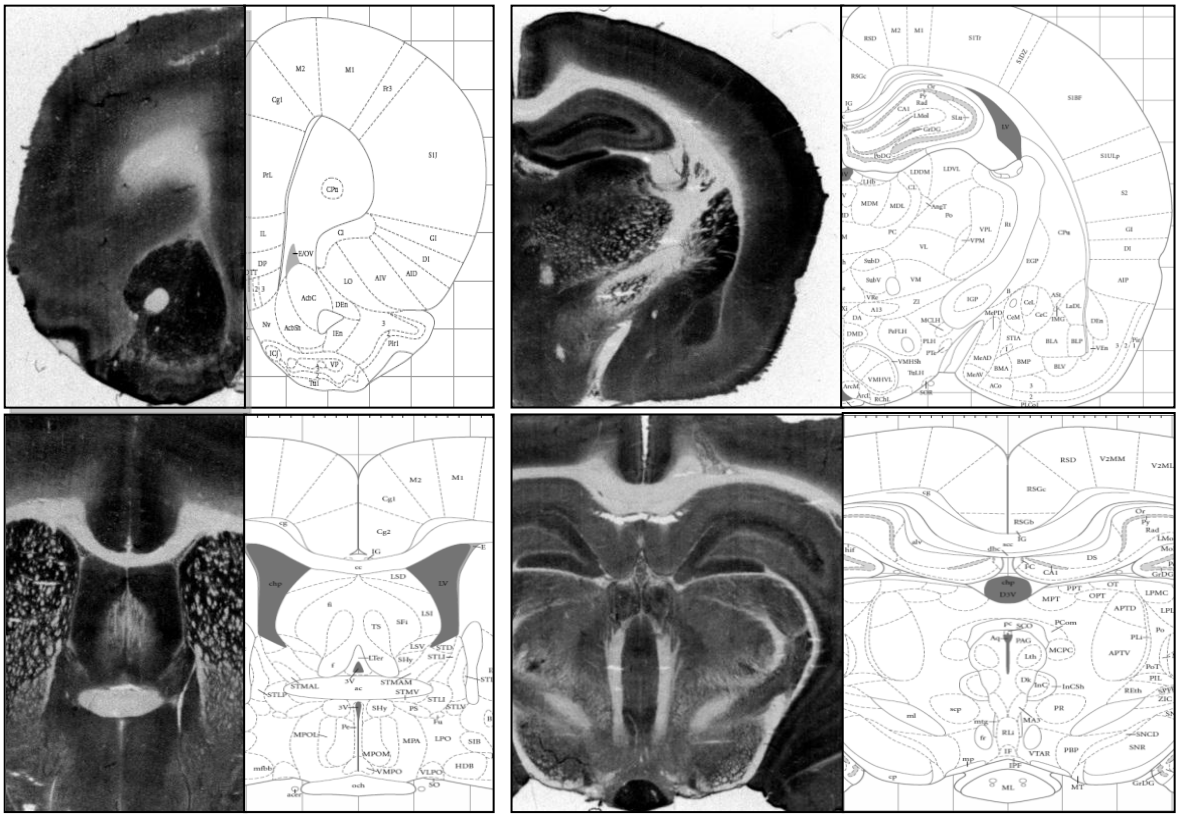


Figure 13: Microphotographs showing coronal CO stained sections and schematic representations of the selected brain regions.

8.3. Discussion

The aim of the present study was to examine the functional brain networks involved in the acquisition of nausea-induced conditioned disgust reactions. For this purpose, a latent inhibition (LI) paradigm was used to measure, at both the behavioral and neuronal level, the effect of non-reinforced flavor exposure on the subsequent establishment of a conditioned taste aversion. Behavioral data revealed that non-reinforced exposure to a saccharin solution prior to its pairing with nausea attenuates the development of conditioned disgust reactions displayed to the paired flavor. This effect is consistent with previous LI studies using a consumption test (Lubow, 1989, 2009) and from our own laboratory using the TR procedure (López et al., 2010). Similar results were found in Chapter 7 where we examined the microstructure of the licking behavior during the voluntary intake of the solution paired with the nausea, an alternative method to assess changes in palatability (see for instance Dwyer, 2012). We found that non-reinforced exposure to a flavor attenuated the subsequent taste aversion as measured by the size of the licking cluster (which is associated with changes in palatability) as well as the reduced consumption of the conditioned solution. As stated previously, measurement of changes in the palatability of both familiar and novel solutions associated with the state of nausea provides a useful tool to evaluate taste aversion learning. A further aim of this study was to provide a useful supplement to the behavioral information concerning taste aversion by exploring the metabolic changes that might occur as a result of this learning process.

With respect to CO activity, the results obtained in the present study showed a reduction of brain CO activity in BLA, thalamus, BNST, PBN, VTA and prefrontal areas in groups that have experienced saccharin-lithium pairings (PE and NPE). In addition, analysis of the connectivity between brain regions revealed different brain

networks in each group. Thus, group PEC, which had repeated experience with a pleasant taste, revealed two main brain networks (see Figure 9). First, a positive correlation was shown between the PBN and VTA. This is consistent with the suggestion that palatable foods such as saccharin or sucrose activate VTA neurons (Kosobud, Harris, & Chapin, 1994; Park & Carr, 1998) and its lesions selectively reduce consumption of a preferred sucrose solution (Shimura, Kamada, & Yamamoto, 2002). This relationship may be due to the parabrachial axons that pass through the VTA (Norgren, 1976) and the fact that this area is also connected with other limbic structures (Oades & Halliday, 1987). Indeed, the PBN might influence limbic structures such as the Nacb via direct connections with the VTA (Hajnal & Norgren, 2005).

The second network comprised the Nacb (shell and core), CeA and Cg interactions, all of which showed negative correlations with the thalamus nuclei. Several studies have shown that Nacb receives afferent inputs from mPFC and amygdaloid structures (see for instance, Yamamoto, 2006). Indeed, long-lasting increase in dopamine release in Nacb is indirectly modulated by amygdala-induced dopamine responses in mPFC (Hajnal & Norgren, 2005). NAcb has been associated with reinforced learning, i.e., an association between pleasant flavors (or unpleasant) and its consequences (Berridge, 2009). In particular, NAcb activity increased with intraoral infusions of pleasant flavors (Mark, Blander, & Hoebel, 1991). Our data suggest that brain regions involved in reward (NAcb), regions that send information of sensorial properties of flavors (thalamus) to the main candidates for integrate reward and sensory information (amygdala and mPFC) (Yamamoto & Ueji, 2011), were functionally paired when a familiar and pleasant flavor is intraorally administered.

Brain networks involved in the processing of familiar pleasant solutions differ from those involved in processing novel solutions. For group NPEC, two brain

networks may be established, a PBN-amygdala interaction, and a second network that comprises the VTA, NAcb, Cg and thalamus. These results are compatible with other research studies (Berridge & Robinson, 1998) in which the neural pathway of the brain reward system is described as being mainly comprised of the VTA and NAcb. As previously mentioned NAcb is interconnected with mPFC which is associated with feeding control and receives afferents from the thalamus via IC. Thalamus (VPM) on the other hand, is related to the processing of novel flavors, given that its lesions disrupt neophobia (Bures et al., 1998). Taken together, our results may suggest that information from the reward pathway (VTA and NAcb) and sensorial information (thalamus) regarding flavor novelty may converge in the mPFC to be integrated for the formation of flavor memories. Additionally, we have found a positive correlation between PBN and BLA. Previous studies revealed that exposure to a novel, but not familiar, saccharin solution induced robust increases of c-fos activity in the PBN and CeA but not in the BLA (Kho, Wilkins & Bernstein, 2003). However, the BLA may be involved in neophobia (Nachman & Ashe, 1974), and therefore in the processing of flavor novelty, given that injury in this area disrupts US-CS associations with a novel, but not familiar CS (Reilly & Bornovalova, 2005 for a review). It is also accepted that initial integration of gustatory and visceral signals takes place in PBN (Bures & Buresova, 1990; Reilly, 1999; Yamamoto, 2006) and as well as the amygdala, PBN plays a crucial role in the processing of novel flavors and the consequences derived from their intake (Agüero, Arnedo, Gallo, & Puerto, 1993; Reilly, Grigson, & Norgren, 1993; Sakai & Yamamoto, 1997).

Finally, our data shows a positive correlation between the PBN and VTA in both conditioned groups, that is, groups PE ad NPE. It should be noted that groups PE and NPE showed decreased CO activity in the PBN and VTA compared to the remaining

groups. As already indicated, PBN is the candidate structure for US-CS associations, whereas VTA is involved in fluid reward (regardless of negative or positive hedonic fluid value) (Reilly et al., 1993). Additionally, data show a positive correlation between the mPFC subregions for the novel CS-US association, and a PrL- Ilc subregions correlation in familiar CS-US associations. This may indicate that CTA learning requires the involvement of all subregions of the mPFC for taste memory formation. This is consistent with other lesion studies in which an impairment of CTA is observed as a result of mPFC injures (Hernádi et al., 2000; Karádi et al., 2005). In addition, we found a negative correlation between Nacb and thalamus nuclei in group PE, indicating that both structures may mediate the hedonic shift from positive to negative, and the data almost implicate the involvement of NAcb in this shift (Yamamoto & Ueji, 2011). But more interesting is the amygdala-NAcb interaction found in the proper taste aversion group, that is, Group NPE. It is known that both regions are essential for CTA expression (Yamamoto, 2006). Some studies have associated the amygdala with the processing of visceral information, given that LiCl injections increase c-fos activity in this region (Ferreira et al., 2006), linking its function to the recognition of US information. However, as mentioned above, other studies suggest a central role for this structure in the formation of CS-US association, relating BLA with aversive conditioning (Schafe & Bernstein, 1996) and with the formation of fear learning in general. But CeA is the main output route for other brain regions related to the expression of specific emotional responses (Ledoux, 2000), and its specific role remains unclear. Our data may link this region to the activity of the NAcb in the formation of taste aversion memory. However, since a similar relation was found in the PEC group, it may be the case that an amygdala-NAcb connection may be involved in both appetitive and aversive rewards.

It is important to emphasize that the CO histochemistry technique assesses changes throughout the learning procedure, and its temporality remains unknown. This means that in contrast to other techniques (early gene expression, for instance) in which activity changes are measured at a specified point in time, the COx technique allows for the measurement of accumulative changes. This may result in unchanged activity in brain regions traditionally related to IL in CTA, or alternatively, these brain regions may not elicit long-term changes in CO activity.

In conclusion, the present study provides the first demonstration of oxidative metabolic activity in brain regions involved in nausea-induced conditioned disgust. In particular, saccharin conditioning with lithium decreased CO activity in the PBN, VTA, BLA, BNST and mPFC areas. In addition, we found novel activation patterns of brain networks thought to be involved in the processing of flavors and their hedonic value. We highlighted a parabrachial-ventral tegmental area interaction and an accumbens-amygdala interaction that may be involved in both appetitive and aversive reward.

Capítulo 9

Actividad c-Fos asociada al efecto de preexposición al EC en aversión al sabor

c-Fos activity associated to the CS-preexposure effect in CTA

9.1. Introduction

As previously discussed in chapters 7 and 8, when rats are given nonreinforced exposure to a flavor prior to conditioning with LiCl, the resulting aversion is abolished or attenuated, a phenomenon referred as the latent inhibition effect (Lubow, 1989, 2009). In a recent study of our laboratory conducted to evaluate whether flavor exposure concurrently attenuates the effects of taste aversion on both fluid consumption and disgust reactions as an index of taste palatability, we found that preexposure not only disrupts suppressed consumption of the LiCl-paired taste, but also attenuates the establishment of conditioned disgust reactions in the TR test (López et al., 2010). Analyzing the microstructure of licking behavior during voluntary consumption as an alternative method to assess taste palatability in rats (see Dwyer, 2012), we have also reported that hedonic responses produced during taste aversion learning can be influenced by prior exposure to the to-be conditioned flavor (Chapter 7). Based upon this finding, in the present study we examined neural activity correlated with hedonic reactivity to taste stimuli in flavor aversion learning as a function of taste novelty. Despite the evidence that taste familiarity influences neural activity in CTA (for reviews, see Bernstein et al., 2009; Nuñez-Jaramillo et al., 2010), much less is known about the brain regions mediating hedonic reactions in this learning paradigm.

Concerning taste familiarity, differential c-Fos protein expression has been reported as a function of the novelty of the taste stimuli in brain regions identified as being involved in CTA including insular cortex (IC) and nucleus accumbens (NAcb). Taste aversion for a novel solution induces a greater increase in c-Fos expression in the IC relative to the same taste when it has become familiar (e.g., Koh & Bernstein, 2005; Koh et al., 2003). There is also evidence from brain lesion studies showing that IC lesions disrupt taste aversion learning depending on the taste novelty. For example,

lesions of the IC attenuate taste aversion learning when the taste stimulus is novel but not following consumption of a familiar taste (e.g., Kiefer & Braun, 1977). However, it has been recently suggested that the IC might be involved in taste neophobia (i.e., reluctance to consume a novel and potentially dangerous taste stimulus) rather than in CTA (e.g., Roman, Lin & Reilly, 2009; Roman & Reilly, 2007). It has been reported that IC lesions produce a decrease in taste neophobia, producing a latent inhibition effect (Lin et al., 2012; Roman & Rilley, 2007). Accordingly, a different pattern of c-Fos expression has been observed depending of the taste novelty. A significant elevation in c-Fos expression emerges in the IC following the exposure to a novel saccharin solution relative to the same taste when it becomes familiar (e.g., Lin et al., 2012), suggesting that IC has a critical role in the neophobic reaction to novel tastes. Likewise, other studies using in vivo microdialysis have reported that a novel taste induces a marked increase in the release of acetylcholine (ACh) relative to a familiar taste (e.g., Miranda, Ramírez-Lugo & Bermudez-Rattoni, 2000). These findings are congruent with those from lesion studies (e.g., Moraga-Amaro et al., 2014; Stehberg, Moraga-Amaro & Simon, 2011) suggesting a dissociation of brain areas involved in CTA and taste familiarity learning. There is also some evidence that IC may play a role in processing affective aspects of the gustatory experience. For example, recent studies using *in vivo* optical imaging have found plastic changes in the gustatory cortex after inducing a shift in the hedonic value (from positive to negative) of a taste stimulus (e.g., Accolla & Carleton, 2008; Carleton, Acolla & Simon, 2010).

Relative to the NAcb, while it has been found that taste aversion induces Fos activation in the NAcb shell following consumption of a novel taste (e.g., Ferreira et al., 2006; Yasoshima et al., 2006), there is no solid evidence that prior nonreinforced exposure to the taste attenuates c-Fos activity in the NAcb. In this regard, Turgeon and

Reichstein (2002) found no differences in c-Fos levels in either the shell or the core of the nucleus accumbens depending of prior taste experience. Concerning the role played by the NAcb in the hedonic reactivity to gustatory stimuli, it has been reported that the appetitive and aversive value of taste stimuli is encoded by opposite changes in NAcb neural activity (e.g., Carlezon & Thomas, 2009; Roitman, Wheeler, Tiesinga, Roitman, & Carelli, 2010). For example, Roitman et al. (2010) using electrophysiological recording techniques have examined NAcb activity during the expression of a CTA. These authors reported an increase in the firing rate of NAcb neurons in response to intraoral infusions of a sucrose solution previously paired with LiCl, indicating a reduction in the hedonic value or palatability of the taste. Interestingly, a similar shift toward excitatory responses in NAcb neurons is observed after the infusion of an unpalatable taste such as quinine (e.g., Roitman, Wheeler, & Carelli, 2005). Conversely, infusion of sucrose induces a reduction in firing rate of NAcb neurons, suggesting that inhibition of the NAcb activity is associated with positive hedonic reactions to taste stimuli (e.g., Roitman et al., 2005; Taha & Fields, 2005).

Based on these findings, in the current experiment we examined c-Fos expression in the insular cortex and the nucleus accumbens (shell and core nuclei) following the acquisition of LiCl-induced conditioned disgust. We also examined whether the elicited neural activation by taste aversion in these brain areas depends on prior nonreinforced exposure to the saccharin. It is hypothesized that attenuation of conditioned disgust, as an index of taste palatability, following nonreinforced flavor exposure in CTA is related to the neural activity in brain areas involved in coding the hedonic value of taste stimuli.

9.2. Experiment 9

9.2.1. Materials and methods

9.2.1.1. Subjects, fluids, apparatus and cannulation surgery

Thirty two Wistar rats, which were bred in our animal facility (University of Oviedo, Spain), were used in this experiment[4]. Their weights before beginning of the study ranged from 190 to 339 g, with a mean weight of 258 g. Upon arrival, the rats were individually housed in opaque plastic cages, under constant temperature (21° C), and a 12 hr light-dark cycle (light on at 08:00 h). All experimental manipulations performed during the light portion of the cycle. Throughout the experiment, water and food were always available in the home cages. The experimental procedures were carried out in accordance with the guidelines for care and use of experimental animals of the Spanish regulation (RD 1201/2005) and the European Communities Council Directive (86/609/EEC). Except otherwise stated, deprivation conditions apparatus, cannulation surgery and other procedural detail were the same as in Chapter 5, 6 and 8.

The fluids used were solutions of LiCl (0.15 *M*) and saccharin (0.1% w/v). LiCl was administered intraperitoneally (i.p.) at a volume of 20 ml/kg of body weight. The saccharin solution was intraorally infused for 5 min at the rate of 1 ml/min and the orofacial reactions displayed by rats were recorded.

9.2.1.2. Behavioral procedure

During the course of the experiment, one rat lost the cannula and was remove from it. The remaining rats were randomly assigned to four groups of subjects as follows: Pre-Paired (n = 8), Non-Paired (n = 8), Pre-Unpaired (n = 7) and Non-Unpaired (n = 8). Three days after the surgery, the rats were habituated to the intraoral method of fluid presentation. They were placed in the conditioning chamber (i.e., the taste

[4] Animals in this chapter were the same used in Chapter 8 for Co analysis. Brain sections were obtained in order to analyze c-fos and CO activity.

reactivity apparatus) with their cannula attached to the infusion pump for fluid delivery, and IO with water for 5 min at the rate of 1 ml/min. The next four days constituted the taste preexposure phase. On these sessions, the subjects in Groups Pre-Paired and Pre-Unpaired (preexposed groups) were placed in the conditioning chamber and intraorally infused with 0.1% saccharin for 5 min at a rate of 1 ml/min, whereas those in Groups Non-Paired and Non-Unpaired (nonpreexposed groups) were given 5-min intraoral infusion of distilled water. The day following the final preexposure session, the conditioning trial was carried out. In this session, all rats were placed in the conditioning chamber and intraorally infused with 0.1% saccharin for 5 min at a rate of 1 ml/min while their orofacial responses were video-recorded. Immediately after the infusion of saccharin, the rats in Groups Pre-Paired and Non-Paired were injected with LiCl (0.15 M; 20 ml/kg), whereas those in Groups Pre-Unpaired and Non-Unpaired received the injection of LiCl 24 hr after the infusion of saccharin. After a recovery day in the home cages, the taste reactivity (TR) test was carried out. On this session, all rats were infused with the 0.1% saccharin solution in the TR chamber for a period of 5 min while their orofacial responses were recorded. The rats were returned to their home cages following the TR test.

9.2.1.3. Tissue preparation and c-Fos quantification

Ninety minutes after completing the behavioral test, the rats were decapitated and their brains were removed to be frozen rapidly in isopentane (Sigma-Aldrich, Germany) and stored at -40˚ C. Coronal 30 μm-thick brain sections were obtained using a cryostat (Leica CM1900, Germany). The sections were mounted on gelatinized slides, which were post-fixed in buffered 4% paraformaldehyde (0.1 M, pH 7.4) during 30 min. Subsequently all slides were rinsed in phosphate buffered saline (PBS) (0.1M, pH 7.4). Slides were incubated for 15 min with 3% hydrogen peroxidase in PBS and were

washed in PBS two times. They were introduced in a PBS solution with 10% Triton X-100 (PBS-T) (Sigma, USA) and 3% bovine serum albumin for 30 min. The slides were washed twice in PBS before incubated with a rabbit polyclonal anti c-Fos solution (1:10000) (Santa Cruz Biotech, Sc-52, USA) diluted in PBS-T for 24 h at 4˚C in a humid chamber.

The following day, slides were washed three times in PBS and incubated in a goat anti-rabbit biotinylated IgG secondary antibody (Pierce, USA; diluted 1:200 in incubating solution) for 2 h at room temperature. The slides were then washed three times in PBS, and later on the sections were reacted with avidin-biotin peroxidase complex (Vectastain ABC ultrasensitive Elite Kit, Pierce) for 1 h and followed by two additional washes. The reaction was visualized treating the sections for about three min in a commercial nickel-cobalt-intensified diaminobenzidine Kit (Pierce). Finally, the reaction was finalized by washing the section twice in PBS, and dehydrated through a series of degraded alcohol baths, cleared with xylene and coverslipped with Entellan (Merck, USA) for microscopic observation.

c-Fos expression was measured in forebrain regions previously selected: the insular cortex, amygdala (central and basolateral nuclei), and nucleus accumbens (core and shell regions). For each brain region, the total number of c-Fos positive nuclei was quantified using three representative sections according to the Paxinos and Watson atlas (2005). The profiles of the selected brain regions were first outlined in the slides using a permanent marker. The quantification was carried out by systematically sampling each of the selected brain regions using counting frames superimposed over the region. Each counting frame used for quantification had a total area of 0.0225 mm. Cell counting was performed using a microscope (Olympus BH-2 Japan) and a TV monitor (300 x total magnifications). c-Fos positive nuclei were defined based on homogenous gray-black

stained elements with a well-defined border. In order to calculate the numerical density, the area of a counting frame was multiplied by the total number of counting frames used for each region. Finally, the mean density of c-Fos positive nuclei was calculated dividing the total number of c-Fos positive nuclei by the area quantified.

9.2.2. Data analysis

The orofacial reactions displayed by the rats during the preexposure phase were analyzed by a repeated measures analysis of variance (ANOVA) with group and trial as the factors. Differences between groups in taste reactivity during conditioning and testing were evaluated by means of two-way ANOVAs, with preexposure and conditioning as the factors, and post hoc comparisons by Student-Newman-Keuls tests. The behavioral data entered into analysis were the total number of disgust reactions (gapes, chin rubs, and paw treads summated) and the duration of appetitive reactions (tongue protrusions and mouth movements summated). Significant differences in the density of c-Fos positive nuclei between groups for each brain region selected were also calculated using two-way ANOVA analysis. Data are presented as the mean $\pm$ standard error of the mean. All data were analyzed using SPSS 15.0 for Windows. A significant level of $p < 0.05$ was adopted.

9.3. Results

9.3.1. Behavioral data

The Figure 14 (panel B) presents the animals´orofacial reactions registered during the preexposure phase. As shown in the figure, the rats infused with saccharin (Pre-Paired and Pre-Unpaired) displayed a higher rate of appetitive responses (tongue protrusions and mouth movements) than rats infused with water (Non-Paired and Non-Unpaired). Furthermore, the number of appetitive responses in the preexposed groups did not increase across the sessions, indicating that there was no evidence of neophobic

reaction to the saccharin solution. A 4 (group) x 4 (trial) repeated measures ANOVA conducted on these data showed a significant main effect of group, F (3,27) = 40.91; p < 0.001, but no effect of trial, F(3,81) = 1.65; p = 0.184, nor a significant interaction between these two factors, F(9,81) = 1.27; p = 0.264. Subsequent ANOVAs for each trial revealed a significant effect of group in trials 1-4 [*Fs* (3,27) >16.06; *ps* < 0.001]. The post-hoc analyses (Student-Newman-Keuls tests) confirmed that subjects in Groups Pre-Paired and Pre-Unpaired displayed appetitive reactions at a higher rate than did the Groups Non-Paired and Non-Unpaired over the 4 preexposure trials (*ps* < 0.05).

A two-way ANOVA, with preexposure (saccharin *vs* water) and conditioning (paired *vs* unpaired) as the factors, for the aversive reactions elicited by the infusion of saccharin during the conditioning session revealed no significant effects of preexposure and conditioning, nor an interaction between these two factors [*Fs* (1,27) < 2.86; *ps* > 0.121], reflecting the absence of any evidence of aversive reactions to the saccharin. The mean number of aversive responses displayed by the different groups in this session was: Pre-Paired: 1.12 (± 0.22); Non-Paired: 1.10 (± 0.26); Pre-Unpaired: 0.42 (± 0.22); Non-Unpaired: 0.75 (± 0.25).

A similar analysis of the appetitive reactions during this session revealed that there were no main effects of preexposure and conditioning, and that there was no interaction between these factors (*Fs* < 1), indicating that taste reactivity during conditioning was not influenced by preexposure phase. The mean duration of appetitive responses elicited by the infusion of saccharin during this session for the four groups in this session was: Pre-Paired: 36.38 (± 1.40); Non-Paired: 36.55 (± 1.95); Pre-Unpaired: 34.88 (± 2.85); Non-Unpaired: 35.54 (± 1.67).

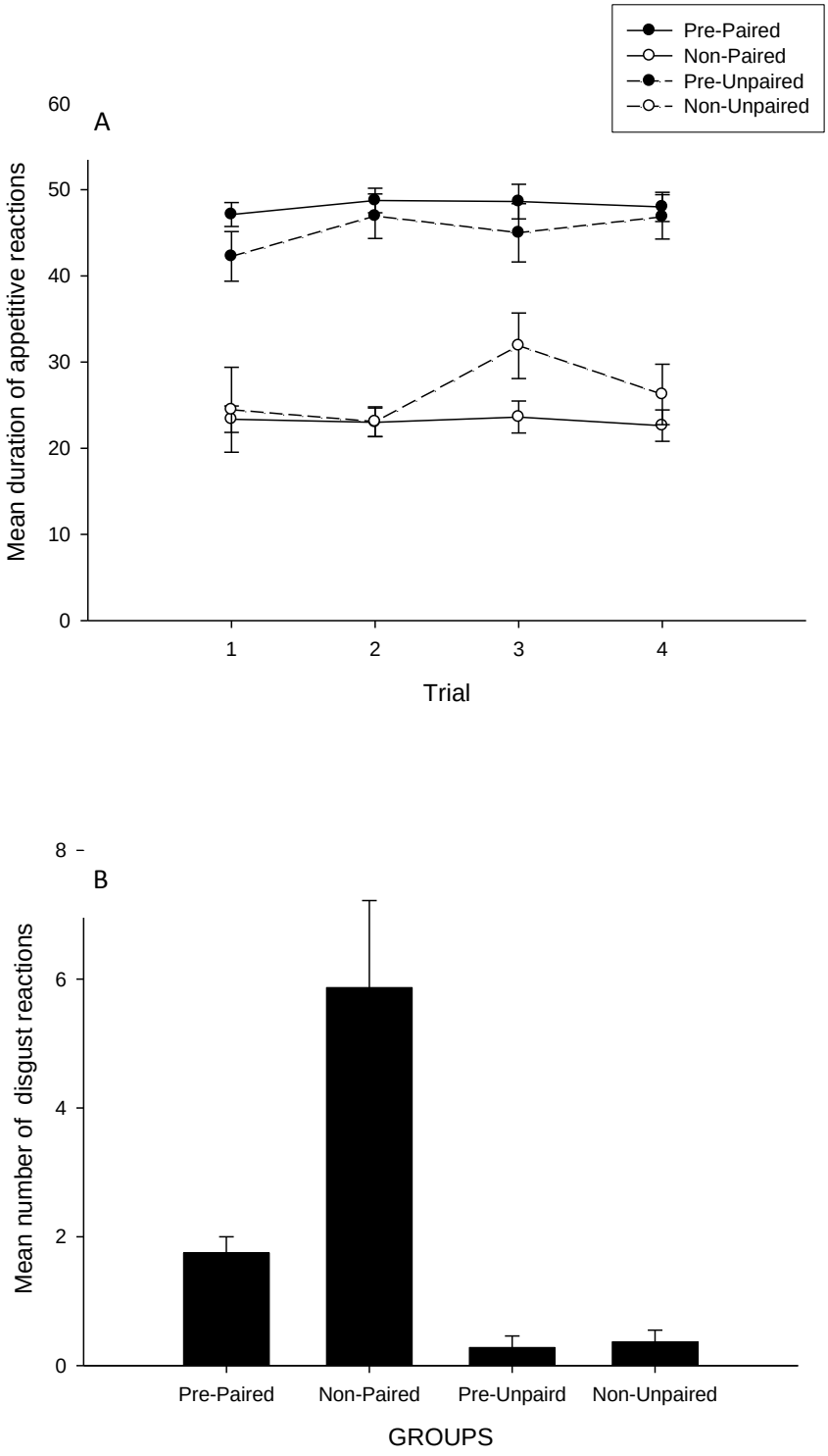


Figure 14: Experiment 9. Pannel A: Mean number of appetitive reactions elicited by the infusion of saccharin during the preexposure sessions for the different groups. *Panel B:* Mean number of disgust reactions displayed by the different groups during the infusion of saccharin in the taste reactivity test (error bars indicate *SEMs*).

Figure 14 (panel B) presents the mean number of disgust reactions elicited by the infusion of saccharin during the taste reactivity test. It shows that rats in Group Pre-Paired displayed less aversive responses (gaping, chin rubbing and paw treading) than rats in Group Non-Paired, indicating that preexposure to saccharin attenuated the effects of taste aversion (i.e., a latent inhibition effect) as assessed by conditioned disgust reactions. It also shows that Group Pre-Paired did not differ in its number of aversive reactions from Groups Pre-Unpaired and Non-Unpaired. The 2 (preexposure: saccharin *vs* water) x 2 (conditioning: paired *vs* unpaired) ANOVA conducted with these data revealed significant main effects of preexposure, $F(1,27) = 8.44$; $p = .007$, and conditioning, $F(1,27) = 23.05$; $p < .001$, and a significant interaction between these two factors, $F(1,27) = 7.74$; $p = .010$. Simple main effects analysis of the interaction showed a significant difference among the groups, $F(3,27) = 13.27$; $p < .001$). Subsequent comparisons among the groups using the Student-Newman-Keuls test confirmed that the Group Non-Paired displayed significantly more disgust reactions than the remaining groups, which did not significantly differ from each other. As well, the analysis of the appetitive reactions displayed by the rats during this session revealed significant main effects of preexposure and conditioning, and a significant interaction between these two factors, *Fs* (1,27) > 15.32; *ps* < .001). The one-way ANOVA conducted with these scores showed a significant effect of group, $F(3,27) = 12.30$; $p < .001$). The post hoc analysis (Student-Newman Keuls) showed that the Group Non-Paired differed significantly from each of the other three groups, which did not themselves differ. The mean duration of appetitive responses for the different groups in this session was: Pre-Paired: 35.22 ($\pm$ 1.66); Pre-Unpaired: 19.31 ($\pm$ 1.68); Non-Paired: 37.24 ($\pm$ 2.77); Non-Unpaired: 34.58 ($\pm$ 2.09).

9.3.1. c-Fos immunohistochemistry

Of the 31 rats used for the behavioral test, 6 rats per group contributed to the c-Fos quantification[5]. Figure 15 (panel A) shows the mean number of Fos-positive nuclei per 0.1 µ (relative quantified area selected) in the IC for the different groups (see also Figure 15 showing representative photomicrograhs).

Inspection of Figure 15 suggests that the number of positive nuclei was lower for the preexposed groups (Pre-Paired and Pre-Unpaired) than for the non-preexposed groups (Non-Paired and Non-Unpaired), indicating that c-Fos expression in the IC was attenuated in animals receiving nonreinforced exposure to saccharin. Confirming this description of the data, a 2 x 2 ANOVA, with preexposure and conditioning as the factors, showed a significant effect of the preexposure factor, $F(1,20) = 27.82$; $p < .001$, but no effect of conditioning, nor a significant interaction between these two factors (*Fs* <1). A one-way ANOVA performed on these data showed a significant difference among the groups, $F(3,20) = 9.77$; $p < .001$. Post hoc comparisons (Student-Newman-Keuls) indicated that c-Fos expression in the IC was significantly lower in the groups preexposed to the saccharin (Pre-Paired and Pre-Unpaired) as compared to the non-preexposed groups.

Panel B of figure 15 presents the number of positive nuclei in the core and shell regions of the nucleus accumbens. In both NAcb regions, the density of Fos-positive nuclei was significantly lower in Group Pre-Paired than in all of the other groups, indicating that Fos activation in these nuclei following taste aversion depended mainly on prior exposure to the taste to be conditioned.

[5] The analysis conducted with the taste reactivity scores from these rats showed the same pattern of results than that described in the previous section for the entire sample of subjects.

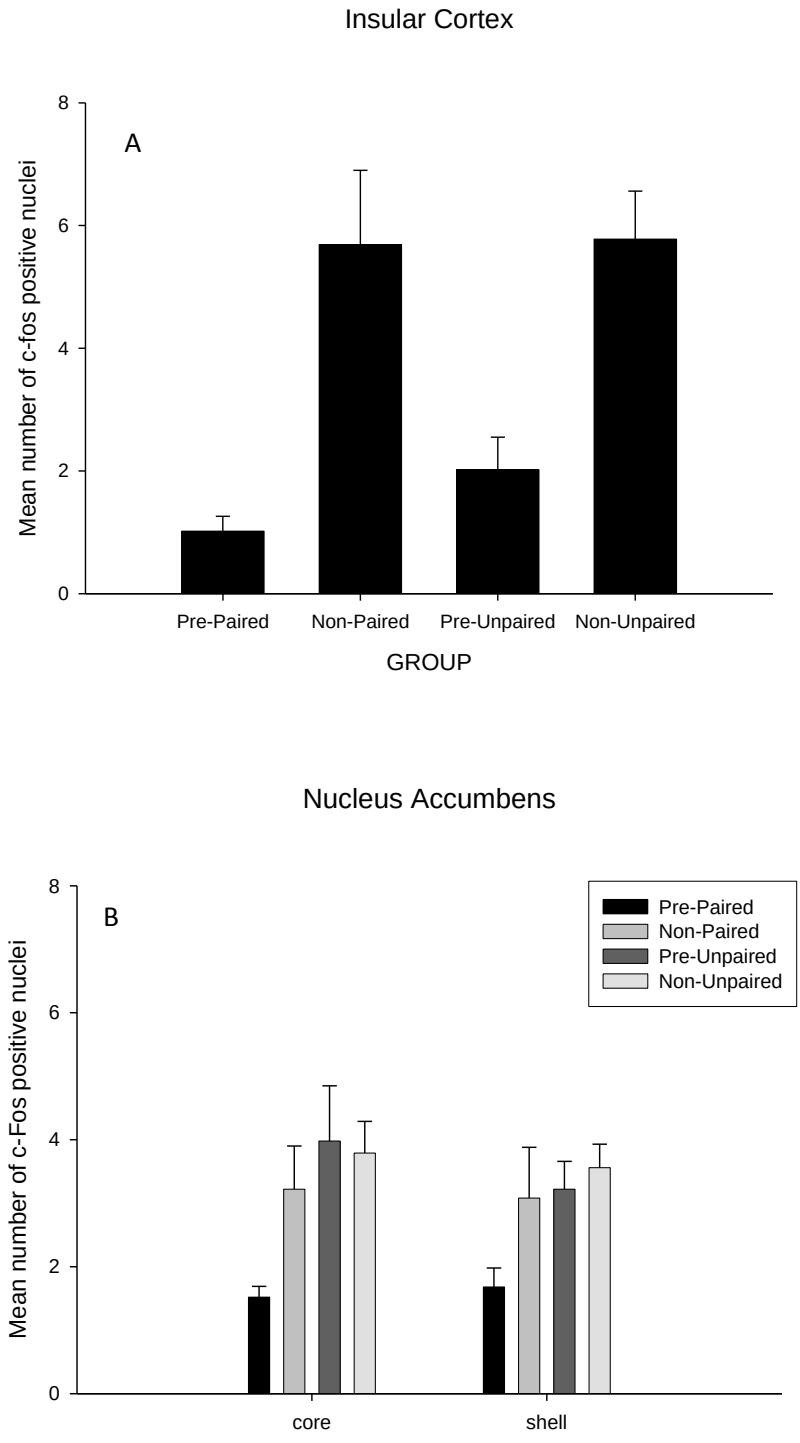
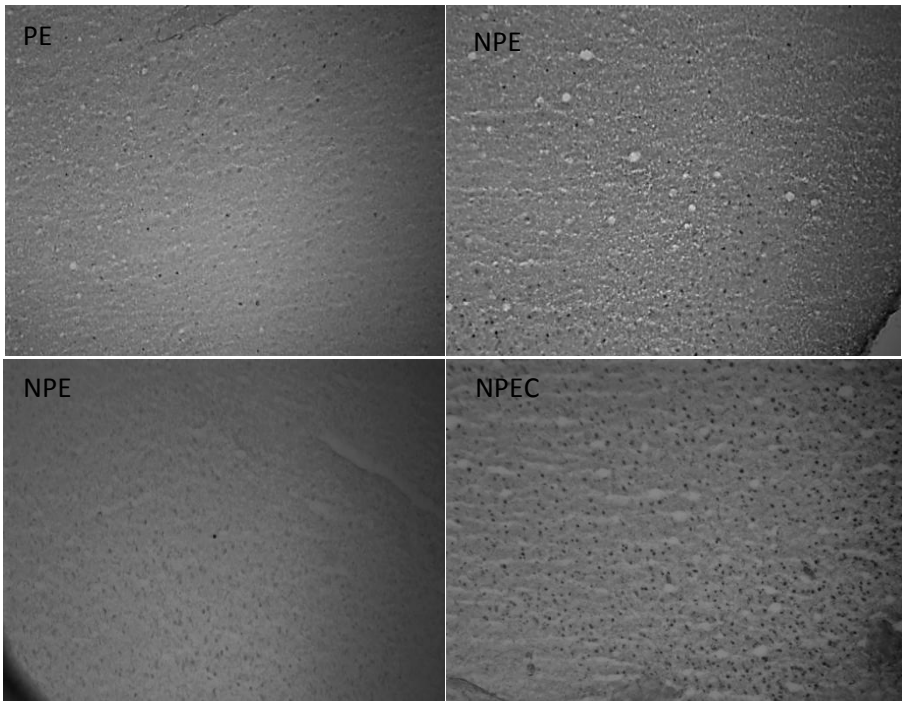


Figure 15: Experiment 9: Mean number of Fos-positive nuclei in 0.1 µ in the IC (A), and the NAcb shell and core (B) (error bars indicate *SEMs*).

A) Insular cortex



B) Accumbens

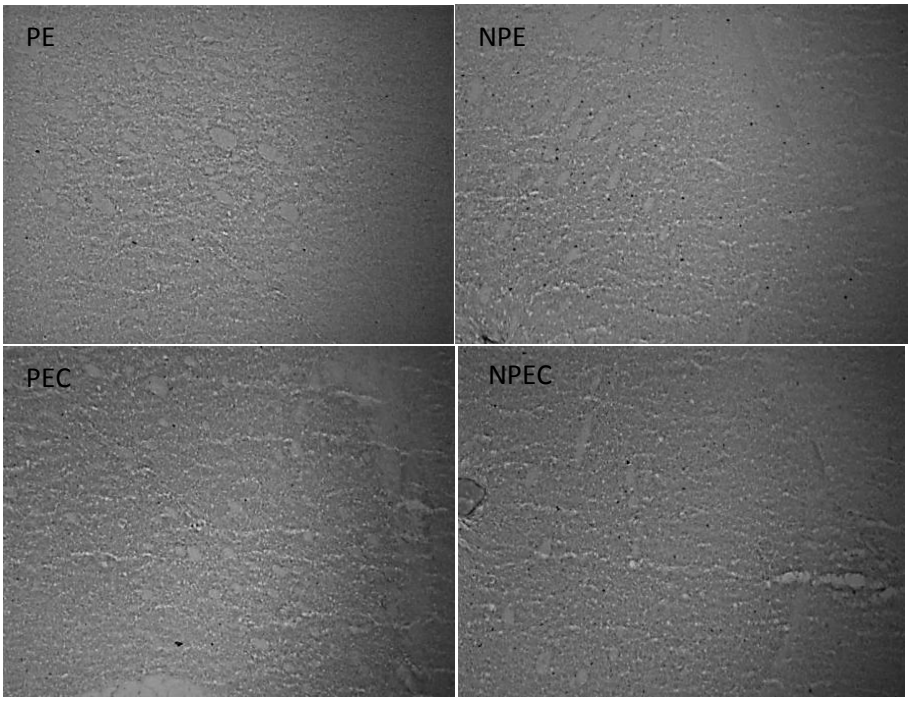


Figure 16: Note: Representative photomicrographs of c-Fos immunoreactivity in the IC (A), and the core (B) and shell (C) nuclei of NAcb 90 min following the saccharin infusion in the taste reactivity test.

Concerning the NAcb core, a two-way ANOVA (preexposure x conditioning) performed on the mean number of c-Fos positive nuclei showed no effect of preexposure, $F(1,20) = 1.16$; $p = 0.293$, a significant effect of conditioning, $F(1,20) = 8.51$; $p = .008$, and a significant interaction between these factors, $F(1,20) = 5.68$; $p = .027$. A further one-way ANOVA revealed a significant difference among the groups, $F(3,20) = 5.12$; $p = .009$. Post hoc comparisons showed that the number of positive nuclei in the NAcb core was lower only in the Group Pre-Paired, which had been preexposed to saccharin and injected with LiCl. As well, the 2 x 2 ANOVA concuted with the number of positive nuclei in the NAcb shell revealed a significant main effect of preexposure, $F(1,20) = 9.63$; $p = .009$, but no effect of conditioning, $F(1,20) = 3.54$; $p = .074$, nor a significant interaction between these two factors, $F(1,20) = 5.40$; $p = .003$. A subsequent one-way ANOVA conducted with these scores revealed a significant effect of group, $F(3,20) = 6.19$; $p = .004$. The post hoc analysis (Student-Newman Keuls) showed that Groups Pre-Paired had a significant lower c-Fos activity than the remaining groups, which did not differ from each other.

In short, the pattern of neural activation observed in this study indicates that repeated exposure to saccharin before aversive conditioning with LiCl attenuated c-Fos expression in the core and shell regions of the NAcb. In contrast, nonreinforced exposure to the taste before conditioning did not influence the level of IC activation.

9.4. Discussion

The behavioral data obtained in this experiment show that nonreinforced exposure to saccharin prior to the acquisition of a LiCl-induced saccharin aversion attenuates the development of conditioned disgust reactions, indicating that the effects of taste aversion on hedonic taste reactivity had been reduced. This result is consistent

with previous experiments using the taste reactivity test to examine changes in taste palatability following flavor preexposure in taste aversion learning. As already noted, López et al. (2010) found that nonreinforced exposure to a flavor prior to conditioning with LiCl attenuates both suppressed consumption and aversive taste reactions, suggesting that the attenuating effects of flavor preexposure in taste aversion learning (i.e., the latent inhibition effect) can be assessed using both voluntary fluid intake and the display of disgust reactions in the taste reactivity test. This conclusion converges with that of Chapter 7 analyzing the microstructure of licking behavior during voluntary consumption as an alternative method to assess taste palatability in rats (see Dwyer, 2012). In this test, larger lick cluster sizes (i.e., sustained runs of rapidly occurring rhythmic licks separated by pauses) are associated with the consumption of palatable tastes, and lower lick cluster sizes with unpalatable solutions or taste stimuli previously paired with LiCl. Using this method, we found that flavor preexposure also attenuates the effects of taste aversion on the size of licking clusters. Therefore, hedonic shifts produced during taste aversion learning can be influenced by prior taste experience.

The results of primary interest in this study are those from the neural activity associated with conditioned disgust reactions in the brain regions examined. Concerning the insular cortex, our results show that c-Fos expression in this area did not depend on the establishment of a taste aversion, but rather on the taste novelty. Non-reinforced exposure to saccharin (Groups Pre-Paired and Pre-Unpaired) reduced c-Fos activation as compared with the non-preexposed groups (Groups Non-Paired and Non-Unpaired). Therefore our results supported the hypothesis that IC is mainly involved in processing taste familiarity. As suggested by Roman and colleagues (e.g., Roman et al., 2009; Roman & Reilly, 2007) it may be that the insular cortex is involved in the detection of taste novelty rather than in CTA acquisition. These authors reported that IC bilateral

lesions retarded the acquisition of a taste aversion regardless of prior taste experience, suggesting that lesions of the insular cortex attenuated taste neophobia given that the pattern of behavioral deficits obtained cannot be interpreted as disruption of taste detection or stimulus intensity. Thus, rats with IC lesions treated a novel taste as if it was familiar and consequently CTA was retarded. In line with this, recent experiments using in vivo microdialysis have found that a novel taste induces a marked increase in the release of acetylcholine (ACh) in the IC, but the magnitude of ACh release in the IC decreases as the taste become familiar (Miranda & Bermúdez-Rattoni, 1999; Miranda et al., 2000). As the present results, these studies support the view that the IC is involved in coding taste familiarity.

As for the role of NAcb, our results show differential c-Fos activity in the NAcb core and shell regions after taste aversion learning depending on prior taste experience. More specifically, we found that taste preexposure resulted in reduced c-Fos expression in the NAcb core and shell as compared with a novel flavor paired with LiCl. The present finding is in accordance with electrophysiological experiments showing that the NAcb is highly involved in coding the hedonic value of taste stimuli. As previously noted, it has been reported that NAcb differentially encodes the hedonic value of the same taste stimulus based on learned associations (e.g., Carlezon & Thomas, 2009; Roitman et al., 2010). For example, the intraoral infusion of a sucrose solution previously paired with LiCl results in increased firing rate of NAcb neurons, reflecting a reduction in the hedonic value or palatability of the solution. In contrast, the infusion of sucrose induces a reduction in firing rate of NAcb neurons, suggesting that inhibition of the NAc activity is associated with positive hedonic reactions to taste stimuli (e.g., Roitman et al., 2005; Taha & Fields, 2005).

There is also pharmacological evidence showing that the intraoral infusion of taste stimuli induces changes in the release of dopamine (DA) in some NAcb regions depending on the hedonic value and novelty of the taste. In this regard, Bassareo, De Luca, and Di Chiara (2002) found that the infusion of appetitive and aversive taste stimuli increased DA release in the NAcb core and shell, independently of their novelty. In the NAcb shell, however, DA release increased in response to the infusion of an appetitive novel taste, but was unaffected by the infusion of an aversive taste. In addition, intraoral saccharin increases DA release in the NAcb shell before but decreases it after pairing with LiCl (e.g., Fenu, Bassareo, & Di Chiara, 2001; Mark et al., 1991). It has been also reported that NMDA receptors in the NAcb have an important role in the assignment of hedonic value to taste stimuli. In this regard, the consumption of a familiar taste, either appetitive or aversive, decreases after NMDA activation (e.g., Nuñez-Jaramillo et al., 2012), and blockade of these receptors increases positive hedonic responses to palatable tastes (e.g., Peciña & Berridge, 2005). Also, recent studies using magnetic resonance imaging (MRI) techniques have shown that the activity of NAcb neurons is involved in the display of aversive orofacial reactions after acquisition of CTA (e.g., Inui, Inui-Yamamoto, Yoshioka, Ohzawa, & Shimura, 2011). In line with these findings, our results suggest that activation of NAcb is correlated with LiCl-induced conditioned disgust reactions, but a reduced NAcb activity appears to be associated with attenuated conditioned disgust produced by prior nonreinforced exposure to the taste.

To summarize, this experiment extended previous works (results from Chapter 7) showing that latent inhibition attenuates the effects of CTA on taste palatability. That is, nonreinforced exposure to a taste-to-be associated with LiCl resulted in reduced conditioned disgust reactions. In addition, the results of this study reveal a role of the

NAcb, but not the IC, in the hedonic shift from appetitive to aversive in taste aversion learning. The IC appears to be more specifically involved in processing taste familiarity. Further studies will be needed to determine conclusively the neural circuitry underlying hedonic shifts in taste aversion learning, and how factors such as the fluid delivery method or the unconditioned hedonic value of taste stimuli can have effects on the patterns of neural activation.

Capítulo 10

Aportaciones del trabajo experimental y conclusiones

10.1. Aportaciones del trabajo experimental

Como se señaló anteriormente, esta tesis doctoral tuvo como objetivo principal el estudio de las respuestas hedónicas en el aprendizaje de aversión al sabor. Se pretendía analizar el efecto de la preexposición a los estímulos en el condicionamiento de respuestas de náusea. Se utilizó la técnica de reactividad al sabor para examinar los cambios hedónicos producidos tras la preexposición a los estímulos en el AAG. Adicionalmente se estudiaron las bases neurales del AAG mediante el uso de técnicas histoquímicas e inmunohistoquímicas. A modo de resumen, se plantearon estos objetivos con los siguientes hallazgos principales:

Tras le revisión teórica del tema y la exposición de los objetivos específicos de la tesis, en el capítulo 5 se examina si las exposiciones repetidas al LiCl en un contexto novedoso interferían con la adquisición posterior de respuestas condicionadas de náusea a una solución de sacarina. El efecto de preexposición al EI se ha estudiado tradicionalmente mediante el uso de pruebas de consumo (Batson y Best, 1979; Randich y Lolordo, 1979; ver Riley y Simpson, 2001 para una revisión) pero su efecto en el condicionamiento de las repuestas de náusea no se había analizado. Los resultados del Experimento 1 muestran que la presentación repetida del litio en un contexto novedoso atenuó el condicionamiento posterior de las respuestas de disgusto a una solución condicionada administrada en ese contexto. Cabe interpretar los resultados de este experimento en términos de bloqueo por el contexto. Esta explicación defiende que el retraso en el condicionamiento de la solución gustativa se debe a que la asociación formada entre las claves contextuales y el malestar inducido por el LiCl interfiere con el establecimiento de la nueva asociación sacarina-náusea. Sin embargo este primer estudio presenta alguna limitación: no se utiliza un procedimiento estricto de bloqueo

que pudiese corroborar la hipótesis planteada, además de la carencia de controles del condicionamiento de la respuesta de disgusto. El experimento 2 se diseñó para responder a estas limitaciones. Finalmente los datos del experimento 2 confirmaron que, efectivamente, la administración repetida de litio en un contexto novedoso dota a ese contexto de la capacidad de evocar respuestas condicionadas de náusea, y por lo tanto, con la capacidad de bloquear el consiguiente condicionamiento de un sabor administrado en presencia de las claves. Si esto es cierto, la extinción del condicionamiento de las claves contextuales antes del condicionamiento de la solución debería eliminar el efecto de preexposición al EI. Esta hipótesis se comprobó en el Experimento 3, otorgándole un papel modulador a las claves relacionadas con el contexto en la presencia o ausencia de respuestas de disgusto a un sabor novedoso emparejado con el litio.

Atendiendo a los resultados obtenidos en los experimentos descritos en el capítulo 5, podemos concluir que las claves contextuales pueden evocar náuseas condicionadas y que éstas no se limitan al consumo de un fluido emparejado con el malestar derivado de la administración de una droga emética. Además este estudio indica que la asociación entre las claves contextuales y la náusea puede interferir con el establecimiento de reacciones de disgusto producidas por la infusión de una solución emparejada con el litio. Este efecto de bloqueo no puede explicarse en términos de habituación a los efectos de la administración repetida del litio, sino en términos de bloqueo por el contexto. Las claves contextuales van adquiriendo progresivamente más fuerza excitatoria lo que dificulta la posterior asociación EC-EI.

Estudios anteriores habían demostrado que otro tipo de claves no gustativas podrían funcionar como un EC predictor de las consecuencias derivadas de la presencia de un EI. Por ejemplo, las claves relacionadas con el manejo de los animales y con las

inyecciones podrían funcionar como un EC capaz de asociarse con el malestar derivado de la administración intraperitoneal de litio (Willner, 1978; De Brugada, 2004). Estos autores demostraron que el efecto clásico de preexposición al EI desaparecía si se intercalaban inyecciones de salino y de LiCl durante la fase de preexposición. Por otro lado, nuestros estudios habían demostrado que las claves relacionadas con el ambiente también juegan un papel modulador en la adquisición de respuestas de disgusto. Esto llevo a plantearnos el rol que podría estar desempeñando las claves relacionadas con la infusión intraoral en la atenuación de las respuestas de disgusto. Si es cierto que otro tipo de claves pueden evocar nauseas condicionadas, es posible que la estimulación derivada de la infusión también adquiera propiedades aversivas y medie en alguna medida el condicionamiento posterior de una solución gustativa.

En el capítulo 6 evaluamos esta cuestión. Específicamente el experimento 4 se evalúa si la admnistración de infusiones intraorales de agua antes de una inyección de LiCl puede interferir con el condicionamiento posterior de una solución de sacarina. Si el contexto general adquiere propiedades aversivas, como se ha descrito en el capítulo 5, el grupo con experiencia repetida al LiCl debería mostrar menos reacciones aversivas a la infusión de la solución palatable en comparación con un grupo sin experiencia previa. Sin embargo, si las claves intraorales se han condicionado en alguna medida, no debería haber diferencias entre ambos grupos cuando se infunde sacarina a los animales. Los resultados de este experimento apoyan esta hipótesis. Sin embargo, una prueba concluyente de la hipótesis implicaría la evaluación de las claves relacionadas con la infusión en ausencia de la clave gustativa. Debido a que la evaluación de las respuestas hedónicas de los animales mediante la técnica de la reactividad facial requiere, necesariamente, la infusión IO del sabor, una alternativa es evaluar las claves relacionadas con la infusión sin la sacarina. Quizás lo más prudente hubiera sido llevar a

cabo una prueba de consumo de la sacarina en ausencia de las claves contextuales que han sido asociadas al malestar. Sin embargo, como se ha venido defendiendo en la presente tesis, la prueba de consumo no es una prueba directa del cambio en la palatabilidad, sino un método que evalúa la evitación del fluido (asociado al miedo condicionado y más sensible a los cambios en el estado fisiológico del animal). Los resultados del experimento 5 mostraron que los animales expuestos repetidamente a una infusión de agua seguida de LiCl, expresaban más reacciones de disgusto cuando eran infundidos nuevamente con agua que los animales sin experiencia previa. Esto supone que los resultados que se observaron durante la prueba con la sacarina era el reflejo del condicionamiento de las claves relacionadas con la infusión. Además, las ratas preexpuestas a infusiones de agua emparejadas con el litio expresaron más reacciones de disgusto a la solución de sacarina cuando ésta era administrada por primera vez que los animales no preexpuestos. Este resultado apoyaría la hipótesis planteada en estos experimentos. Es importante mencionar que el condicionamiento de las claves relacionadas con la infusión puede potenciarse por la presencia del agua durante las infusiones, como ha sido demostrado en estudios anteriores (Mitchell y Heyes, 1996; Symonds et al., 1998). Por lo tanto, el capítulo 6 proporciona evidencia a favor de que claves relacionadas con el ambiente sean capaces de adquirir fuerza asociativa. Tanto las claves exteroceptivas generales del ambiente (capítulo 5), como claves interoceptivas relacionadas con el método de infusión, pueden funcionar como un EC capaz de evocar náuseas condicionas.

En estudios anteriores de nuestro laboratorio se había argumentado que las claves derivadas de la administración IO de fluidos podían constituir un contexto específico capaz de modular respuestas aversivas en un paradigma de preexposición al EC (López et al., 2010). En este trabajo se evaluaba el papel de exposiciones no

reforzadas a un fluido en el posterior condicionamiento de respuestas de náusea a esa solución. Este trabajo demostró por primera vez que la preexposición no solamente atenúa el descenso en el consumo de una solución, sino que también afecta a las respuestas condicionadas de nausea. Este efecto fue replicado en los estudios incluidos en los capítulos 8 y 9. Sin embargo, el efecto de IL depende del uso de la misma metodología de administración de los fluidos entre las sesiones . Es decir, cuando la preexposición se realiza a través del consumo voluntario de la solución, el efecto de inhibición latente solo se observa en pruebas posteriores de consumo tras el condicionamiento. Sin embargo, si la preexposición se realiza de manera intraoral, el efecto de inhibición latente se observa en la prueba posterior de reactividad facial, pero no en la prueba de consumo. Por lo tanto, un cambio en la modalidad de presentación de los fluidos atenua el efecto de inhibición latente. El método de presentación, es decir, las claves relacionadas con la infusión forman parte de un contexto específico capaz de modular el efecto de inhibición latente, y como se ha descrito en el capítulo 6, también el efecto de preexposición al EI.

Si las claves relacionadas con la infusión pueden modular los cambios en la palatabilidad en los paradigmas de preexposición al EI, nos planteamos eliminar este factor. Los experimentos recogidos en el capítulo 7 examinan la inhibición latente de respuestas condicionadas de disgusto cuando se elimina el posible efecto derivado de la infusión de los sabores. Para eliminar este factor, utilizamos una medida alternativa de la palatabilidad, el análisis del patrón de ingesta (ver por ejemplo Dwyer, 2012). Los resultados del experimento 6 demostraron que la exposición repetida a la sacarina antes de su condicionamiento aumentó tanto la tasa de consumo de la sacarina como la valoración hedónica del fluido (tamaño del cluster), en comparación con un grupo de ratas sin experiencia previa con la solución. Sin embargo, las diferencias entre los

animales preexpuestos y no preexpuestos se mantuvieron constantes a lo largo de las sesiones, lo que podría sugerir que los resultados obtenidos no responden a un efecto de inhibición latente, sino a un efecto de atenuación de la respuesta neofóbica. La posibilidad de que la preexposición no afectase al aprendizaje EC-EI sino que proporcionase una línea base de consumo mayor en el grupo preexpuesto se evaluó en el experimento 7 a través de un diseño intrasujeto, en el que se añadió un sabor no condicionado.

Los resultados del experimento 7 muestran que la exposición repetida a un fluido atenúa el descenso en el consumo de esa solución tras su condicionamiento, así como también el cambio en su palatabilidad. Además el uso de un diseño intrasujeto elimina la posibilidad de explicar los resultados en términos de atenuación del efecto de neofobia. La preexposición a dos sabores antes de emparejar uno de ellos con un estado de náusea disminuye las diferencias en el consumo entre los sabores, así como también disminuye los cambios en la palatabilidad de la sustancia emparejada. Adicionalmente, se encontró que los efectos del condicionamiento en los cambios en la palatabilidad se extinguen más rápidamente que aquellos producidos en el consumo de la solución, lo que supone una prueba más de que la aversión al sabor (cambio en el valor hedónico del fluido) y la evitación del fluido son dos procesos diferenciables en el aprendizaje de la aversión al sabor. Por otro lado, la preexposición a los fluidos no tuvo efecto en la rapidez de la extinción ni de los cambios hedónicos ni en la supresión del consumo, lo que puede indicar que la inhibición latente afecta a cuanto se aprende y no a qué se aprende de la asociación EC-EI.

En los experimentos del capítulo 7 se estudia el efecto de inhibición latente tanto en consumo como en los cambios en la palatabilidad eliminando el posible papel que juegan las claves IO en la adquisición y expresión de repuestas condicionadas de

nausea. En este sentido, se ha descrito en trabajos anteriores que un cambio en la modalidad de presentación de los fluidos puede eliminar el efecto de inhibición latente (López et al., 2010). En este sentido ambos trabajos demuestran el efecto de inhibición latente usando diferentes métodos de medidam de la palatabilidad de los fluidos, sugiriendo que ambos métodos pueden ser complementarios para el análisis de las respuestas hedónicas.

Finalmente los experimentos presentados en los capítulos 8 y 9 de la presente tesis doctoral tenían como objetivo principal analizar las bases neurológicas de las respuestas condicionadas de náusea en el aprendizaje de la aversión al sabor. En el capítulo 8 se midió el metabolismo cerebral de las principales regiones que se saben media la formación de la memoria de la aversión al sabor. Este trabajo representa la primera demostración sobre los cambios producidos en la enzima mitocondrial CO asociados a cambios en palatabilidad de los sabores inducidos por la administración de una droga emética. Aunque el uso de esta técnica suponga una novedad en el estudio de la aversión al sabor, ha demostrado su efectividad en el estudio de otros procesos como en tareas de memoria espacial (Conejo et al., 2010; Conejo et al., 2013; Fidalgo et al., 2012), en respuestas de estrés crónico y vulnerabilidad a la depresión (Harro et al., 2014) y en respuestas de ansiedad (Sampedro-Piquero et al., 2013). El uso de la técnica histoquímica de la CO ofrece ciertas ventajas a la hora de estudiar estructuras cerebrales relacionadas con el condicionamiento de respuestas de náusea a un fluido. Entre ellas, además de las ventajas metodológicas, podemos destacar que permite evaluar los cambios que se producen a lo largo de todo el proceso de aprendizaje, en este caso, los cambios que se producen a largo plazo en la formación de memorias gustativas. Adicionalmente, nos permite establecer un conjunto de redes neuronales que están asociadas a la aversión al sabor y al procesamiento de sabores familiares y novedosos.

Con este propósito se utilizó un paradigma de inhibición latente, dado que ofrece ciertas ventajas frente a otros paradigmas. Por ejemplo, permite evaluar el establecimiento de aversiones a sustancias de las que ya se ha formado una memoria gustativa (sabores familiares) o por el contrario supone una novedad para el sujeto. En este sentido, los datos del capítulo 8 mostraron la actividad conjunta de ciertas estructuras cerebrales como resultado de la asociación de un fluido con las consecuencias derivadas de su ingesta. Destacamos la interacción de la actividad entre la amígdala y el núcleo accumbens en los grupos para los cuales la solución era altamente palatable o altamente aversiva. En este sentido podía establecerse una relación de ambas regiones en el procesamiento de la información de las representaciones hedónicas de los fluidos. Estudios anteriores apoyan la hipótesis de la implicación de estas estructuras en la integración de información sobre las consecuencias derivadas del consumo de un fluido y sus cualidades sensoriales (Nuñez-Jaramillo et al., 2012; Reilly y Bornovalova, 2005; Schafe y Bernstein, 1996).

Otras de las redes neuronales que podemos destacar es la que se produce como resultado del procesamiento de sabores novedosos. En este sentido, encontramos que se produce una actividad conjunta del área tegmental ventral, el núcleo accumbens, tálamo y corteza cingulada en el prefrontal. Apoyándonos en estudios anteriores (Berridge y Robinson, 1998; Yamamoto, 2006; Yamamoto y Ueji, 2011) nuestros datos podrían sugerir que el área tegemental ventral y núcleo accumbens (principales regiones del sistema de la recompensa) envían la información relacionada con las consecuencias del consumo de la solución a zonas de integración de la corteza prefrontal, que podría recibir a su vez la información procedente del tálamo sobre las cualidades sensoriales del fluido.

Los sistemas cerebrales implicados en la aversión al sabor se han estudiado utilizando otro tipo de técnicas donde se estudian los cambios en actividad a corto plazo. Una de las más utilizadas es la técnica inmunohistoquímica de la c-fos. En el capítulo 9 evaluamos la actividad c-fos de dos de las principales regiones relacionadas con la inhibición latente en el aprendizaje aversivo gustativo, esto es, la corteza insular y el núcleo accumbens. Los resultados obtenidos otorgan un papel diferencial para ambas estructuras en el procesamiento de los sabores asociados al malestar. La actividad c-fos fue menor en la corteza insular en los dos grupos para los cuales la solución era familiar, con independencia de las consecuencias derivadas de su consumo (aversivas o no). Estos resultados podrían sugerir que la corteza insular es una estructura crucial en la memoria de los sabores, sugiriendo que está implicada en la detección de sabores familiares o novedosos más que en el aprendizaje de la aversión al sabor (Domjan, 1976; Roman et al., 2009).

En contraposición los datos del capítulo 9 revelaron que el grupo de inhibición latente mostró un descenso en la actividad cerebral del núcleo accumbens en las dos subregiones (corteza y núcleo). Estos resultados podrán sugerir un papel del núcleo accumbens en el procesamiento del cambio hedónico de positivo a negativo en la versión del sabor. De hecho, los resultados de los experimentos recogidos en el capítulo 9 apoyan trabajos previos que han relacionado el acumbens con la codificación del valor hedónico del estímulo (por ejemplo, Carlezon y Thomas, 2009; Roitman et al., 2010).

En resumen, los datos experimentales presentados en esta tesis doctoral indican que las respuestas hedónicas producidas durante el aprendizaje de la aversión al sabor puede estar influenciadas por la exposición previa al estímulo incondicionado (capítulo 5 y 6) así como al estímulo condicionado (capítulo 7,8 y 9). Además se establecieron las redes neuronales asociadas a las respuestas hedónicas elicitadas por la infusión de

sabores apetitivos y aversivos (capítulo 8). Finalmente, los resultados sugieren una se implicación de la corteza insular en la detección de la familiaridad de los sabores, así como la implicación del núcleo accumbens en la inhibición latente de las respuestas condicionadas de disgusto (capítulo 9).

10.2. Implicaciones prácticas

Los resultados del trabajo de investigación aquí presentado aportan alguna información sobre la adquisición de nauseas condicionadas por parte de las claves ambientales presentes durante el establecimiento de la aversión. Los datos obtenidos aquí pueden contribuir al desarrollo de los modelos animales de náuseas anticipatorias que desarrollan los enfermos de cáncer durante los tratamientos de quimioterapia. Uno de los efectos secundarios más desagradables de los que han informado estos pacientes son precisamente las nauseas y vómitos que se desarrollan tras los primeros ciclos de tratamiento. Si bien hay tratamientos farmacológicos efectivos en el tratamiento de los vómitos, no lo hay para las náuseas anticipatorias.

En la presente tesis doctoral mostramos evidencia de que el contexto es capaz de elicitar nauseas condicionadas. El conocimiento de los mecanismos implicados en el condicionamiento de náuseas a las claves contextuales puede constribuir a la identificación de nuevos fármacos que puedan reducir dichos efectos derivados de los tratamientos contra el cáncer. A este respecto, se están estudiando la capacidad de los derivados del cannabis como el Δ^9-tetrahidrocannabinol y otros componentes no-psicotrópicos (cannabidiol) en la atenuación de las respuestas de nausea derivadas de la administración de drogas eméticas (ver Parker, 2014 para una revisión reciente). Además el estudio de los substratos neuroanatómicos de las náuseas condicionadas supondría un avance para la investigación de posibles dianas terapéuticas. En este sentido, se han estudiado ampliamente las regiones cerebrales implicadas en la evitación

de los fluidos (ver Reilly, 2009 para revisión), pero pocos trabajos han investigado los mecanismos neuronales implicados en las respuesta de disgusto. Toda información aportada sobre la adquisición y expresión de respuestas de disgusto nos acercará más a posibles soluciones que atenúen los efectos derivados de los tratamientos de quimioterapia. Además este modelo puede servir para evaluar el efecto de diferentes procedimientos de aprendizaje en la disminución de las náuseas anticipatorias, como por ejemplo la inhibición latente, el ensombrecimiento o el bloqueo.

En resumen, el modelo animal de nauseas anticipatorias permitirá ensayar con ratas en el laboratorio y en situaciones bien establecidas distintos tratamientos terapéuticos que podrían ser posteriormente aplicados a enfermos de cáncer.

10.3. Conclusions

In summary, the results reported in this thesis suggest:

1. A context previously paired with lithium chloride (LiCl) acquires the ability to evoke conditioned nausea, and therefore it could interfere with the subsequent establishment of disgust reactions to a palatable solution.

2. In addition to environmental cues, infusion-related cues associated with LiCl can modulate the US-preexposure effect in taste aversion learning.

3. Non-reinforced exposure to a flavor attenuates the effect of taste aversion learning on cue palatability as measured by both taste reactivity and licking analysis.

4. Suppressed consumption appears to be more resistant to extinction than changes in taste palatability, in spite of prior exposure to flavors.

5. CO activity results provide the first demonstration of decreased metabolic activity in the PBN, VTA; BLA, BNST and mPFC, all of which are linked to changes in palatability in taste aversion learning.

6. Novel activation patterns in brain networks were described, one of which is highlighted as an acumbens-amygdala interaction that may be involved in the processing of the hedonic value of taste stimuli.

7. c-Fos immunohistochemistr suggest that NAcb is involved in the response to the hedonic value of taste stimuli and that the IC is more specifically involved in the processing of taste novelty.

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