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EFFECTIVENESS OF NONREINFORCED TRIALS ON CONDITIONED FLAVOUR PREFERENCE BASED ON NUTRIENT: EXTINCTION AND LATENT INHIBITION

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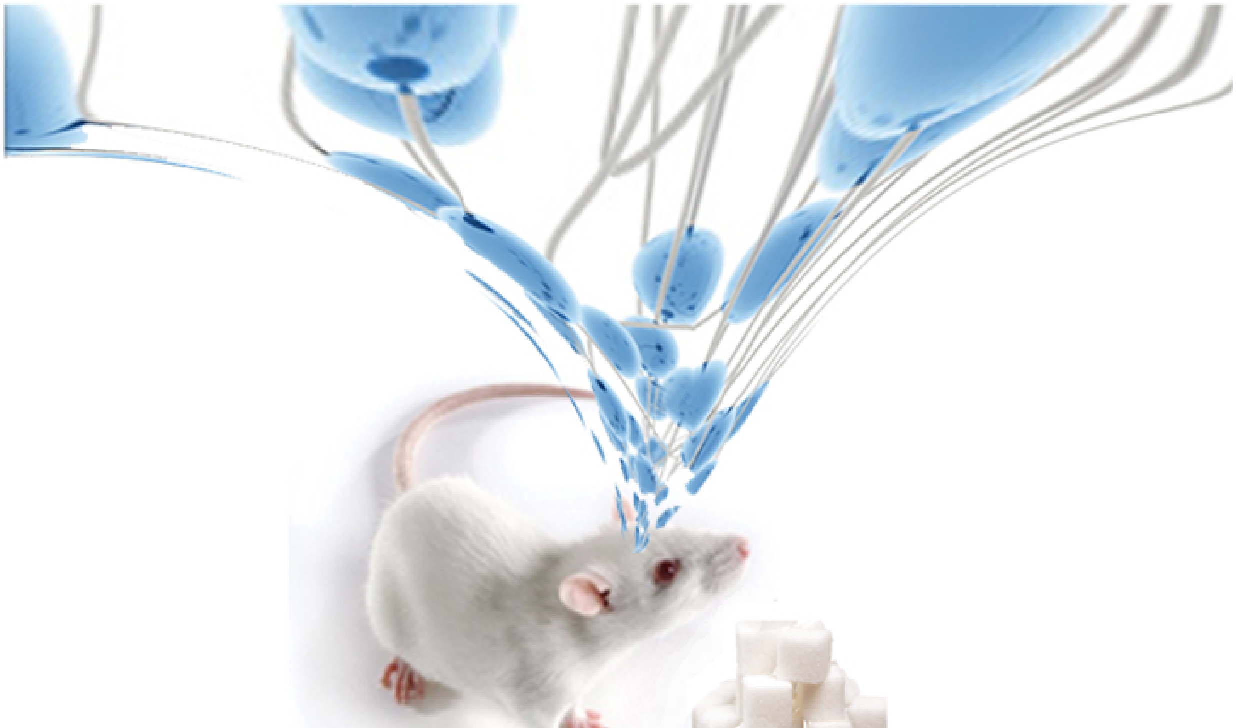

DOCTORAL THESIS

EFFECTIVENESS OF NONREINFORCED TRIALS ON CONDITIONED FLAVOUR PREFERENCE BASED ON NUTRIENT: EXTINCTION AND LATENT INHIBITION

Efectividad de los ensayos no reforzados sobre la preferencia condicionada al sabor basada en nutriente: extinción e inhibición latente

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Dña. FELISA GONZÁLEZ REYES, PROFESORA TITULAR DE PSICOLOGÍA BÁSICA DE LA UNIVERSIDAD DE GRANADA CERTIFICA:

Que el trabajo de investigación titulado "EFFECTIVENESS OF NONREINFORCED TRIALS ON CONDITIONED FLAVOUR PREFERENCE BASED ON NUTRIENT: EXTINCTION AND LATENT INHIBITION" (Efectividad de los ensayos no reforzados sobre la preferencia condicionada al sabor basada en nutriente: extinción e inhibición latente) ha sido realizado por D. DAVID GARCÍA BURGOS para optar al grado de Doctor Europeo en Psicología en el Departamento de Psicología Experimental y Fisiología del Comportamiento de la Facultad de Psicología de la Universidad de Granada, bajo su dirección.

Y para que conste donde proceda se firma este certificado en Granada a 30 de junio de 2011.

Fdo. Felisa González Reyes   Fdo. David García Burgos

EFFECTIVENESS OF NONREINFORCED TRIALS ON CONDITIONED FLAVOUR PREFERENCE BASED ON NUTRIENT: EXTINCTION AND LATENT INHIBITION

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A mis hermanos, Daniel y Mª Ángeles.

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Garcia-Burgos, D., González, F., & Hall, G. (2011). *Motivational control of latent inhibition in flavor preference conditioning.* Manuscript in preparation.

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ABBREVIATIONS / ABREVIATURAS

CFP: Conditioned flavour preference.

CS: Conditioned stimulus.

EC: Estímulo condicionado (in English, CS).

EI: Estímulo incondicionado (in English, US).

Exp.: Experiment.

i.g.: Intragastric.

IL: Inhibición latent (in English, LI)

i.p.: Intraperitoneal.

LI: Latent inhibition.

LiCl: Lithium chloride.

M: Maltodextrin.

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

US: Unconditioned stimulus.

RESUMEN

CAPÍTULO 1: INTRODUCCIÓN

Un área que ha recibido considerable atención en el estudio de la selección de alimentos desde la perspectiva del aprendizaje es la adquisición de preferencias alimentarias en el laboratorio. Por ejemplo, está bien establecido que preferencias robustas por sabores pueden obtenerse a medida que los animales experimentan los efectos positivos de la comida, tales como su valor hedónico o sus propiedades nutritivas post-ingesta. Conocer cómo estas preferencias se desarrollan debería ayudarnos a comprender cómo y por qué determinados productos son preferidos y seleccionados sobre otros, dando lugar a persistentes, y con frecuencia poco saludables, patrones alimentarios. Sin embargo, poco se sabe sobre los procesos que subyacen a la adquisición y modificación de estas preferencia aprendidas. Proporcionar conocimiento sobre las preferencias condicionadas a los alimentos a través del paradigma de preferencia condicionada al sabor es el principal propósito de esta disertación.

CAPÍTULO 2: PREFERENCIA CONDICIONADA AL SABOR

La búsqueda de comida y la selección de alimentos son actividades frecuentes y necesarias para la supervivencia. Dada la relevancia de identificar fuentes de alimentos, evitar venenos e ingerir contenidos nutritivos, la selección de alimentos representa uno de los mayores retos evolutivos para multitud de animales (Rozin y Schulkin, 1990), entre ellos el ser humano. Frente a las muchas fuentes potenciales de comida disponibles en la naturaleza, los animales deben decidir qué comer y qué rechazar. Para superar estas presiones, los animales han desarrollado una serie de poderosos mecanismos dirigidos a optimizar la conducta ingestiva. Uno de estos mecanismos destinados a mejorar el proceso de selección de alimentos es el sistema del sabor (Stevenson, 2009), como parte de una estrategia de defensa encargada de proteger el medio interno del organismo (Prescott, 1999). Este sistema funcional de

alto valor adaptativo estaría orientado a la identificación correcta de alimentos y tóxicos a partir del análisis de las propiedades sensoriales de la comida.

Los individuos muestran preferencias alimentarias, es decir, la selección de una sustancia sobre otras, basándose en los sabores de las mismas. Además de mostrar una predisposición genética a preferir sustancias dulces y saladas y a rechazar aquéllas que son ácidas y amargas, así como a rechazar sabores novedosos (Birch, 1999), los animales refinan sus preferencias gustativas a través de la experiencia. De hecho, la amplia variedad de preferencias por sabores que muestran los adultos frente a los recién nacidos, la plasticidad en las preferencias a lo largo de la vida y el rango de diferencias individuales se atribuyen al efecto del aprendizaje (Capaldi, 1996; Myers y Sclafani, 2006). Diferentes procedimientos de aprendizaje están implicados en la aparición de preferencias por un sabor particular. El más simple consiste en el efecto de familiaridad, también denominado efecto de mera exposición, a través del cual se incrementa la aceptación y preferencia por un sabor tras exposiciones repetidas (Hill, 1978). Aunque la mera exposición puede reducir la neofobia, este efecto no produce preferencias robustas ni estimula el sobreconsumo de sustancias. Estas últimas respuestas se atribuyen más bien a procesos asociativos de aprendizaje (Rozin y Zellner, 1985).

En el laboratorio, el procedimiento estándar más usado en el estudio experimental en ratas de preferencias adquiridas es el paradigma de preferencia condicionada al sabor (PCS), en el que a los animales se les presenta un sabor nuevo (el estímulo condicionado, EC) emparejado con un segundo sabor palatable (p.ej., Holman, 1975) o un nutriente (p.ej., Capaldi, Campbell, Sheffer y Bradford, 1987; Sclafani y Nissenbaum, 1988), el cual sirve de estímulo incondicionado (EI). Aunque un solo ensayo puede ser suficiente, tras varios ensayos de condicionamiento se evalúa la preferencia por el sabor mediante un test de dos botellas, observándose una preferencia por el sabor EC+ (emparejado con el EI) sobre otro sabor EC- (no emparejado con el EI) (p.ej., Drucker, Ackroff y Sclafani, 1994), así como por el EC+ sobre agua (p.ej., Harris, Gorissen, Bailey y Westbrook, 2000; Pérez, Lucas y Sclafani, 1998).

Existen variaciones del paradigma básico de PCS, especialmente en función de la naturaleza de los estímulos condicionados e incondicionados utilizados. Según la modalidad sensorial del estímulo condicionado, se han usado sabores básicos como ECs (p.ej., Drucker, Ackroff y Sclafani, 1994), sabores complejos (p.ej., Sclafani y Ackroff, 2006) y claves olfatorias aisladas (p.ej., Lucas y Sclafani, 1995). En cuanto a la naturaleza del EI, se han utilizado tanto nutrientes específicos (véase revisión en Sclafani, 1999) como sabores palatables (p.ej., Holman, 1975), observándose en ambos casos preferencias condicionadas al sabor.

Por otro lado, se han aplicado distintos métodos de administración del EI, tales como administración de nutrientes intragástrica (p.ej., Azzara y Sclafani, 1998), intraduodenal (p.ej., Drucker y Sclafani, 1997), intrayeyunal (p.ej., Ackroff, Yiin y Sclafani, 2010) o portal-hepática (p.ej., Tordoff y Friedman, 1986). En estos métodos, dado que el nutriente evita la cavidad oronasal, el sabor de éste no interfiere con la asociación entre el sabor del EC y las consecuencias reforzantes post-ingesta del nutriente. El EI también puede ser proporcionado oralmente (p.ej., Fanselow & Birk, 1982). En este caso, los animales adquieren preferencias por el sabor condicionado cuando éste se presenta en solución formando un compuesto bien con un nutriente, bien con un sabor palatable.

En cuanto al procedimiento de condicionamiento, a diferencia de otros paradigmas que utilizan principalmente el condicionamiento hacia delante, el procedimiento simultáneo aparece con frecuencia en PCS (p.ej., Mehiel y Bolles, 1988). La presentación oral simultánea del sabor y el nutriente palatable suele facilitar el condicionamiento, incluso potenciarlo (p.ej., Capaldi y Privitera, 2008; Higgins y Rescorla, 2004). Otro de los procedimientos usados en PCS es el procedimiento demorado, que introduce un intervalo temporal entre la presentación del EC y el EI. En este caso, la preferencia basada en palatabilidad sólo ocurre cuando el lapso entre el sabor condicionado y el reforzador palatable es mínimo, no así cuando la preferencia está basada en los efectos nutritivos del reforzador, observándose entonces preferencia con demoras de incluso 1-5 horas (p.ej., Capaldi y Sheffer, 1992).

En el desarrollo de preferencias condicionadas, los animales pueden ser entrenados o probados con/sin privación de comida y/o agua. Aunque la privación de comida podría facilitar el condicionamiento al sabor cuando se usan nutrientes como EI al potenciar el valor reforzante del nutriente por incremento de la necesidad energética de los animales (Davidson, 1998), autores como Yiin, Ackroff y Sclafani (2005b) ha demostrado que la privación de comida sólo potencia la expresión de la preferencia por un sabor emparejado con los efectos post-ingesta de un nutriente, pero no la adquisición de dicha preferencia. Se ha constatado también que preferencias basadas en sabores palatables son independientes del estado motivacional durante la prueba (Fedorchak y Bolles, 1987).

En términos procedimentales, otra cuestión de relevancia es la manera de medir la preferencia condicionada. En la mayoría de estudios, los animales reciben dos tipos diferentes de ensayos durante el condicionamiento: ensayos reforzados en los que un sabor está emparejado con el EI (EC+) y ensayos no reforzados en los que un segundo sabor se presenta en ausencia del EI (EC-). El resultado del condicionamiento se mide en un test de dos botellas CS+ vs. CS-. Sin embargo, esta medida de condicionamiento ha sido cuestionada (p.ej., Delamater, 2007) dado que no descarta que la preferencia obtenida por el EC+ sea parcialmente debida a una evitación aprendida del EC-, el cual se ha sido explícitamente no emparejado con el EI (Harris, Shand, Carroll y Westbrook, 2004).

En suma, la PCS se ha estudiado usando una variedad de estímulos y métodos. Las preferencias por un sabor neutro pueden estar asociadas tanto al sabor palatable de una sustancia (nutritiva o no) como a las acciones post-ingesta de un nutriente. En ambos casos se observa una adquisición rápida, aunque sólo la expresión de la preferencia basada en nutrientes se ve potenciada por la privación de comida. Finalmente, la forma de medir dicha preferencia condicionada al sabor es importante, siendo el test de elección de dos botellas CS+ vs. CS- un procedimiento que presenta problemas de interpretación.

Preferencia condicionada al sabor usando un nutriente palatable como EI

La PCS usando un nutriente palatable (p.ej., la sacarosa) como EI puede ser considerada como preferencia basada en la palatabilidad (valor hedónico del sabor del nutriente) por un lado, y preferencia basada en las consecuencias post-ingesta del nutriente, por otro (p.ej., Sclafani y Ackroff, 1994). Ambos tipos de preferencia se han disociado usando manipulaciones específicas. El uso de sustancias hedónicamente positivas sin valor nutricional ha permitido el estudio exclusivo de la preferencia basada en palatabilidad (p.ej., Fansenlow y Birk, 1982), mientras que el uso de infusiones intragástricas ha posibilitado el estudio de la preferencia basada en nutriente (p.ej., Elizalde y Sclafani, 1990). Esta división no sólo implica procedimientos distintos, sino también parece ser sustentada por dos procesos diferentes (Dwyer, 2005; Fedorchak y Bolles, 1987). Según la propiedad reforzante del nutriente, se han distinguido dos tipos de aprendizajes, uno de ellos basado en la evaluación hedónica del sabor del nutriente conocido como aprendizaje sabor-sabor (o condicionamiento sabor-sabor) y otro basado en las expectativas de consecuencias post-ingesta, denominado aprendizaje sabor-nutriente (o condicionamiento sabor-nutriente) (Capaldi, 1996). Por ejemplo, cuando se usa la sacarosa como EI, el sabor del nutriente incluye propiedades sensoriales específicas (i.e., el dulzor de la sacarosa) así como las propiedades hedónicas positivas (i.e., las respuestas afectivas positivas). Es posible, por tanto, que cuando un sabor EC se presenta en compuesto con la sacarosa, el sabor establezca asociaciones tanto con las propiedades sensoriales como con las respuestas hedónicas. Usando la técnica de devaluación del EI se ha puesto de manifiesto la existencia de asociaciones sensoriales sabor-sabor (Delamater, 2007; Dwyer, 2005), que consisten en asociaciones entre el sabor condicionado y las propiedades sensoriales del EI. Una segunda asociación es la establecida entre el sabor inicialmente neutro y el valor hedónico del sabor del EI, estudiada a través de en técnicas como el análisis microestructual de la respuesta de *licking* (p.ej., Dwyer, 2008) y el análisis de respuestas orofaciales en el test de reactividad al sabor (p.ej., Forestell y LoLordo, 2003). Además de poseer un sabor dulce palatable, una segunda propiedad reforzante de la sacarosa son sus propiedades positivas post-ingesta, lo que posibilita el establecimiento de una tercera asociación entre sabor EC y las consecuencias nutritivas post-ingesta (Delamater, Campese, LoLordo y Sclafani, 2006). Esta tercera asociación sabor-nutriente se ha visto favorecida por el uso de infusiones intragástricas de sustancias con valor nutritivo (p.ej., Sclafani y Nissembaum, 1988). Como sugieren Owens, Capaldi y Sheffer (1993), las asociaciones con las propiedades reforzantes del nutriente, es decir las asociaciones sabor-valor hedónico y sabor-nutriente, permiten establecer preferencias aprendidas por sabores neutros, estando sustentadas por mecanismos distintos. En particular, la asociación sabor-valor hedónico estaría relacionada con mecanismos de aprendizaje sabor-sabor, mientras que la asociación sabor-nutriente estaría relacionada con el mecanismo de aprendizaje sabor-nutriente.

Diferentes disociaciones experimentales apuntan a la independencia de ambos tipos de mecanismos de aprendizaje (Myers y Sclafani, 2006). Se ha demostrado que, a diferencia del aprendizaje sabor-sabor, la preferencia basada en sabor-nutriente depende del estado motivacional de prueba, donde el hambre selecciona el aprendizaje sabor-nutriente (Harris et al., 2000), y es sensible al procedimiento de extinción (Harris et al., 2004), donde la manipulación de la contingencia EC-EI tiene repercusión sobre el nivel de preferencia observado. Estas disociaciones han promovido una explicación de la PCS en términos de dos mecanismos (p.ej., Campbell, Capaldi, Sheffer y Bradford, 1988; Drucker et al., 1994; Dwyer y Quirk, 2008; Fedorchak, 1997; Harris y Thein, 2005). Tomando en consideración el papel que juega la expectativa de consecuencias post-ingesta y la relación de contingencia EC-EI como uno de los rasgos más importantes que distingue el aprendizaje sabor-sabor del aprendizaje sabor-nutriente, la idea central es que mientras que en el aprendizaje sabor-nutriente los animales aprenden que el sabor es una señal de las consecuencias nutritivas post-ingesta del nutriente, en el aprendizaje sabor-sabor el sabor no se comporta como una señal de palatabilidad. El aprendizaje sabor-nutriente puede implicar un aprendizaje de expectativas (es decir, el sabor condicionado se convierte en una señal de nutrientes), mientras que el aprendizaje sabor-sabor puede implicar un aprendizaje hedónico (es decir, el sabor condicionado adquiere las propiedades hedónicas del sabor del reforzador) (Drucker et al., 1994). Esta distinción entre expectativa y valor hedónico ofrecida por Drucker et al. implica que en el primer tipo de aprendizaje se produce la adquisición de conocimiento sobre la relación de contingencia entre el sabor y las acciones nutritivas post-ingesta dando lugar a un aprendizaje de tipo predictivo, sensible a cambios en contingencia, mientras que en el segundo tipo de aprendizaje se produce un proceso de transferencia de las propiedades hedónicas al sabor condicionado afectado por la relación de contigüidad entre el sabor y el reforzador y no por la relación de contingencia, conformando un tipo de aprendizaje no predictivo.

Se han propuesto distintos modelos para explicar las características de estos dos tipos de aprendizaje. La aproximación configuracional (Pearce, 2002) es una de las propuestas que está recibiendo más apoyo (p.ej., Dwyer, Haselgrove y Jones 2011; Harris y Thein, 2005). Este modelo permite explicar ambos tipos de mecanismos, planteando que en el caso del aprendizaje sabor-sabor lo que se produce es la formación de una representación configuracional. Este proceso configuracional es automático e inmediato y no resulta afectado por la relación de contingencia entre el sabor y el nutriente palatable. Por otro lado, la representación configuracional se asocia excitatoriamente con las consecuencias nutritivas. Así, la formación de una representación configuracional sabor-sabor y el establecimiento de una asociación entre la representación configuracional y las propiedades post-ingesta del nutriente no sólo permiten explicar las características del aprendizaje sabor-nutriente, sino que también permiten predecir los rasgos más importantes del aprendizaje sabor-sabor, tales como la insensibilidad a la manipulación de la relación de contingencia EC-EI y la mayor efectividad del procedimiento de condicionamiento simultáneo a la hora de obtener la respuesta condicionada. Otras aproximaciones teóricas que intentan explicar la peculiaridades del aprendizaje sabor-sabor son el modelo de aprendizaje evaluativo (como una extensión del condicionamiento evaluativo en humanos; De Houwer, Thomas y Baeyens, 2001), y las basadas en el condicionamiento de segundo orden (p.ej., Fedorchak y Bolles, 1987), en la formación de asociaciones intracompuesto (Capaldi, 1996; en términos propuestos por Rescorla y Cunninham, 1978) o en el aprendizaje estímulo-respuesta (Harris et a., 2004; Rozin y Zellner, 1985).

A modo de conclusión, la revisión anterior muestra cómo la preferencia basada en palatabilidad y la preferencia basada en nutriente parecen disociarse en términos de procedimiento, contenido de aprendizaje y mecanismos implicados.

Regulación motivacional del aprendizaje en la PCS usando nutrientes

La expresión de ambos tipos de aprendizaje parece estar regulada por el estado motivacional de prueba. Se observa una preferencia basada en el aprendizaje sabor-sabor cuando los animales no están privados durante el test; por el contrario, la privación de comida selecciona el aprendizaje sabor-nutriente, observándose una preferencia basada en las propiedades post-ingesta cuando los animales están hambrientos en el test (Harris et al., 2000). Es importante resaltar, sin embargo, que el estado motivacional parece no afectar a la adquisición de ambos tipos de aprendizaje durante el condicionamiento, sino sólo a su expresión (p.ej., Fedorchak y Bolles, 1987; Yiin, Ackroff y Sclafani, 2005a, 2005b).

CAPÍTULO 3: EFECTIVIDAD DE LOS ENSAYOS NO REFORZADOS EN PREFERENCIA CONDICIONADA AL SABOR BASADA EN NUTRIENTE

Una característica central que distingue al aprendizaje predictivo sabor-nutriente del aprendizaje no predictivo sabor-sabor es la sensibilidad a la relación de contingencia entre el EC y EI. En concreto, mientras que el sabor EC en el aprendizaje sabor-nutriente predice el reforzador y, por tanto es sensible a los cambios de contingencia EC-EI entre distintas fases experimentales, en el aprendizaje sabor-sabor la presentación aislada del sabor parece no tener efecto sobre la preferencia condicionada.

Esta conclusión se ha visto apoyada por los estudios sobre extinción de preferencias condicionadas al sabor, que ha mostrado una persistencia de la preferencia a pesar de las repetidas exposiciones al sabor no reforzado (p.ej. Albertella y Boakes, 2006; Drucker et al., 1994). Estudios más refinados han puesto de manifiesto que esta resistencia a la extinción desaparece cuando se mide la preferencia con test de dos botellas sabor vs. agua en lugar de comparaciones EC+ vs. EC- y se han introducido periodos de privación de comida (Drucker et al., 1994, Exp. 3). Harris et al. (2004, Exps. 2A y 2B), manipulando el nivel de hambre de ratas durante la prueba, demostró que la preferencia por el sabor mostrada por animales hambrientos desciende tras presentaciones no reforzadas del mismo, a diferencia de la preferencia mostrada por animales saciados. Dado que el sabor emparejado con sacarosa forma distintas asociaciones, sabor-sabor y sabor-nutriente, y que el estado de hambre en el test selecciona la asociación sabor-nutriente, el decremento en preferencia condicionada sólo en animales hambrientos durante el entrenamiento o durante el test proporcionó evidencia de que la presentación de sabor no reforzado sólo es efectiva en la preferencia basada en el aprendizaje sabor-nutriente, pero no en la preferencia basada en el aprendizaje sabor-sabor.

Otra conclusión interesante del estudio de Harris et al. (2004, Exp. 3) está relacionada con los efectos de la devaluación del EI. Los resultados mostraron que la exposición al sabor fuera del compuesto sabor-sacarosa tras el condicionamiento, pero antes de la devaluación de la sacarosa, tuvo impacto en la asociación sabor-sabor. En concreto, el efecto de devaluación del EI no se encontró, a diferencia de lo ocurrido en un grupo devaluado pero no expuesto. Un resultado similar fue presentado por Delamater (2007), quien además de observar un debilitamiento de esta asociación (Exp. 1), también informó que la asociación sabor-nutriente parecía haberse debilitado tras la exposición al sabor tras el condicionamiento y previa a la devaluación. Sin embargo, estos resultados no parecen acomodarse a la postura dominante actual que defiende que la extinción no tiene impacto sobre la fuerza de las asociación EC-EI (p.ej., Delamater, 1996; Rescorla, 1996). Este importante hallazgo, junto con los resultados encontrados por Higgins y Rescorla (2004) sobre la dificultad de conseguir la readquisición de la respuesta condicionada tras la exposición después del condicionamiento simultáneo con un nutriente, pone en duda que dicho decremento de la preferencia observada en PCS basada en nutriente sea debida a extinción. Una explicación alternativa en términos de inhibición condicionada se ofrecerá en el Capítulo 5 y se examinará en el Capítulo 6.

Respecto a la efectividad de los ensayos no reforzados durante el condicionamiento de preferencias al sabor, los reducidos datos de los que actualmente se disponen utilizando procedimientos de reforzamiento parcial (Delamater, en prensa, Exp. 1) y discriminación bicondicional utilizando claves contextuales (Dwyer y Quirk, 2008; pero ver Campbell, Capaldi, Sheffer y Bradford, 1988, Exp. 3) no permiten establecer conclusiones definitivas sobre el impacto de la presentación no reforzada del sabor sobre los aprendizaje sabor-sabor y sabor-nutriente.

Finalmente, los estudios sobre inhibición latente (IL) parecen reforzar la idea de que sólo el aprendizaje sabor-nutriente es sensible a los cambios de contingencia EC-EI. Los resultados de De la Casa, Márquez y Lubow (2009) con animales hambrientos y los de Delamater (en prensa, Exp. 2) con animales sedientos parecen mostrar que mientras que el efecto IL ha sido encontrado claramente utilizando animales hambrientos los resultados con animales sedientos parecen menos claros, al observarse sólo tras exposiciones adicionales a la sacarosa tras la fase de preexposición y condicionamiento y antes de la prueba. Por tanto, parece que preexposición al sabor produce un efecto claro de IL en preferencia basada en las consecuencias post-ingesta del nutriente, resultando más difícil de observar cuando la preferencia está basada en las propiedades hedónicas del sabor del nutriente.

Especialmente interesante resulta el hecho de que la presentación no reforzada del sabor tras (extinción) o antes (IL) del condicionamiento producen resultados similares. En concreto, la presentación no reforzada parece ser efectiva únicamente en el aprendizaje sabor-nutriente e inefectiva en el aprendizaje sabor-sabor Sin embargo, no existen estudios específicamente diseñados para examinar la contribución de cada uno de estos mecanismos sobre el efecto de IL en el paradigma de PCS, como sí ocurre en el caso de la extinción (Harris et al., 2004. Ese fue el objetivo del Capítulo 7.

CAPÍTULO 4: JUSTIFICACIÓN Y OBJETIVOS

Sobre la base de las consideraciones anteriormente mencionadas, uno de los propósitos de la presente disertación es investigar la naturaleza del proceso que produce una reducción en preferencia cuando el sabor no reforzado se presenta repetidamente tras el condicionamiento simultáneo con la sacarosa como EI (bajo condiciones similares al procedimiento usado por Harris et al., 2004, Exp. 2B).

Además, dado que el cambio de contingencia EC-EI en el procedimiento de extinción afecta a la preferencia basada en nutriente pero no a la preferencia basada en palatabilidad (Harris et al., 2004, Exp. 2B), la presente disertación también examinó si el efecto de los ensayos no reforzados puede extenderse al procedimiento de IL. Concretamente, se manipularon diferentes estados motivacionales de hambre y sed presentes durante el entrenamiento (preexposición y condicionamiento) y el test, con el fin de evaluar la efectividad de la presentación repetida del sabor no reforzado antes del condicionamiento.

Dos hipótesis de trabajo se plantearon.

Hipótesis 1. Si la reducción en preferencia condicionada al sabor en animales hambrientos expuestos al sabor no reforzado tras el condicionamiento simultáneo sabor-sacarosa mientras están sedientos no es producida por extinción, sino por las propiedades inhibitorias adquiridas por el sabor, entonces el sabor no debería exhibir ninguno de los fenómenos relacionados con la recuperación de respuestas extinguidas, ni mostrar efecto de devaluación del EI, y debería pasar las pruebas de sumación y retraso.

Asumiendo que el decremento en PCS basada en nutriente encontrado en animales hambrientos expuestos al sabor tras condicionamiento simultáneo sabor-sacarosa mientras están sedientos no es debido a extinción, la primera predicción es la ausencia de evidencias de recuperación de la preferencia al sabor en los tests de recuperación espontánea, reinstauración o renovación (Exp. 2, 3 y 4), así como la ausencia de efecto de devaluación del EI (Exp. 6). La segunda predicción es que el sabor mostrará retraso en la readquisición de la preferencia durante el reentrenamiento tras la fase de exposición al sabor (Exp. 7), así como un descenso en la preferencia por un segundo sabor previamente emparejado simultáneamente con sacarosa cuando ambos sabores se presenten formando un compuesto no reforzado (Exp. 8).

Hipótesis 2. Si los animales privados de agua o agua y comida durante el entrenamiento adquieren las asociaciones sabor-sabor (aprendizaje no predictivo) y sabor-nutriente (aprendizaje predictivo), siendo el estado motivacional de prueba el que selecciona la expresión de ambos tipos de aprendizaje, entonces la IL debería aparecer en animales probados hambrientos, pero no probados sedientos, con independencia del estado motivacional durante el entrenamiento.

La predicción en este caso es que si la atenuación de la preferencia al sabor sólo ocurre en el aprendizaje predictivo sabor-nutriente, que es seleccionado por el estado de hambre durante el test, entonces la IL aparecerá como una menor razón de preferencia en los animales preexpuestos al sabor comparados con los no preexpuestos cuando los éstos estén hambrientos y sedientos durante todo el experimento, pero no cuando estén sólo sedientos (Exp. 9A). Además, la IL no aparecerá en ratas probadas sedientas aunque se entrenen hambrientas (Exp. 10), pero sí en ratas entrenadas sedientas pero probadas hambrientas (Exp. 11).

CAPÍTULO 5: FENÓMENOS RELACIONADOS CON LA EXTINCIÓN

El propósito de este capítulo fue evaluar la naturaleza del decremento de la PCS basada en nutrientes durante la exposición repetida al sabor no reforzado en ratas hambrientas tras el condicionamiento simultáneo sabor-sacarosa mientras estaban sedientas. Dado que este decremento ha sido considerado previamente como extinción, se evaluaron los fenómenos de recuperación de la respuesta extinguida.

Para ello, inicialmente se realizó un estudio preliminar previo con el objetivo de poner de manifiesto la disociación entre la preferencia basada en nutriente y la preferencia basada en palatabilidad. Así, los Experimentos 0A y 0B mostraron la ausencia de preferencia condicionada al sabor en animales sedientos tras el condicionamiento simultáneo usando un sabor no preferido como EC y un nutriente palatable como EI. Por el contrario, una robusta preferencia por el mismo sabor se observó cuando las consecuencias post-ingesta del EI fueron relevantes al probar a los animales hambrientos. Ambos resultados confirmaron que el aprendizaje sabor-sabor y sabor-nutriente pueden ser efectivamente disociados manipulando el estado motivacional en el momento de la prueba.

A continuación se evaluó el efecto básico referido al decremento en preferencia condicionada al sabor tras la exposición al sabor no reforzado en ratas hambrientas y se examinó la idoneidad de la condición serial como grupo control (Exp. 1), demostrando que el entrenamiento simultáneo fue más efectivo que el serial, y que la exposiciones al sabor tras el entrenamiento en animales hambrientos y sedientos produce una reducción en la preferencia condicionada. Aunque este descenso significativo en preferencia se encontró en el grupo simultáneo (grupo experimental) en cada experimento de esta serie destinada a evaluar los fenómenos relacionados con la extinción, no hubo evidencia de recuperación espontánea (Exp. 2) ni de reinstauración (Exp. 3). Además, se encontró un efecto en sentido opuesto en renovación contextual (Exp. 4). Finalmente, se comprobó que la exposición al sabor tras el condicionamiento y antes de la devaluación del EI debilitó la asociación original sabor-sacarosa, no encontrándose el efecto de devaluación del EI que sí apareció en ausencia de exposición (Exp. 5).

Estos resultados ponen en cuestión que el descenso en preferencia observado en animales hambrientos tras repetidas exposiciones al sabor en solitario después del condicionamiento simultáneo pueda ser debido a la extinción de la respuesta condicionada. Por el contrario, si se considera que durante el condicionamiento se produce una configuración sensorial formada por el sabor neutro + el sabor de la sacarosa que se asocia con las propiedades reforzantes de la sacarosa (propiedades nutritivas y hedónicas), la presentación aislada del sabor en animales hambrientos podría activar la representación de ambas propiedades reforzantes, especialmente las propiedades nutritivas que ahora estarían ausentes. Bajo estas condiciones, el sabor aislado podría actuar como una señal de la ausencia de consecuencias positivas post-ingesta en ratas hambrientas. Según apuntan Higgins y Rescorla (2004), la exposición al sabor neutro y al sabor de la sacarosa formaría un compuesto durante el condicionamiento que sería difícil de separar en sus elementos. En la fase en la que se presenta el EC solo, lo que parecería extinción podría reflejar la formación de la representación de uno de los componentes del compuesto. A medida que se presenta repetidamente el sabor en solitario, la representación de este componente se diferenciaría de la representación el compuesto. Finalmente, se asume que el resultado final sería una asociación inhibitoria entre dicha representación del sabor aislado y las propiedades nutritivas. De ser así, el sabor debería mostrar las propiedades de un inhibidor neto, pasando las pruebas de retraso y sumación.

CAPÍTULO 6: INHIBICIÓN CONDICIONADA EN PREFERENCIA CONDICIONADA AL SABOR BASADA EN NUTRIENTE

Los experimentos realizados en este capítulo tuvieron como propósito evaluar las posibles propiedades inhibitorias adquiridas por un sabor durante las exposiciones repetidas en solitario tras el condicionamiento, usando los tests de retraso y sumación. Antes de ello, se estudió la posibilidad de obtener condicionamiento inhibitorio en preferencia basada en nutriente utilizando un procedimiento de inhibición condicionada pavloviana (Exp. 6), dada la ausencia de trabajos publicados al respecto. Posteriormente, dos experimentos de preferencia condicionada al sabor evaluaron el efecto de la exposición al sabor en solitario en ratas hambrientas que habían sido condicionadas de forma simultánea con el nutriente estando sedientas (el mismo procedimiento utilizado en los experimentos anteriores, Exps. 1-5). Los resultados mostraron un retraso en el condicionamiento del sabor tras la exposición del EC (Exp. 7). Asimismo, el sabor disminuyó la preferencia mostrada por un sabor previamente emparejado de manera simultánea con sacarosa cuando ambos sabores fueron presentados formando un compuesto no reforzado en el test de sumación (Exp. 8). Por el contrario, ninguno de estos efectos se encontró en el grupo control, que había recibido presentaciones seriales sabor → nutriente. Tomados en conjunto, estos resultados sugieren que el sabor presentado inicialmente en compuesto con sacarosa adquiere las propiedades de un inhibidor neto cuando es presentado posteriormente fuera del compuesto estando los animales hambrientos, sugiriendo que el mecanismo responsable del descenso de la preferencia condicionada es el establecimiento de un condicionamiento inhibitorio EC-EI.

CAPÍTULO 7: CONTROL MOTIVACIONAL DE LA INHIBICIÓN LATENTE EN PREFERENCIA CONDICIONADA AL SABOR

Dado que la presentación no reforzada del sabor tras el condicionamiento con sacarosa afecta a la preferencia basada en la asociación sabor-nutriente (animales hambrientos durante el test), pero no a la preferencia basada en la asociación sabor-sabor (animales sedientos durante el test) (Harris et al, 2004, Exp. 2B), en este capítulo se examinó si el efecto de los ensayos no reforzados podría extenderse al procedimiento de IL. Específicamente, se evaluó el control motivacional de la IL en PCS. Para ello, se manipularon diferentes estados de hambre o sed bien durante el entrenamiento (preexposición-condicionamiento) bien durante el test y se examinó la efectividad de las preexposición del sabor sobre el condicionamiento sabor-sacarosa.

Los resultados mostraron que la IL sólo apareció en animales entrenados y probados hambrientos (Exp. 9A y 10). La ausencia de LI en animales sedientos no se debió a un déficit en la adquisición de preferencia condicionada (Exp. 9B). No obstante, los resultados mostraron que el entrenamiento en animales hambrientos no era condición suficiente (Exp. 10) ni necesaria (Exp. 11) para obtener IL. Por tanto, independientemente del estado motivacional durante el entrenamiento, el efecto de IL se obtuvo cuando los animales se probaron hambrientos. Tomados en conjunto, estos resultados apoyan la idea de que los animales aprenden sobre la ausencia de propiedades nutritivas durante la preexposición al sabor, tanto cuando están hambrientos como cuando están sedientos. Este aprendizaje parece interferir con el aprendizaje asociativo entre el sabor y las propiedades nutritivas durante el condicionamiento. No obstante, esta interferencia sólo se pone de manifiesto cuando el aprendizaje sabor-nutriente es el que controla la ejecución durante la prueba: cuando los animales están privados de comida en esta fase. Por el contrario, no se observa el efecto de IL sobre el aprendizaje sabor-sabor, cuando los animales están solamente sedientos. Finalmente, los datos apoyan la hipótesis principal defendida en esta disertación que afirma que solamente el aprendizaje sabor-nutriente, de naturaleza predictiva, se ve afectado por los ensayos no reforzados previos al condicionamiento.

CAPÍTULO 8: CONCLUSIONES

El examen de las propiedades inhibitorias adquiridas por un sabor presentado repetidamente en solitario en ratas hambrientas después de ser condicionado de forma simultánea con el nutriente estando sedientas confirmó que dicho sabor se comportó como un inhibidor condicionado, pasando las pruebas de sumación y retraso.

Estas propiedades inhibitorias permiten explicar las polémicas conclusiones encontradas en los estudios de “extinción” de preferencia condicionada al sabor. Por ejemplo, Harris et al. (2004) y Delamater (2007) informaron que el procedimiento de extinción en PCS debilitó la asociación sabor-sacarosa: una conclusión que está en contra de los resultados obtenidos en estudios de extinción que han usado paradigmas de aprendizaje más convencionales (Delamater, 2007). Por el contrario, si una asociación inhibitoria entre el sabor y la sacarosa que dota al sabor de las propiedades de un inhibidor neto se desarrolla tras la exposición del sabor después de condicionamiento, sería esperable la ausencia de efecto de devaluación y la ausencia de fenómenos relacionados con la recuperación de la preferencia condicionada, pues el sabor no debería activar la representación del EI.

Dado que diferentes aproximaciones teóricas han supuesto que la extinción es el resultado de un proceso inhibitorio que reduce la excitación durante el condicionamiento, y que el presente examen de las propiedades inhibitorias del sabor expuesto tras el condicionamiento ocurren en el contexto de lo que se ha considerado extinción, resulta conveniente resaltar que esta disertación no propone que la exposición al sabor después del condicionamiento convierta a un estímulo “extinguido” en un inhibidor neto. De hecho, se sugiere una posibilidad, siguiendo la propuesta de Higgins y Rescorla (2004), compatible con las propiedades inhibitorias netas del sabor y el aparente procedimiento de extinción. Esta explicación propone que ratas expuestas al compuesto sabor-sacarosa pueden no identificar separadamente el sabor neutro y el sabor de la sacarosa como elementos aislados, formando más bien una representación unitaria del compuesto sabor-sabor de la sacarosa. Dado que la sacarosa posee propiedades post-ingesta, dicha representación unitaria del compuesto podría asociarse con las propiedades nutritivas post-ingesta de la sacarosa. Entonces, lo que parece ser extinción de la preferencia condicionada por presentaciones repetidas del sabor en solitario podría ser en realidad la formación de la representación del sabor por diferenciación perceptual. Asimismo, mientras se presenta el sabor sin la sacarosa en animales hambrientos, el sabor podría activar la representación de las propiedades reforzantes del EI (las acciones post-ingesta y el valor hedónico), que ahora estarían ausentes, especialmente las acciones nutritivas post-ingesta. Esta activación podría explicarse, por ejemplo, a través del modelo SOP de Wagner (Wagner, 1981). Asumiendo que hay cierta generalización de la fuerza excitatoria desde el compuesto al sabor durante las primeras presentaciones del sabor, podría explicarse el aprendizaje inhibitorio neto al sabor. De hecho, Wagner argumentó que una asociación inhibitoria se forma cuando la representación del EC en el estado A1 se asocia con la representación del EI en el estado A2. Por tanto, si la presentación del sabor en solitario activa su representación en A1 y vía generalización con el compuesto el sabor produce la activación asociativa de la representación de las acciones nutritivas post-ingesta de la sacarosa en A2, esta situación podría dar lugar a la adquisición de propiedades inhibitorias por parte del sabor EC.

Aunque este proceso es meramente especulativo por el momento, representa una explicación sobre la adquisición de propiedades inhibitorias por parte del sabor sin entrar en contradicción con explicaciones actuales de la extinción en condicionamiento pavloviano. En consonancia con Bouton (2007), se asume que el sabor EC no llega a convertirse en un inhibidor condicionado neto tras la extinción y que un estímulo extinguido se parece más a un excitador que a un inhibidor. Esto es congruente con los modelos de extinción que aun implicando procesos de aprendizaje inhibitorio (p.ej., Bouton, 1993; Delamater, 1996) no asumen que un estímulo extinguido pueda llegar a convertirse en un inhibidor neto.

Respecto a los resultados observados sobre IL en PCS dependientes del estado motivacional de los animales durante el test, es importante subrayas que el ausencia de IL en animales probados sedientos es sorprendente y no se acomoda fácilmente a las teorías clásicas de la IL basadas en la atención/asociabilidad (p.ej., McLaren y Mackintosh, 2000; Pearce y Hall, 1980), en la asociación contextual del modelo de Wagner (1981), la interferencia asociativa (p.ej., Hall y Rodriguez, 2010) o los modelos basados en la ejecución (p.ej., Bouton, 1993,1997).

Desde la perspectiva propuesta en esta disertación, la ausencia de IL explica desde la aproximación de los modelos de déficit en la adquisición basado en la interferencia asociativa, no en los basados en la atención/asociabilidad pues el procesamiento del sabor EC se ve afectado durante la preexposición. De hecho, animales sedientos o sedientos y hambrientos aprenden a asociar el sabor y la sacarosa tras la preexposición al sabor, demostrado por la preferencia condicionada al sabor cuando los animales son probados sedientos con independencia de si el sabor ha sido preexpuesto o no. Una posibilidad es que durante la preexposición animales sediento o sediento y hambrientos formen una asociación sabor-no nutriente, debido a la tendencia de los animales a examinar el valor nutricional de cualquier sustancia comestible presente en el ambiente (Day, Kyriazakis, & Rogers, 1998). Este aprendizaje previo podría interferir con la formación posterior de asociaciones entre el sabor y la sacarosa cuando ambos se presentan en compuesto durante el condicionamiento, concretamente con la asociación sabor- nutriente. Finalmente, cuando los animales son probados hambriento durante el test, el aprendizaje predictivo basado en las expectativas de nutriente se selecciona reflejando el efecto de preexposición al sabor (IL). Por otro lado, dado que la sacarosa tiene dos propiedades reforzantes, la palatabilidad y el componente nutritivo, los animales también aprendería la asociación entre el sabor y el valor hedónico del sabor de la sacarosa. Sin embargo, esta última asociación no se vería perjudicada por la asociación previa sabor-no nutriente debido a la independencia de sus contenidos. Así, cuando los animales se probaron sedientos, el aprendizaje no predictivo basado en palatabilidad se seleccionó, reflejando el fallo del efecto de preexposición al sabor (no LI). La implicación más importante de los resultados presentados es que el aprendizaje en la IL depende de la clase de reforzador y que la expresión de la IL –o la ausencia de IL– depende del estado motivacional durante el test.

Estas conclusiones apoyan la idea de que manipulaciones de la contingencia EC-EI a través de las presentaciones no reforzadas del EC ya sea antes o después del condicionamiento son efectivas sólo en el aprendizaje sabor-nutriente, sugiriendo que se basa de hecho en un aprendizaje predictivo. El mecanismo de aprendizaje sabor-sabor, el cual no está basado en la expectativa de la ocurrencia del EI, parece ser insensible a este tipo de manipulación, lo que sugiere al mismo tiempo que se trataría de un mecanismo no predictivo.

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Part 1

THEORICAL INTRODUCTION

Chapter 1

INTRODUCTION

1.1. INTRODUCTION

1.1.1. MOTIVATION

Healthy eating, dieting, and eating-related problems such as overweight, obesity and eating disorders have increased our interest in eating behaviour. The best evidence of this fast growing interest of eating behaviour and eating process is reflected by the exponential growth in scientific literature during the last few decades.

Research on eating behaviour has mainly been focused around two broad topics. The first one is control of intake, which concerns the parameters of when and how much an organism eats. The second one is food selection, in terms of what foods an organism likes and chooses to eat. The study of food intake has addressed a variety of major issues such as factors responsible for the initiation of feeding or the termination of meals. By contrast, factors such as palatability, food choice, avoidance of substances, and social influence have received attention from the study of food selection. In both areas, much of the research has highlighted learning determinants of eating behaviour.

In the domains of food selection, development of eating patterns, and modification of dysfunctional eating habits, an area that has received considerable attention from learning is the acquisition of food preferences in the laboratory. For instance, it is now well established that robust food preferences can also be observed as animals experience the positive effects of foods such as the hedonic value of their taste or the post-ingestive properties of their macronutrients. Most interestingly, the study of acquisition of food preference has provided a framework for understanding the role of learning in the regulation of eating, appetite, and diet choice. Knowing how these preferences develop should help us to understand how and why specific products are selected over others, giving rise to the formation of persistent, and frequently unhealthy, learned food preferences.

Nevertheless, we still know little about the basic processes underlying the acquisition and modification of preference for foods. To provide further investigation of the conditioned food preference through the conditioned flavour preference paradigm using rats as subjects is the principal aim of this dissertation.

1.1.2. ORGANIZATION OF THE DISSERTATION

Having provided the motivation for the research, we proceed to outline the contents of the present work. This dissertation research examines the effectiveness of non-reinforced trials in conditioned flavour preference (CFP) through the procedures of extinction and latent inhibition (LI) using an orally consumed nutrient (sucrose) as unconditioned stimulus (US). Given the specific characteristics of the CFP, Chapter 2 describes the different stimuli and variations of this paradigm that have traditionally been used, and the procedural parameters that influence this phenomenon. Moreover, it reviews the CFP based on nutrient in terms of procedure, content of learning, and mechanisms. Finally, it examines the duality of mechanisms proposed on CFP paradigm, where preference based on the taste of the nutrient appears to be a case of non-predictive learning, whereas preference based on the post-ingestive effect of nutrient appears to be a case of predictive learning.

Chapter 3 reviews the studies that describe the effectiveness of non-reinforced conditioned stimulus (CS) presentation upon the CS-US contingency in CFP. Specifically, it examines the effect of non-reinforcement of the flavour cue after flavour-nutrient pairings, as well as the effect of the flavour presented alone either during or before flavour-nutrient pairings. Chapter 4 contains the justification and objectives of this research.

Chapter 5 explores the nature of the decrement in CFP based on nutrient following the extinction procedure. It examines the phenomena related to the recovery of the conditioned preference lost in rats trained thirsty with a simultaneous flavour-sucrose compound and exposed to the flavour unreinforced while hungry and thirsty. The US devaluation effect is also assessed. Chapter 6 assesses the putative net inhibitory properties acquired by the CS flavour, using the retardation of acquisition and summation tests for conditioned inhibition. Chapter 7 provides empirical evidence of the motivational control of LI in CFP. Different motivational states of hunger or thirst presents during preexposure-conditioning training and testing are used to assess the effectiveness of non-reinforcement flavour presentations before flavour-sucrose pairings.

Finally, Chapter 8 summarizes the research and contributions presented in this dissertation, and discusses the implications of these results in the context of CFP.

Chapter 2

CONDITIONED FLAVOUR PREFERENCE

2.1. INTRODUCTION

The quest for food and food selection are very frequent and necessary activities and probably place more demands on behaviour than any other activity. In fact, food search, identification, selection, and ingestion accounts for most of the waking time of most animals (Rozin & Schulkin, 1990). Among the many potentially edible substances, organisms face the problem of identifying food sources, avoiding toxins, and eating a nutritionally balanced diet. Faced with a variety of potential sources of nutrient available in the environment, animals must decide which to eat and which to reject. In doing so, food selection is perhaps the single most important force in animal evolution (Rozin, 2007), and surely the prominence of food selection holds for humans as well (Rozin & Schulkin, 1990). Under strong selection pressure, the powerful series of interlocking mechanisms to guide this process has evolved in animals. One of these mechanisms is referred to as the flavour system, which is composed of all the senses and processes that are directed at the overarching goal of optimizing food selection (Stevenson, 2009). Given the high survival value of correctly identifying food properties, the flavour system can be seen as part of a defence system to protect our internal environment. Such a system becomes important to decide whether a flavour is an appropriate food or not by means of recognizing nutrient and providing warning signals based on the sensory properties of substances, which might have inherently toxic actions (Prescott, 1999).

The flavour system involves almost all the senses, particularly the senses of smell and taste. Although senses of sight and hearing are implicated in locating and identifying food, olfaction, taste and somatosensation (touch, temperature, irritation, and pain) play a crucial role in detecting features of a food once a decision has been made to place the food in the mouth. Information from these three senses are combined to form an emergent property: flavour (Stevenson, 2009; see review in Auvray & Spencer, 2008). It is well known that the terms *flavour* and *taste* have been used interchangeably. In the strict sense, the term *taste* refers only to those sensations arising from the taste system, whereas the term *flavour* is used to denote the integration of sensory cues, including those arising from olfaction, taste and somatosensorial systems (St. John & Boughter, 2008). To prevent the confusion over terminology that can arise in this setting, the Stevenson, Prescott, and Boakes's (1995, p. 434) convention is adopted here under which they "use *odour* to refer to the sensation arising from active sniffing, *flavour* to refer to the olfactory sensation arising from ingestion of a substance that may or may not include a taste component, and *taste* to a substance such as a sucrose solution or saline that includes no significant odour component".

From an evaluation of the sensory characteristic of the foodstuff, particularly the flavour, animals (e.g., rats) select or reject food. In determining food preference (i.e., the selection of one substance over anothers), genetic predispositions interact with aspects of the environment to produce specific flavour preference. Birch (1999) has suggested that the innate predispositions include the predisposition to prefer sweet tastes (the preference for sucrose emerges in rats over the first 2 week of postnatal life and does not depend on previous experience; Hall & Bryan, 1981) and salty tastes (at 10 days of age rats appear to prefer hypertonic saline solution to water; Bernstein & Courtney, 1987), as well as to reject those that are sour and bitter (Hall & Bryan, 1981; Johanson & Shapiro, 1986). Other innate predispositions are rejecting novel substances (neophobia), and learning preferences for the more familiar (Miller & Holzman, 1981).

Through experience however, animals may refine their flavour preferences. In fact, the preference response to gustatory stimuli changes as a result of experience. This behavioural plasticity in food preference enables organisms to adapt to the special demands of the particular area in which they are located. Thus, unlike the newborn animals that display only a few innate flavour preferences, adults exhibit robust acquired preference for an incredible number of flavours. This tremendous plasticity of flavour preference throughout an individual's lifespan and the range of individual differences in these preferences are attributed to the effects of learning (Myers & Sclafani, 2006). Learning can increase preference for a flavour in different ways. The simplest may be the effect of familiarity –the so-called "mere exposure effect" –

through which flavours become increasing preferred by virtue of repeated exposure (Hill, 1978). However, while mere exposure produces an increase in preference (by reducing inherent neophobia), it does not typically produce robust preferences or does it stimulate the over-consumption that results in positive energy balance. These more significant preference effects are attributed to Pavlovian or classical conditioning, through which an initially arbitrary (o even initially aversive flavour) can become strongly preferred due to learned associations (Myers & Sclafani, 2006; Rozin & Zellner, 1985). A case of preferences attributed to learned associations is the conditioned flavour preferences, which result from the association of food cues with positive consequences of its ingestion. In fact, after repeated opportunities to consume food, animals learn to associate foods' sensory cues with positive effects of ingested substances (Capaldi 1992). This learning plays a crucial role in food choice, since it permits to optimize nutrition when challenged, for example, by a variety of nutrient deficiencies or special energetic demands.

2.2. CLASSICAL CONDITIONING

As pointed out by Tarner, Friedman, and Mehiel (2004), many studies have been conducted on flavour preference learning since LeMagnen in 1955 first suggested it could be due to conditioning. From then onward, classical conditioning has been used to account for learned flavour preference (Rozin & Schulkin, 1990; Rozin & Zellner, 1985). In the laboratory, the most common procedure used in the experimental study of acquired flavour preference is the CFP paradigm (see Sclafani, 1999). At a procedure level, this paradigm involves the presentation of a neutral flavour (CS) paired with the reinforcing properties of a substance (US) that may be either a palatable taste (e.g., Holman, 1975) or post-oral nutrient actions (e.g., Capaldi, Campbell, Sheffer, & Bradford, 1987; Sclafani & Nissenbaum, 1988). After a number of pairings, the flavour preference is assessed in two-bottle choice tests. In these tests, rats display substantial preferences for a conditioned flavour (CS+) either over other flavour non-paired with the US (CS-) or over water.

2.2.1. NATURE OF STIMULI

There are several variations of the CFP paradigm according to the nature of conditioned and unconditioned stimuli used.

Taste as CS

Regarding the sensorial modality of the CS, some studies have used basic tastes –salt (salty), citric acid (sour), saccharin (sweet), and quinine (bitter) – as CSs (e.g., Drucker, Ackroff, & Sclafani, 1994, using a bitter sucrose octaacetate solution or a sour citric acid monohydrate solution with intragastric Polycose infusions), though tastes are generally used as USs (e.g., Fanselow and Birk, 1982). The results point to the fact that rats acquired strong preferences even for initially non-preferred tastes (e.g., Drucker et al., 1994). Although some authors (e.g., Capaldi, Hunter, & Lyn, 1997) have suggested that conditioning preferences to CS tastes may be complex because the affective reaction of tastes might interfere with their functioning as CS, preferences conditioned in compound with the US (e.g., sucrose) are similar to those for extracts (odour) conditioned in the same way (Capaldi & Hunter, 1994).

Complex flavour as CS

Other studies have also reported conditioned flavour preferences with a mixture of taste and extract as CS (e.g., sodium saccharin solutions flavoured with unsweetened Kool-Aid in Ackroff and Sclafani's [2006] experiments).

Odour as CS

Regarding the olfactory stimuli, the role of odour cues in conditioned flavour preference is of particular interest because, whereas animals have innate predispositions to prefer (or avoid) specific tastes, olfactory preferences appear to be primarily acquired through experience (Bartoshuk, 1991; cited in Lucas & Sclafani, 1995, p. 446). Studies that have examined pure conditioned odour preference (e.g., Lucas & Sclafani, 1995) pairing extracts (e.g., almond) with intragastric infusions of Polycose, support the effectiveness of odour cues to produce conditioned flavour preferences in rats.

Nutrient as US

According to the nature of US, different lines of work have utilized specific nutrients to produce conditioned flavour preference (see Sclafani, 1999). The nutrients vary in their ability to produce these learned preferences in rats (see Ackroff, 2008, for a review). Carbohydrate (e.g., Gibson & Booth, 1989; Lucas & Sclafani, 1995), protein (e.g., Baker, Booth, Duggan, & Gibson, 1987), fat (e.g., Ackroff, Lucas, & Sclafani, 2005; Pérez, Lucas, & Sclafani, 1995), ethanol (e.g., Ackroff, Rozental, & Sclafani, 2004), even artificial diet (e.g., Bolles, Hayward, & Crandall, 1981) have all been used in CFP paradigm. Ackroff (2008) suggested that the optimal nutrient for conditioning flavour preferences is glucose (and glucose polymers), which conditions strong preferences in short session procedures.

Palatability as US

A second category of US is the palatable taste of a non-nutritive solution. The importance of *palatability*, defined by Young (1948, p. 310) as "immediate affective reaction of an organism which occurs when a food comes in contact with the head receptors", in conditioned preference and food choice has been such that it has prompted a fuller analysis of the concept of preference (Rozin & Schulkin, 1990). Holman (1975) demonstrated that the conditioned flavour preferences might be reinforced by orosensory properties of substances. In both cases, preferences based on the post-ingestive effect of nutrient and on palatable taste of non-nutrient, the magnitude of the reinforcing properties of the US may be important. For example, a reduced concentration of a palatable taste in solution or a weak nutrient infusion may make the development of flavour preferences more difficult. Another relevant factor that contributes to the reinforcing potency of a nutrient is the rapidity of post-oral effects. For example, the post-absorptive effects of fructose and fat are delayed relative to those of glucose, which may account in part for their weaker effects (Ackroff, 2008).

2.2.2. PROCEDURAL PARAMETERS

Route of US administration. Several methods produce conditioned flavour preference via a route that bypasses the oral cavity. One common non-oral method is pairing the intake of flavoured water with an intragastric (i.g.) nutrient infusion. With the appropriate i.g. infusion variables (volume, concentration, or rate), flavour preferences have been conditioned using different nutrients (e.g., Azzara & Sclafani, 1998; Sclafani, Cardieri, Tucker, Blusk, & Ackroff, 1993; but see Deutsch, Molina, & Puerto, 1976). Other post-oral methods have delivered nutrient into duodenum (e.g., Drucker & Sclafani, 1997), jejunum, ileum (e.g., Ackroff, Yiin, & Sclafani, 2010), or hepatic portal vein (e.g., Tordoff & Friedman, 1986) with disparity of results. Although the mechanism of action by which nutrient condition flavour preference acts is still an unresolved issue, Ackroff et al. (2010) have suggested that duodenum and jejunum are critical sites for glucose-conditioned preferences, because through bypassing the intestine the infusion of nutrient makes it ineffective for conditioning a preference for a flavoured solution. As Capaldi (1995) claimed, in these methods the flavour of the nutrient cannot interfere with the association between the conditioned flavour and the post-ingestive nutrient reinforcement. However, this situation occurs when the nutrient is orally administrated (e.g., Fanselow & Birk, 1982). The oral method is the simplest and most natural way to condition flavour preferences and consists of adding the flavour cue to the US substance which the animals eats through the mouth. This method mimics what normally happens when food is consumed (Sclafani, 1995).

Conditioning procedure. Unlike other conditioning paradigms that mainly utilize the standard forward conditioning procedure, the simultaneous conditioning procedure is frequently found in CFP paradigm; thus, the CS and the US are mixed forming a compound (e.g., Mehiel & Bolles, 1988). Rats can also be trained to consume a flavour cue followed by the delayed presentation of the US (e.g., Holman, 1975; Elizalde & Sclafani, 1988). Holman reported that rats develop a preference for a flavour that was followed 30 min later by a glucose solution, but not for a flavour followed 30 min later by a saccharin solution. However, rats displayed a flavour preference when the saccharin was presented immediately after the flavour or when it was mixed with the flavour. These results suggest that preference based on the palatable taste of the US occurs only if there is little or no delay between the CS flavour and the US taste, whereas preferences based on post-ingestion effects occur even when there is a delay between the CS flavour and the US nutrient (Elizalde & Sclafani, 1988). Significant conditioned flavour preferences have been obtained with delays as long as 1-5 h between flavour cue and US nutrient (e.g., Capaldi & Sheffer, 1992). This capacity to acquired flavour preferences with nutrients using a delayed method mimics the normal dynamic of the physiological function of ingestion and digestion. If anything, several studies have reported that the simultaneous procedure conditions stronger preferences that do the delay/trace procedures (e.g., Sclafani & Ackroff, 1994). In addition, in both the oral-simultaneous and the oral-delay procedures the flavour of the nutrient may interfere with or enhance the conditioning of the neutral flavour. In the oral-simultaneous procedure, the interference with the conditioning of a stimulus can be due to overshadowing (e.g., Capaldi & Hunter, 1994). On the other hand, the enhancement can occur between olfactory and gustative stimuli in compound through the potentiation effect (e.g., Capaldi & Privitera, 2008). As suggested by Dwyer, Haselgrove, and Jones (2011), unlike potentiation, it is possible that overshadowing is more likely to occur when the compound comprises elements that are from the same sensory modality, when stimuli are of equal salience, or when the generalization between a compound and its elements is weak. If anything, mixing the flavour cues with the reinforcers seems to actually facilitate, rather than interfere with, the conditioning of preferences (e.g., Boakes, Rossi-Arnaud, & Garcia-Hoz, 1987).

Number of pairings. Although prior work has documented robust effects after extensive training, other studies with hungry rats found a significant increase in glucose-based flavour preference (measured as CS+ vs. CS-) for a flavour paired only once with an i.g. glucose infusion during 10-min (Myers, 2007) or 30-min training sessions (Ackroff, Yiin, & Sclafani, 2009). This has implications for the ecological and adaptive significance of preferences based on nutrients, in that a single flavour-nutrient training trial provides an experience comparable to what could normally occur in a single meal. On the other hand, preferences based on tastes also are formed quickly (e.g., Boakes et al., 1987). Therefore, the conditioned flavour preferences appear to be established rapidly.

Motivational state. In the CFP paradigm, animals can be trained or tested under food and/or water deprivation or under non-deprived conditions. When nutrients are used as US, some investigators have postulated that the post-ingestive actions of nutrients are reinforcing during training only if the animals experience some level of hunger by either water (water deprivation reduces dry food intake; Bolles, 1961) or food deprivation. For example, Harris, Gorissen, Bailey, and Westbrook (2000) suggested that rats failed to learn about the calorie properties of a nutrient because they were not hungry, being neither food nor water deprived. In contrast, others maintain that nutrient reinforcement does not require an energy deprivation state (e.g., Yiin, Ackroff, & Sclafani, 2005b). Drucker, Ackroff, and Sclafani (1993) demonstrated a strong preference for a flavour (over water) paired with intragastric infusion of 32% Polycose in rats with ad libitum access to food and 23 h/day access to fluid solutions. These different considerations of the role of food deprivation might be explained by the fact that small amounts of nutrient in brief training sessions produce a weak US, as could be the case in the study by Harris et al. (2000); they trained animals with a dilute 4% sucrose during 10 min/day sessions. Although food restriction could facilitate flavour conditioning because of enhancement of the reinforcing value of nutrient by increasing the animal's energy need (Davidson, 1998; Capaldi, Owens, & Palmer, 1994), Yiin et al., (2005b) have demonstrated that food deprivation enhances the expression but not the acquisition of flavour preference for a flavour paired with the post-oral action of a nutrient in rats. In turn, Yiin, Ackroff, and Sclafani (2005a) demonstrated that the post-oral actions of the nutrient were reinforcing in both food-restricted and food-ad-libitum rats. Fedorchak and Bolles (1987) examined the effects of food deprivation on rat's preference for flavours paired with saccharin and sucrose. The results showed that the flavour paired with sucrose was highly preferred when rats were tested in a food-deprived state and that these preferences decreased when testing occurred after 24 hr of ad libitum feeding. This effect was not found when saccharin was used as the US. Flavour paired with saccharin was preferred regardless of the hunger level manipulation during testing. Likewise, a preference based primarily on a taste may be more stable across different physiological states and longer lasting than a preference depending on consequences of ingestion (Rozin & Zellner, 1985), and seems to be independent of test-time hunger level (Fedorchak & Bolles, 1987). Overall, there is considerable evidence that animals learn about both oral and post-oral properties of nutrient at the same time within an oral simultaneous conditioning procedure, and that conditioned preferences reinforced by sucrose and other caloric substances seem to be highly sensitive to post-conditioning hunger level manipulations (hunger-sensitive preference). However, the same hunger changes have no effect on non-caloric mediated preferences (hunger-insensitive preference).

Control conditions. In most studies of CFP paradigm using within-subject designs, each animal receives two different types of CS trials during conditioning: CS+ (the flavour paired with the US) and CS- (the flavour paired either a different US, water, or unpaired with US), in combination with control procedures such as counterbalancing of the assignment of CS among animals. The result of conditioning is measured by a subsequent two-bottle CS+ vs. CS- choice test. It has been argued however that this measure of conditioning is inadequate because they cannot definitely rule out the possibility that preferential intake of the CS+ is partly due to learned avoidance of the CS- flavour, which had been unpaired with the US (see section 3.2.1). In other cases, between-subject designs have used groups given various control conditions during the initial training phase. For instance, in the unpaired condition, the target flavour and the nutrient are available on different days. As in the within-subject designs mentioned above, this negative contingency has the potential to produce inhibitory learning (if flavour, then not sucrose) that might reduce preference for the flavour (Albertella & Boakes, 2006). Thus, this control group may be misleading when it is compared with a group given a paired condition (e.g., access to a compound of the flavour cue and nutrient as US) and a further control conditions are absent. Another control group consists in using a group given simple exposure to a flavour cue. This latter group may not be a satisfactory control because it does not have the same number of US experiences as the paired condition; therefore, any differences between conditions can be attributed to differences in experience with the nutrient. Another control condition used is a blocked one in which rats receive either several flavour cue sessions in succession, followed by a block of nutrient sessions, or vice versa (e.g., Dwyer, Bennett, & Mackintosh, 2001).

In sum, the CFP has been demonstrated using a variety of stimuli and methods. The preferences for new flavours are enhanced when they are associated with already preferred tastes. Likewise, they appear capable of associating novel flavours with nutrient and develop strong preferences. More importantly, the data reviewed here show that flavour conditioned preference are established rapidly, the expression of preference based on positive post-ingestive consequences depends on the motivational state during testing, and the assessment of flavour preferences depends on the type of control conditions.

2.3. CONDITIONED FLAVOUR PREFERENCE USING A PALATABLE NUTRIENT AS US

In this point, it is important to indicate that the CFP using a palatable nutrient as US can be considered separately as preferences based on palatable or hedonic taste of a nutrient, on the one hand, and preferences based on the post-ingestive consequences of a nutrient, on the other (e.g., Sclafani & Ackroff, 1994). In procedural terms, both types of preferences have been experimentally separated with specific manipulations. Thus, the use of a palatable taste of non-nutritive substance has made possible the study of preference based on palatability (e.g., Fanselow & Birk, 1982), whereas the use of i.g. nutrient infusion has made possible the study of preference based on nutrient (e.g., Elizalde & Sclafani, 1990). In contrast, when a flavour and a nutrient are orally presented both types of conditioned preference can occur, as many nutrients also have palatable tastes (e.g., the sucrose). For instance, a novel flavour added to a sucrose solution may pair with both the sweet taste of the sucrose, and the post-oral nutrient effects of the sugar. We will discuss the characteristics and nature of conditioned flavour preferences with orally administrated nutrient in the following sections.

2.3.1. FLAVOUR-TASTE LEARNING VERSUS FLAVOUR-NUTRIENT LEARNING

The distinction between palatability-based and nutrient-based preferences does not only involve two different procedures, but also seems to underpin different processes (Dwyer, 2005; Fedorchak & Bolles, 1987). According to the reinforcing property of a nutrient, two types of learning have been differentiated in CFP. One is based upon hedonic evaluation of the taste of the nutrient (similar to the learning based on non-nutrient palatable US), phenomenon known as *flavour-taste learning* (also called *flavour-flavour learning*, *flavour-taste conditioning*, or *flavour-flavour conditioning*) (Capaldi, 1992, 1996). The other is based upon expected outcomes, phenomenon known as *flavour-nutrient learning* (also called *flavour-nutrient conditioning*) (Capaldi, 1992, 1996; Capaldi & Privitera, 2007; Sclafani, 1999; Sclafani & Ackroff, 1994).

As pointed out by both Dwyer (2005) and Myers and Sclafani (2006), several lines of evidence support the idea that flavour-taste learning and flavour-nutrient learning actually differ at a processes level. For instance, they produce behavioural change by affecting different sub-components of the feeding behaviour sequence (Myers & Hall, 1998). Specifically, preference based on palatability produces conditioning of appetitive responding (olfactory orienting) to the flavour, while preference based on nutrient produces conditioning of consummatory responding (oral responsiveness) (but see Myers & Hall, 2000). They are differently sensitive to temporal parameters during training (Lyn & Capaldi, 1994). Unlike nutrient-based preference, palatability-based preference is not formed when there is a delay between the flavour cue and the palatable reinforcer (Sclafani & Ackroff, 1994). Although both palatability-based and nutrient-based preferences appear to be sensitive to reinforcer-specific devaluation effect, they seem to differ with respect to how immediate and direct the effect is. This is also consistent with the idea that these types of learning are underpinned by different mechanisms (Dwyer, 2005). Furthermore, they are differently affected by motivational state. Despite evidence for the simultaneous operation of multiple mechanisms within a single conditioning procedure and with the same reinforcing nutrient, food restriction only seems to facilitate the expression of nutrient-based preference by means of stimulating the intake of the conditioned flavour during the preference testing (Fedorchak & Bolles, 1987; Yiin et al., 2005a). For example, Harris et al. (2000) found that thirsty rats given the flavour in simultaneous compound with sucrose learned these two kinds of associations during training, and that motivational state selected which one controlled performance at the time of testing: food deprivation controlled nutrient-based but not palatability-based preference. Finally, they are thought to differ in their susceptibility to extinction and other CS-US contingency manipulations; in particular, the resistance to extinction has received attention (see section 3.2.) (e.g., Harris, Shand, Carroll, & Westbrook, 2004). These experimental dissociations seem to be inconsistent with a simple mechanism account and suggest that, despite procedural similarities, flavour-taste learning and flavour-nutrient learning may rely on different processes that operate independently (Myers & Sclafani, 2006; Warwick & Weingarten, 1994).

Although the mechanisms that underlie nutrient-conditioned flavour preference are not actually well known (Myers and Sclafani, 2003) and are still a matter of debate, the dissociations reviewed above on flavour-taste learning and flavour-nutrient learning have promoted the thinking about a dual-mechanism account in the underlying nature of conditioned flavour preference learning (e.g., Campbell, Capaldi, Sheffer, & Bradford, 1988; Drucker et al., 1994; Dwyer & Quirk, 2008; Fedorchak, 1997; Harris et al., 2004; Harris & Thein, 2005; Tarner et al., 2004). Nevertheless, before discussing the mechanisms proposed to explain the flavour preference learning, we will further examine the CFP paradigm in terms of content of learning.

2.3.2. CONTENT OF LEARNING IN CONDITIONED FLAVOUR PREFERENCE USING A PALATABLE NUTRIENT AS US

Delamater & Oakeshott (2007) suggested that the CS becomes potentially associated with a number of different properties of the US, such as its sensory (e.g., gustatory or olfactory components of the US), hedonic (i.e., how attractive the US is), motivational (i.e., such as calories when the subject is hungry), and temporal properties (i.e., how much time elapses between the CS and the presentation of the US), as well as the overt response components of the S-R association (Figure 1).

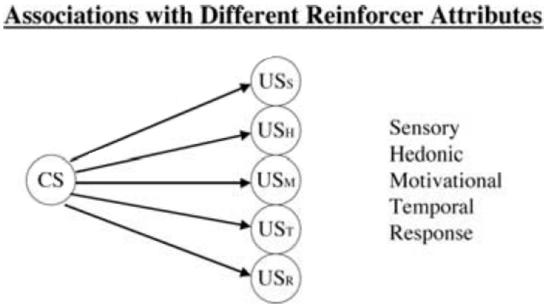

Figure 1. Example of possible components of Pavlovian US representation, and possible associative links between the CS and those US components. From “Learning about Multiple Attributes of Reward in Pavlovian Conditioning” by A.R. Delamater and S. Oakeshott, 2007, *Annals of the New York Academy of Sciences, 1104*, p. 3.

In CFP, the taste of the nutrient contains specific sensory properties and positive hedonic properties when a palatable nutrient is used as US. This is in consonance with authors such as Galef (1981) that conceived the ontogeny of response to tastes as evoking response in two relatively independent dimensions: a discriminative dimension and an affective one. The discriminative dimension would describe the detectability and identificability of a stimulus, whereas the affective dimension would describe the amount of pleasure or displeasure that a stimulus arouses in a sensing organism. According to these independent dimensions, a neutral flavour paired with sucrose can be associated with the sensory and affective/hedonic properties of the taste of sucrose[1].

Sensory flavour-taste association

Several works using the US devaluation technique[2] have shown the existence of sensory flavour-taste associations (Delamater et al., 2006; Delamater, 2007; Dwyer, 2005; Scaret, Campese, & Delamater, 2009), i.e., the associations formed between the conditioned stimulus (CS) and the sensory properties of the unconditioned stimulus (US). For instance, Dwyer (2005) showed that rats without food or water deprivation associated a grape flavour CS with one outcome (2% sucrose) and a cherry flavour CS with another outcome (2% maltodextrin). In the following phase, one of the USs was presented alone followed by an injection of lithium chloride (LiCl). In the test phase, rats were given a two-bottle choice test, where the two flavours were presented without their US associates. The rats avoided the flavour paired with the devalued US and consumed more of the flavour paired with the non-devalued US. Dwyer's (2005) results support the idea that the conditioned stimuli formed an association with the sensory-specific properties of the nutrient. Hence, when one of the nutrients was devalued by being paired with LiCl, the rats selectively avoided the flavour paired with it, but not the other flavour. If the flavours had merely been associated with non-specific motivational or hedonic properties of the reinforcer, then one would expect no difference in flavour preferences during the two-bottle choice test.

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[1] The current approach implicitly assumes an elemental explanation to Pavlovian conditioning. However, Pearce (2002) suggested that configural accounts might be usefully applied to flavour preference learning (see section 2.4.3.).

[2] The US devaluation technique (or devaluation of the reinforcer procedure) is used to reduce the attractiveness of an unconditioned stimulus, usually achieved by aversion conditioning (e.g., pairing it with i.g. LiCl) or satiation. In Pavlovian conditioning, US devaluation is used to determine whether the conditioned response is mediated by a CS-US association (e.g., Domjan, 2009). In the CFP paradigm, as proposed by Delamater, Campese, Lolordo, and Sclafani (2006), when a nutrient is devaluated with the US devaluation technique, the aversion is established to the sensory-specific properties of the nutrient and not to the more genera hedonic or post-ingestive reinforcing properties that the nutrient in question share with other reinforcing nutrients.

Flavour-hedonic value of the taste association

As cited above, the taste of the nutrient has affective as well as sensory properties. In fact, physiological, psychological, and pharmacological manipulation studies of animals have separated sensory versus affective aspects of taste stimuli (see review in Berridge, 2000). Given that palatable nutrients are used in CFP, a second association is possible: flavour-hedonic value of the taste. This dissociation between emotional and sensory qualities of US is not new. Wagner and Brandon (1989; see also Konorski, 1967) assumed that a US presentation actually activates two US nodes: a sensory node that corresponds to the stimulus's specific sensory qualities, and an affective node that corresponds to its hedonic properties. During conditioning, the CS becomes associated with both of these nodes. Myers and Sclafani (2001b) remarked different measures to make inferences about the unconditioned and conditioned hedonic responses of rats to a flavoured solution, especially the microstructure of licking behaviour (e.g., Davis & Smith, 1992; Dwyer, 2008) and the affective orofacial responses in taste reactivity test (e.g., Grill & Norgren, 1978; Forestell & LoLordo, 2003). For example, taste reactivity testing has been used to show that preferences of CS flavours paired with palatable substances are associated with an increase in hedonic reactions to the CS flavour (e.g., Forestell & LoLordo, 2003).

Flavour-nutrient association

Finally, when using multifaceted USs as sucrose which has sensory, hedonic, and nutritional properties, a flavour CS paired with an orally consumed sucrose solution, the flavour could become associated not only with the sensory (e.g., Dwyer, 2005) or hedonic properties of taste of sucrose (e.g., Dwyer, 2008, Harris et al., 2004), but also with its post-ingestive nutritive actions (e.g., Sclafani & Nissembaum, 1988). In the same way that the use of strategies such as the US-devaluation technique, microstructural analysis of licking, and taste reactivity test have permitted a specific assessment of sensory flavour-taste or flavour-hedonic value of the taste associations, the intragastric nutrient infusions have made possible a direct assessment of the association between flavour cue and the post-ingestive effects of nutrient (e.g., Sclafani & Nissembaum, 1988). The advantage of this procedure is that the nutrient is untasted. It is well established that rats acquire preference for arbitrary flavours when they are followed by the intragastric nutrient infusion (see Capaldi, 1999).

Although Myers and Sclafani (2001b) demonstrated that a flavour paired with i.g. glucose infusion might show a learned shift in the hedonic evaluation or palatability of the flavour, strong nutrient-conditioned flavour preferences with intragastric procedure are not always associated with increased flavour palatability. Myers and Sclafani (2003) trained rats with a CS+ flavour (sour citric acid or bitter sucrose octaacetate) paired with intragastric 16% glucose infusion, and the opposite flavour (CS-) paired with intragastric water. Glucose conditioning produced a strong CS+ preference in two-bottle choice test, but taste reactivity responses to intraoral infusions of the two CS flavours did not differ even after extensive testing. Likewise, learned preferences for hedonically negative flavours can be readily observed when thirsty animals are tested after conditioning under food deprivation, but not when rats have ad lib access to food on test, suggesting that flavour-nutrient, but no flavour-taste or flavour-hedonic association, controls performance (e.g., González, Garcia-Burgos, de Brugada, & Gil, 2010). These findings support the view that flavour-hedonic value of taste and flavour-nutrient associations are independent, and that the conditioned changes in CFP can occur independently of conditioned changes in the hedonic value of the flavour.

I.g. method permits not only separating taste or palatability-based CFP and nutrient-based CFP at a procedural level, but it also allow the study of what do rats learn (i.e., at a content of learning level). As claimed by Myers and Whitney (2011), pairing a flavour and a nutrient ensures that rats associate the flavour CS with the post-ingestive consequences of the US, instead of associating the CS flavour with the reward value of the nutrient’s inherently attractive taste. “Such flavour-flavour or flavour-taste associations can also establish learned preferences for CS flavours, but are mechanistically and psychologically distinct from flavour-nutrient learning”

(Myers & Whitney, 2011, p. 466). Moreover, it has been suggested that flavour-taste and flavour-nutrient associations correspond to the flavour-taste learning and flavour-nutrient learning mechanisms respectively (as suggested by Owens, Capaldi, & Sheffer, 1993).

2.3.3. MECHANISMS INVOLVED IN CONDITIONED FLAVOUR PREFERENCE USING A PALATABLE NUTRIENT AS US

One of the more important features that differentiate this dual mechanism account relies on the role of expectancy of consequences. The basic idea is that, whereas in preference based on flavour-nutrient learning the subject learns that the conditioned flavour *is* a signal for the post-ingestive action of the nutrient (US), the conditioned flavour is not treated as a signal for palatability (US) in preference based on flavour-taste learning.

Drucker et al. (1994) argued that pairing a cue flavour with a nutrient may involve expectancy learning (i.e., the flavour comes to signal nutrition), as well as hedonic learning (i.e., the flavour comes to "taste better"). In descriptive terms, the "expectancy-hedonic" distinction offered by Drucker et al. (1994) means that expectancy learning implies the acquisition of knowledge about the contingency relationship between the flavour cue and the post-ingestive consequences of the nutrient (CS predicts US), whereas hedonic learning refers to a process by which the mere presentation of the neutral flavour with an hedonic stimuli changes the valence of the originally neutral stimulus. Furthermore, according to Capaldi (1992, p. 25), "the basic result of flavour-flavour learning is that any affective tone of one of the flavours transfers to the other flavour". Authors as Rozin & Zellner (1985) supported this distinction and argued that preference changes as a result of simultaneous flavour-taste pairings cannot be explained in terms of anticipated consequences, since the CS does not "predict" the US. In addition, Capaldi (1992) suggested that flavour-flavour learning does not easily fit the CS→US framework, given that flavour-flavour learning is not possible with a delay between flavours, and it is optimum when the flavours are given in solution together. Consequently, the hedonic learning would be affected by the contiguity between CS and US, and not by contingency manipulations or cue competition. These suggestions point out that flavour-taste learning is mediated by a mechanism distinct from the one that mediates flavour-nutrient learning which results in expectancy learning (i.e., the Pavlovian learning of signal relationship). What follows is a review of different accounts for explaining these two types of learning.

Stimulus generalization and configural account

One explanation is that both mechanisms are based on configural learning (e.g., Pearce, 2002; see also Capaldi & Hunter, 1994; Dwyer 2008; Dwyer, et al., 2011; Dwyer & Quirk, 2008; Harris & Thein, 2005). Specifically (see Figure 2), Pearce suggested that when a neutral flavour is paired with an attractive flavour without nutritional value (e.g., saccharin) in the same solution during flavour-flavour learning, both stimuli excite a configural representation of the compound, comprising elements of both stimuli. Subsequent presentation of one element of the compound via activation of the configural unit activates the input unit of the other[3]; thus, they acquire the capacity to activate each other's sensory representations. Therefore, the presentation of the neutral flavour will activate the input unit for saccharin through the configural unit. Because of its innate hedonic value, saccharin will be connected to an output unit (i.e., palatability) that will be responsible for sustaining preference. On the other hand, a more complex network will be developed if the neutral flavour is presented in solution with a palatable nutrient (e.g., sucrose). In particular, "the configural unit will gradually enter into an excitatory association with the nutritional consequences of consuming the solution" (Pearce, 2002, p. 95).

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[3] In his connectionist theory of configural learning, Pearce (2002) involves a layer of inputs unit, which are activated by CSs and sensory properties of USs, a layer of output units, which are activated by reinforcing properties of USs, and an additional layer of configurational units interposed between the input and the output units. In addition, he assumed that input units and configural units are connected by biderectional links (see Pearce, 2002, p. 89).

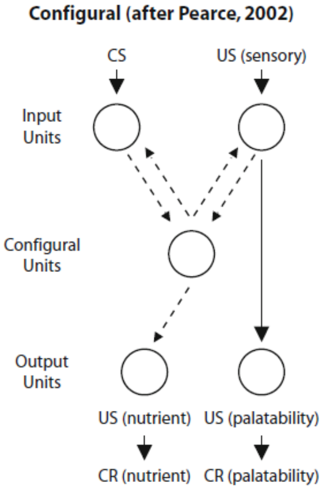

Figure 2. Example of configural network proposed to underpin flavour conditioning with palatable nutrient according to Pearce (2002). Adapted from "Microstructural analysis of conditioned and unconditioned responses to maltodextrin" by Dwyer, 2008, *Learning & Behavior, 36*, p. 150. Note: –→ unconditioned excitatory connections; - - → acquired excitatory connections.

In flavour-flavour learning, one a flavour (CS) has acquired excitatory connections due to pairings with a palatable taste (US), the hedonic shift observed in the flavour during test, $E_{flavour,}$ is due to the level of activation of the flavour-taste compound, ${}_{flavour}S_{compound}$, multiplied by the strength of the connection between the compound configural unit and the palatability unit, $V_{compound}$, in accord with Equation 1 (Pearce, 2002, Equation 3):

$$E_{flavour} = {}_{flavour}S_{compound} \times V_{compound} \qquad \text{(Equation 1)}$$

Pearce (2002) suggested that this learning is acquired very rapidly, since the degree to which the initially neutral flavour is able to activate the configural representation increases rapidly, and that it is highly resistant to an extinction procedure (e.g., evidences supporting this prediction comes from Harris et al., 2004, Exp. 2A and 2B, in rats without food deprivation). This stimulus generalization model explains the irrelevance of contingency in flavour-taste learning by stating that this learning depends on the formation of a configural representation. Once an input unit is connected to a configural unit, the strength of that connection will be unaffected by subsequent experience. This latter process of configuring is thought to occur automatically, relying solely on the co-activation of the representations of the presented stimuli. In fact, Pearce (1994) describes the formation of a configural representation as an automatic and immediate process, as opposed to the gradual formation of a Pavlovian association between stimuli, process supposed in flavour-nutrient learning. This model predicts three of the more important features of the flavour-taste learning. That is, CS-US contingency manipulations should exert no effect on the flavour-taste learning, simultaneous presentation of the CS and the US should be the most effective procedure (e.g., evidences comes from Holman, 1975), and perceptual similarity between sensory properties of flavour and compound play a key role in generalization of conditioned response from US to the CS (e.g., evidences comes from Capaldi & Hunter, 1994, who demonstrated that when a flavour-taste compound was conditioned, conditioning did generalize to the flavour cue presented alone).

Second-order conditioning

As mentioned by Díaz and De la Casa (2011), other approach that has also explained flavour-taste learning involves a second order form of conditioning in which the preferred taste acts as a prepared CS that generates an expectancy, possibly innate, of nutritional post-ingestive effect (e.g., Fedorchak and Bolles, 1987). Fedorchak (1997) suggested that flavour-taste learning might best be viewed not as a case of first order conditioning, with the taste of nutrient representing a US, but rather as a case of second-order conditioning, with taste playing the role of an “innate” first-order CS.

Evaluative conditioning account

Another approach is the evaluative learning mechanism (proposed in human evaluative conditioning, see De Houwer, Thomas, & Baeyens, 2001) that does not involve the development of expectancy learning. As a result of the number of common elements between flavour-flavour learning and evaluative conditioning, such as the importance of contiguity between the CS and US, the irrelevance of contingency, or resistance of extinction, evaluative conditioning has been put forward as a model for flavour-taste learning in rats (e.g., Myers & Sclafani, 2006). However, the failures of this model to provide a complete account of flavour-taste learning and to generate specific predictions present a serious problem for this account. In this sense, it should be noted that the development of more specific models of evaluative conditioning has given rise to a configural approach in terms of Pearce's model of stimulus generalization and configural learning (see Havermans & Jansen, 2007), in the similar way to flavour-taste learning.

Formation of within-compound association account

Capaldi (1996), taking into account the study by Rescorla and Cunningham (1978) and Durlach and Rescorla (1980; see also Rescorla & Durlach, 1981), suggested that flavour-taste learning involves a process of formation of within-compound associations, a type of learning that can occur whenever two stimuli are presented in compound. Thus, when taste and flavour are combined, the animals have an opportunity to associate them. Therefore, any conditioning that might accrue to the taste is readily transferred to the odour. Although Durlach and Rescorla (1980) found that taste extinction (or odour-taste in Durlach and Rescorla parlance) reduced aversion to the odour in a conditioned taste aversion paradigm, our understanding of the extinction of flavour-taste conditioning in CFP paradigm is still incomplete.

Stimulus-response learning account

From another elemental approach to Pavlovian conditioning, it has been suggested that flavour-taste learning may be produced by stimulus-response learning. In fact, there is a possibility that conditioning can take the form of stimulus-stimulus or stimulus-response learning (Figure 3) in different conditioning systems and preparations (see Holland, 1990). For instance, Harris et al. (2004; see also Rozin & Zellner, 1985) suggested that the persistence of conditioned flavour preference after an extinction procedure in sated rats was not based on the association with the sweet taste of sucrose, but rather the flavour became imbued with the capacity to directly elicit the hedonic reaction previously provoked by sucrose. Thus, if the preference based on palatability is produce because the CS flavour had been associated with some positive hedonic reaction to the taste of the reinforcer, then post-training CS alone presentations may not influence the conditioned preference. One explanation points out that if presentation of a CS activates a representation of the US (e.g., palatability) during extinction procedure, “then the functional CS-US pairing would occur because of the ‘self-reinforcement’ of the CS by the US representative evoked by that CS” (Holland, 1990, p. 121). However, we have evidence that event representatives do not affect the stimuli that activated them, avoiding the self-reinforcement of the CS by the US representative evoked by that CS (Holland, 1990).

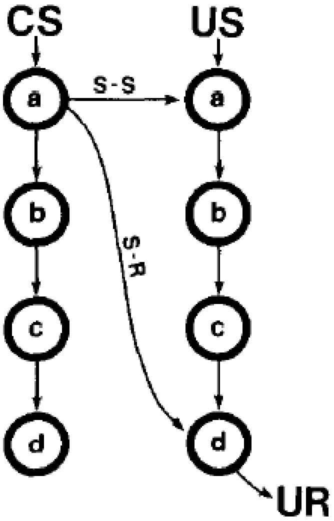

Figure 3. Example of elemental network proposed to underpin flavour conditioning. Adapted from “Event representation in Pavlovian conditioning: Image and action” by Holland, 1990, Cognition, 37, p. 108. Note: The nodes “a”, “b”, “c”, and “d” represent units of sensory, elaborative, and motor systems activated by the CS and the US, beginning with purely sensory processing and ending with motor processing. The S-S and S-R represent acquired excitatory connections between conditioned stimulus (CS) and unconditioned stimulus (US). UR = unconditioned response.

Conclusion

Despite advances in the debate about numerous models proposed for CFP, a conclusion is gaining some consensus: the Pavlovian mechanisms can support flavour preference learning (Dwyer & Quirk, 2008). As pointed out by these author in terms of a dual mechanism account, flavour-taste learning might rely largely on configural mechanism (e.g., Pearce, 2002), while flavour-nutrient learning could be equally well served by elemental[4] (e.g., Rescorla & Wagner, 1972; Mackintosh, 1975; Pearce & Hall, 1980; Wagner, 1981) or configural mechanisms (e.g., Pearce, 2002). In both types of learning, it is not necessary to assume special mechanisms apart from Pavlovian conditioning processes to explain the "special" attributes of learned flavour preferences, such as the insensitivity to contingency manipulations (i.e., the resistance to extinction procedure of palatability-based preferences).

Finally, we can draw a framework where palatability based-preference and nutrient based-preference can be dissociated in terms of procedures, content of learning, and mechanisms.

2.3.4. MOTIVATIONAL REGULATION OF CONTENTS OF LEARNING IN CONDITIONED FLAVOUR PREFERENCE USING A PALATABLE NUTRIENT AS US

It is established that food deprivation primarily affects the expression of conditioned flavour preference based on nutrient in rats, but does not fundamentally alter the learned associations between the conditioned flavour and the nutrient (e.g., Fedorchak & Bolles, 1987; Yiin et al., 2005a, 2005b; but see Capaldi et al., 1994; Harris et al. 2000). As noted above, Yiin et al. (2005a) argued that the post-oral actions of nutrient were reinforcing in both food-restricted and food-ad-libitum

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[4] The nature of elemental theories is made evident by the assumption that a compound conditioning trial provides the opportunity for a change in the associative strength of each element of the compound.

trained rats. Concretely, the evidence suggests that animals learn about oral and post-oral properties of nutrient in a parallel way during the conditioning procedure, and that conditioned preferences relying on nutrient, but not relying on taste, seem to be highly sensitive to post-conditioning hunger level manipulations.

It should be made clear however that in all these studies rats learned about the association between the flavour and nutritive actions if they experienced some level of hunger during training, by either food deprivation or water deprivation (i.e. latent hunger). Moreover, Harris et al. (2000) claimed that the rats formed flavour-taste and flavour-nutrient associations when they were water or food deprived, and that their motivational state on test determined which of these associations controlled performance.

Since in all experiments of the present dissertation animals were either water or both water and food deprived, we have assumed that animals acquire both flavour-taste and flavour-nutrient associations during training (according to González et al., 2010; Harris et al., 2000; Yiin et al., 2005a; 2005b), and that hunger level in testing controls the expression of flavour-taste and flavour-nutrient associations (Harris et al., 2000). In addition, we further assume that motivational state at time of testing selects which mechanism, flavour-taste learning or flavour-nutrient learning mechanisms, controls the performance.

Chapter 3

EFFECTIVENESS OF NON-REINFORCED TRIALS ON CONDITIONED FLAVOUR PREFERENCE BASED ON NUTRIENT

3.1. INTRODUCTION

Among the multiple differences noted above, one basic feature of flavour-taste learning distinguishes it from predictive flavour-nutrient learning: the contingency relationship, which refers to the degree to which one stimulus predicts another (i.e., flavour predicts/is contingent with the reinforcer). As noted above, unlike flavour-taste learning, the CS flavour in flavour-nutrient learning predicts the occurrence of the reinforcer. The present Chapter reviews studies that describe the effectiveness of non-reinforced CS presentation upon the CS-US contingency in CFP, and also analyses the content of learning. Concretely, it examines the effect of non-reinforcement of the flavour cue after flavour-nutrient pairings (extinction), as well as the effect of the flavour presented alone either during (partial reinforcement and context conditional flavour preference) or before flavour-nutrient pairings (latent inhibition, LI). Moreover, the motivational state of the animals at time of testing is further considered, as it selects which mechanism, flavour-taste learning or flavour-nutrient learning, controls performance (Harris et al., 2000).

3.2. EFFECTIVENESS OF NON-REINFORCED TRIALS AFTER FLAVOUR PREFERENCE CONDITIONING

Traditionally, repeated non-reinforced exposure to the flavour after conditioning is said to have little effect on CFP. In fact, a property of CFP that appears to be independent of how it is acquired is resistance to extinction (e.g., Albertella & Boakes, 2006; Capaldi, Myers, Campbell, & Sheffer, 1983; Drucker et al., 1994; Elizalde & Sclafani, 1990; Fedorchak, 1997). For instance, Capaldi et al. showed the persistence of conditioned preference to a flavour paired with a sweet taste throughout 28 days of extinction tests. Drucker et al. (1994) trained to associate a CS+ flavour with i.g. Polycose infusion and a CS- flavour with i.g. water during 23h/day sessions and then observed the preference for the CS+ over CS- using an extinction procedure. Animals displayed a significant persistence CS+ over CS- across 12 days of extinction. These findings were very similar to those obtained in other studies in which rats also displayed a preference for CS+ over CS- across repeated extinction tests (e.g., Elizalde & Sclafani, 1990).

3.2.1. THE CS+ VERSUS CS- CHOICE TESTS

One problem with these studies conducted on extinction of CFP is that the method used for assessing such phenomenon, CS+ vs. CS- two-bottle choice test, might not be optimal (Delamater, 2007). For instance, with more sensitive testing procedures it has been possible to observe effects of conditioned flavour alone presentations on CFP after conditioning. Delamater (2007, Exp. 1) established preference for two different flavours cues by pairing them with the same palatable nutrient (sucrose) on separate occasions. Then, one of these flavours was extinguished (Fe) before a choice test was given between the extinguished (Fe) and the nonextinguished flavour (Fne). The results showed that the extinction procedure reduced the preference for Fe when it was assessed against Fne (see also Tarner et al., 2004; Díaz & De la Casa, 2011; which have also obtained evidence of extinction in CFP).

Harris et al. (2004) suggested that when the preference is assessed by a choice between a flavour that had been paired with the reinforcer (CS+) and a flavour explicitly unpaired with the same reinforcer (CS-), the preference measured confounds selection of the paired flavour with avoidance of the unpaired flavour. This is consistent with the avoidance observed in rats for a flavour that has been explicitly unpaired with an attractive reinforcer (see Boakes, Colagiuri, & Mahon, 2010; Harris et al., 2000). Moreover, as claimed by Harris et al. (2004), under this CS+ vs. CS- choice test the preference could be maintained across testing because the inhibitory properties of the CS- flavour might not be reduced or extinguished by presentation of the flavour in absence of reinforcer (Zimmer-Hart & Rescorla, 1974) and because the presence of CS- during testing could protect the flavour CS+ from undergoing any associative loss during repeated extinction tests (Rescorla, 2003).

If this analysis is correct, a decrement in conditioned flavour preference should be observed when the test for that preference is not confounded by avoidance of a second flavour that has acquired inhibitory properties. This evidence was found in the Drucker et al.'s (1994) Experiment 3, in which the authors observed that the CS+ preference was reduced with repeated testing in the absence of reinforcement when the tests involved a choice between CS+ versus water but it was quite persistent when the choice was between the CS+ vs. CS-.

3.2.2. MOTIVATIONAL STATE AND EXTINCTION OF CONDITIONING FLAVOUR PREFERENCE

To avoid the problem of CS+ vs. CS- choice tests during extinction procedure, Harris et al. (2004) examined the persistence of preference for a flavour by testing rats with a choice between flavour and water in different experiments. In all of them, rats were exposed to a flavour-sucrose compound and then animals were repeatedly tested. However, Harris et al. went one step further. They specifically manipulated the rats' level of hunger during test to investigate the differential contributions of the motivational state on extinction of CFP. The results (Exps. 2A and 2B, see below for more detail) showed that the preferences acquired and displayed by hungry rats differed from those of sated rats in their sensitivity to extinction by repeated flavour CS-alone. Concretely, if rats were trained and tested while maintained on ad-libitum access to food, their preference for the flavour associated with sucrose was resistant to extinction procedure. By contrast, if rats were food deprived during training or testing, their preference for the flavour showed a decrement.

Given that a flavour paired with sucrose formed different associations (i.e., flavour-taste and flavour-nutrient), and that the hunger on test selected the flavour-nutrient association (Harris et al., 2000), the decrement of flavour preference in hungry animals during testing demonstrated that the flavour-nutrient association extinguished whereas flavour-taste association did not. In other words, these findings provide evidence that repeated non-reinforced presentations of the flavour are effective in preference based on predictive flavour-nutrient learning, but not in preference based on flavour-taste learning.

To account for the resistance to extinction procedure in preference based on flavour-taste learning, different explanations have been proposed such as inadvertent reinforcement by food, and hydrating effect of the flavour alone in fluid-deprived rats. However, these possibilities have been ruled out. For instance, Albertella and Boakes (2006) examined whether access to food following an extinction session produced unnoticeable flavour-food associations that might maintain flavour preference under extinction conditions. After examining this hypothesis with variations of the interval between experimental sessions and the access to the food, the authors demonstrated that a putative flavour-food association made no contribution to resistance to extinction.

The account for the persistence of the preference based on flavour-taste association suggested by Harris et al. (2004) claimed that preference conditioning resulted in a change in the palatability of the conditioned flavour, and that this change was maintained over repeated tests (but see Dwyer, Pincham, Thein, & Harris, 2009); perhaps animals failed to detect the discrepancy between the expected hedonic value of the reinforcer and that elicited by the flavour. At this point, it should be noted that although the flavour CS exposure does not reduce preference, its presentation after conditioning has effect on the flavour-sucrose associations (see following section).

3.2.3. RESULTS FOR EXTINCTION OF CONDITIONED FLAVOUR PREFERENCE

Another important aspect of the Harris et al.' (2004) study is related to US-devaluation results in subjects given extinction. In Experiment 3, rats deprived of water throughout the experiment were given simultaneous almond-sucrose compound during the conditioning stage. In the extinction stage, half the rats received exposure to almond across 20 trials, while the remaining rats were exposed to water. Then, the sucrose was paired with injections of LiCl for half of the rats in each of these conditions. Finally, rats were given an almond vs. water choice test.

The results showed that exposure to almond outside the compound after conditioning but before sucrose devaluation had impact on the sensory almond-sucrose association; the effect of the sucrose devaluation was itself reduced in the extinguished group compared to the non-extinguished group. Since stimuli given extinction are as sensitive to the devaluation procedure as those not extinguished (e.g., Rescorla, 1996), the rats given paired exposures to sucrose and LiCl (either extinguished or non-extinguished conditions) should have acquired a similar aversion to the sucrose and rejected the almond on test. The authors considered that the association between the flavour and the taste of sucrose was weakened during the exposure of the flavour. A similar result has been reported by Delamater (2007) who suggested that the association formed between the flavour CS and the specific sensory properties of an orally presented nutrient US in thirsty rats (Exp.1), and between the flavour CS and the motivational consequences of this nutrient (i.e., their calories) in hungry rats (Exp. 3) were weakened by extinction.

However, a clear demonstration of the preservation of Pavlovian associations through extinction is provided by Rescorla (1996). For instance, in Experiment 3 (see Figure 4A) all animals received conditioning with four stimuli, two auditory (S1 and S2) and two visual (S3 and S4) stimuli. One stimulus within each modality resulted in US1 and one in US2 (USs were pellet and sucrose counterbalanced). Then, for half the animals both auditory stimuli were extinguished, and for the other half both visual stimuli were extinguished. This was followed by the pairing of all four stimuli with US3 (Polycose). Then US1 was paired with LiCl, and all four stimuli were tested. The results showed that the CS whose US had been devalued (Deval) showed a lower level of performance, independently of whether the stimuli had or had not received extinction. That is, there was no evidence that extinction weakened the CS-US association. By contrast, Delamater (2007, Exp.1, see Figure 4B) trained two distinct flavour paired separately with the same reinforcer (sucrose) in simultaneous compound. Then one of the flavours was repeatedly presented alone without sucrose. Then the subjects were divided into two subgroups. One subgroup received US-devaluation (Gp Dev), but the other subgroup did not (Gp Not Dev). Finally, all subjects were tested for their preference between the two sucrose-paired flavours. The results showed that the extinction treatment reduced preference for the extinguished stimulus. However, after US-devaluation, rats preferred the extinguished to the non-extinguished flavour. These results were interpreted as evidence that extinction treatment weakened the association flavour-sucrose.

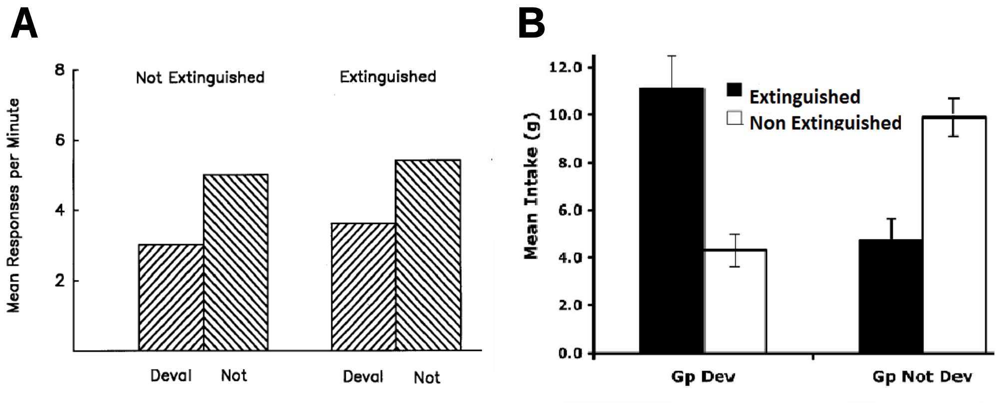

Figure 4. Performance for conditioned stimuli that had or had not received extinction when their original US had or had not been devaluated in a traditional Pavlovian paradigm (A) or in a CFP (B). Note: A is adapted from "Preservation of Pavlovian associations through extinction", by R. A. Rescorla, 1996, *Quarterly Journal of Experimental Psychology, 49*, p. 255. B is adapted from "Extinction of conditioned flavor preferences", by A. R. Delamater, 2007, *Journal of Experimental Psychology: Animal Behavior Processes, 33*, p. 164.

However, assuming that the extinction procedure may impair the association between the flavour and the sensory properties of sucrose, and the association between the flavour and the more general motivational attributes of the sucrose (i.e., post-ingestive action) is problematic. The dominant view of extinction is that it has no impact on the strength of the CS-US association (Delamater, 1996; Rescorla, 1996; see Delamater, 2004; Rescorla, 2001, for reviews). It can be asked whether proposing that extinction may weaken the association between the flavour and the components of the nutrient is the best way to view the decrease in conditioned preference after post-training flavour exposure. Given that the studies described above failed to show any impact of extinction on CS-US associations (see Delamater, 1996; Rescorla, 1996), perhaps the decrease in preference observed in the CFP studies might be due to a different cause compatible with the absence of a US-devaluation effect.

Another surprising finding of interest was found by Higgins and Rescorla (2004) in hungry animals. They found that, although exposure to the flavour after simultaneous pairing of almond and 20% Polycose solution produced a decrease in flavour consumption, there was relatively little indication that retraining produced reconditioning. In fact, Rescorla (Exp. 3) compared the relative magnitudes of retraining with sequential and simultaneous conditioning. The results confirmed that re-establishing the flavour-nutrient relation readily re-established the response only in the case of sequential presentation, in spite of the conditioning being more successful with simultaneous than with sequential presentation. However, it is assumed that when new CS-US pairings are introduced after extinction, the reacquisition of responding appears even more rapidly than the acquisition with a novel CS (e.g., Ricker & Bouton, 1996).

Taken together, all this empirical evidence raises doubts about the fact that the decrement of flavour preference in CFP based on nutrient is due to extinction. At the very least, it opens the door to alternative explanations that should be examined before stating that this decrement in conditioned flavour preference found in hungry rats exposed to a flavour alone following simultaneous flavour-sucrose conditioning (e.g., in Harris et al.'s [2004] Exp. 2B) is due to extinction. One alternative explanation of this finding in terms of inhibitory learning will be presented in the General Discussion of Chapter 5 and examined in Chapter 6.

3.3. EFFECTIVENESS OF NON-REINFORCED TRIALS DURING FLAVOUR PREFERENCE CONDITIONING

Few studies in rats have explored the effects of non-reinforced exposures to a flavour cue during conditioning in CFP and, to the best of my knowledge, none of them has examined the differential contributions of flavour-taste learning and flavour-nutrient learning mechanisms. For instance, a partial reinforcement procedure in CFP was used by Delamater (in press, Exp. 1) to explore the effect of non-reinforcement over the control of sensory-specific flavour-sucrose associations in thirsty rats. In this Experiment, rats learned to prefer a flavour cue (F2) paired consistently with 8% sucrose over one flavour (F1) paired with sucrose the same number of times but also presented without sucrose on other occasions. In the following phase, one subgroup of rats received a sucrose-devaluation treatment by LiCl and a second subgroup of rats received sucrose and LiCl unpaired, serving as a non- devaluated control group. To assess the preference, a choice between F1 and F2 was given on test. For non-devaluated groups, the results showed that the F2 was preferred over the F1. In contrast, devaluated groups displayed a preference for F1 over F2. These findings were interpreted as a reduced ability of the F1 to activate the sensory flavour-sucrose association by non-reinforcement exposure during conditioning, and they were consistent with the fact that non-reinforcement impairs the sensory flavour-taste association. However, one problem of this work was that the animals were not experimentally naïve, as they had had previous experience with 20% sucrose US while food deprived. Therefore, it is complicated to determine what the animals have learned, as preexposure to sucrose could have affected the subsequent formation of flavour-sucrose associations and thus interact with the experimental manipulation (i.e., the effect of partial reinforcement).

Another procedure that manipulates the CS-US contingency during training (across the contexts) is the context conditional flavour preference. Campbell et al. (1988, Exp. 3) assessed the acquisition of context-specific flavour-consequence expectancies in hungry animals. In this Experiment, flavour (F1) was followed by sucrose and a second flavour (F2) was followed by quinine in Context 1 (C1), whereas in C2 F1 was followed by quinine and F2 was followed by sucrose. Preference was measured through a two-bottle F1 vs. F2 choice test, and latency to approach the flavour-consequence tube was also measured; the results showed that animals did learn to expect different consequences associated with the flavour cues in the different training context, approaching quicker to the F1; however, they did not show a context-specific conditioned preference. That is, the animals approached the consequence flavour more quickly when they were in the context in which that flavour predicted the consequences, but the amount of the flavours consumed was not affected by the context in which they were tested. However, using a more sensitive procedure, Dwyer and Quirk (2008) demonstrated that hungry rats could learn preference based on taste (fructose US) and preference based on nutrient (maltodextrin US) that were conditional to the training context. Unpublished data obtained in our lab have also shown that animals trained thirsty and tested hungry can solve a bi-conditional discrimination using contexts as conditional cues, flavours as CSs, and sucrose as US (González, Garcia-Burgos, & Hall, 2010).

3.4. EFFECTIVENESS OF NON-REINFORCED TRIALS BEFORE CONDITIONED FLAVOUR PREFERENCE: LATENT INHIBITION

Regarding the effect of non-reinforcement flavour presentation before flavour-nutrient pairings in CFP, a recent study by De la Casa, Márquez, and Lubow (2009) has demonstrated a LI effect in flavour preference learning based on nutrient. In Experiment 1, three groups of rats, both water and food deprived, were preexposed to water, almond, or citrus. Then, all rats were conditioned by pairing the almond and the citrus solutions with sucrose. Finally, in a two-bottle almond vs. citrus choice test, the preexposed flavoured solutions were consumed less than the non-preexposed flavours (i.e., LI effect). Given that hunger selects the expression of flavour-nutrient association to control performance on test (Harris et al., 2000); these data demonstrated that preference based on flavour-nutrient association was sensitive to a change in CS-US contingency between the flavour-alone and flavour-sucrose pairings phases.

Delamater (in press, Exp. 2) explored the effects of non-reinforcement on flavour-sucrose associations in CFP using a LI procedure with thirsty animals. In this case, all non-reinforced F1 presentations occurred prior to the conditioning phase in which F1 + sucrose and F2 + sucrose pairings were given. Then, one subgroup of rats received a sucrose-devaluation treatment by pairing sucrose consumption with the effects of LiCl whereas a second subgroup of rats received the sucrose and LiCl unpaired, serving as a non-devaluated control group. On test, a choice between F1 and F2 was given to assess preference. The results showed the absence of devaluation effect in CFP in the first F1 vs. F2 choice test, and therefore absence of LI in the non-devaluated group (note that animals were thirsty throughout the experiment). Only when additional sucrose vs. water choice tests were given (i.e., exposure to sucrose before F1 vs. F2 tests), the expected patterns of data emerged: the non-devaluated group preferred F2 over F1, but that devaluated group preferred F1 over F2. Given that exposure to sucrose between tests may play an important role in the results, this demonstration of LI in thirsty rats seem to be rather uncompelling. Exposure to sucrose after flavour-sucrose pairings weakens the flavour-taste of sucrose association in thirsty rats, and leads to a reduction of the conditioned flavour preference when the CS is presented alone after sucrose exposure, a preference which, as noted above, is otherwise quite persistent (Harris et al., 2004).

3.4.1. MOTIVATION EFFECT ON LATENT INHIBITION

Killcross and Balleine (1996) demonstrated the role of motivational factors in what the animals learn during CS preexposure in appetitive LI procedure. They argued that preexposure to a CS results in LI only when the reinforcer was relevant to the motivational state in which CS preexposure was conducted. In their work, rats were preexposed to one stimulus when hungry (CS1) and to another when thirsty (CS2). For one group of thirsty and hungry animals both stimuli were paired with food, whereas for a second group of thirsty and hungry animals both stimuli were paired with fluid reinforcer. These authors found that the conditioning for CS1 was retarded when it was paired with food, but the conditioning for CS2 was retarded when it was paired with fluid. They concluded that during preexposure phase rats learned that the preexposed stimulus was unrelated to evens of relevance to their current motivational state; that is, rats did learn CS-no nutrient or CS-no fluid associations. These findings demonstrated the influence of motivation not only in performance, but also in learning, allowing a specification of the content of learning during preexposure, instead of using the terms CS-“no US” or the all-encompassing CS-“nothing”.

It is worth noting that some interpretations by Killcross and Balleine in terms of content of learning of these results are in conflict with the interpretation that Harris et al. (2000) proposed in their study about how motivational states regulate the content of learned flavour preferences. According to Harris et al. (2000, 2004), animals with some level of hunger learn both flavour-taste and flavour-nutrient associations at the same time during conditioning; this is also true for water deprived animals, as they suffer from latent hunger. When these rats are finally tested, food deprived rats show a preference based predominantly on the flavour-nutrient association. Therefore, animals reinforced with either saline or pellets during conditioning in the Killcross and Balleine's experiment should have shown retardation in the acquisition of magazine approach for the CS preexposed while the animals were thirsty, and not only animals reinforced with saline during the conditioning phase. That is, animals water-deprived should have learned the CS-no fluid and CS-no food associations (induced by latent hunger), and not only the CS-no fluid associations during preexposure; this learning should have affected conditioning with both reinforcers. Furthermore, given that the animals were thirsty and hungry during conditioning, the CS-no food association has been enhanced, giving rise to an even greater interference with the CS-pellets association than saline in thirsty preexposed animals. Given that the role of motivational state of thirst upon the content of learning applied to LI procedure on CFP have not been examined in thirsty-trained and hungry-tested rats, it would be premature to conclude on the basis of Killcross and Balleine's (1996) that the animals do not learn the flavour-nutrient association when they are trained thirsty and tested hungry. Moreover, by assessing this possibility, we will provide more information about the acquisition and expression of flavour-taste learning and flavour-nutrient learning on CFP.

3.4.2. CONCLUDING REMARKS ON LATENT INHIBITION

Another point of interest concerns the effect of CS-US contingency manipulations in the LI procedure. The data from De la Casa et al. (2009) with hungry animals and from Delamater (in press, Exp. 2) with thirsty animals, seem to support the differential sensitivity of contingency manipulations in flavour-nutrient and flavour-taste learning mechanisms respectively. It seems clear that flavour CS exposure produces a LI effect on preference based on post-ingestive effects of nutrient, i.e. when animals are hungry. By contrast, when the animals are thirsty, the LI effect is harder to see. More interestingly, the effect of non-reinforced presentation of the flavour observed in the extinction of CFP seems to extend to other procedures that also involve exposing the rats to contingent CS-US pairings as well as to presentation of the CS alone. In fact, commonalities between extinction and LI in terms of mechanisms have also been underlined by some theories (e.g., Kraemer & Spear, 1992; Bouton, 1993). For instance, Kraemer and Spear (1992) involved a similar mechanism of retrieval processes in both LI and extinction. However, to the best of my knowledge, unlike extinction, no studies have been specifically designed to examine the differential contributions of flavour-taste and flavour-nutrient learning over LI effect on CFP. This examination of motivational control of LI is the objective of Chapter 7.

Chapter 4

JUSTIFICATION AND OBJECTIVES

4.1. JUSTIFICATION AND OBJECTIVES

Based on the considerations examined above, there are important reasons to be cautious about the explanation of decrement of conditioned flavour preferences based on nutrient in terms of extinction. Therefore, the purpose of the present dissertation is to provide a further investigation of the phenomenon that reduces the conditioned preference in CFP based on nutrient when non-reinforced flavour trials are presented after flavour-nutrient pairings. Concretely, the first objective is to examine the decrement in nutrient-based conditioned flavour preference found in hungry rats exposed to a flavour following simultaneous flavour-sucrose conditioning whilst thirsty (under the similar conditions used by Harris et al., 2004, Exp. 2B).

In addition, given that the change in CS-US contingency in extinction procedure affects CFP based on nutrient, but not CFP based on taste (e.g., Harris et al., 2004, Exp. 2B), this dissertation also examines whether the effect of non-reinforced trials can extend to LI procedure. Concretely, different motivational states of hunger or thirst present during preexposure-conditioning training and testing are used to assess the effectiveness of non-reinforcement flavour presentations before flavour-sucrose pairing.

4.1.1. WORKING HYPOTHESES

The working hypotheses that have been set for this dissertation can be stated as follows:

First objective

Hypothesis 1. If the loss of the conditioned flavour preference in nutrient-based CFP found in hungry rats exposed to a flavour following simultaneous flavour-sucrose conditioning whilst thirsty is not produced by the extinction of the conditioned preference, but by the acquisition of the properties of a net inhibitor by the flavour acting as an element, then a) the flavour should not show extinction-related phenomena of recovery of the extinguished response, b) nor should it show the US-devaluation effect, and c) flavour should pass the retardation and summation tests.

Testable implications 1. If the decrement in nutrient-based conditioned flavour preference found in hungry rats exposed to a flavour following simultaneous flavour-sucrose conditioning whilst thirsty is not due to extinction, no evidences of recovery of flavour preference will be observed in spontaneous recovery, reinstatement, or renewal tests (Exps. 2, 3, and 4). Accordingly, no evidence of a US devaluation effect should be found (Exp. 5). The experiments included in Chapter 5 examined spontaneous recovery, reinstatement, and renewal phenomena after the decrement of conditioned flavour preference, as well as the study of the US devaluation effect after CS exposure.

Testable implications 2. Given that the decrement in nutrient-based conditioned flavour preference found in hungry rats exposed to a flavour following simultaneous flavour-sucrose conditioning whilst thirsty is due to conditioned inhibition, the flavour will show retardation of preference reacquisition when retrained after the exposure phase (retardation test, Exp. 7), and a decrease in the preference for a second flavour previously paired simultaneously with sucrose when both flavours are presented forming an unreinforced compound (summation test, Exp. 8). Therefore, the experiments presented in Chapter 6 had the main objective of providing empirical evidence of inhibitory learning using retardation and summation tests for conditioned inhibition.

Second objective

Hypothesis 2. If animals either water or both water and food deprived during training acquire both flavour-taste (non-predictive learning) and flavour-nutrient associations (predictive learning), the motivational state at the time of testing being that which controls the expression of both types of learning, then LI should appear in animals tested hungry, but not in rats tested thirsty, independently of the motivational state during training.

Testable implications 3. Given that an attenuation of flavour preference only occurs in the predictive flavour-nutrient learning and this is selected by hunger state at the time of testing, then LI will appear as lesser preference for flavour in the flavour-preexposed group compared to the water-preexposed group when the animals are hungry and thirsty throughout the experiment, but not when they are thirsty (Exp. 9A), the LI will not manifest in rats tested thirsty though they are trained hungry (Exp. 10), and LI will appear in rats trained thirsty but tested hungry (Exp.11). The experiments included in Chapter 7 had the main objective of providing empirical evidence of the motivational control of LI in CFP.

Part 2

EXPERIMENTAL STUDIES

Chapter 5

EXTINCTION RELATED PHENOMENA

Experiments 0A and 0B from manuscript published as:

González, F., Garcia-Burgos, D., Brugada, I., & Gil, M. (2010). Learned preference for a hedonically negative flavor is observed after pairings with positive post-ingestion consequences rather than with a palatable flavor. *Learning and Motivation, 41*, 141-149.

Experiment 1 from manuscript published as:

Garcia-Burgos, D. & González, F. (in press). Evidence of predictive learning in nutrient-based flavor conditioning in rats using the summation test for conditioned inhibition. *Psicothema*.

Experiments 2, 3, 4, and 5 from manuscript to be submitted as:

Garcia-Burgos, D. & González, F. (2011). *Absence of extinction-related phenomena after post-training flavor exposure in nutrient-based conditioned flavor preference*. Manuscript to be submitted for publication.

5.1. INTRODUCTION

The purpose of the present set of experiments was to provide further investigation on the nature of the decrement in CFP based on nutrient after CS exposure following conditioning. Concretely, these experiments examined the phenomena related to the recovery of the conditioned preference lost in rats trained thirsty with a simultaneous flavour-sucrose compound and exposed to the flavour unreinforced while hungry and thirsty. The US devaluation effect was also assessed.

The present Chapter is organized in three sections. The first section presents a preliminary study previous to the core of the dissertation research, though as an auxiliary research for establishing the effectiveness of the motivational manipulation. Experiments 0A and 0B examined the differential contribution of hunger levels during preference test on the expression of the preference for flavour paired with sucrose during conditioning. In the second section, Experiment 1 examined the basic effect of post-training flavour exposure in CFP based on nutrient. In the third section, Experiments 2, 3, and 4, the phenomena of spontaneous recovery, reinstatement, and renewal using motivational states as contexts in CFP based on nutrient were assessed. In this same section, the specificity of the effect of post-training flavour exposure on particular components of the flavour-sucrose learning using the US devaluation technique was also studied, Experiment 5.

5.2. PRELIMINAR STUDY

Experiment 0A: Preliminary study

The goal of this experiment was to find evidence of dissociation between flavour-taste and flavour-nutrient learning using a CS that is not normally preferred by rats with the aim of increasing the sensitivity of the motivational manipulation. Thus, an unsweetened 0.2% (2 g/L) Kool–Aid fruit-flavoured solution in tap water was used as CS. This solution at lower concentrations is often presented in compound with saccharin to increase its acceptability (e.g., Myers & Sclafani, 2006). The Kool–Aid-solution concentration used here is 2–4 times higher than that used in other studies, but not so high as to prevent an enhanced consumption under hunger using a one-bottle test (e.g., Balleine, Espinet, & González, 2005). The experiment was designed to compare the preference under different motivational states by giving extended exposure to the conditioning compound (eight trials) in group Simultaneous, and using a high sucrose solution concentration as the US (200 g/L). Animals in group Unpaired received the flavour and the sucrose at different times, one solution in the morning and the other in the afternoon. After training, conditioned preference was assessed by two choice tests (flavour vs. water), one with animals thirsty, and the other with animals thirsty and hungry.

Methods

Subjects and apparatus

The subjects were 16 female Wistar rats at least 110 days old at the start of the experiment. They had previously participated in a flavour preference experiment with 1% (v/v) almond, 1% (v/v) vanilla, and sucrose (200 g/L), but were orthogonally assigned to both groups in order to equalize experience with these stimuli. Animals were housed in individual home cages and kept in a large colony room on a 12-h light/12-h dark schedule (light coming on at 7 am). All experiments of the present dissertation were run at the University of Granada, with the exception of Experiments 9A and 11 (Chapter 7). All training sessions took place twice a day in the home cage during the light cycle (at approximately 11:00 am and 5:00 am), whereas tests were conducted during the morning session. Rats had continuous access to food throughout the experiment with the exceptions mentioned below. They were maintained on a water deprivation schedule with daily 30-min access to fluid (flavoured solution or water). Fluids were administered at room temperature in two 50-ml plastic tubes with a rubber stopper fitted with stainless steel ball-bearing tipped spouts. Fluid consumption was measured by weighing the tubes before and after fluid presentation to the nearest 0.1 g. The US was a 20% (200 g/L) sucrose solution, and the CS (flavour A) was a Kool–Aid cherry-flavoured unsweetened solution at 0.2% (2 g/l) concentration. When flavour A and sucrose were presented forming a compound, 200 g of sucrose and 2 g of Kool–Aid flavour were added to a litre of water. Fresh solutions were made every day using room temperature tap water. For this and the following experiments, all the experimental procedures were approved by the University of Granada Ethics Committee, and were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Procedure

Before the start of training, rats were water deprived for 4 days during which they had free access to water for 30 min once a day during the morning session. Afterwards, they were divided into two groups matched in water consumption (group Simultaneous, n = 8; group Unpaired, n = 8). On days 1–8, group Simultaneous received a conditioning trial, during the morning session on days 2, 5, 7, and 8, and during the afternoon session on days 1, 3, 4, and 6. In each session, the rats had access to 10 g of flavour A mixed with sucrose solution for 15 min. Rats also received 10 g of water in the alternative session every day. Rats in group Unpaired received 10 g of the sucrose solution for 15 min at the same time of the day that group Simultaneous received the compound solution, and 10 g of flavour A for 15 min at the same time of the day that group Simultaneous received water. This procedure equalized experience with the calorific US. On day 9, all animals had the conditioning test during the morning session in which they had access to two bottles for 15 min, one containing 20 g of flavour A solution and the other 20 g of water. The positions of the bottles (i.e., left and right) were counterbalanced by group and day. Animals received 10 g of water for 15 min during the afternoon session. At the end of this session, food was removed from the cages. On days 10–12, animals received 90-min access to both water and food during the morning session. On day

13, another two-bottle test identical to the conditioning test of day 9 was conducted, differing only in that all animals were also food deprived.

Results

Statistical analysis. For all the analyses, a significance level of p < .05 was adopted. The data were analyzed using analysis of variance (ANOVA), followed by post hoc Tukey's tests where appropriate to determine the locus of significant main effects and interactions. Two-tailed t-tests were used to evaluate data not involving multiple comparisons. The test data for each rat were also converted into preference ratios, calculated as the intake of the target flavour over the total amounts consumed.

Conditioning phase. Consumption of the compound in group Simultaneous, and of both the sucrose and the flavour A solutions in group Unpaired, was analyzed through three repeated-measures ANOVAs with day as the factor. Consumption of the compound in group Simultaneous differed among days, F(7, 49) = 4.09. Post hoc Tukey's tests revealed that consumption on day 2 was lower than on the other days, which did not differ among themselves (means: 7.77, 6.75, 8.83, 7.98, 9.37, 8.41, 8.95, and 9.57 g). Regarding consumption of the sucrose solution in group Unpaired, the ANOVA revealed no differences among days, F(7, 49) = 1.10 (mean: 9.52 g). However, the ANOVA conducted on the consumption of flavour A in this group showed differences among days, F(7, 49) = 16.47. Post hoc Tukey's tests confirmed that consumption on days 2, 5, 7, and 8 —the days in which animals drank flavour A during the afternoon session— was significantly lower than that on days 1, 3, 4, and 6, probably because they were less thirsty (means for 4 days were 6.92 and 5.10 g, respectively).

Conditioning tests. Preference ratios were analyzed through a mixed two-way ANOVA with group (Simultaneous, Unpaired) as a between-subjects factor, and test (thirst, thirst plus hunger) as a within-subjects factor (see Figure 5). The ANOVA revealed significant main effects of group, F(1, 14) = 12.15, test, F(1, 14) = 7.25, and the group x test interaction, F(1, 14) = 23.74. The t-tests revealed that groups did not differ on the test under thirst, *t*(14) = 0.65, but that they did differ on the test under both thirst and hunger, *t*(14) = 7.66. The preference in group Simultaneous was larger than the preference in group Unpaired. In addition, there was no change in preference in group Unpaired between tests, *t*(7) = 1.38, but preference increased in group Simultaneous on the test under thirst and hunger, *t*(7) = 6.12.

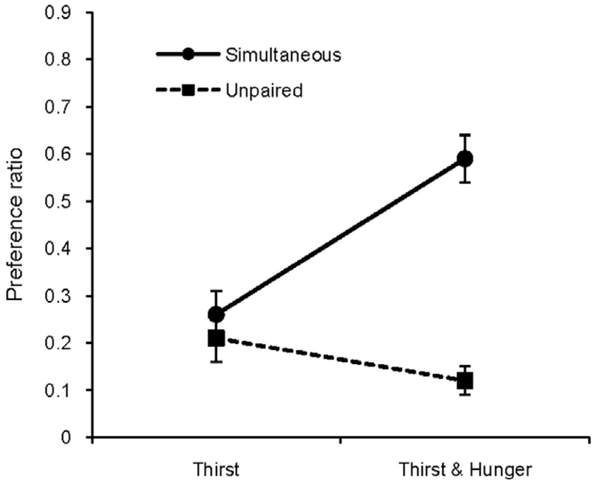

Figure 5. Experiment 0A. Mean preference ratio (flavour A) for groups Simultaneous and Unpaired during the conditioning tests under thirst (left side) and thirst plus hunger (right side). Group Simultaneous received eight flavour–sucrose compounds during training while group Unpaired received flavour and sucrose several hours apart. Rats were water but not food deprived during training. Error bars represent SEM.

Discussion

The results from Experiment 0A showed that pairing a non-preferred flavour with sucrose did not produce any evidence of a conditioned preference when animals were tested just thirsty. Preference ratios for both groups, Simultaneous and Unpaired, were virtually identical. One possible explanation for this lack of a difference between groups is generalization decrement from the compound to the flavour in group Simultaneous. Rats might not recognize flavour A when presented separately from the compound. However, a conditioned preference in group Simultaneous was readily observed when animals were also hungry, a finding that is at odds with the generalization decrement account. We concluded that animals refused to drink flavour A in the first test when it was presented apart from sucrose because they still found it aversive. Although the flavour did not become more palatable as a result of training, it could, none the less, become a cue associated with caloric intake. Under the assumption that animals will accept an unpleasant flavour associated with caloric intake when they are hungry, the motivational change introduced for the second test could have produced an increment in preference.

Experiment 0B: Preliminary study

The absence of a preference for flavour A in the test given when the rats were just thirsty suggests that they really are averse to the flavour, even after it had been paired eight times with a strong sucrose solution. Nevertheless, this result does not demonstrate that a flavour–taste association was not formed during training. To address this issue we made use of the US devaluation technique in Experiment 0B. Thirsty rats were trained under conditions similar to those used for group Simultaneous in Experiment 0A, with the exception that they received two daily conditioning sessions over 4 days instead of a single conditioning session on each of the 8 days. Afterwards, animals in group Dev (devaluation) received two cycles of sucrose–lithium chloride (LiCl) pairings before being tested under thirst, whereas animals in group NonDev received sucrose and LiCl unpaired. If a flavour–sweetness association formed during training, a decrease in preference should be observed in group Dev but not in group NonDev.

Methods

Subjects and apparatus

The subjects were 16 female Wistar rats at least 110 days old at the start of the experiment. They had previously participated in a flavour preference experiment with 1% (v/v) almond and 20% (200 g/l) sucrose, but were orthogonally assigned to both groups in order to equalize experience with these stimuli. They were housed and maintained under the same conditions as were used in Experiment 1, with the exception that they were never food deprived. The US was again a 20% (200 g/l) sucrose solution in tap water. The CS (flavour A) was this time a Kool–Aid artic-green-apple-flavoured unsweetened solution in tap water at 0.2% (2 g/l) concentration. During the devaluation phase, animals received intraperitoneal (i.p.) injections of 0.15 M LiCl at 20 ml/kg of body weight.

Procedure

Before the start of training, rats were water deprived for 4 days during which they had free access to water for 30 min during the morning session. On days 1–4, animals received two daily conditioning trials, one in the morning and the other in the afternoon. In each one, rats had access to flavour A mixed with 20% sucrose solution for 15 min. No additional water was supplied in this phase. The conditioning test occurred on day 5. Animals were given one 15-min two-bottle choice test (20 g of flavour A vs. 20 g of water) during the morning session, and 15-min access to water during the afternoon.

They were also weighed at the end of the session. Rats were subsequently divided into two equal-sized groups matched in preference ratio. The US devaluation phase took place during days 6–11, in which either sucrose solution or water was presented in a single bottle. Animals in group Dev (n = 8) received two 2-day cycles of sucrose devaluation (days 6–9), and then a 2-day cycle of fluid consumption (days 10–11). On the first day of each sucrose devaluation cycle, rats were given access to 20 g of sucrose solution for 15 min followed by an i.p. LiCl injection, while on the second day they received 15 min access to water only. Animals in group NonDev (n = 8) were also injected with LiCl on the first day of each cycle, but they were given access to water instead of sucrose. On the second day, they had access to 20 g of sucrose for 15 min. Both groups received 15 min of water access in the afternoons. On days 10–11, animals in group Dev drank sucrose on the first day and water on the second. Animals in group NonDev drank water on the first day and sucrose on the second. Preference tests, identical to the conditioning test conducted on day 5, took place during the mornings of days 12–14. Animals were given an additional 15 min of water access during the afternoons of days 12 and 13.

Results

Conditioning phase. Compound consumption in each of the morning and afternoon sessions was averaged to obtain a daily value that was then analyzed (means: 6.83, 7.93, 8.80, and 9.09 g). An ANOVA conducted over the consumptions on the 4 days of training yielded a significant effect of day, F(3, 45) = 22.11. Pairwise comparisons using Tukey's test showed that consumption on days 1 and 2 differed from that on days 3 and 4, but that day 1 did not differ from day 2 and that day 3 did not differ from day 4.

Conditioning test. After this phase, animals were randomly divided into two halves and the preference ratios were analyzed (Figure 6, left side). Since there was no difference between the two subgroups, *t*(14) = 0.96, they formed the groups Dev and NonDev for the following phase (the mean preference ratios were 0.28 and 0.35, respectively).

Devaluation phase. An ANOVA on sucrose consumption during the 3 days of this phase with group and day as factors, yielded main effects of both group, F(1, 14) = 35.47, and day, F(2, 28) = 5.20, and their interaction, F(2, 28) = 4.33. Post hoc Tukey's tests showed that the average consumption of sucrose differed between groups on day 3 (NonDev: 11.39, 10.35, and 10.89 g; Dev: 9.07, 7.25, and 3.06 g). Two separate ANOVAs with day as factor, showed a significant effect of day in group Dev, F(2, 14) = 7.21, but not in group NonDev, F < 1. Regarding differences in consumption in group Dev, Tukey's tests showed that the sucrose consumption on day 1 differed from that on day 3, and marginally from that on day 2 (p = 0.05).

Preference tests after US devaluation. Mean preference ratios for flavour A after the devaluation phase for each group appear in Figure 6 (right side). Preference in group Dev seems to be much lower than in group NonDev. The ANOVA with group and day as factors confirmed this impression, yielding a significant main effect of group, F(1, 14) = 28.64. No other main effect or interaction was significant. Although US devaluation might be expected to reduce preference in group Dev, it may be noted that the preference ratios shown by group NonDev are in fact quite high compared with that of the conditioning test. To test for this, an ANOVA with group and day (conditioning test vs. first devaluation test) as factors was conducted.

The analysis yielded a significant main effect of group, F(1, 14) = 23.29, and a significant group x day interaction, F(1, 14) = 23.77. Mean comparisons in each group revealed that both groups changed preference after devaluation. In group Dev, there was a significant decrease, *t*(7) = 2.74, whereas in group NonDev the preference increased, *t*(7) = 3.99.

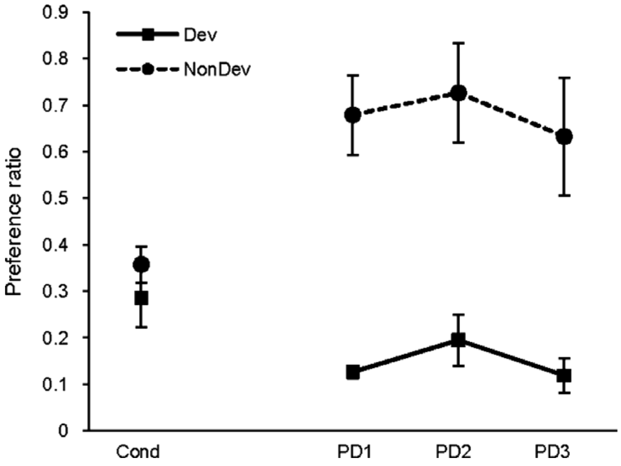

Figure 6. Experiment 0B. Mean preference ratio (flavour A) for groups Dev and NonDev on conditioning and three post-devaluation tests. Both consumed eight simultaneous flavour–sucrose pairings during training. Group Dev received two sucrose–LiCl pairings after conditioning, whereas sucrose and LiCl injections were unpaired in group NonDev. Error bars represent SEM.

Discussion

Performance on the conditioning test in Experiment 0B was similar to that obtained in Experiment 0A for group Simultaneous when rats were tested thirsty, and thus replicates the absence of a preference. In order to detect a flavour–taste association, the taste of the US was devalued by pairing the sucrose solution with two i.p. LiCl injections in group Dev. A decrement in preference for flavour A, which remained through the three days of testing, was observed. This result proves that the absence of a preference in thirsty rats is not due to a deficit in flavour–taste learning acquisition, and might be attributed to performance factors. The flavour–sweetness taste association could not be effective in producing a CS hedonic shift because the intensity of the unpalatable flavour was so high as to counteract the effect of the associate sweetness. Thus, rats preferred water to an unpalatable solution paired with calories when thirsty, and only showed a preference for the flavoured solution when they were hungry (Exp. 0A).

General discussion

Taken together, the results of the two experiments showed the absence of a conditioned flavour preference in thirsty rats after simultaneous pairings of a highly non-preferred flavour (CS) and a nutrient with a palatable taste (US). Nevertheless, the preference is observed when the positive consequences of the US consumption are relevant to the motivational state. In Experiment 0A, preference was achieved by a motivational change. Thirsty rats did not show any evidence of a preference on the conditioning test. However, when rats were tested also hungry, the preference ratio for the CS in group Simultaneous increased significantly over that shown by group Unpaired, suggesting the existence of a flavour–nutrient association acquired during training. The fact that this preference was only observed when rats were hungry suggests that hunger modulates the expression of learning acquired during conditioning (Harris et al., 2000; Yiin et al., 2005a, 2005b). Hunger may promote the consumption of a flavour paired with a nutrient even if the flavour is unpalatable. Regarding Experiment 0B, preference for flavour A decreased when its associate sucrose was paired with i.p. LiCl injections (group Dev), providing evidence of a CS-flavour–taste association formed during training. This association however was insufficient to produce a preference on the conditioning test, probably due to the initial low palatability of the flavour.

Therefore, using a hedonically negative flavour, thirsty rats acquire both flavour–taste and flavour–nutrient learning during training. That a flavour–taste association was formed is shown by the US devaluation effect found in group Dev (Exp. 0B). On the other hand, the increase in preference observed in group Simultaneous (Exp. 0A) after a motivational change to hunger suggests that animals also formed a flavour–nutrient association. It is worth noting that rats seem to acquire flavour–nutrient learning in the absence of explicit food deprivation, suggesting that this learning occurs after pairing a flavour with the post-ingestive effects of sucrose, even if animals are not food-deprived during training (see also Yiin et al., 2005a, 2005b). These results also suggest that both flavour-taste and flavour-nutrient learning were acquired by water deprived rats and that they may indeed be dissociated by manipulating the motivational state at time of testing.

5.3. EXTINCTION PROCEDURE IN CONDITIONED FLAVOUR PREFERENCE BASED ON NUTRIENT: BASIC EFFECT

The immediate background to the present set of experiments lies in Harris et al.'s (2004) experiments, which carried out extensive work on the resistance to extinction of conditioned flavour preferences. In those experiments, a decrement in conditioned preference after post-training flavour exposure was found in hungry rats. In their procedure, thirsty rats drank a mixture of the flavour cue and sucrose solution during conditioning, and were subsequently tested under food deprivation using flavour vs. water choice tests (Harris et al., 2004, Exp. 2B).

Similar to Harris et al., and in order to apply an ecological and adaptive approach that mimics the sources of reinforcement of food preferences, a CFP with orally consumed nutrient (sucrose) procedure was used throughout the experiments in the present dissertation. Since the optimal nutrient for conditioning flavour preferences is glucose (Ackroff, 2008), we used a glucose-based carbohydrate such as sucrose as US. Neutral flavour cues (e.g., odour) were used as CS because flavour preferences appear to be primarily acquired through experience, unlike the innate predispositions to prefer (or avoid) tastes. The presentation of the CS and the US was in simultaneous solutions for the experimental groups, because several studies have reported that the simultaneous procedure conditions stronger preferences that the delay/trace procedures (e.g., Sclafani & Ackroff, 1994). Finally, the flavour preference was assessed in two-bottle choice flavour CS vs. water tests, as CS+ vs. CS- choice tests cannot rule out the possibility that the preference for CS+ is due to learned avoidance of the CS- flavour, which had been unpaired with the US (e.g., Boakes et al., 2010; Harris et al., 2000).

Experiment 1: Basic effect

The goal of Experiment 1 was to replicate the basic effect of decrement in conditioned flavour preference when rats trained thirsty received post-training flavour exposure whereas hungry, and to set up an optimal control condition for excitatory conditioning to be used in following experiments.

Method

Subjects and solutions

Sixteen naïve female Wistar rats at least 110 days old at the start of the experiment were housed in individual home cages and kept in a large colony room with a 12-hour light/12-hour dark schedule. This and all subsequent experiments took place in the home cages and were conducted during the light cycle at approximately 9:30 am. Rats were water deprived and had continuous access to food (Global Diet 2014 Chow; Harlan, Barcelona, Spain) throughout the experiment, with the exceptions mentioned below. Fluids were administered in 50-ml plastic tubes with a rubber stopper fitted with a stainless steel ball-bearing tipped spout. Fresh solutions were made daily with tap water and administered at room temperature. Consumption was estimated by weighing the tubes before and after fluid presentation to the nearest 0.1 g. The US was a 20% (wt/vol) sucrose solution. The target flavour, flavour A, was a

1% (vol/vol) almond solution in tap water (almond flavouring supplied by SuperCook, Leeds, UK).

Procedure

The water bottles were removed from the home cages 24 hr before the start of the experiment, and animals were assigned to two weight-matched groups (n = 8).

During the pre-conditioning phase (Days 1-4), rats in the both groups were given 4 days to accommodate to the water deprivation. They were permitted to drink tap water for 30 min in the tubes subsequently used to present the flavoured solutions.

During the conditioning phase (Days 5-8), rats in the group Simultaneous were given 6 ml of the almond-sucrose compound for 5 min (6 ml were provided to guarantee a 5ml consumption to compensate for possible fluid spillage), whereas the rats in the group Serial were given 6 ml of almond presented for 5 min followed by 5-min access to 6 ml of sucrose. After each conditioning session, animals had access to supplementary water for 25 min in group Simultaneous and 20 min in group Serial.

After the conditioning phase, animals were also food deprived by being given limited access to both water and food for 90 min per day at the end of each session. During Days 9-11, the rats were given 3 days to accommodate to the food deprivation to guarantee fluid intake on test, as we had previously detected a reluctance to consume unreinforced flavours immediately after food deprivation. Likewise, all groups received water in two tubes for 30 min on each of these three days to adapt them to the test conditions used in the extinction phase that was to follow.

The flavour-exposure phase occurred over the next 6 days (Days 12-18). All rats were given 10 two-bottle almond vs. water tests for 30 min, each bottle containing 20 ml of the correspondent fluid. The position of bottles (i.e., left and right) was counterbalanced within each group and alternated across days.

Results

Conditioning phase. Consumption of the compound in group Simultaneous, and of both the sucrose and the flavour A solutions in group Serial, was analyzed through three repeated-measures ANOVAs with Trial as the factor. Consumption of the compound differed among trials in group Simultaneous, F(3, 21) = 15.19, consumption of the first day being lower than that of the others, which did not differ among themselves (means: 3.52, 5.92, 5.62, and 5.96 g). A similar pattern was found in the consumption of the sucrose solution in group Serial, F(3, 21) = 38.11 (means: 3.73, 5.89, 6.15, and 6.06 g). These patterns suggest that fluids containing the dense sucrose solution were affected by neophobia on the first trial in both groups. On the contrary, consumption of flavour A in group Serial proceeded smoothly with no differences among trials F(3, 21) = 1.88 (means: 4.25, 4.62, 4.56, and 5.25 g).

Flavour-exposure phase. Preference ratios on the test phase (see Figure 7) were analyzed through a repeated-measures ANOVA with Group as the between-subjects factor and Day as the within-subjects factor. There were main effects of both Group, F(1, 14) =12.44, and Day, F(5, 70) = 2.74, and the interaction was also significant, F(5, 70) = 2.97. Two separate one-way ANOVAs were conducted to analyze differences among days for each group. There were no differences in group Serial, F< 1, but preference ratios differed in group Simultaneous, F(5, 35) = 4.81. Post hoc Tukey's tests showed that the average preference ratio of test 6 was significantly lower than that of the tests 1-5, which did not differ among themselves. Regarding differences between groups in each test, the average preference ratios of group Simultaneous were significantly higher than those of group Serial on day 1, *t*(14) = 3.98, day 2, *t*(14) = 4.73, and marginally higher on day 3, *t*(14) = 1.93, p = 0.07. In addition, the preference ratio on the first day (conditioning test) was significantly higher than 0.5 in group Simultaneous, *t*(7) = 11.93 (p < .0001), but not in group Serial, in which the preference ratio did not differ from 0.5, *t*(7) = 1.75. Therefore, it seems that conditioned preference for flavour A developed only in group Simultaneous and decreased by day 4 compared to the control group. This decrease is in agreement with the results of Harris et al. (2004), showing that resistance to extinction of nutrient-based conditioned preference is not observed when rats are repeatedly tested under food deprivation. These results also replicate our previous observations and thus show that serial flavour-sucrose training constitutes a good control condition for excitatory conditioning, eliminating the flavour avoidance problems arising from both the differential and the unpaired training procedures.

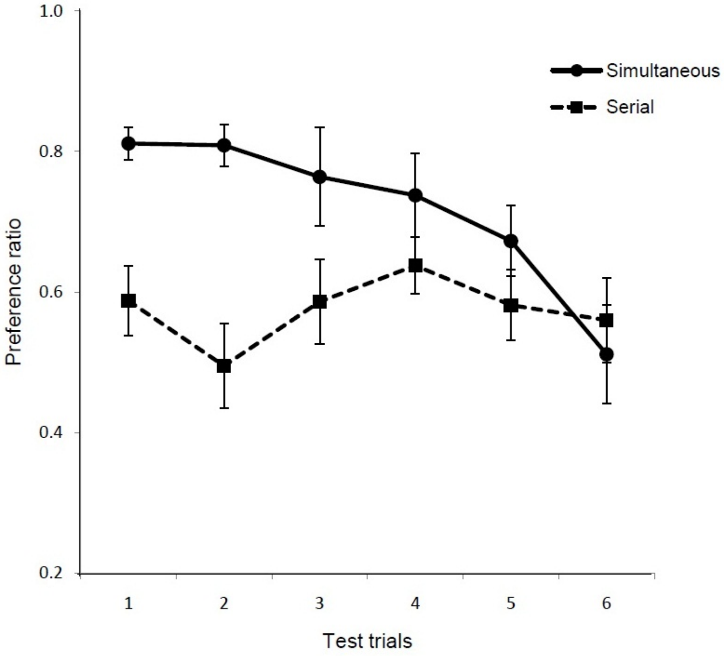

Figure 7. Experiment 1. Mean preference ratios on conditioning and extinction tests for groups Simultaneous and Serial. Error bar represents SEM.

Discussion

The results of Experiment 1 demonstrated that simultaneous training was more effective than a serial procedure. It suggests that a flavour simultaneously paired with a sucrose solution during training activates a representation of the sucrose on test that in turn produces a preference for the flavour over plain water. It also confirmed that subsequently, when the flavour was repeatedly experienced in the absence of sucrose under both hunger and thirst, preference for the flavour declined to the same level as that of the controls. On the other hand, Experiment 1 corroborated a previous observation from our lab showing that serial flavour-sucrose presentations to thirsty rats did not produce evidence of a preference over plain water when tested hungry. Thus serial condition was used as a control procedure for flavour conditioning –indeed, a conservative one.

5.4. EXTINCTION RELATED PHENOMENA IN CONDITIONED FLAVOUR PREFERENCE BASED ON NUTRIENT

To the best of my knowledge, only two studies have been aimed at obtaining evidence of phenomena of recovery of the conditioned preference following flavour-exposure after training. Díaz and De la Casa (2010) found spontaneous recovery and renewal using a taste-taste preference paradigm based on the sensory-affective properties of saccharin using an initially non-preferred taste (citric acid) as the CS. It is possible, however, that exposing a flavour previously paired with a nutrient to hungry animals might involve a different mechanism that exposing a flavour which has been paired with saccharin in thirsty animals (Harris et al., 2000). On the other hand, Tarner et al. (2004) found spontaneous recovery of nutrient-based conditioned preference although, as commented in the Discussion of Experiment 4, the lack of proper comparisons with the control groups makes this evidence rather uncompelling.

Experiment 2: Spontaneous recovery

It is well established since Pavlov's times that the extinguished responses can recover if time is allowed to pass following extinction. The aim of this experiment was to evaluate whether the reduced preference after post-training flavour exposure in hungry rats could be recovered following the passage of time. The design is shown in Table 1 (Exp. 2). Two groups of thirsty rats (Simultaneous and Serial) were trained during several daily sessions. Group Simultaneous, given access to almond-sucrose simultaneous compound, was compared with a Serial condition in which the sucrose solution was presented after the consumption of almond. The flavour-exposure treatment was identical for each group and consisted of repeated 2-bottle almond vs. water tests while animals were hungry and thirsty. Thus, both groups had an equivalent history of exposure to almond and sucrose, but differed with respect to the CS-US temporal relationship during training, simultaneous or serial, respectively. Two weeks after the flavour-exposure phase, subjects were tested for spontaneous recovery.

Table 1. Designs for spontaneous recovery, reinstatement, and renewal experiments.

Groups	**Conditioning** *Thirsty*	**Pre-extinction** *Thirsty & Hungry*	**Extinction** *Thirsty & Hungry*		
Experiment 2				**Spontaneous recovery** (2 weeks later) *Thirsty & Hungry*	
Simultaneous	4 A +	3 water vs. water	10 A vs. water	A vs. water	
Serial	4 A → +				
Experiment 3				**Reinstatement** *Thirsty & Hungry*	
Simultaneous	4 A +	3 water vs. water	10 A vs. water	+	A vs. water
Serial	4 A → +				
Experiment 4				**Renewal** **Motivational context**	
Simultaneous_ABA	4 A +	3 water vs. water	10 A vs. water	A vs. water (*Thirsty*)	
Simultaneous_ABB				A vs. water (*Thirsty & Hungry*)	
Serial_ABA	4 A → +			A vs. water (*Thirsty*)	
Serial_ABB				A vs. water (*Thirsty & Hungry*)	

Note. A = CS flavour; + = US sucrose; Simultaneous = group given almond-sucrose compound; Serial = control condition in which the sucrose solution was presented after the consumption of almond; ABA/ABB = renewal test either in context of conditioning A (ABA) or in context of extinction B (ABB).

Method

Subjects and apparatus

The subjects were 16 experimentally naïve female Wistar rats with a mean body weight of 245 g at the start of the experiment. Housing, food, and water supply, as well as the apparatus and solutions, were the same as in Experiment 1.

Procedure

Conditioning and flavour-exposure ensued in the same manner as that in Experiment 1, with the following exceptions. After conditioning, all animals had access to supplementary water for 30 min and the flavour-exposure phase occurred over the next 10 days. On the 14th day after the flavour-exposure phase, all groups received a two-bottle spontaneous recovery test.

Results

Conditioning phase. The mean daily intakes of almond-sucrose compound consumed across the conditioning days were 2.56, 5.72, 5.9, and 5.79 g for group Simultaneous. A similar pattern was found in the consumption of the almond solution, means: 4.95, 5.67, 5.57, and 5.63 g., and of the sucrose, means: 1.45, 4.6, 5.5, and 5.61 g., in group Serial. These patterns suggest that fluids containing the almond and the sucrose solutions were affected by neophobia on the first trial, which disappeared on subsequent trials. Regarding differences between groups in the conditioning test (first flavour-exposure test), the average preference ratio of group Simultaneous (0.91) was significantly higher than that of group Serial (0.57) [$t(14) = 12.33$], which did not differ from the preference reference-value 0.5 [$t(7) < 1$].

Flavour-exposure phase. The results from the flavour-exposure phase on almond preference and almond intake are shown in Figure 8 (top and bottom left), demonstrating the mean ratio of almond preference and the mean intake of almond in both groups during this phase (2-day blocks). The 2 group x 5 block ANOVA of the preference ratios revealed a marginally main effect of group [$F(1, 14) = 4.01$, $p = 0.06$], and a significant effect of block [$F(4, 56) = 7.62$], which once analyzed showed a decrement from block 1 to blocks 4 and 5. The data of most interest came from almond consumption, as the statistical analysis of water intake revealed neither main effect of group [$F(1, 14) = 3.28$], block [$F < 1$], nor group x block interaction [$F(4, 56) = 1.12$]. The 2 group x 5 block ANOVA of the almond intake revealed a main effect of block [$F(4, 56) = 10.56$] and a significant group x block interaction [$F(4, 56)$

= 4.59]. The statistical analysis revealed that group Simultaneous exhibited significantly lower consumption of almond in blocks 3, 4, and 5 than in block 1, and that group Serial did not exhibit significant differences between blocks 1 and 5 but showed a higher almond intake in block 2 compared to block 4. Finally, the group Simultaneous exhibited higher almond consumption than Serial in block 1.

Spontaneous recovery test. The preference and almond-intake results of the spontaneous recovery test can be found in Figure 8 (top and bottom right). The two groups were tested for spontaneous recovery 14 days after flavour-exposure comparing the average preference ratio of the last test (test 10) with that of the spontaneous recovery test. The 2 group x 2 day ANOVA of the preference ratios failed to detect any main effect or significant interaction (Fs < 1.17). Similarly, the 2 group x 2 day ANOVAs of the consumption revealed neither significant main effects nor interactions either in almond or water intake (Fs < 2.6).

Discussion

The analyses of the conditioning test showed that rats developed a preference for the flavour only when it was simultaneously paired with sucrose (group Simultaneous). Although preference ratios did decrease in both groups, a more detailed inspection of fluids consumption revealed that almond intake differed from block 1 to blocks 3-5 in the experimental group, whereas this was not the case for group serial, which showed some fluctuations in almond consumption along this phase. No differences were found in water consumption.

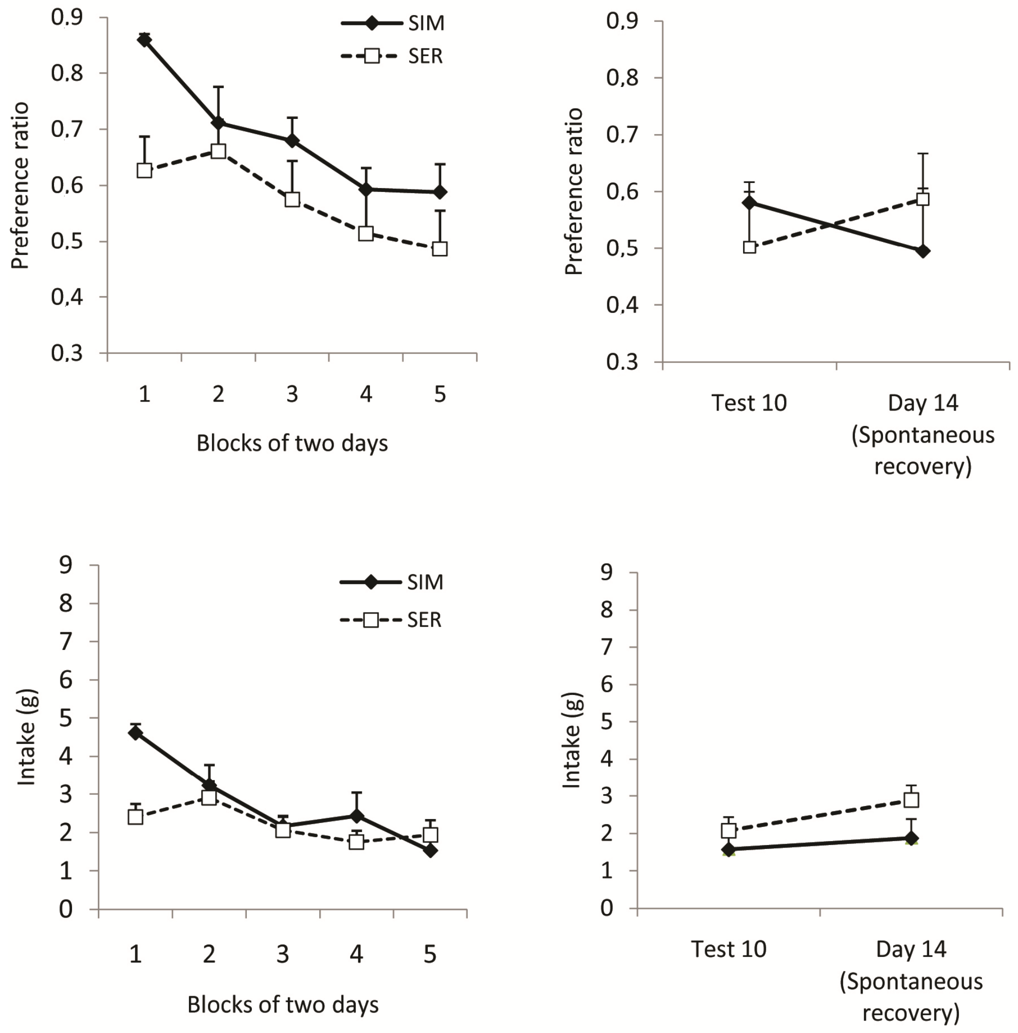

Figure 8. Experiment 2. Top left: Average preference ratio of almond (as ratio of almond intake over total intake) on each of the 5 extinction blocks of two days for the two groups: simultaneous (SIM) and serial (SER). Top right: Average preference ratio of almond on the last extinction exposure to almond (Test 10) and day of spontaneous recovery test (14 days after). Bottom left: Average intake of almond on each of the 5 extinction blocks of two days for both groups. Bottom right: Average intake of almond on the last extinction exposure to almond and day of spontaneous recovery test. Error bars represent SEMs.

Following a retention interval of 14 days, the almond solution was again pitted against water. There was not any significant increase in the preference ratio or flavour intake when tested for spontaneous recovery. The absence of spontaneous recovery after a 2-week interval replicates previous results obtained in our lab using mere exposure and unpaired control groups (González, de Brugada, & Gil, 2008; Exp. 3).

This lack of spontaneous recovery is somehow surprising, as it is a widespread phenomenon, both in terms of the variety of learning paradigms in which it happens and the frequency with which it is reported, including taste-taste learning (Díaz & De la Casa, 2010), conditioned taste avoidance (e.g., Rosas and Bouton, 1996), spatial preference learning (e.g. Lattal, Mullen, & Abel, 2003), fear conditioning (e.g. Quirk, 2002), instrumental learning (e.g. López-Romero & García-Barraza, 2010), drug seeking (e.g. Di Ciano & Everitt, 2002), and consummatory behaviour (e.g. Norris, Daniel, & Papini, 2008).

To the best of our knowledge, only one previous study has assessed spontaneous recovery using flavour-nutrient learning. Tarner et al. (2004, Exp. 2) found spontaneous recovery of conditioned flavour preference based on calories using a differential training procedure (CS+ paired with sucrose and CS- paired with saccharin) after 7, 14 and 21 days in hungry rats. It is difficult to determine the sources of the dissimilarities with our results, due to the differences in procedure and measures. For instance, their training procedure consisted of 10 days during which the CSs were exposed for 23 hr without additional available water, and CS+ vs. CS – instead of CS+ vs. water preference was assessed during pre- and post-extinction tests. Although they did not report a decrease in CS+ consumption during the one-bottle extinction phase, they found a decrease in the percent of CS+ consumed over CS- in a post extinction test in the experimental group, as well as a subsequent increase after each of the three temporal intervals. However, as they did not conduct comparisons with the two control groups, which were not evaluated during the spontaneous recovery phase, it is not clear whether these results can be interpreted in terms of the manipulation conducted in the experimental group.

Experiment 3: Reinstatement

Another experimental manipulation that can recover the conditioned response after extinction is reinstatement, in which the extinguished response returns if the animal is merely re-exposed to the unconditioned stimulus after extinction (Bouton, 2004).

The aim of Experiment 3 was to test for the reinstatement effect after giving rats repeated two-bottle almond vs. water tests, as in Experiment 1 and 2. The design is shown in Table 1 (Exp. 3). If the decrement in conditioned flavour preference is due to extinction, then the extinguished preference should be restored by the simple re-exposure to the original US (sucrose) in the absence of further presentations of the conditioned stimulus.

Method

Subjects and apparatus

The subjects were 16 experimentally naïve female Wistar rats with a mean body weight of 245 g at the start of the experiment. Housing, food and water supply, as well as the apparatus and solutions, were the same as in Experiment 1.

Procedure

Conditioning and flavour-exposure ensued in the same manner as that in Experiment 2. During the reinstatement phase, conducted one day after the last flavour-exposure test, rats received 6 g of sucrose solution for 5 min followed by 30 min of water access; the next day animals were tested for reinstatement with identical preference tests to those given during the flavour-exposure phase.

Results

Conditioning phase. The mean daily intakes of almond-sucrose compound consumed across the conditioning days were 3.93, 5.89, 5.9, and 5.97 g for the group Simultaneous. A similar pattern was found in the consumption of the sucrose in group Serial, means 1.66, 4.77, 5.65, and 5.75 g, with a lesser consumption in day 1 compared with the others. Finally, the mean consumptions of the almond in the group Serial were 5.64, 5.55, 5.4, and 5.15 g. Regarding differences between groups in the conditioning test (first flavour-exposure test), the average preference ratio of group Simultaneous (0.84) was significantly higher than that of group Serial (0.67) [*t*(14) = 9.32], which was significantly higher than 0.5 [*t*(7) = 3.33].

Flavour-exposure phase. The results from the flavour-exposure phase on both almond preference ratio and intake are shown in Figure 9 (top and bottom left), demonstrating the average ratios and intakes in the two groups across this phase (2-day blocks). The 2 group x 5 block ANOVA of the preference ratios revealed a main effect of group [F(1, 14) = 18.98], and block [F(4, 56) = 4.47]. Regarding main effect of group, group Simultaneous showed a greater preference than group Serial. On the other hand, flavour preference showed a decrement from block 1 to blocks 3 and 5. For more detailed exploration of preferences, both almond and water consumptions were analyzed. The 2 group x 5 block ANOVA of the almond intake revealed a main effect of group [F(1, 4) = 9.35], and block [F(4, 56) = 11.56]. The statistical analysis revealed that group Simultaneous exhibited a higher consumption than group Serial. On the other hand, rats consumed more on Block 1 than Blocks 2, 3, 4, and 5. Statistical analysis of water intake revealed that there were neither main effect of group [F(1, 14) = 2.01], block [F(4, 56) = 2.37], nor group x block interaction [F(4, 56) = 1.54].

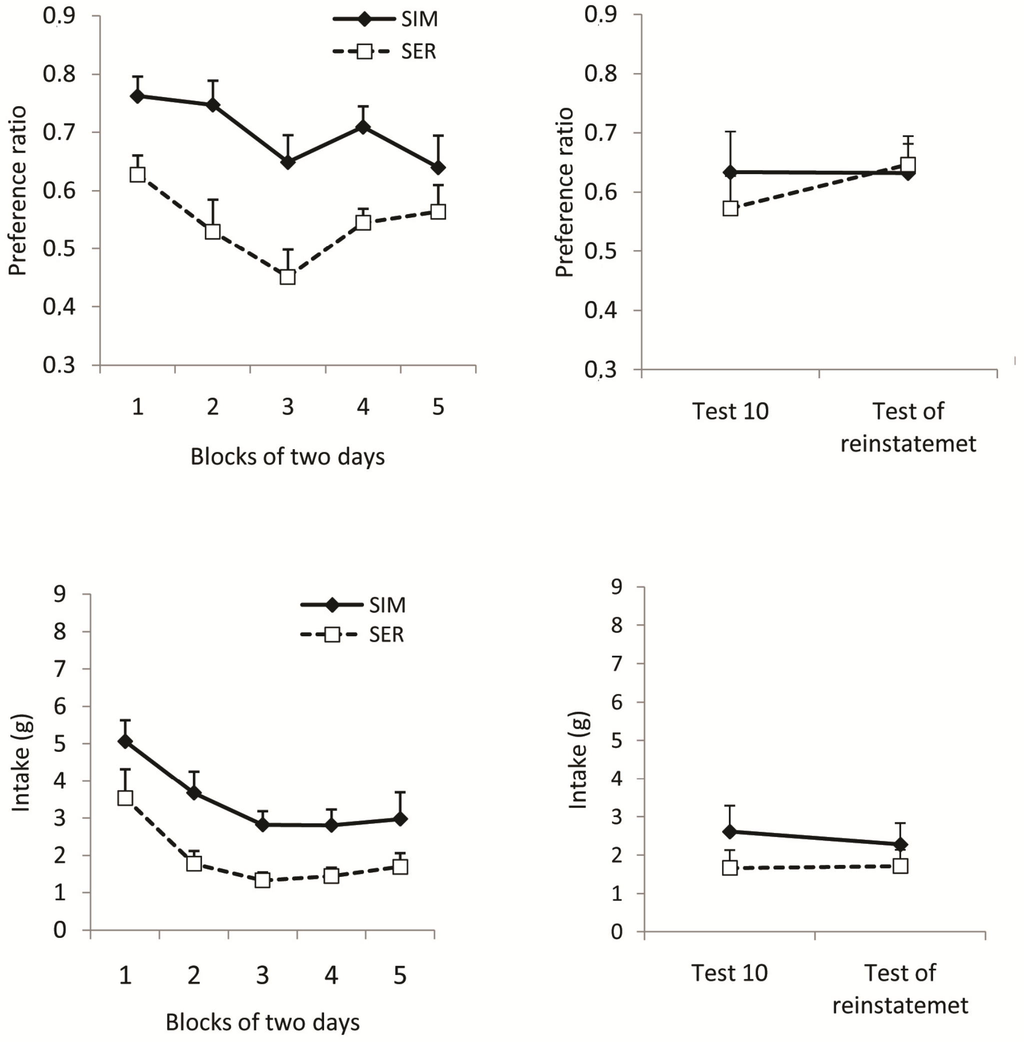

Figure 9. Experiment 3. Top left: Average preference ratio of almond (as ratio of almond intake over total intake) on each of the 5 extinction blocks of two days for the two groups: simultaneous (SIM) and serial (SER). Top right: Average preference ratio of almond on the last extinction exposure to almond (Test 10) and day of reinstatement test. Bottom left: Average intake of almond on each of the 5 extinction blocks of two days for the both groups. Bottom right: Average intake of almond on the last extinction exposure to almond and day of reinstatement test. Error bars represent SEMs.

Reinstatement test. Before the reinstatement test, all rats were given 6 ml of a sucrose solution that they consumed completely. Preference ratio and almond intake can be found in Figure 9 (top and bottom right). Both groups were tested for reinstatement comparing performance on the last flavour-exposure test with that of the reinstatement test. The 2 group x 2 day ANOVA of preference ratios failed to detect either main effects or a significant interaction (Fs < 1). Similarly, the 2 group x 2 day ANOVAs of the consumption revealed that there were neither significant main effects nor interaction in either almond or water intake (Fs < 2.27).

Discussion

The conditioning data of Experiment 3 were consistent with that of Experiment 1 and 2, in which rats developed a higher preference for the flavour in group Simultaneous than in group Serial. In this experiment, group Serial exhibited a preference ratio greater than 0.5. Although this level of preference in group Serial had not been observed in other Experiments, this data suggests that this procedure, under the conditions used in the present experiments, could be a weakly effective form of conditioning; alternatively, it could also be due to mere exposure to flavour (Capaldi, 1996).

Other works using different procedural conditions have found that gustatory stimuli can be conditioned with both simultaneous and sequential flavour-nutrient presentation. For instance, Higgins and Rescorla (2004) found an increase in flavour consumption after serial presentations of a flavour mixed in 2% Polycose followed by 20% Polycose during the 18-day training, a considerable longer training procedure that ours. It is possible that serial presentations of a flavour and 20% Polycose in hungry animals, as well as mixing the flavour with the nutrient even at low doses, might produce more robust conditioning.

Regarding the flavour-exposure phase, repeated non-reinforced presentation of almond reduced both preference and intake from the first block to the last block. This happened, again, in both groups. However, there was no sign of reinstatement of preference or consumption of the almond after the US had been presented in a non-contingent fashion. It is true that this result involves accepting a null result, but this occurred despite the fact that a variety of different procedures readily produced reinstatement including behavioural pharmacology preparations as a model of relapse (e.g. for review, see Katz and Higgins, 2003), fear conditioning (e.g. Bouton & Bolles, 1979), appetitive conditioning (e.g. Bouton & Peck, 1989), and conditioned taste aversion (e.g. Schachtman, Brown, & Miller, 1985).

Experiment 4: Contextual renewal

Experiment 4 tested the possibility of restoring the reduced preference by shifting the animals to a context different from that of the flavour exposure. An important fact about the renewal effect is that it appears to be supported by many kinds of contexts (Bouton, 2004). Although physical contexts have been studied most often, a variety of different kinds of events can play the role of context, among them interoceptive contexts such as hormonal state (Ahlers & Richardson, 1985), mood state (Eich, 1985), drug state (Bouton, Kenney, & Rosengard, 1990), and the state of food deprivation (Davidson,1998). In the present experiment, the contexts were primary motivational states obtained by manipulating the rats' level of food deprivation during the different experimental phases.

Specifically, the conditioning phase was conducted in one context (context A: thirst) whereas extinction was then conducted in a second one (context B: thirst and hunger), as in the previous experiments. Afterward, testing could occur either in context A (ABA procedure, renewal) or in context B (ABB procedure). The design is shown in Table 1 (Exp. 4) consisting of a 2 (Simultaneous, Serial) x 2 (ABA, ABB) factorial. Half the rats received exposures to a simultaneous compound of almond and sucrose while the remaining rats were exposed to serial flavour-sucrose presentations. After the last session of flavour-exposure and prior to renewal, the rats in both the Simultaneous and Serial groups were further divided into two groups regarding the motivational context during testing: Simultaneous_ABA, Simultaneous_ABB, Serial_ABA, and Serial_ABB. If the decrease in preference is due to extinction, an increase in flavour preference should only occur in group Simultaneous_ABA, since the renewal test was conducted outside the extinction context.

Method

Subjects and apparatus

The subjects were 32 experimentally naïve female Wistar rats with a mean body weight of 205 g at the start of the experiment. Housing, food and water supply, general maintenance as well as apparatus and solutions were the same as in Experiment 1.

Procedure

Pre-conditioning, conditioning, and flavour-exposure phases ensued in the same manner as in Experiment 2. After the last session of flavour-exposure and prior to renewal, the rats in both Simultaneous (n = 16) and Serial (n = 16) groups were matched for preference taking into account the preference ratio of the last flavour-exposure trial and further divided into two groups: Simultaneous_ABA (n = 8), Simultaneous_ABB (n = 8), Serial_ABA (n = 8), and Serial_ABB (n = 8). The contextual renewal was run in a single day in which the rats in groups ABA received the two-bottle renewal test in the motivational context of conditioning (thirst) whereas rats in groups ABB received the two-bottle renewal test in the motivational context of flavour-exposure (thirst and hunger). The two-bottle test was given in the cages for 30 min.

Results

Conditioning phase. The mean daily intakes of almond-sucrose compound consumed across the conditioning days were 3.21, 5.85, 5.9, and 5.91 g for the group Simultaneous. The consumption of the first day was lower than that of the others. A similar pattern was found in intake of the sucrose (means: 2.29, 5.28, 5.35, and 6.0 g) and almond solution (means: 3.77, 5.62, 6.0, and 5.98 g) in group Serial. Regarding differences between groups in the conditioning test (first test of flavour-exposure), the average preference ratio of group Simultaneous (0.81) was significantly higher than that of group Serial (0.59) [*t*(30) = 24.24], which did not differ significantly from preference reference value 0.5 [*t*(15) = 2.1].

Flavour-exposure phase. Figure 10 (top and bottom left) shows the average almond preference ratio and intake of both groups during the flavour-exposure phase (2-day blocks). The 2 group x 5 block ANOVA of the preference ratios revealed a main effect of group [F(1, 30) = 12.38], and block [F(4, 120) = 6.85], and a significant group x block interaction [F(4, 120) = 3.41]. Separate one-way ANOVAs revealed a greater preference for group Simultaneous than in group Serial in blocks 1 [F(1, 30) = 21.03], 2 [F(1, 30) = 9.81], and 3 [F(1, 30) = 4.83]. On the other hand, there was an effect of block in group Simultaneous [F(4, 60) = 12.48], with a higher preference in block 1 with respect to blocks 2, 3, 4, and 5, and block 2 compared to block 3 and 5. The ANOVA with block as factor showed no significant effect in group Serial (F< 1). Statistical analyses of the almond consumption data offered similar results. The 2 group x 5 block ANOVA of the almond intake revealed a main effect of group [F(1, 30) = 10.16], and block [F(4, 120) = 34.30], and a significant group x block interaction [F(4, 120) = 11.38]. Separate one-way ANOVAs revealed a greater intake in group Simultaneous than in group Serial in blocks 1 [F(1, 30) = 26.30], 2 [F(1, 30) = 4.91], and 4 [F(1, 30) = 5. 31]. On the other hand, there was an effect of block in the Simultaneous [F(4, 64) = 39.06], with a higher consumption in Block 1 with respect to Blocks 2, 3, 4, and 5, and Block 2 compared to Blocks 3 and 5. The ANOVA with block as factor showed a significant effect in group Serial [F(4, 64) = 6.5], with a greater intake in Blocks 1-2 than Blocks 4-5. Statistical analysis of water intake revealed that there were neither main effect of group [F(1, 30) = 3.6], block [F(4, 120) = 1.52] nor group x block interaction [F(4, 120) = 1.24].

Motivational renewal test. The preference and almond intake results of the motivational renewal test can be found in Figure 10 (top and bottom right). In both groups contextual renewal was assessed comparing almond preference ratio and intake of the 10th flavour-exposure test with those of the renewal test. Regarding preference ratio, the 2 group x 2 context x 2 day ANOVA revealed a significant group x context interaction [F(1, 28) = 6.4]. No other effects were significant. Separate ANOVAs revealed a context effect only in the case of groups Simultaneous [F(1, 14) = 8.88], with a lower preference in group Simultaneous_ABA than in group Simultaneous_ ABB.

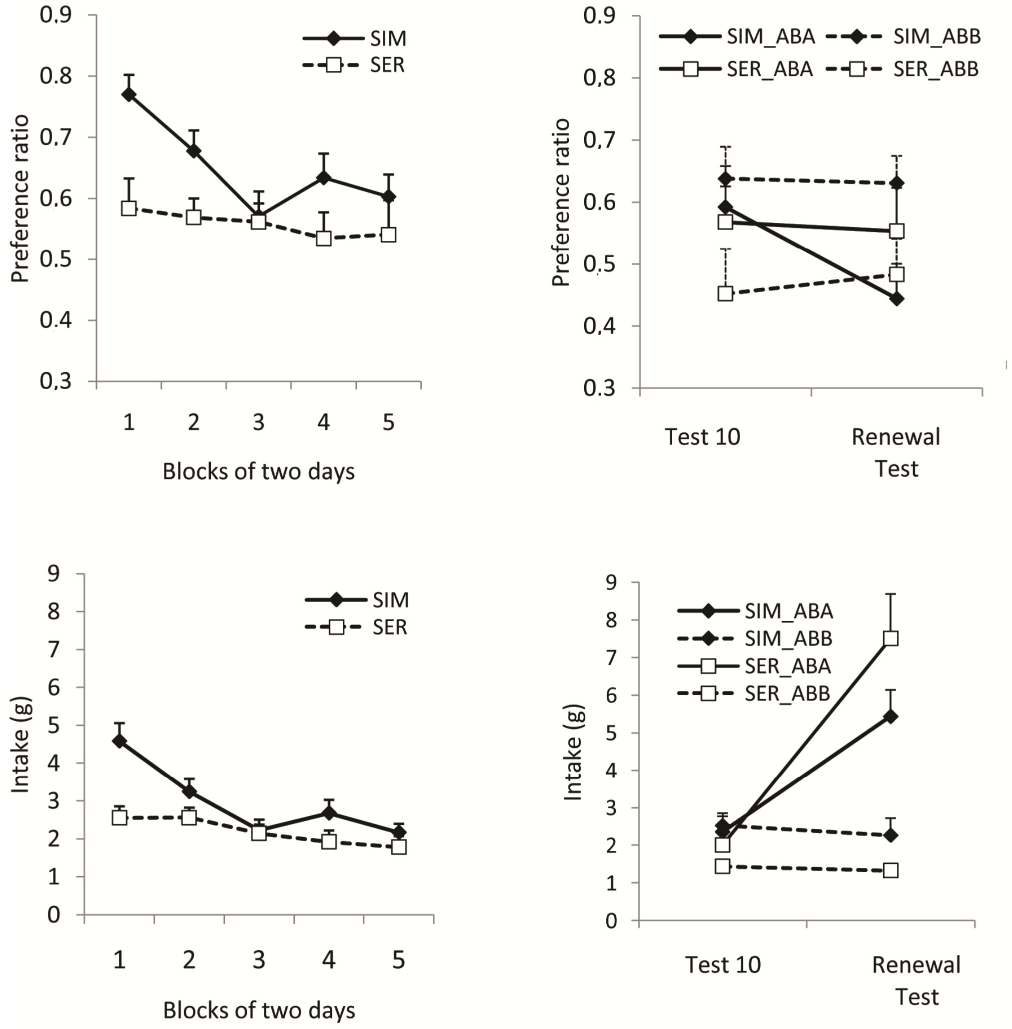

Figure 10. Experiment 4. Top left: Average preference ratio of almond (as ratio of almond intake over total intake) on each of the 5 extinction blocks of two days for the two groups: simultaneous (SIM) and serial (SER). Top right: Average preference ratio of almond on the last extinction exposure to almond (Test 10) and day of renewal test for four conditions: SIM or SER and ABA or ABB . Bottom left: Average intake of almond on each of the 5 extinction blocks of two days for the both groups. Bottom right: Average intake of almond on the last extinction exposure to almond and day of renewal test for all conditions. Error bars represent SEMs.

On the other hand, the 2 group x 2 context x 2 day ANOVAs of the almond consumption revealed a significant context x day interaction [F(1, 28) = 34.7]. Separate ANOVAs revealed a higher almond consumption for groups ABA than groups ABB. Finally, 2 group x 2 context x 2 day ANOVAs of the water consumption also revealed a significant context x day interaction [F(1, 28) = 49.3]. Note that in the previous experiments no differences in water consumption were found. Separate ANOVAs revealed, once again, higher water consumption in groups ABA than groups ABB. Thus it seems that shifting animals from the motivational state of hunger and thirst to that of thirst (groups ABA) produces an unspecific increase in fluid consumption, both of almond and water, and in both Simultaneous and Serial groups. Although an increment in almond intake was observed in the ABA groups, a similar increment in water consumption was also found, consequently preference for the flavour remained at the same levels as in the previous phase; in other words, there was no evidence of renewal in these animals, neither in group Simultaneous nor in group Serial.

Discussion

The conditioning test showed that rats developed conditioned preference only in group Simultaneous, which differed significantly from group Serial in the conditioning test. This time, a decrement in the preference ratio was observed only in group Simultaneous during the flavour-exposure phase, although a decrement in almond intake was shown by both groups. Thus, the effect of the motivational state at the time of extinction again affected both groups in some way, this effect being more evident in the experimental one.

Regarding motivational renewal, testing thirsty animals (groups ABA) produced an unspecific increase in fluid intake, as both consumption of almond and water was higher than those of groups ABB. However, preference ratio gave precise information about the relative consumption of almond related to that of water. In contrast to the results that one would expect to see (i.e., an increment in preference ratio in group Simultaneous_ABA) a significant effect in the opposite direction to renewal was found, with a lower preference in group Simultaneous_ABA than in group Simultaneous_ ABB. This result merits at least a tentative explanation. Since motivational state determines what component of the flavour-sucrose association controls responding (Harris et al., 2000), we may accept that performance during the renewal test in group Simultaneous_ ABA, in which animals are just thirsty but not hungry, may be controlled by the flavour-taste association. Harris et al. (2004) suggested that preference in thirsty animals might also be controlled by the association between the flavour and the hedonic responses produced by sucrose during training. It is then possible that the aversive signals of the state of hunger experienced by the animals during the flavour-exposure phase and the emotional responses produced by the absence of food in a context in which calories are expected endowed the flavour with a negative hedonic value. Accordingly, preference may decrease when tested again under thirst because performance would reflect the hedonic value of the flavour which was changed during the flavour-exposure phase under hunger.

The lack of renewal is surprising, as it has been observed in virtually every conditioning preparation in which it has been investigated, including fear conditioning (Bouton & Bolles, 1979), appetitive conditioning (Bouton & Peck, 1989), taste aversion learning (Rosas & Bouton, 1998), taste-taste learning (Díaz & De la Casa, 2010), drug seeking (Crombag & Shaham, 2002), and operant conditioning (Nakajima, Urushihara, & Masaki, 2000). Although in most of these studies environmental contexts have been used, several lines of evidence suggest that internal contexts may be very effective in the renewal of extinguished conditioned responses. For instance, many internal emotional and physiological cues, rather than external context, are relevant for relapse in drug abuse (Otto, O'Cleirigh, & Pollack, 2007). Furthermore, robust renewal effects have been obtained in animals after changes in internal context provided by food deprivation (Davidson, 1993). The present study, in which an opposite effect to renewal has been shown, provides further evidence of the effectiveness of the motivational cues in the control of flavour preferences.

Experiment 5: US-devaluation effect

Although unmasking procedures (spontaneous recovery, reinstatement, and renewal) can be effective in restoring extinguished performance, they are limited in the inferences they permit, as they are not especially powerful in estimating the strength of the association relative to associations that have not undergone extinction (Rescorla, 2001). It is well known that extinguished stimulus show the US-devaluation effect, and that the magnitude of the effect is indistinguishable from that observed with stimuli that had not received extinction (Delamater, 2004; Rescorla, 2001). This suggests that there is excellent preservation of the CS-US associations through an extinction procedure.

This experiment had two aims. Like in the Harris et al.'s (2004, Exp. 3) study, the first aim was to determine whether rats exposed to almond after conditioning and prior to the development of a sucrose aversion will reject the almond on test in the same way as non-exposed rats. Unlike Harris et al., who used thirsty animals throughout the experiment, we trained animals thirsty but they were also food-deprived during the flavour-exposure and subsequent phases, in a similar way to our previous experiments. As the experimental evidence suggests that different motivational states at the time of testing may result in preferences based on different associations (Harris et al, 2000), we wanted to assess the absence of the sucrose devaluation effect in flavour-exposed animals when a motivational context change between conditioning and the other phases of the experiment was conducted.

The present experiment also introduced a potentially important difference with that of Delamater (2007; Exp. 3). Although in both our experiment and that of Delamater (2007) animals were exposed to the target flavour and devaluated while hungry, in our experiment rats were conditioned while thirsty. Thus, the main difference between our experiment and those of Harris et al. and Delamater is the introduction of a motivational change between conditioning and the rest of phases.

Experiment 5 examined the specificity of the effects of flavour exposure on particular components of CFP using the US-devaluation technique and the motivational states during training and flavour-exposure used in the previous experiments. The design is shown in Table 2 and consists of a 2 x 2 factorial. Subjects in this experiment were initially trained with the simultaneous almond-sucrose compound while thirsty. Subsequently animals were food deprived; half the rats received exposures to the almond and water while the remaining rats were exposed to water alone during the extinction phase (Factor 1). Group Ext was given 6 repeated two-bottle almond vs. water choice tests, whereas group NonExt was given water in the two bottles during this time. Sucrose was then paired with lithium chloride (LiCl) for half of the rats in each of these conditions, but not for the remaining rats, which received water paired with LiCl (Factor 2). Finally, all rats were given an almond vs. water choice test.

Table 2. Design for US-devaluation effect. Experiment 5.

Groups	**Conditioning**	**Pre-extinction**	**Conditioning Test**	**Extinction**	**Devaluation**		**Sucrose Devaluation Test**	**Post-Devaluation Test**
	Thirsty	*Thirsty & Hungry*	*Thirsty & Hungry*	*Thirsty & Hungry*	*Thirsty & Hungry*		*Thirsty & Hungry*	*Thirsty & Hungry*
Ext_ Dev	2 A +	3 water vs. water	A vs. water	6 A vs. water	+ → LiCl	water	+, water	A vs. water
Ext_ Nondev					water → LiCl	+	water, +	
Nonext_ Dev				6 water vs. water	+ → LiCl	water	+, water	
Nonext_ Nondev					water → LiCl	+	water, +	

Note. A = CS flavour; + = US sucrose; LiCl = lithium chloride; Ext_Dev, Ext_Nondev, Nonext_Dev, Nonext_Nondev = four conditions according extinction factor (Extinction or Non-extinction) and US-devaluation factor (Devaluation or Non-devaluation).

Method

Subjects and apparatus

The subjects were 32 experimentally naïve female Wistar rats with a mean body weight of 260 g at the start of the experiment. Housing, solutions, apparatus and general maintenance were the same as in Experiment 3. During the sucrose devaluation phase, animals received intraperitoneal (i. p.) injections of 0.15M LiCl at 20 ml/kg of body weight.

Procedure

Preconditioning (Days 1-4) and conditioning procedures were similar to those used in Experiment 3, with the exception that there was no serial conditioning group, and that two rather than four conditional trials were conducted. The reason for this was to reduce exposure to sucrose before the devaluation phase, thus making sucrose aversion easier. During the conditioning phase, the CS flavour was presented forming a simultaneous compound with sucrose over Days 5-6. In each one, rats had access for 5 min to 6 ml of almond mixed with sucrose. Additional 30-min period of water was supplied in this phase. After the conditioning phase, animals were both water and food deprived. The rats were given 3 days to accommodate to the food and water deprivation (Days 7-9), by restricting food access to 90 min per day to both commodities. After 3 days of accommodation to food and water deprivation, the conditioning test (Day 10) took place (a 30-min two-bottle test, 20 ml of flavour A against 20 ml of water).

The extinction occurred over the next 6 days (Days 11-16). Before this phase, animals were divided into two groups, Ext and NonExt, matched in preference ratio. During the extinction phase, group Ext was given 6 two-bottle almond vs. water choice tests for 30 min, while the other received 6 two-bottle exposures to water. Rats in each condition were further divided into two groups, Dev and NonDev, thus forming four groups (n = 8; see Table 2). The US devaluation took place during Days 17-18, in which either sucrose solution or water was presented in a single bottle. Animals in groups Dev were given access to 20 ml of sucrose solution for 15 min followed by a LiCl injection (i. p.), while on the second day they received 15 min access to water only. Animals in groups NonDev were also injected with LiCl on the first day, but they were given access to water instead of sucrose, and only on the second day had access to 20 ml of sucrose for 15 min. Both groups received 90 min of water and food access at the end of each session.

The sucrose test took place on Days 19-20, in which animals in groups Dev drank sucrose on the first day and water on the second. Animals in groups NonDev drank water on the first and sucrose on the second. Preference test after devaluation (post-devaluation test) took place on Day 21. Post-devaluation test ensued in the same manner as the pre-devaluation test. Note that the pre-devaluation test was the conditioning test for the NonExt groups, and the last extinction test for the Ext groups.

Results

The data from the acquisition, extinction, and devaluation phases proceeded mostly as expected.

Conditioning phase. The intake of almond and sucrose solutions during the two acquisition trials increased significantly from a mean of 3.37 g on first day to 5.33 g on the second day [*t*(31) = 68.74]. Regarding the conditioning test, the average preference ratio for almond was 0.83, very similar to the conditioning level obtained in previous experiments with four conditioning trials.

Extinction phase. Preference ratios for almond decreased during the extinction phase in group Ext. The statistical analysis revealed a decrement from a mean preference ratio of 0.76 on the first extinction test to 0.54 on the sixth [*t*(15) = 13.71]. This was due to a decrement in almond consumption from a mean of 4.75 g to 1.91g, *t*(15) = 14.58, but not to changes in water consumption, *t*(15) < 1.

Devaluation phase. Analysis of the consumption of both almond and water are not reported for this phase as preliminary analysis showed a triple extinction x devaluation x test interaction in water consumption, F(1, 28) = 6.47; therefore preference ratio gave a clearer information of the relative almond/water consumption. The results from sucrose devaluation test are shown in Figure 11 (top). The 2 extinction x 2 devaluation ANOVA revealed a main effect of devaluation [F(1, 28) = 128.47]. No other effects or interactions were significant.

The data of most interest are presented in Figure 11 (bottom), which shows almond preference ratios for the four groups in pre- and post-devaluation tests. It is clear that rats in group NonExt_Dev showed a lesser preference for the flavour after sucrose devaluation, whereas the other three groups showed similar preference levels for the flavour before and after devaluation. The statistical analysis confirmed these impressions. The 2 extinction x 2 devaluation x 2 test ANOVA revealed a main effect of extinction [F(1, 28) = 16.93], devaluation [F(1, 28) = 6.7], test [F(1, 28) = 7.36), and significant extinction x test [F(1, 28) = 16.13], and extinction x devaluation x test interactions [F(1, 28) = 6.09]. To explore the three-way interaction, separate 2 devaluation x 2 test ANOVAs were conducted for each extinction condition. The analysis revealed a significant devaluation x test interaction only in the NonExt condition [F(1, 14) = 13.3]. No effects were significant in the Extinction condition. The one-way ANOVA with day as factor showed a lower preference in group NonExt_Dev in the post-devaluation test with respect to the pre-devaluation test [F(1, 7) = 32.07]. Such a difference was not significant in group NonExt_NonDev.

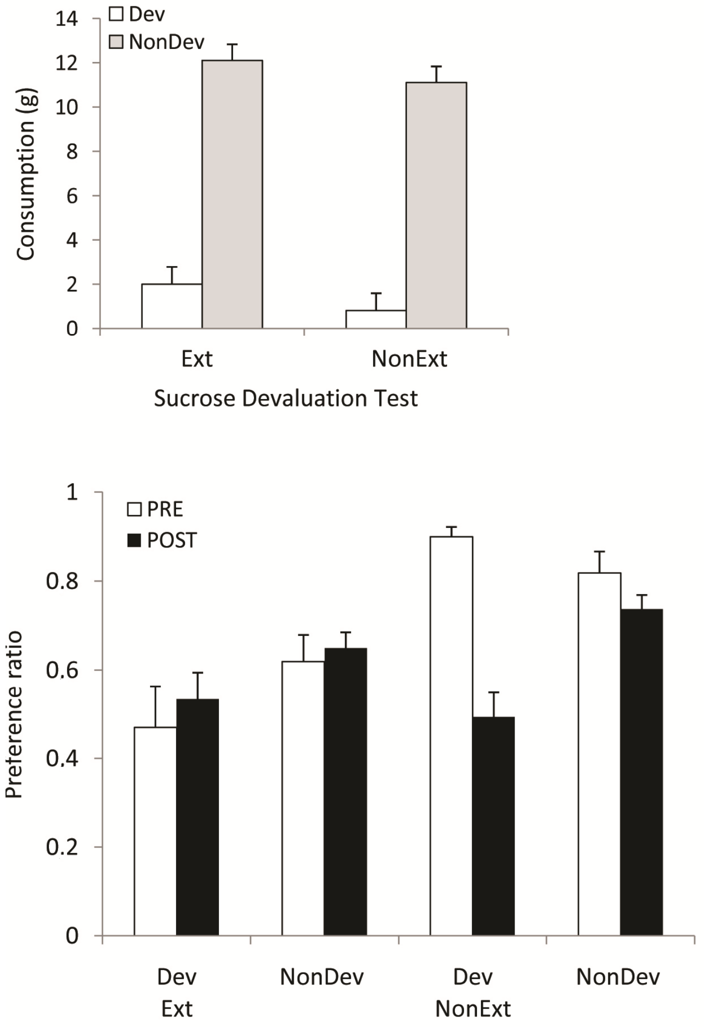

Figure 11. Experiment 5. The top graph shows mean intake (g) of sucrose for the extinguished and devaluated (Ext_Dev), extinguished and non-devaluated (Ext_NonDev), non extinguished and devaluated (NonExt_Dev), and non-extinguished and non-devaluated (NonExt_NonDev) conditions in Experiment 4. The bottom showed the preference ratio for the flavour cue in the same four conditions before (PRE) and after (POST) sucrose devaluation. Error bars represent SEMs.

Discussion

The data from Experiment 5 confirmed that only group NonExt_Dev showed a US-devaluation effect. No decrease in almond preference after sucrose devaluation was observed among rats trained thirsty that had received intervening exposures to almond while hungry prior to the establishment of a conditioned aversion to sucrose. These results agree with other studies in which no motivational shift has been introduced between conditioning and flavour exposure as those reported by Harris et al. (2004) with thirsty rats, and Delamater (2007), both with thirsty and hungry rats. However, the locus of the US-devaluation effects in hungry animals is unclear. On the one hand, if the preference among food-deprived rats is based exclusively on an association between the flavour and the calories (Harris et al., 2000), and taking into account that only the NonExt_Dev showed a US devaluation effect, then we must assume that the exposure to the almond alone after training in hungry rats had impact on flavour-calorie association (in agreement with Delamater, 2007, Exp. 3). On the other hand, it seems plausible that when an aversion is established to the nutrient, the aversion is established to the sensory properties of the nutrient and not to the more general post-ingestive reinforcing properties. This claim is congruent with the failure to find evidence that a flavour CS associated with an intragastrically presented nutrient is sensitive to nutrient devaluation achieved in this manner (see Delamater, Campese, Lolordo, & Sclafani, 2006). We must therefore assume that the post-training flavour exposure weakens the flavour-taste association, and consequently the preference in hungry rats might not be based exclusively on an association between the flavour and the calories. In any case, exposure to almond after training seems to weaken the association formed between the flavour and the sucrose, which is against the dominant view of extinction, as commented above.

General Discussion

Findings obtained in the current set of experiments suggest that the decrease in preference observed in hungry rats after flavour-exposure following simultaneous conditioning with sucrose may not be due to extinction. The aim of these experiments was to check whether well-known extinction related phenomena could be found in nutrient-based flavour preference in the same way they have been widely described using more standard Pavlovian conditioning paradigms. Decrease of the conditioned preference for a flavour simultaneously paired with sucrose during training was found in each experiment when it was subsequently exposed alone to hungry animals. In the experiments in which this simultaneous group was compared against a serial group (Exps. 1, 2, 3, and 4), the preference ratio for group Simultaneous in the conditioning test was always significantly higher than that of group Serial, which was only greater than the reference value (0.5) in Experiment 3. Thus, these results point out that conditioning was particularly strong in group Simultaneous, and that conditioned preference decreased after post-training CS exposure. A decrease in preference ratio was also found in group Serial in Experiments 2 and 3, even when no evidence of conditioned flavour preference was found (i.e., Exp. 2). It seems that using this procedure preference for the flavour was weaker, and it might be due to increased experience with it (i.e., mere exposure). Nevertheless, the state of hunger also produced a decrease in flavour preference and consumption. Although hunger might have produced an unspecific decrease in flavour intake due to the discomfort produced to animals, an effect of weak conditioning in group Serial cannot be totally ruled out.

The results of more interest were those involving the procedures known to restore the extinguished conditioned response. First, no evidence of spontaneous recovery was found when the flavour preference was tested 2 weeks after the flavour-exposure phase (Exp. 2). Second, no evidence of reinstatement was found either when the US was re-exposed after the flavour-exposure phase in the absence of presentation of almond (Exp. 3). Third, there was an effect in the opposite direction to contextual renewal using motivational states as contexts. We found that if post-training flavour exposure was conducted in one motivational context (thirst and hunger), the preference was indeed lower in the Simultaneous group when tested in the motivational context of thirst (Exp. 4). Finally, only the non-extinguished and devalued group showed a sucrose devaluation effect, whilst the extinguished and devalued group failed to show it (Exp. 5). These results encourage the view that the decrease in preference after post-training flavour exposure might not be due to extinction and, at the same time, raise the question about the underlying learning mechanism that produced the decrease in preference observed when hungry animals were repeatedly exposed to the flavour alone after simultaneous conditioning. Interestingly, Higgins and Rescorla (2004) found that, although exposure to the flavour after simultaneous pairing of almond and 20% Polycose solution produced a decrease in the flavour consumption, there was relatively little indication that retraining produced reconditioning.

Since sucrose has multiple features —sensory, hedonic, and nutritive— the flavour may borrow properties of the sucrose after simultaneous almond-sucrose pairings. Specifically, while drinking the flavour-sucrose compound, thirsty rats may learn three distinct associations: almond-taste, almond-calorie, and probably an association between almond and the hedonic reactions resulting from the ingestion of the compound (see Harris et al., 2004). Consider the phase in which the flavour is repeatedly presented outside the flavour-sucrose compound —for the first time— after conditioning, the rats now being hungry. According to Harris et al. (2000) hungry rats base their preference for almond on its association with the calories provided by sucrose, but the nutrient calories, as well as the sweetness, are absent. That the almond-sweetness association is impaired after this treatment is revealed by the absence of a US devaluation effect in Experiment 5. It is reasonable to think, as well, that the almond-nutrient association is also impaired (see Delamater 2007). This probably applies to almond-hedonic reactions as well (but see Dwyer et al., 2009).

It may be the case that the exposure of the flavour whilst animals are hungry after the simultaneous pairing with sucrose endows the flavour with inhibitory properties. If we consider that during training there is a sensory compound (almond + sweetness) followed by both nutrient properties and hedonic reactions, presenting the almond alone during testing under hunger could activate the representations of both US properties which would be absent at that moment. This procedure could be envisioned more as AT+, A- (being A the flavour, T the taste of sucrose, and + the affective and motivational properties of sucrose) than A+, A- which is the usual extinction procedure. Under these training conditions, it is likely that the flavour presented alone acts to signal the absence of the expected relevant US, which among hungry rats are calories (Harris et al., 2000). Alternatively, it is also possible to consider the effect of presenting the flavour alone after simultaneous conditioning without appealing to within-compound associations. Exposing the flavour and the taste of sucrose forming a compound during training, the rat may not separately identify flavour and taste of sucrose as elements, and may form a representation of a unitary AT compound (see Figure 12). That which appears to be extinction will be the formation of the representation of a component. As repeated presentations of the flavour alone proceeds, a representation of the element is formed producing a discrimination between the flavour and the compound (see Higgins & Rescorla, 2004), with the former signaling the absence of the nutrient. Assuming that there should be some generalization of the excitatory strength from the compound to the flavour in the first stages of the flavour exposure, net inhibitory learning may occur.

If these assumptions are correct the flavour should pass the summation and retardation tests for conditioned inhibition. This was the purpose of the experiments presented in Chapter 6.

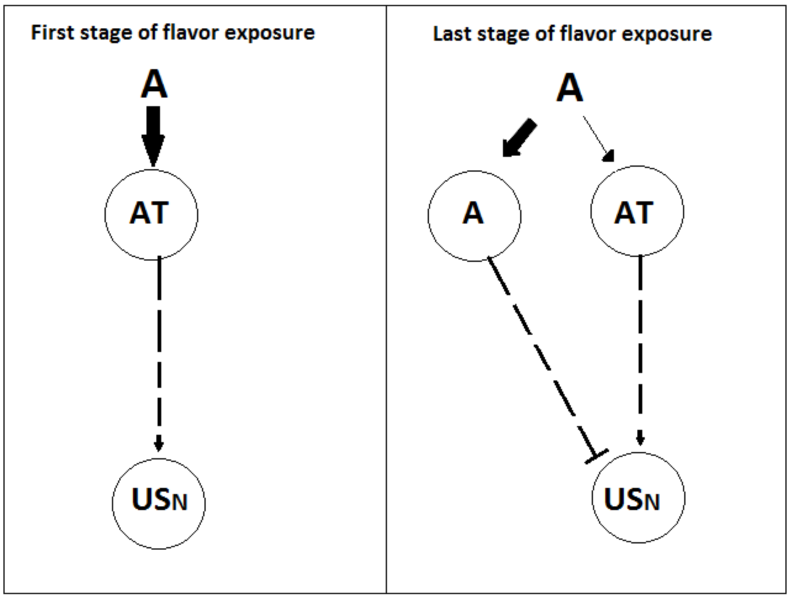

Figure 12. Proposal of associative structure partially based on Higgins & Rescorla (2004). In left panel, the node AT represents the flavour A and the Taste of sucrose forming a representation of a unitary AT compound with an excitatory association with nutrient (USN). As repeated presentations of the flavour alone proceeds, a representation of the element is formed producing a discrimination between the flavour and the compound (right panel). Which appears to be extinction will be the formation of the representation of a component (see Higgins & Rescorla, 2004, pp. 217-218). During that last stage, assuming that there should be some generalization of the excitatory strength from the compound to the flavour in the first stages of the flavour exposure, we suggest that the flavour A representation may acquire inhibitory properties, as it signals the absence of the nutrient. Note. - -┤ acquired inhibitory connection; - → acquired excitatory connection.

Chapter 6

CONDITIONED INHIBITION IN CONDITIONED FLAVOUR PREFERENCE BASED ON NUTRIENT

Experiment 6 from manuscript published as:

Garcia-Burgos, D. & González, F. (in press). Evidence of predictive learning in nutrient-based flavor conditioning in rats using the summation test for conditioned inhibition. *Psicothema*.

Experiments 7 and 8 from manuscript to be submitted as:

Garcia-Burgos, D. & González, F. (2011). *CS exposure after simultaneous training with a nutrient converts the CS into a conditioned inhibitor: Evidence from retardation and summation tests.* Manuscript to be submitted for publication.

6.1. INTRODUCTION

The present set of experiments was designed to examine the putative net inhibitory properties acquired by the CS flavour. According to several theoretical accounts, conditioned inhibition occurs whenever a cue signals the absence of an otherwise expected US (e.g., Rescorla & Wagner, 1972). Assuming that CFP based on nutrient is due to predictive learning, and that conditioned inhibition involves the activation of the US expectancy during training, there are reasons for expecting conditioned inhibition in flavour-nutrient learning after post-training CS exposure.

It is generally accepted that an inhibitor should pass both the summation and retardation tests for conditioned inhibition (Rescorla, 1969), thus the goal of the following experiments was to provide empirical evidence of inhibitory learning using retardation (Exp. 7) and summation (Exp. 8) tests for conditioned inhibition. In each experiment, rats were trained thirsty and subsequently exposed to the flavour while hungry and thirsty, as in previous experiments. These conditions should produce a decrease in conditioned preference according to Harris et al. (2004; Exp. 2B). However, given the fact that no evidence of conditioned inhibition was reported at the time that these objectives were proposed, the possibility of getting conditioned inhibition in CFP based on nutrient was previously assessed through conditioned Pavlovian inhibition procedure (Exp. 6).

Since the examination of the inhibitory properties of a CS exposed after training (Exps. 7 and 8) occurs in the context of what has been considered extinction, it should be noted that this dissertation does not propose that the post-training flavour-exposure in CFP converts an “extinguished” flavour CS into a net inhibitor. As we assume that what appears to be extinction of flavour preference may be the formation of the representation of the flavour by means of perceptual differentiation and the formation of an inhibitory association of this element with the US, our hypothesis about the acquisition of net inhibitory properties by the flavour enters in no contradiction with the current accounts of extinction in Pavlovian conditioning.

These consider that extinction may involve some form of inhibitory learning process (e.g., Bouton, 1993; Delamater, 1996; Konorsky, 1948), but consider this process self-limiting (at least according to error-correction models) and so should not lead to a net inhibitory, by which is meant a stimulus having properties antagonistic to those possessed by an excitory CS (Aguado, de Brugada, & Hall, 2001).

Experiment 6: Evidence of conditioned inhibition

This Experiment made use of the Pavlovian conditioned inhibition procedure in which stimulus A is always reinforced when presented alone, but unreinforced when presented forming a compound with stimulus B. Under these conditions, B becomes a conditioned inhibitor (CS-) signalling the absence of an otherwise expected US (i.e., the US expectation activated by stimulus A). An independent second excitor (C+) was also trained for the summation test. The rationale underlying Experiment 6 is as follows. If during training flavour A activates an expectancy of the US occurrence, flavour B should become a conditioned inhibitor as the US does not occur in its presence; therefore consumption of flavour C should decrease when presented forming a simultaneous compound with stimulus B in the summation test (i.e., C vs. BC). Since flavour preference among food-deprived rats is based exclusively on flavour-nutrient association (Harris et al., 2000), rats were food deprived on test. Performance on the summation test was compared with that of a control group in which no activation of the US occurrence by flavour A was expected (i.e., consequently flavour B should not became an inhibitor).

Two groups of thirsty rats, Simultaneous and Blocked, were trained during several daily sessions. In both groups, flavours A and B were presented unreinforced simultaneously (AB-) during six sessions. There were two critical differences between the groups: the training procedure for flavour A (simultaneous in the case of groups Simultaneous and serial in the case of group Blocked); and the location of the AB- trials (presented at the beginning of training in groups Blocked instead of intermixed throughout training as was the case for group Simultaneous). This latter manipulation was intended to minimize any possible negative contingency-relationship between flavour B and sucrose in the control group. Given that the serial A→+ presentations produces null (or very poor) conditioning using this training procedure, the manipulation cannot be considered to produce sensory preconditioning learning to flavour B. A third flavour (C+) was trained as an independent excitor for the summation test in both groups. Taking into account the results from serial group in earlier experiments, activation of the US expectancy by flavour A is not expected during training in group Blocked and, therefore, flavour B should not acquire inhibitory properties. Consequently, flavour B should decrease consumption of flavour C in the summation test only in group Simultaneous. Table 3 summarizes the experimental design of this Experiment.

Method

Subjects and apparatus

The subjects were 16 female Wistar rats at least 110 days old at the start of the experiment. They had previously participated in a conditioned flavour preference experiment with 1% (vol/vol) almond and 20% (wt/vol) sucrose, but were orthogonally assigned to both groups in order to equate experience with those stimuli. Animals were housed and maintained in a similar way as in Experiment 1. For flavour A, the US was 6 ml of a 20% (wt/vol) sucrose solution, whereas for flavour C the US was 10 ml of a 10% (wt/vol) sucrose solution. Flavour A was a 1% (vol/vol) mint solution. Flavours B and C were 1% (vol/vol) solutions flavoured with either banana or vanilla, counterbalanced (mint, banana, and vanilla flavourings supplied by SuperCook, Leeds, UK). Fresh solutions were made every day using room-temperature tap water. Fluid and food deprivation are detailed below.

Procedure

Animals were water deprived by giving a daily 30-min period of free access to tap water for four days before the start of the experiment. Afterwards they were divided into two halves equated in body weight (n = 8). Training sessions took place daily in the home cages during the light cycle at approximately 09:30 am. The two testing sessions were scheduled at approximately 12:00 noon (see below). Group Simultaneous received conditioning to flavour C on days 1-3 and 14, consisting of 10-min access to 10 ml of a simultaneous compound of flavour C and sucrose solution. On days 5, 7, 9, and 11 they received 6 ml of simultaneous compound of flavour A and sucrose for 5 min. On days 4, 6, 8, 10, 12, and 13 animals had 10-min access to 10 ml of simultaneous unreinforced compound of flavours A and B. The training schedule for group Blocked was similar, with two important exceptions: a) on days 10-13, 6 ml of flavour A were presented for 5 min immediately followed by 5-min access to 6 ml of sucrose (serial A→+ presentations); and b) on days 1-6 they received the six unreinforced AB presentations (blocked trials at the beginning of training). Flavour C was trained in a similar way to that of group Simultaneous on days 7-9, and 14. After training, both groups were treated identically. During days 15-17, animals were both water and food deprived by limiting access to both commodities to 90 min. On the afternoon of day 17, water bottles were returned to the home cages and removed on day 18 at 09:00 am, 3 h before testing began. As one-bottle tests were used, this manipulation was aimed to maintain animals hungry but not very thirsty during testing and thus increase the sensitivity of the measure; rats should drink the solution as long as it was a cue for a nutrient and not because it was a fluid. Summation testing took place on days 18-19. The order of presentation for the two tests, C or CB, was counterbalanced across the two days in each group. The first summation test took place on day 18 at 12:00 noon. At the end of the session, the animals were given free access to water and 90-min access to food. On day 19, water bottles were removed at 09:00 am, and the second session of the summation test took place at 12:00 noon. Each test session lasted 10 min.

Table 3. Design of Experiment 6.

Groups	**Training**	**Summation test**
	Thirsty	*Mildly Thirsty & Hungry*
Simultaneous	3 C+, AB-, A+, AB-, A+, AB-, A+, AB-, A+, AB-, AB-, C+	C; CB
Blocked	6 AB-, 3 C+, 4 A → +, C +	

Note. A, B and C = flavours; + = US sucrose; - = non reinforcement; Simultaneous = group given flavour A-sucrose compound during training; Blocked = control condition given the six unreinforced AB at the beginning of training (blocked trials), and the sucrose solution after the consumption of flavour A (serial presentation).

Results

Training phase. Consumption of the different solutions proceeded smoothly and similarly in both groups. The average intakes (for group Simultaneous and Blocked, respectively) were 9.33 and 9.32 g for the C+ compound, and 7.94 and 8.05 g for the AB- unreinforced compound. The mean consumption of the A+ compound in group Simultaneous was 5.21 g, whereas consumption of Flavour A and sucrose solution, respectively, were 4.77 and 5.17 g for group Blocked.

Summation test. Consumptions of both C and CB flavoured solutions were transformed into acceptance scores (Biederman & Davey, 1997) in the form of intake suppression ratios according to $a/(a+b)$, where a and b are, respectively, the amounts of the CB compound flavour and flavour C consumed in the test. Acceptance score under 0.5 shows that animals are drinking less of the compound than of the excitor (i.e., summation effect). Using acceptance scores rather than flavour consumption scores did not change the pattern of results but did increase statistical sensitivity by factoring out individual differences in amount of fluid intake. The average acceptance scores and the absolute consumptions of flavour C and CB after 10 min of testing appear in Figure 13 (main figure and inset, respectively).

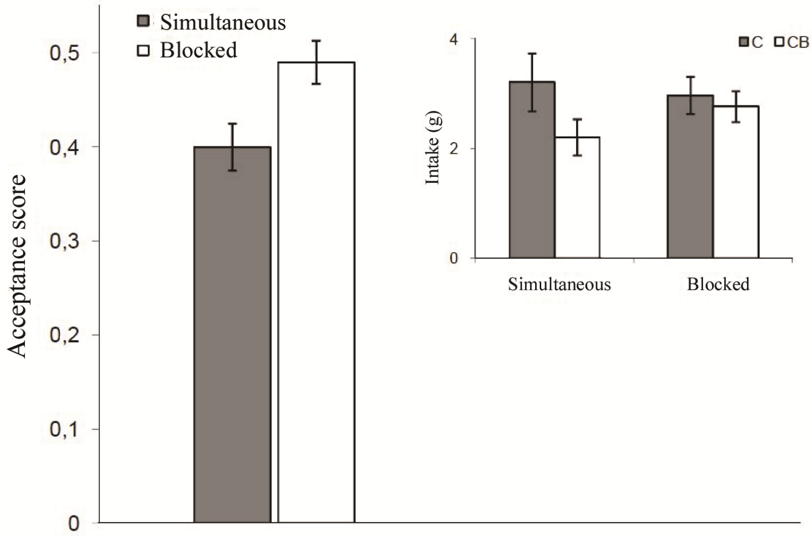

Figure 13. Experiment 6. Mean acceptance score on 10-min summation test for groups Simultaneous and Blocked in Experiment 2. The inset shows the average consumption of flavour C and CB flavour compound for both groups in the test. Error bar represents SEM.

There was a significant difference between groups in average acceptance score, *t*(14) = 2.87 (means 0.40 and 0.49, for group Simultaneous and Blocked, respectively). Regarding the ANOVA on the average absolute consumptions, there was a main effect of solution, F(1, 14) = 8.49, and the group x solution interaction was close to the significance level, F(1, 14) = 3.50, p = 0.07. The main effect of group was not significant, F < 1. Comparisons between C and CB consumptions for each group showed that the difference was reliable for group Simultaneous, *t*(7) = 3.57, but not for group Blocked, *t*(7) = 0.51.

Taken together, these results suggest that flavour B acted as a conditioned inhibitor for the nutrient US in group Simultaneous. The acceptance score was significantly lower than that of group Blocked, which was virtually equal to 0.5, revealing that the consumption of the CB compound was lower than the consumption of the excitor C in the experimental group. The analysis of the total consumption was somewhat less sensitive, showing a marginally significant Group x Summation interaction, which once explored, revealed that only in group Simultaneous was consumption of the CB compound lower than that of flavour C alone. Therefore, it seems safe to conclude that flavour B passed the summation test for inhibition in the experimental group.

Discussion

The main goal of this Experiment was to obtain evidence of conditioned inhibition in CFP based on nutrient previously assessed through conditioned Pavlovian inhibition procedure. To achieve this goal, we make use of the summation test for conditioned inhibition; if the nutrient expectancy was activated during training, the absence of the nutrient in presence of a flavour should render that flavour a conditioned inhibitor.

Experiment 6 revealed that adding flavour B to the excitor flavour C produced a reduction in the consumption when comparing with the consumption of the excitor alone (i.e., summation effect). However this effect was only found in group Simultaneous. Using serial flavour A-sucrose pairings and arranging the unreinforced AB trials at the beginning of training in group Blocked, precluded the possibility of the acquisition of inhibitory properties by flavour B. The absence of the summation effect in this group discards an explanation of the decrease in compound consumption in group Simultaneous in terms of generalization decrement. Taken together, these results point out that animals in group Simultaneous did in fact learn about the absence of an otherwise expected US during training (i.e., predictive learning).

Experiment 7: Retardation test

In this Experiment we used an experimental group where rats were exposed to a simultaneous compound of a target flavour and sucrose during conditioning phase (Simultaneous) and a control group (Serial) where rats were given the same amount of the target flavour followed by sucrose (similar to Experiments 1, 2, 3, and 4). The post-training flavour-exposure phase was identical for both groups, consisting of four sessions in which the flavour was presented alone in a single bottle. Given that acquisition rate depends on the degree of exposure to the stimulus and that in CFP the degree of exposure is determined by the consumption of the fluid, the control animals may show faster reacquisition simply because they had received less exposure to flavour before the retardation test. In order to guarantee that the Simultaneous and Serial groups were similarly exposed to the target flavour after this second phase, and taking into account that experimental animals drank more of the CS flavour during the four days of the exposure phase, animals in group Serial received an additional exposure day to the flavour, whereas group Simultaneous received water. In the retardation test, and according to the study by Higgins and Rescorla (2004), we expected retardation in reacquisition in group Simultaneous compared with the acquisition rate of group Serial. Table 4 (Exp. 7) summarizes the experimental design of this experiment.

Method

Subjects and apparatus

The subjects were 16 experimentally naïve female Wistar rats with a mean body weight of 255 g at the start of the experiment. Animals were housed in individual home cages and kept in a large colony room maintained on a fixed 12-hr light-dark cycle. The experiment took place in the home cages and was conducted during the light cycle in the morning. Rats were water deprived throughout the experiment, with the exceptions mentioned below. Fluids were administered at room temperature in a 50-ml plastic tube with a rubber stopper fitted with a stainless steel ball-bearing tipped spout, and fresh solutions were prepared every day. Consumption was estimated by weighing the tubes before and after fluid presentation to the nearest 0.1 g. The solutions used were made up with tap water consisting of 1% (vol/vol) almond (SuperCook, Leeds, UK), 20% (wt/vol) sucrose, and 1% almond and 20% sucrose compound.

Procedure

Before the start of the experiment, the animals were assigned to two weight-matched groups (n = 8). The water bottles were removed from the home cage 24 hr before the start of the experiment. Rats were then adapted to a water deprivation schedule for 4 days (Days 1-4), during which animals were allowed free access to water for 30 min on each morning in the tubes subsequently used to present the flavoured solutions.

During the conditioning phase (Days 5-6, 8-9), rats in group Simultaneous were given 6 ml of the almond-sucrose compound for 5 min (6 ml were provided to guarantee a 5 ml consumption to compensate for possible fluid spillage), whereas rats in the group Serial were given a 6 ml of almond presented for 5 min followed by 5-min access to 6 ml of sucrose. After each conditioning session, all animals had access to supplementary water for 30 min. After the second conditioning trial and before the third, rats were given a two-bottle almond vs. water tests for 30 min, each bottle containing 20 ml of the correspondent fluid. This preference test was introduced with the hope that two conditioning trials were enough to produce conditioned flavour preference in group Simultaneous yet not so many as to produce latent inhibition in group Serial. However, a total of four conditioning trials were finally necessary.

After the conditioning phase, the rats were given 3 days (Days 10-12) to accommodate them to the motivational conditions used in the conditioning test of food and water deprivation. All groups received water in two tubes for 30 min followed by access to both food and water for 90 min in each of these three days. The procedure for the conditioning test (Day 13) involved a two-bottle almond vs. water choice test for 30 min, each bottle containing 20 ml of the corresponding fluid, followed by limited access to food for 90 min. In all two-bottle tests given in this and the following experiment, the position of bottles was counterbalanced within each group.

During the post-training flavour exposure phase (Days 14-17), water bottles were replaced overnight and removed at 8:30 am, exposing animals to 20 ml of CS flavour at 10:00 am during 30 min using a single tube. This manipulation was intended to have rats hungry but not very thirsty thus increasing the sensitivity of the measure; animals should drink as far as they associate the solution with calories and not just because they were very thirsty. Note that water was not concurrently available during the CS exposure sessions. The post-training flavour exposure test (Day 18) was conducted under the same conditions of the conditioning test; thirsty and hungry rats were given one two-bottle almond vs. water test for 30 min, each bottle containing 20 ml of the corresponding fluid, followed by limited access to water for 90 min and free to food. In order to guarantee that both groups were similarly exposed to the target flavour before this test, group Serial received one additional exposure day (Day 19) to 20 ml of flavour for 30 min, whereas the former received water.

Table 4. Designs of Experiment 7 (Retardation test) and Experiment 8 (Summation test).

Groups	**Conditioning** *Thirsty*	**Conditioning Test** *Thirsty & Hungry*	**Post-training A-Exposure** *Mildly Thirsty & Hungry*	**Test A** *Thirsty & Hungry*		
Exp. 7					**Retardation of Acquisition (3 cycles)**	
					Reacquisition *Thirsty*	Test *Thirsty & Hungry*
Simultaneous	4 A+	A vs. W	4 A-	A vs. W	2 A+	A vs. W
Serial	4 A → +					
Exp. 8					**Summation test** *Thirsty & Hungry*	
Simultaneous	4 A+, 4 B+	A vs. W B vs. W	4 A-	A vs. W	B vs. W AB vs. W	
Serial	4 A → +, 4 B+					

Note. A and B = CS flavours; + = US sucrose; - = non reinforcement; W = water; Simultaneous = group given flavour A-sucrose compound; Serial = control condition in which the sucrose solution was presented after the consumption of flavour A.

The retardation test phase occurred over the next 9 days (Days 20-28). It was run in three 3-day cycles. In each cycle, rats received 2 simultaneous almond-sucrose pairings on the first two days, and one almond vs. water two-bottle test on the third. To equate the motivational conditions of the reacquisition phase to those of the conditioning phase, food was returned before the reacquisition training sessions and removed after the second day of reacquisition. Thus rats were thirsty during the acquisition trials and hungry and thirsty during testing.

Results

Conditioning phase. The mean daily intakes of almond-sucrose compound consumed across the conditioning days were 1.52, 5.18, 5.32, and 5.58 g for group Simultaneous. A similar pattern was found in group Serial in the consumption of the almond solution (means: 4.81, 4.65, 5.08, and 5.01 g), and of the sucrose solution (means: 2.52, 4.27, 4.7, and 5.15 g). Repeated-measures ANOVAs confirmed the effect of trial on almond-sucrose consumption [F(3, 21) = 100.82] in group Simultaneous, and on sucrose consumption [F(3, 21) = 12.99] in group Serial, showing that rats consumed less of the solution on the first trial showing neophobia to the dense sucrose solutions. There were no significant differences among trials in the consumption of almond alone in group Serial (F < 1). Regarding differences between groups in the conditioning test, the average preference ratio of group Simultaneous (0.71) was significantly higher than that of group Serial (0.46) [*t*(14) = 12.24]. The average preference ratio for flavour A conditioning test, post-training flavour exposure test, and retardation of acquisition tests are shown in Figure 14.

Post-training flavour exposure phase. The mean daily intakes of almond consumed across the four exposure days were 6.26, 3.8, 4.57, and 3.25 g for group Simultaneous, and 2.65, 2.6, 3.05, and 2.38 g for group Serial. The 2 group x 4 day ANOVA revealed a significant group x day interaction [F(3, 42) = 8.47]. The statistical analysis revealed that group Simultaneous exhibited significantly lower consumption of almond on days 2, 3, 4, and 5 than on day 1, and that group Serial did not exhibit significant differences across days. Finally, group Simultaneous exhibited higher almond consumption than group Serial on day 1. Regarding the post-training flavour-exposure test, no significant differences were found between the preference ratio of group Simultaneous (0.48) and group Serial (0.44) [*t*(14) < 1]. The mean intake of almond consumed was 17.89 g for group Simultaneous, and 20.7 g for the group Serial (adding the additional exposure day in order to guarantee that both groups were similarly exposed to the target flavour). There were no differences on almond intake between groups before retardation test [*t*(14) < 1].

Retardation test phase. The results of the almond preference ratio test during this phase included three reacquisition trials. The 2 group x 3 trial ANOVA of the preference ratios showed a main effect of group [F(1, 14) = 4.7], that revealed a higher preference in group Serial. It is important to note that the difference between groups emerged after the third reacquisition test. No other main effects or interactions were significant (Fs< 1).

Discussion

Group Simultaneous acquired a conditioned preference for almond after training whereas group Serial did not, acting effectively as a control. During the CS exposure after training, the consumption of the flavour presented alone decreased in the first group but not in the second. After this phase, the previous difference in preference ratio between groups observed after conditioning disappeared. Taken together, these data suggest that the conditioned flavour preference in group Simultaneous decreased as a result of the flavour exposure after training. However, the results of more interest are those of the retardation test.

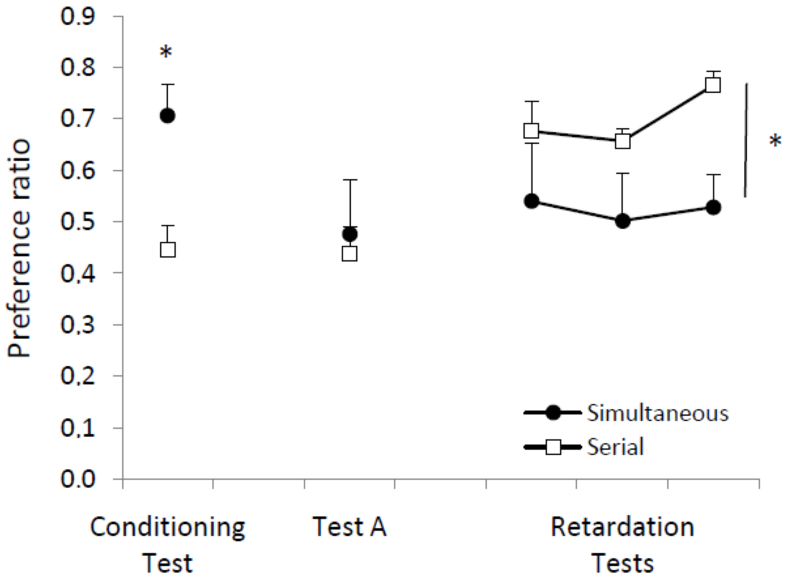

Figure 14. Experiment 7. Average preference ratio of flavour A on conditioning test, post-training flavour exposure test, and retardation tests for groups Simultaneous and Serial. Error bars represent SEMs. * indicates a significant between-group difference ($p < 0.05$).

The between-group comparison confirmed that reacquisition of preference after post-training flavour exposure was retarded in group Simultaneous. It is important to note that both groups had a similar level of flavour exposition before this test, thus it is difficult to attribute the difference in acquisition rate to differential latent inhibition. Therefore, these results are consistent with the suggestion that the flavour preference decrement in the present experiment is a result of inhibitory learning. The results also agree with the failure in retraining in the simultaneous group found by Higgins and Rescorla (2004).

Experiment 8: Summation test

Experiment 8 attempted to check the potential of a conditioned flavour which has been given post-simultaneous conditioning flavour exposure to pass a summation test, compared with a serial group. Table 4 (Exp. 8) provides a description of the present experimental design. During the conditioning phase, thirsty animals either received initial simultaneous or serial training of a flavour (A) and sucrose, and simultaneous conditioning of a second flavour (B), which served as the independent excitor for the summation test. Animals were subsequently food deprived and given non-reinforced exposures to flavour A in a similar way as in the previous experiment. During the summation test, animals were given a choice between the excitor B presented in compound with flavour A in a two-bottle compound vs. water test. All animals also received a two-bottle flavour B vs. water choice to assess the preference governed by the test excitor. The order of these tests, which were conducted on different days, was counterbalanced. Thus, the present study was designed to examine the summation effect by within-subject as well as between-group comparisons. For this reason the size of groups was increased (from 8 to 16 animals). On the basis of the results of Experiment 7, we expected to find a lesser preference for the AB compound than that for the excitor B presented alone in group Simultaneous, but not in group Serial. Additionally, no difference between groups was expected in the preference exhibited for flavour B, whereas a lesser preference for the AB compound in group Simultaneous than in group Serial was expected.

Method

Subjects and apparatus

The subjects were 32 experimentally naïve female Wistar rats with a mean body weight of 251 g at the start of the experiment. Animals were housed and maintained in a similar way as in Experiment 6. The solutions were those of Experiment 6 with one exception. In addition to flavour A and sucrose (20% [w/v]), a second flavour (B) was used; flavour A and B were almond or vanilla in tap water at 1% (vol/vol) concentration, counterbalanced (almond, and vanilla flavourings supplied by SuperCook, Leeds, UK). Fresh solutions were prepared every day using room temperature tap water. Animals were fluid and food deprived as detailed below.

Procedure

Before the start of the experiment, the animals were assigned to two weight-matched groups (n = 16). The rats were adapted to a water deprivation schedule for 4 days

(Days 1-4), removing the water bottles from the home cage 24 hr before the start of the experiment. During these four days, the animals were allowed free access to water in the drinking tubes for 30 min each morning in the tubes subsequently used to present the flavoured solutions.

During the conditioning phase (Days 5-12), group Simultaneous received conditioning to flavour B on Days 5, 6, 9, and 12, consisting of 5-min access to 6 ml of a simultaneous compound of flavour B and sucrose solution. On Days 7, 8, 10, 11 they received 6 ml of simultaneous compound of flavour A and sucrose solution for 5 min. The conditioning schedule for group Serial was similar, except that the 5-min access to 6 ml of flavour A were followed by 5-min access to 6 ml of sucrose (serial A→+ presentations). After each conditioning session, all animals had access to supplementary water for 30 min.

After this conditioning phase, the rats were given 3 days (Days 13-15) to accommodate them to the test conditions used in the conditioning test of the food and water deprivation as in Experiment 7. The procedure for the conditioning test for flavours A and B (Days 16 and 17) was one two-bottle flavour vs. water test for 30 min, each bottle containing 20 ml of the correspondent fluid during two days, order counterbalanced.

Post-training flavour exposure phase. Post-training flavour exposure phase (Days 18-21) and post-training flavour exposure test (Day 22) ensued in the same manner as Experiment 7.

Summation test. The summation test occurred over the next 2 days (Days 23 and 24) under food and water deprivation. As mentioned above, the order of flavour presentation during summation tests across two days, B or AB, was counterbalanced.

Results

Conditioning phase. The mean daily intakes of flavour B-sucrose compound consumed across the conditioning days were 3.73, 5.36, 5.6, and 5.56 g for group Simultaneous, and 3.76, 5.32, 5.46, and 5.73 for the group Serial. The 2 group x 4 trials ANOVA of the flavour B-sucrose intake revealed a significant main effect of trial [F(3, 90) = 52.6]. The statistical analysis revealed a lower consumption on the first day. The mean daily intakes of flavour A-sucrose compound consumed across the conditioning days were 5.12, 5.46, 5.55, and 5.45 g for group Simultaneous. A repeated-measures ANOVA confirmed an effect of trial [F(3, 45) = 3.51], showing a lower consumption on the first day compared to the third. A similar pattern was found for the group Serial in the consumption of the flavour A solution [F(3, 45) = 7.96], means: 4.39, 5.13, 5.05, and 5.14 g., and of the sucrose [F(3, 45) = 4.59], means: 4.63, 5.27, 5.28, and 5.31 g.

Regarding differences between groups in the conditioning of flavour A, the average preference ratio of group Simultaneous (0.73) was significantly higher than that of group Serial (0.43) [*t*(30) = 49.71]; regarding comparison between groups in the conditioning of flavour B, no differences were found between the average preference ratio of group Simultaneous (0.77) and that of group Serial (0.72) [*t*(30) = 2.69]. The average preference ratios for flavour A and B conditioning test, post-training flavour A exposure test, and summation test are shown in Figure 15.

Post-training flavour-exposure phase. The mean daily intakes of almond consumed across the four exposure days were 6.3, 5.12, 3.19, and 2.4 g for group Simultaneous; and 4.67, 3.31, 3.36, and 2.32 g for the group Serial. The 2 group x 4 day ANOVA of the flavour A intake revealed a significant group x day interaction [F(3, 90) = 5.34]. The statistical analysis revealed that group Simultaneous exhibited significantly lower consumption of flavour A on days 3, and 4 than on day 1; and that group Serial exhibited significantly lower consumption on days 2, 3, and 4 than on day 1. Finally, the group Simultaneous exhibited higher flavour A consumption than Serial on days 1 and 2. Regarding the post-training flavour A exposure test, no significant differences were found between the preference ratio of group Simultaneous (0.48) and group Serial (0.48) [*t*(30) < 1].

Summation test. The 2 group x 2 trial ANOVA of the preference ratios showed a significant group x trial interaction [F(1, 30) = 7.28], which once explored revealed a lower preference for the AB compound in group Simultaneous than in group Serial [*t*(30) = 17.58]. No differences between groups were found in preference for flavour B. On the other hand, only group Simultaneous showed a significant decrement in consumption of the AB compound compared with that of flavour B alone [*t*(15) = 11.45].

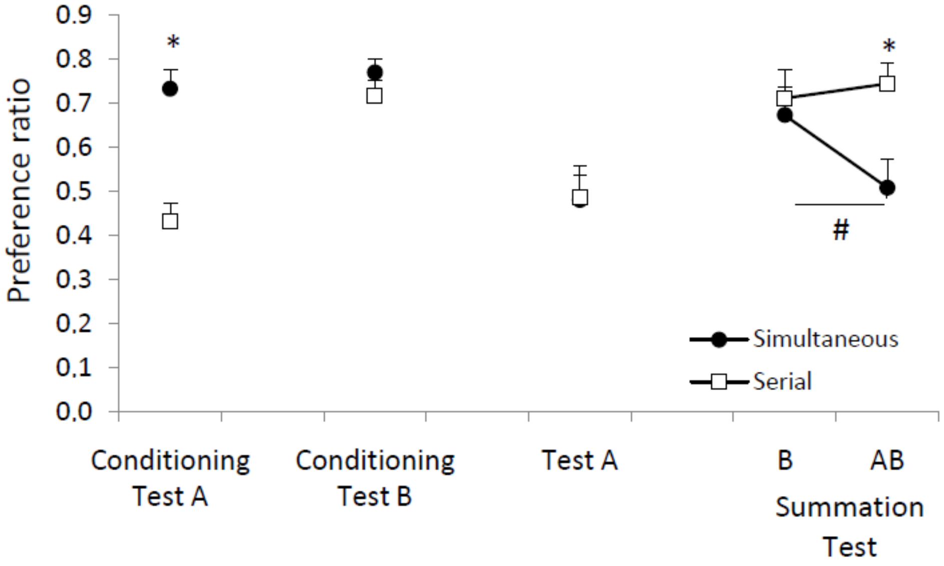

Figure 15. Experiment 8. Average preference ratio of flavour A, flavour B, and AB compound) on conditioning (Cond.) test for A, conditioning (Cond.) test for B, post-training flavour A exposure test, and summation test for groups Simultaneous and Serial. Error bars represent SEMs. * indicates a significant between-group difference, and # a significant within-subject difference in the summation test ($p < 0.05$).

Discussion

Group Simultaneous acquired a conditioned preference for flavour A whereas group Serial did not; both, however, showed a similar conditioned preference for flavour B. During the exposure to flavour A after training, both groups decreased the consumption of the flavour presented alone. After this phase, the previously observed difference in preference ratio for flavour A between groups disappeared. Taken together, these data suggest that the conditioned flavour preference in group Simultaneous decreased as a result of the flavour exposure after training. Regarding the summation test, there was no decrease in preference for the flavour B when presented forming a compound with flavour A in group Serial, an effect that was detected in group Simultaneous. In addition, group Simultaneous showed a lesser preference for the compound than group Serial. The absence of an effect in group Serial indicated that the decrease in preference for the compound in group Simultaneous was not due to a generalization decrement. In sum, flavour A passed the summation test of conditioned inhibition in group Simultaneous, but not in group Serial.

General Discussion

The present set of Experiments reported in Chapter 6 showed evidence of that conditioned inhibition in nutrient-based flavour learning (Exp. 6). It also confirms that a flavour paired simultaneously with a nutrient during conditioning in thirsty rats acquired inhibitory properties after being subsequently presented alone repeatedly with animals being food deprived, showing the properties of an inhibitor. Evidence consisting of both retardation of reacquisition of the conditioned preference after flavour exposure (Exp. 7), and reduction in the preference for an excitor flavour when it was presented forming a compound with the target flavour (Exp. 8). Retardation and summation tests are routinely regarded as the empirical proofs for conditioned inhibition (Rescorla, 1969). These results suggest that the decrease in preference observed in hungry animals when they are exposed to the CS after training may be due to the acquisition by the flavour of the proprieties of a net inhibitor.

Conditioned inhibition in nutrient-based flavour learning has recently been reported by Boakes et al. (2010). They found that hungry rats learned to avoid a flavour that signalled the absence of otherwise expected nutrient using both an unpaired condition (see also Harris et al., 2000) and a differential conditioning procedure, an effect they have termed the “missing calorie effect”. Boakes et al. compared preference measured through two-bottle choice tests in a group given training consisting of intermixed sessions of the target flavour in 2% maltodextrin (M) and of unflavoured 20% M –Unpaired- with a group given a block of sessions in which the flavour was mixed with 2% M and then a block of sessions in which they were given only unflavoured 20% M –Blocked. Then, for both groups, the training stage was followed by excitatory training in which the target flavour was added to 20% M, as a retardation test. The experiment confirmed unpaired produced flavour avoidance, and that excitatory training produced some retardation. Likewise, the authors examined the flavour avoidance of a flavour which had served as a CS- in a differential conditioning procedure, whereby a flavour was paired with a 20% M (CS+) and a control flavour was paired with a 2% M (CS-). In the test, a relatively novel solution of unflavoured 6% M was used as the common test solution in a two bottle choice test adding the target flavour to one bottle as a summation test. The results confirmed, as the flavour passed retardation and summation tests, that the differential conditioning in nutrient-based flavour learning can produce inhibitory conditioning in hungry animals in the form of avoidance of the CS- flavour (Boakes et al., 2010).

However, to the best of our knowledge, there are no previous studies that evaluate the possibility of the development of conditioned inhibition after post-training CS exposure in nutrient-based conditioned flavour preference. As the result of our assessment is positive, the empirical evidence does not support the view that the decrement of flavour preference is due to extinction by which is meant a process that restores the CS associative strenght to a zero level or renders the CS as associatively neutral (Aguado et al., 2001). It should be noted that although previous studies using conditioned taste aversion reported evidence in favor of the acquisition of such properties for an extinguished CS (Calton, Mitchell, & Schachtman, 1996; Hart, Bourne, & Schachtman, 1995), more refined designs have proved that this was not the case (Aguado et al., 2001; see also Brooks, Bowker, Anderson, & Palmatier, 2003). Modern theories of extinction suppose that some form of inhibitory learning occurs during extinction, but none of them supposes that the extinction will convert the CS into a net inhibitor, rather the extinction will render the CS associatively neutral (e.g., Bouton, 1991; Pearce & Hall, 1980; Wagner, 1981; Rescorla & Wagner, 1972). Therefore, it seems there are neither theoretical nor empirical grounds for that claim.

Additionally, several facts invoke exercising caution before accepting that the detected decrease in preference during post-training CS exposure reflects extinction of the conditioned preference for the flavour. Firstly, one feature of the extinguished stimulus is that they exhibit rapid reacquisition (Bouton, 2004); the results present here are against this general observation (see also Higgins & Rescorla, 2004). Even equating the total amount of flavour exposure before the retardation test, group Simultaneous not only showed no retardation compared with group Serial, but also gave no signs of reacquisition throughout the three reacquisition trials. Secondly, it is well known that extinguished stimuli are sensitive to the US devaluation effect, but this does not seem to be the case in conditioned flavour preference (Delamater, 2007; Harris et al., 2004). We have also replicated this absence of the US devaluation effect in Experiment 5 using a procedure quite similar to the one used in the experiments presented in this Chapter; whereas the effect was readily obtained in a group in which no extinction treatment was given, devaluated and extinguished animals failed to show the effect. The absence of a US devaluation effect suggests that the CS-US association has been impaired, and it is at odds with studies of extinction that have used more conventional learning paradigms (e.g., Delamater, 1996; Rescorla, 1996). Thus, for the reasons mentioned above, it seems reasonable to consider that the observed decrease in preference after post-training CS exposure in the present experiments might not be due to extinction procedure, but to the development of net inhibitory learning. If this assumption is right, there is no necessity to consider that extinction acts under different rules in this paradigm as it has been suggested (e.g., Delamater, 2007). It also raises the question of which is the particular mechanism which produces conditioned inhibition, an issue that falls beyond the scope of the present experiments aimed at finding empirical evidence of conditioned inhibition but not at analyzing the precise mechanism underlying such learning. Therefore, we recognize that at this point such an analysis has to be rather speculative.

We have pointed out that the procedure used in this and other investigations —in which the flavour CS is presented forming a simultaneous compound with sucrose during conditioning before being exposed alone— needs to be considered further. During conditioning, there are two sensory cues—the target flavour (A) and the taste of sucrose (T)—which are paired with the nutrient consequences of sucrose (i.e., calories), the relevant US (+) during the CS exposure phase when animals are hungry (Harris et al., 2000). After simultaneous conditioning, the flavour is presented alone without the calories previously provided by the sucrose. This (i.e., AT+; A-) is not the usual extinction procedure, in which usually a single cue is followed by the US before the cue is presented alone (e.g., A→ +; A -). After the extinction procedure, the stimulus acquires two different and opposite associations, both of which seem to subsist and compete for performance (Bouton, 1993). However, the AT+; A- procedure has similarities with the first phases of retrospective revaluation training, specifically unovershadowing, a retrospective cue competition phenomenon in which more than ambiguity, there is redundancy in the prediction of the outcome which is solved in the second phase of the procedure. The result of this process is thought to affect the associative strength of the redundant cue according to some theoretical accounts. Retrospective revaluation using the principles of Pavlovian conditioning has been previously analyzed in the field of human causality judgments (e.g., Dickinson & Burke, 1996). Interestingly, this phenomenon depends on the development of strong within-compound associations, and the implications of the modified Wagner's (1981) SOP model developed to account for it (e.g., Aitken & Dickinson, 2005; Dickinson & Burke, 1996) has also been successfully applied to conditioned flavour preference experiments with rats as subjects (e.g., Balleine et al., 2005; Dwyer, Mackintosh, & Boakes, 1998), a paradigm which involves presentations of flavours in compounds which are known to produce strong within-compound associations (e.g., Rescorla & Cunningham, 1978). However, our procedure overlaps only partially with that of retrospective revaluation, as we do not measure the changes in the associative strength of the taste T; rather our study focuses on what happens during post-training exposure to flavour A.

During the CS exposure phase, flavour A is expected to activate the representations of both the taste of sucrose (T) and the nutrient US appealing to the formation of within-compound associations (e.g. Durlach & Rescorla, 1980). According to this view, pairing the AT compound with calories results in the formation of associations between these stimuli and the US, plus reciprocal associations between A and T. However, the sweet taste is absent and, since animals are food deprived, so is the relevant US (i.e., calories). Accordingly, their representations will be associatively activated in presence of flavour A through the within-compound associations. Under these conditions, flavour A should form inhibitory associations with both the taste T and the US according to both the original and the modified Wagner's models (Aitken & Dickinson, 2005; Wagner, 1981); none of these analyses however supposes that flavour A will become a net inhibitor. The results from the present study suggest that this might be the case when a flavour presented in compound with a palatable nutrient to thirsty rats is subsequently presented alone under food deprivation.

Alternatively, as commented previously, it is also possible according to Higgins and Rescorla (2004) that during training a unitary representation of the flavour-taste compound is formed (AT), and that the exposure of the flavour alone after this phase produced the formation of the representation of the flavour as a separate element (A). The decrease in preference for the flavour observed during the flavour exposure might reflect discrimination between the flavour and the compound, rather than extinction (Higgins and Rescorla, 2004). With the auxiliary assumption that there should be some generalization of the excitatory strength from the compound to the flavour in the first stage of the flavour exposure, inhibitory learning may occur as the flavour, as separate element, acts to signal the absence of the nutrient to hungry rats.

The procedure used in Experiment 8 for group Simultaneous, in which both flavours A and B were presented forming a simultaneous compound with the taste of sucrose T, may lead to an additional source of inhibitory learning which would not be present in Experiment 7[5]. Exposing animals to AT and BT compounds during the conditioning phase might have produced an inhibitory association between flavours A and B. During flavour B conditioning, a within-compound BT association was formed. The common element T might have activated the representation of flavour B in its absence during the conditioning of flavour A presented in compound with the taste of sucrose. This inhibitory association between flavours A and B could have also contributed to the decrease in the consumption of the AB compound during the summation test in group Simultaneous but not in group Serial, which was not exposed to the AT compound.

The acquisition by flavour A of the properties of a net inhibitor signalling the absence of both the sweet taste and the calories might explain why the sucrose devaluation effect is absent when the flavour is presented on its own after compound conditioning (Delamater, 2007; Harris et al., 2004), as well as both the retardation in flavour-nutrient reacquisition (see also Higgins & Rescorla, 2004) and the decrease in the preference for a excitor mixed with the flavour found in the present study. Furthermore, the absence of the expected nutrient in hungry animals might have activated a negative motivational state (Dickinson & Dearing, 1979) which could have endowed the CS with a negative affective value. It is then possible that the three factors (absence of an expected nutrient when animals are hungry; absence of the associate sweet taste; and the negative hedonic value associate to the flavour) might have each contributed to convert the flavour into a conditioned inhibitor. The present results do not allow us to assess the relative impact of these possible factors or even to support this proposal. More research is needed to specify the precise underlying learning mechanisms involved in the present results.

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[5] The autors are grateful to Prof. Dr. G. Hall and Prof. Dr. R. F. Westbrook for this suggestion in a previous reading of the manuscript.

The experiments presented here provide empirical evidence showing that a flavour previously paired simultaneously with sucrose acquired inhibitory proprieties after being presented alone when animals were hungry: the flavour showed retardation in subsequent reacquisition and a decrease in the preference for an excitor when presented in a simultaneous compound with it. To which extent similar results may be found in other more conventional paradigms of Pavlovian conditioning is an issue subject to empirical verification, but the principles under which conditioned inhibition might arise could be common to these paradigms.

Chapter 7

MOTIVATIONAL CONTROL OF LATENT INHIBITION IN CONDITIONED FLAVOUR PREFERENCE

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The experiments included in this Chapter have been carried out in collaboration with Prof. Geoffrey Hall.

Experiments 9A and 11 were run in his Lab at the University of York.

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Experiments 9A, 9B, 10, and 11 from manuscript in preparation as:

Garcia-Burgos, D., González, F., & Hall, G. (2011). *Motivational control of latent inhibition in flavor preference conditioning.* Manuscript in preparation.

7.1. INTRODUCTION

Given that unreinforced presentation of the flavour after flavour-sucrose pairing affects to CFP based on nutrient, but not to CFP based on taste (e.g., Harris et al., 2004, Exp. 2B), this Chapter focuses on the effect of non-reinforced trials in LI procedure (see review of LI in Lubow & Weiner, 2010). In fact, both procedures may procedurally be considered symmetrical, as both expose the subject to contingent CS-US pairing as well as to presentation of the CS alone, but in reverse order. Concretely, the present set of experiments had the main objective of providing empirical evidence of the motivational control of LI in CFP. For this reason, different motivational states of hunger or thirst presented during preexposure-conditioning training and testing were used to assess the effectiveness of non-reinforcement flavour presentations before flavour-sucrose pairings. In turn, the results will allow a further investigation of the nature of the flavour-taste and flavour-nutrient mechanisms on CFP.

Experiment 9A firstly examined the basic effect of two motivational states, thirst or thirst and hunger, on LI in conditioned flavour preference, whereas Experiment 9B assessed whether the failure of finding a LI effect in trained and tested thirsty rats was due to the absence of the formation of the flavour-sucrose association during conditioning. Experiment 10 examined whether the LI effect was specific to the motivational state of training. Finally, Experiment 11 tested the possibility that LI found on CFP could be accounted for in terms of state dependency between training and testing.

7.2. MOTIVATIONAL CONTROL OF LATENT INHIBITION IN CONDITIONED FLAVOUR PREFERENCE

Different learning theories have been proposed to explain the LI effect (e.g., Bouton, 1997; Lubow, Weiner, & Schnur, 1981; Mackintosh, 1975; Pearce & Hall, 1980; Wagner, 1981), defined as retarded conditioning/performance when a stimulus is presented previously without consequences compared to a novel stimulus on a conditioning task.

Although there have been many explanations of the LI, current theories reside within two major categories: association-deficit models and retrieval-interference models (Lubow & Gewirtz, 1995). Association-deficit models of LI make the assumption that stimulus preexposure impairs the attention to/associability of that stimulus during conditioning phase (e.g., Mackintosh, 1975; Pearce & Hall, 1980; Wagner, 1981). In this category, as mentioned by De la Casa & Pineño (2010), attentional models argue that the exposure to the CS in the absence of the consequences will result in a progressive loss of attention to the CS. In turn, since attention to the CS determines its ability to acquire associative strength, the effectiveness of subsequent CS-US pairing will be diminished relative to a conditioning with no CS-preexposure. For instance, Mackintosh's attentional modes (1975) explained LI by assuming that the associability of the CS declines during its preexposure because the CS is no better at predicting biologically significant events (US) that other CSs that are also present on a trial. Therefore, animals learn that the preexposed stimulus signals nothing of importance. Another attentional model was proposed by Pearce and Hall (1980). In this model, the attention to CS decreases as the CS becomes a good predictor of the outcome, the absence of the US. Therefore, animals learn that the preexposed stimulus signals nothing. Wagner's (1981) SOP model also accounts for LI as an associative deficit. This model explained LI by association between the CS and the context formed during CS-preexposure. As the CS is predicted by context at the start of subsequent conditioning, the CS is not surprising and it is not processed for conditioning. In fact, preexposed stimulus suffers a context-specific loss of associability.

In contrast, retrieval deficit models claim that there is no impairment of stimulus associability but that preexposed and non-preexposed groups enter the conditioning phase with the same capability for forming new associations with the preexposed stimulus (Lubow & Gewirtz, 1995). According to retrieval interference models, the stimulus exposures affect conditioned responding to this stimulus, but do not interfere with the acquisition of the CS-US association during conditioning. In other words, the association formed during preexposure competes for expression with the association formed in the conditioning phase on test (e.g., Bouton, 1993). For instance, Bouton proposed, in line with Pearce & Hall (1980), that during CS-preexposure the animal learns a CS–nothing association. This CS–nothing association then interferes with the retrieval of the CS–US association at test, resulting in the LI effect.

Despite their multiple merits, these models of LI based information processing are unable to explain the effects of the motivational factors in the learned associations between the CS and the US. For example, the associability-deficit theories assume that the associability of CS will be low with the preexposure regardless of motivational factors (e.g., Hall & Rodriguez, 2010). As opposed to these theories, Killcross and Balleine's (1996) study (outlined in section 3.4.1.) demonstrated the relevance of motivational factors in the acquisition of conditioning in a LI procedure. In fact, they argued that LI depended on the motivational state in which the preexposure was conducted. As claimed by Killcross and Balleine (1996), these results are not accommodated easily into the classic models of latent inhibition based on attention/information processing of conditioning. The authors manipulated the motivational states during preexposure and conditioning, showing that animals learn that the preexposed stimulus was unrelated to the events of relevance to their current motivational state. However, unlike the Killcross and Balleine (1996), the experiments of the present Chapter examined the effect of motivational factors on the expression of flavour preference in a LI procedure.

It is assumed that animals either water or both water and food deprived during training acquire both flavour-taste (non-predictive learning non affected by CS preexposure) and flavour-nutrient associations (predictive learning though to be affected by CS preexposure), being the motivational state at the time of learning which controls the expression of both types of learning (Harris et al. 2000). Therefore, LI should appear in animals tested hungry, given that only hunger on test selects the flavour-nutrient association, but not in rats tested thirsty, independently of the motivational state during training. To avoid that the LI effect was attenuated or interrupted by a change in context between preexposure and conditioning (i.e., state dependent effects in LI, e.g., Hall & Channell, 1986), the motivational state of preexposure and conditioning phases was the same across all the experiments.

Experiment 9A: Basic effect

This Experiment was designed to compare the influence of CS preexposure on flavour-sucrose conditioning when the animals were either just thirsty or thirsty and hungry throughout the experiment. The design appears in the uppers of Table 5 (Exp. 9A). Animals were allocated to one of two motivational states, one in which rats were water deprived (group Th) and other in which rats were food and water deprived (group H). In the preexposure phase, rats in both groups were further subdivided into two conditions, where rats were preexposed to either almond (groups PE/Th and PE/H) or water (groups NPE/Th and NPE/H).

If LI effect only occurs in the predictive flavour-nutrient learning and this is selected by hunger state at the time of testing, then LI effect should appear as lesser preference for almond in the almond-preexposed group compared to the water-preexposed group when the animals are hungry and thirsty on test, but not when they are thirsty. If, however, the LI does not depend on the motivational state at the time of testing, the conditioned flavour preference should be equal in both almond-preexposed and water-preexposed groups, independently of motivational state on test.

Table 5. Experimental designs of Experiments 9A, 9B, 10, and 11.

	Group	Preexp.	Condit.	Test
EXP 9A			2 cycles	
	PE/Th	*Th*: 8 A^{o}	*Th*: 2 A+	*Th*: A vs. water
	NPE/Th	*Th*: 8 water		
	PE/H	*H*: 8 A^{o}	*H*: 2 A+	*H*: A vs. water
	NPE/H	*H*: 8 water		
EXP 9B			2 cycles	
		Thirsty	*Thirsty*	*Thirsty*
	PE/PA	8 A^{o}	2 A+	A vs. water
	PE/UN		2 A/+	
	NPE/PA	8 water	2 A+	
	NPE/UN		2 A/+	
EXP 10		*Hungry*	*Hungry*	
	PE/Th	8 A^{o}	2 A+	*Th*: A vs. water
	PE/H			*H*: A vs. water
	NPE/Th	8 water		*Th*: A vs. water
	NPE/H			*H*: A vs. water
EXP 11			2 cycles	
		Thirsty	*Thirsty*	
	PE/Th	8 A^{o}	2 A+	*Th*: A vs. water
	PE/H			*H*: A vs. water
	NPE/Th	8 water		*Th*: A vs. water
	NPE/H			*H*: A vs. water

Note. PE = preexposed, NPE = non-preexposed, PA = paired presentations of flavour and sucrose, UN = unpaired presentation of flavour and sucrose, A = almond, + = sucrose, Th = thirsty, H = hungry & thirsty.

Method

Subjects and apparatus

The subjects were 32 male Lister hooded rats at least 14 weeks old with a mean body weight of 519 g at the start of the experiment. All subjects had earlier participated in conditioned suppression experiments. They were housed in individual home cages and kept in a colony room that was lit from 8:00 am to 8:00 am each day. The present experiment was run at the University of York. This and all subsequent experiments took place in the home cages. Inverted 50-ml plastic tubes equipped with stainless steel ball-bearing-tipped spouts were used to present fluids in these cages. Consumption was estimated by weighing the tubes before and after fluid presentation to the nearest 0.1 g. The solutions used were made up with tap water and consisting of 1% (vol/vol) almond essence (Silver Spoon, London, UK) and 1% almond essence and 10% sucrose (wt/vol) compound.

Procedure

The water or food and water, according to the group, were removed from the home cage 24 hr before the start of the experiment. Rats were weighed and randomly allocated to two weight-matched groups: group Th (n = 16) and group H (n = 16), where half of the rats were placed on a water deprivation schedule, and the remaining subjects were placed on food and water deprivation schedules. Then, all rats were given 3 days to accommodate to the water and food deprivation schedules, in which access to water was allowed for 30 min at 9:30 am, and water (group Th) or food and water (group H) for 90 min at 2:00 am Both deprivation regimes were maintained across the entire duration of the experiment. Then, rats were subsequently divided into four groups: PE/Th (n = 8), PE/H (n = 8), NPE/Th (n = 8), and NPE/H (n = 8). The preexposure phase occurred over the next four days. This phase consisted of two daily trials (9:30 am and 2:00 am) administered on each of four consecutive days. Each trial consisted of 10 min access to 10 ml (first two trials) or 6 ml (remaining six trials) of almond (for animals in the PE conditions) or water (for animals in the NPE conditions). The preexposed amount of fluids was reduced from 10 to 6 ml in order to equalize consumption between groups as animals in group Th drank more almond than animals in group H.

The conditioning and test phases were run in 2 cycles. In each cycle, conditioning occurred over 2 days with one trial each day at 9:30 am. In each trial, animals had 10-min access to 10 ml of an almond + sucrose compound. On the third day of the cycle animals were tested; the test consisted of 15-min access to two bottles at 9:30 am, one containing 20 ml of the almond solution and the other 20 ml of water. The positions of the bottles were counterbalanced across subjects and cycle.

Results

Preexposure phase. Mean consumption (g) of almond was 6.05 for group PE/Th; and 4.69 for group PE/H. Mean consumption of water was 5.89 for group NPE/Th; and 4.07 for group NPE/H. A 2 (Preexposure) x 2 (Motivational state) ANOVA provided a significant main effect of motivational state [F(1, 28) = 77.38] and preexposure [F(1, 28) = 4.57]. No other significant main effect or interaction was found [largest F(1, 28) = 1.79, p = 0.19]. The main effect of preexposure reflected a higher fluid consumption in almond-preexposed groups comparing with water-preexposed groups. On the other hand, thirsty animals drank significantly more fluid than hungry animals.

Conditioning phase. Mean consumptions (g) of almond + sucrose compound across conditioning days were 10, 9.38, 9.81, and 9.81 for group PE/Th; 9.72, 9.48, 9.67, and 9.95 for group PE/H; 10, 9.61, 9.8, and 9.97 for group NPE/Th; and 9.6, 9.75, 9.9, and 10 for group NPE/H. A 2 (Preexposure) x 2 (Motivational state) x 4 (Trial) ANOVA provided a significant main effect of trial [F(3, 84) = 5.05, p < 0.01]. No other main effect or interaction was significant [largest F(3, 84) = 2.22, p > 0.091]. The effect of trials reflected a lesser compound consumption on trial 2 comparing with trial 1.

Test phase. Despite the fact that the differences in preference ratio values between PE/H (0.63) and NPE/H (0.85) were not significant in the first flavour preference test, these data encouraged the running of a second cycle of conditioning and test. Given that there was no effect of test or interaction with test, for the purposes of all statistical analyses, the data were collapsed over the two test sessions. Figure 16 displays the mean almond preference ratio collapsed across the two flavour vs. water test days as a function of almond preexposure (PE vs. NPE) and motivational state (Thirsty vs. Hungry). A 2 (Preexposure) x 2 (Motivational state) ANOVA of almond preference provided a significant main effect of preexposure [F(1, 28) = 12.54, p < 0.01] and a significant preexposure x motivational state interaction [F(1, 28) = 6.82]. No other main effect of interaction was significant [largest F(1, 28) < 1]. The exploration of preexposure x motivational interaction showed a lesser almond preference for group NPE/Th comparing with group NPE/H [*t*(14) = 8.24]; and a lesser almond preference in the group PE/H comparing with group NPE/H [*t*(14) = 15.76, p < 0.01].

Discussion

The results of Experiment 9A revealed a LI effect only in animals trained and tested hungry, i.e., group PE/H showed a lesser almond preference than NPE/H; there was not difference in preference between groups PE and NPE trained and tested thirsty. These results are consistent with the dual mechanism approach. Both thirsty and thirsty and hungry animals acquired flavour-nutrient and flavour-taste learning during conditioning. However, as preexposure is assume to be effective only in nutrient-flavour learning, LI should only be found in groups tested hungry. Note that the motivational state at time of learning selects which mechanism controls performance.

On the other hand, the lesser almond preference for group NPE/Th comparing with group NPE/H is consistent with previous results. As proposed by several investigators (e.g., Drucker et al., 1994; Fedorchak & Bolles, 1987), hunger enhance preference flavour paired with a nutrient.

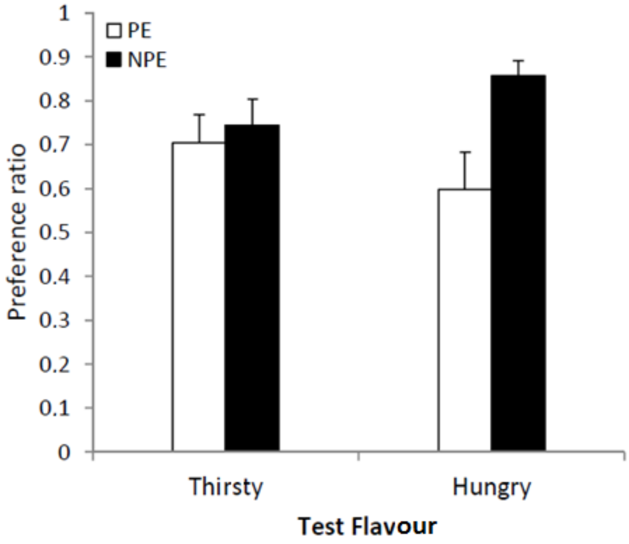

Figure 16. Experiment 9A. Mean almond preference ratios (± SEM) collapsed across the two almond vs. water choice tests shown separately for the preexposed (PE) and non-preexposed (NPE) groups when tested thirsty and when tested hungry.

Nevertheless, it must be admitted that another explanation of the absence of LI in thirsty animals remains as an alternative account of the data. This possibility suggests that the absence of the LI effect in thirsty animals may be due to the failure to form an association between almond and sucrose. Preference observed for almond in these groups might be the consequence of a non-associative process (e.g., mere exposure effect). That is, the absence of a difference in preference may not be due to an ineffective conditioning in the preexposed group, but to the absence of conditioning in both of them. Experiment 9B examined this possibility.

Experiment 9B: Flavour-sucrose association in thirsty animals

Among the different ways to increase preference for a flavour, the simplest one is the "mere exposure" effect, through which flavours become increasing preference by virtue of repeated exposure (e.g. Hill, 1978). While mere exposure produces an increase in preference by reducing inherent neophobia, it does not typically produce robust preferences. These more significant preference effects are attributed to associative learning, through which an initially arbitrary (or even initially aversive flavour) can become preferred due to earned associations (Myers & Sclafani, 2006; Rozin & Zellner, 1985). Experiment 9B was designed to examine whether the flavour became associated with sucrose after conditioning in thirsty animals. To assess this possibility, the same procedure was used here with the exceptions that rats received either a paired or unpaired relation between the almond and sucrose during conditioning phase and that all animals were only water deprived throughout experiment. The design appears in the uppers of Table 5 (Exp. 9B). As in Experiment 9A, one group of rats were exposed to almond alone (preexposed group, PE) while the other group of rats received water (non preexposed group, NPE) during preexposure phase. In the conditioning phase, the sucrose was then paired with almond in simultaneous compound for half of the rats in each of these conditions (groups PE/PA and NPE/PA) and was unpaired (rats received the almond and 5 hr later they received the sucrose) for the remaining rats (groups PE/UN and NPE/UN).

If the results of thirsty rats in Experiment 9A did represent an effect of flavour-sucrose association, then rats' preference that have received flavour paired with sucrose should be higher than in those animals from whom the flavour is unpaired with sucrose. If no differences on flavour preference are observed, we cannot assume that flavour-sucrose association has been acquired. On the other hand, the same pattern demonstrated during Experiment 9A for the preexposed and non-preexposed thirsty rats with paired presentation of flavour and sucrose (i.e., absence of differences and therefore of LI) are expected.

Method

Subjects and apparatus

The subjects were 32 experimentally naïve male Wistar rats at least 14 weeks old with a mean body weight of 274 g at the start of the experiment. Housing, general maintenance, and apparatus (including the solutions) were the same as in Experiment 9A.

Procedure

Animals were maintained under water deprivation and food was freely available throughout the experiment. All animals were given the experimental sessions at 9:30 am (and second sessions at 2:00 pm for the unpaired groups during the conditioning phase), and allowed daily 90 min access to water at 2:00 am approximately. Regarding Experiment 9A, the present Experiment was run with the following differences. Given that subjects did not consume similar quantities of the cue solution according to motivational state, all animals received 5 ml of solutions to equalize the similar quantities of the cue solution consumption during preexposure in this and the following experiments. Likewise, the eight sessions of preexposure phase passed from 4 to 8 days, with an experimental session per day. Water was removed from the home cage 24 hr before the start of the experiment. Then, all rats were given 3 days to accommodate to water deprivation, in which access to water was allowed for 30 min at 9:30 am and 90 min at 2:00 am Then, the rats were weighted and randomly allocated to two weight-matched groups: PE (n = 16), and NPE (n = 16). This preexposure phase consisted of one daily trial, in which rats received 10 min access to 5 ml of almond (in the PE conditions) or water (in the NPE conditions). After preexposure phase, rats were subsequently divided into four groups: PE/PA (n = 8), PE/UN (n = 8), NPE/PA (n = 8), and NPE/UN (n = 8), according the relation between flavour and sucrose (Paired vs. Unpaired).

The conditioning and test phases were the same as in Experiment 9A, except for the unpaired conditions (PE/UN and NPE/UN) which received one exposure (am) to 10 ml of almond during 10 min and one exposure (pm) to 10 ml of sucrose during 10 min. As animals in group PE/PA drank more almond than animals in group PE/UP, the unpaired groups received two additional 10-min exposures to almond on the second day of the first cycle in order to equalize almond intake. This was not necessary on the second cycle.

Results

Preexposure phase. Mean consumption (g) of almond was 4.43 for the group PE; and water was 4.40 for group NPE. No significant differences were found [*t* < 1].

Conditioning phase. Mean consumptions (g) of almond + sucrose compound across conditioning days were 9.26, 9.41, 9.22, and 9.43 for the group PE/PA; and 8.82, 9.36, 9.32, and 9.45 for the group NPE/PA. Mean consumptions of almond alone across conditioning days were 9.06, 9.41, 9.11, and 9.27 for the group PE/UN; and 9.01, 9.41, 9.24, and 9.22 for the group NPE/UN. A 2 (Preexposure) x 2 (Condition) x 4 (Trial) ANOVA was used to test for differences on almond intake among groups, which provide a significant main effect of trial [F(3, 84) = 3.91]. No other main effect or interaction was significant [largest F(3, 84) = 0.84, p = 0.47]. The main effect of trial reflected a lesser fluid intake in trial 1 comparing with trials 2 and 4. On the other hand, mean consumptions of sucrose alone across conditioning days were 8.85, 9.27, 9.21, and 9.26 for the group PE/UN; and 8.87, 9.42, 9.1, and 9.28 for the group NPE/UN. With the data of almond+sucrose compound during conditioning cited above, a 2 (Preexposure) x 2 (Condition) x 4 (Trial) ANOVA was used to test for differences on sucrose intake among groups, which provide a significant main effect of trial [F(3, 84) = 6.05, p < 0.001]. No other main effect or interaction was significant [largest F(1, 28) = 1.16, p = 0.29]. The main effect of trial reflected a lesser fluid intake in trial 1 comparing with trials 2 and 4. These data confirmed that there was no difference among groups in almond and sucrose intakes.

Test phase. Given that there was no effect of test or interaction with test, for the purposes of all statistical analyses, the data were collapsed over the two test sessions. Figure 17 displays the mean almond preference ratio collapsed across the two flavour vs. water test days for paired and unpaired condition. A 2 (Preexposure) x 2 (Condition) ANOVA of almond preference provided a significant main effect of condition [F(1, 28) = 19.46]. No other main effect of interaction was significant [largest F(1, 28) = 1.93, p = 0.17]. The exploration of condition effect showed a higher almond preference for Paired groups comparing with Unpaired groups.

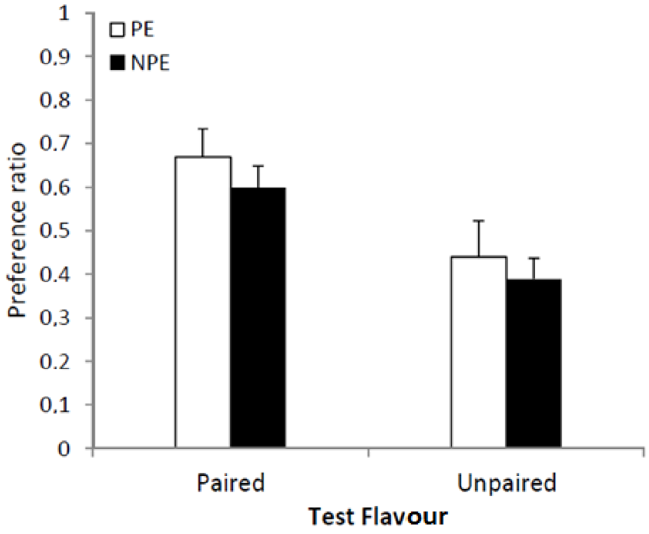

Figure 17. Experiment 9B. Mean almond preference ratios (± SEM) collapsed across the two almond vs. water choice tests shown separately for the paired groups and unpaired groups that received paired or unpaired presentations the almond and sucrose during conditioning. Ad libitum food access was maintained throughout the experiment.

Discussion

Experiment 9B confirmed the finding of the previous experiment: preexposure or non-preexposure to almond in rats with paired presentation of almond and sucrose did not produced differences on flavour preference during test when the animals were maintained with free access to food throughout the experiment. That is, there was no LI effect in thirsty trained and tested animals. However, the more important result is that PA groups showed a higher flavour preference that UN groups, interpreted as evidence for the development of associative learning in the former.

However, one consideration is necessary to UN groups which cannot be overlooked. Given that the preference ratio for almond in the unpaired group revealed a slight aversion (preference ratio of 0.41; below the 0.5 level, *t*(15) = -2.54, p < 0.05), the possibility that this condition reflects some form of inhibitory learning may be argued, as commented in other sections of this dissertation. In fact, the explicitly unpaired control may introduce a negative contingency relationship between almond (CS) and sucrose (US). Thus, instead of the almond being a signal for sucrose, it can become a signal for the absence of sucrose. In this case, the unpaired groups would not be an optimum control. To assess this possibility an additional statistical analysis was carried out in the UN groups comparing the flavour preference on two tests. If some form of inhibitory learning produced this slight aversion, the preference ratio should decrease across unpaired sessions during the two cycles of conditioning-test. With only unpaired groups' test data of tests, a 2 (Preexposure) x 2 (Test) ANOVA showed, however, no significant main effects or interaction [largest $F(1, 14) = 3.22$, $p = 0.094$; from a mean almond preference ratio about 0.36 on first test to 0.47 on the second]. Ruled out the inhibitory learning hypothesis, under these circumstances, thirsty rats might also learn that almond occurred in the absence of the attractive sucrose, creating an anticipatory negative contrast effect that led to avoidance of the almond (see Flaherty, 1982).

Experiment 10: Importance of motivational state on training

Experiment 9A demonstrated that preexposure to a flavour generated LI effect when the animals were preexposed, conditioned and tested hungry, but not when these phases were conducted under and state of thirst. However, these data do not provide the knowledge whether hunger is a relevant factor during training (preexposure-conditioning), during testing, or whether it has to be present in both.

Under the hypothesis that LI is specific to the motivational state of hunger present during preexposure and conditioning, LI should be expected independently of the motivational state at the time of testing. By contrast, if the important event is the motivational state of hunger during testing, which selects the predictive flavour-nutrient learning affected by change of CS-US contingency, the LI effect should not manifest in rats tested thirsty even though they were trained hungry.

To assess this suggestion, Experiment 10 used the similar basic procedure, but with all animals hungry and thirsty during preexposure and conditioning phases. The motivational state of food deprivation was manipulated on test. The design appears in Table 5 (Exp. 10). In this Experiment, hungry and thirsty animals were preexposed to either almond (groups PE) or water (groups NPE) and subsequently given conditioning. During testing, the animals in both groups were further subdivided into two subgroups, one in which rats were water deprived (groups PE/Th and NPE/Th) while in the other, rats were food and water deprived (groups PE/H and NPE/H).

Method

Subjects and apparatus

The subjects were 32 experimentally naïve male Wistar rats at least 14 weeks old with a mean body weight of 263 g at the start of the experiment. Housing, general maintenance, and apparatus (including the solutions) were the same as in Experiment 9A.

Procedure

Initially, animals were placed on a food and water deprivation schedule. This deprivation regime was maintained during preexposure and conditioning phases. All animals were given the experimental session at 9:30 am and allowed daily 90 min access to food and water at 2:00 am The food and water were removed respectively from the home cage 24 hr before the start of the experiment. Then, all rats were given 3 days to accommodate to the water and food deprivation, in which access to water was allowed for 30 min at 9:30 am. Before the start of the experiment, rats were weighed and randomly allocated to two weight-matched groups (n = 16) and the preexposure phase was conducted over 8 days, as in Experiment 9B.

The conditioning occurred over 2 days with one trial each day. In each trial, all animals had 10 min access to 10 ml of almond + sucrose. After this phase, rats were subsequently divided into four groups: PE/Th (n = 8), PE/H (n = 8), NPE/Th (n = 8), and NPE/H (n = 8), according to motivational state on test (Thirsty vs. Hungry).The test phase consisted of simultaneous 15-min access to two bottles at 9:30 am, one containing 20 ml of the almond solution and the other 20 ml of water. The positions of the bottles were counterbalanced across subjects.

Results

Preexposure phase. Mean consumption (g) of almond was 2.21 for the group PE; and water was 2.14 for group NPE. No significant difference was found [*t* < 1].

Conditioning phase. Mean consumption (grams) of almond + sucrose compound across conditioning days were 6.47 and 10 for group PE; and 4.49 and 9.95 for group NPE. A 2 (Preexposure) x 2 (Trial) ANOVA provided a significant main effect of trial [F(1, 30) = 89.2, p < 0.01]. No other main effects or interactions were significant [Fs < 1]. The effect of trials reflected a lesser compound consumption on trial 1 compared with trial 2.

Test phase. Figure 18 displays the mean almond preference ratios on flavour test as a function of almond preexposure (PE vs. NPE) and motivational state (Thirsty vs. Hungry). Given that there was no effect of test or interaction with test, for the purposes of all statistical analyses, the data were collapsed over the two test sessions. A 2 (Preexposure) x 2 (Motivational state) ANOVA of almond preference provided a significant main effect of preexposure [F(1, 28) = 4.33] and a significant preexposure x motivational state interaction [F(1, 28) = 6.82]. No significant main effect of motivational state was found [F(1, 28) < 1]. The exploration of preexposure x motivational interaction showed a lesser almond preference for group NPE/Th comparing with group NPE/H [*t*(14) = 7.76]; and a lesser almond preference in the group PE/H comparing with group NPE/H [*t*(14) = 18.13, p < 0.01].

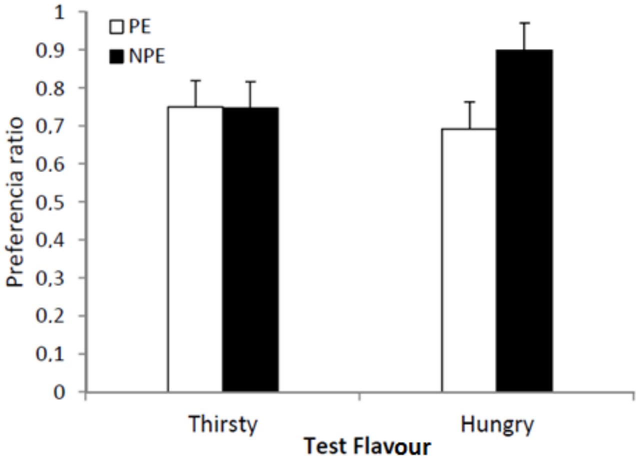

Figure 18. Experiment 10. Mean almond preference ratios (± SEM) for the almond vs. water choice test shown separately for the preexposed (PE) and non-preexposed (NPE) groups when tested thirsty and when tested hungry.

Discussion

The data pattern on test were similar to those of Experiment 9A, in spite of all animals being hungry during preexposure and conditioning phases. These results confirmed that preexposure to almond and conditioning by pairing almond and sucrose under a hunger motivational state produced a LI effect only when rats were hungry on test, but not when they were thirsty on test. On the other hand, the failure to observe the LI effect in rats trained hungry and tested thirsty suggested that the motivational state of hunger during training is not the relevant factor. These results support the fact that the rats given the flavour-sucrose compound learned flavour-taste learning and flavour-nutrient learning, and that motivational state selected which one controlled the performance at time of testing (Harris et al., 2000): food deprivation on test selected the preference based on flavour-nutrient learning, and thus LI was observed. Performance of animals tested thirsty, on the other hand, was based on flavour-taste learning which was not affected by flavour preexposure.

In this experiment, the LI on animals tested hungry was observed with only one conditioning-test cycle, i.e., less almond intake in the group PE/H compared with group NPE/H. This is consistent with the idea that food restriction seems to facilitate nutrient-based preference due to enhancement of the reinforcing value of nutrient by increasing the animal's energy need (Davidson, 1998). However, several studies agree in finding that deprivation state during training has little influence on the acquisition of the conditioned flavour preference (Yiin et al., 2005a).

However, it should be noted that the current data for hungry animals may be explicable in terms of state dependency of LI between training and testing phases. So far, only the animals preexposed, conditioned and tested hungry have shown LI.

If the LI shown by hungry rats on test in Exps. 9A and 10 is due to state-dependency between training and testing (i.e., animals are hungry both on training and on testing), rats trained thirsty but tested hungry should not show LI. However, if LI depends on rats being hungry at the time of testing with independence of the motivational state during training, they should show LI.

Experiment 11: State dependency between training and testing

Experiment 11 was mainly designed to assess the state dependency of LI between training and test and used the basic procedure of Experiment 10, but with 2 conditioning-test cycles (as Experiment 9A). All rats were water deprived during preexposure and conditioning phases. The motivational state of food deprivation was manipulated on test. The design appears in Table 5 (Exp. 11). Thirsty animals were preexposed to either almond (groups PE) or water (groups NPE). During testing, animals in both groups were further subdivided into two conditions, one in which rats were water deprived (groups PE/Th and NPE/Th) while in the other, rats were food and water deprived (groups PE/H and NPE/H).

Method

Subjects and apparatus

The subjects were 32 male Lister hooded rats at least 14 weeks old with a mean body weight of 425 g at the start of the experiment. All subjects had earlier participated in conditioned suppression experiments. The present experiment was run at the University of York. Housing, general maintenance, and apparatus (including the solutions) were the same as in Experiment 9A.

Procedure

Initially, animals were placed on a water deprivation schedule. This deprivation regime was maintained during preexposure and conditioning phases. All animals were given the experimental session at 9:30 am and allowed daily 90 min access to water at 2:00 pm. The water was removed respectively from the home cage 24 hr before the start of the experiment. Then, all rats were given 3 days to accommodate to the water deprivation, in which access to water was allowed for 30 min at 9:30 am. Then, the rats were weighed and randomly allocated to two weight-matched groups (n = 16).

The preexposure phase was conducted as in Experiment 9B.

The conditioning and test phases were run in 2 cycles as in Experiment 9A. After this phase, rats were subsequently given 3 days in which they received water for 30 min each on these three days at 9:30 am, and they were allowed daily 90-min access to water or food and water according to deprivation schedules at 2:00 pm to favour the adaptation of groups H to the food deprivation schedule. Then, they were divided into two subgroups: PE/Th (n = 8), PE/H (n = 8), NPE/Th (n = 8), and NPE/H (n = 8), according to motivational state on test (Thirsty vs. Hungry). The test phase consisted of simultaneous 15-min access to two bottles at 9:30 am, one containing 20 ml of the almond solution and the other 20 ml of water. The positions of the bottles were counterbalanced across subjects.

Results

Preexposure phase. Mean consumption (g) of almond was 4.78 for the group PE; and water was 4.89 for group NPE. A significant difference between groups [*t*(1) = 13.8, $p < 0.01$], showing a lesser almond consumption in group PE than water in group NPE.

Conditioning phase. Mean consumption (g) of almond+sucrose compound across conditioning days were 6.47 and 10 for the group PE; and 4.49 and 9.95 for group NPE. A 2 (Preexposure) x 4 (Trial) ANOVA provided a significant main effect of trial [F(1, 30) = 89.2, $p < 0.01$]. No other main effect or interaction was significant [largest F(1, 30) = 3.05, p = 0.09]. The effect of trials reflected a higher compound consumption on trial 3 compared with trials 2, and 4.

Test phase. Given that there was no effect of test or interaction with test, for the purposes of all statistical analyses, the data were collapsed over the two test sessions. Figure 19 displays the mean almond preference ratio collapsed across the two flavour test days as a function of almond preexposure (PE vs. NPE) and motivational state (Thirsty vs. Hungry). A 2 (Preexposure) x 2 (Motivational state) ANOVA of almond preference provided a significant main effect of motivational state [F(1, 28) = 4.33] and a significant preexposure x motivational state interaction [F(1, 28) = 6.82]. No other main effect of interaction was significant [largest F(1, 28) = 2.8, p = 0.1]. The exploration of preexposure x motivational interaction showed a lesser almond preference for group NPE/Th compared with group NPE/H [*t*(14) = 14, $p < 0.01$]; and a lesser almond preference in the group PE/H compared with group NPE/H [*t*(14) = 7.73].

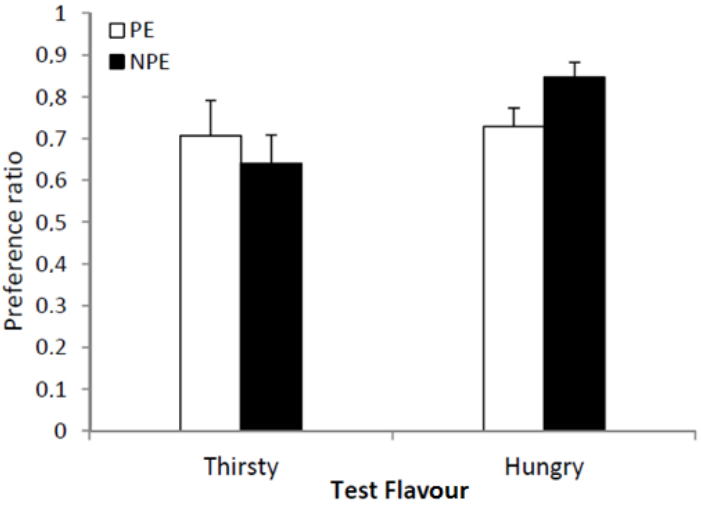

Figure 19. Experiment 11. Mean almond preference ratios (± SEM) collapsed across the two almond vs. water choice tests shown separately for the preexposed (PE) and non-preexposed (NPE) groups when tested thirsty and when tested hungry.

Discussion

Experiment 11 confirmed the previous results about the absence of LI in groups tested thirsty and demonstrated its occurrence in groups tested hungry (as Exps. 9A, 9B and 10). In addition, this experiment indicated that the LI effect in CFP paradigm did not appear to be specific to the motivational state of hunger during training. Therefore, the LI effect for animals tested hungry in Exps. 9A and 10 may not be explicable in terms of state dependency, because animals trained thirsty showed LI when they were tested hungry. On the other hand, the LI in thirsty-trained and hungry-tested rats may not be explained by interference in terms of content of learning according to the Killcross and Balleine's (1996) approach. Rats trained with ad-libitum access to food should only have learned the flavour-hydrating effect association during preexposure, and the flavour-hedonic value of taste association during conditioning, but nothing about flavour-no nutrient association[6].

Therefore, the current data support the assumptions about the action of different mechanisms during conditioning using the same reinforcing nutrient, and the importance of motivational state of hunger on test. In fact, the more important factor in the expression of LI effect was the motivational state during testing (provided that the preexposure and conditioning were run in the same motivational state). Furthermore, hunger on test selected the predictive flavour-nutrient learning mechanism. Unlike flavour-taste learning, this mechanism is sensitive to flavour preexposure in CFP. Finally, the results of this Experiment are consistent with the idea that food deprivation primarily affects the expression rather than the acquisition of flavour-nutrient learning (e.g. Capaldi et al., 1994; Fedorchak & Bolles, 1987; González et al, 2010; Yiin et al., 2005).

General Discussion

The present experiments examined the role of motivational state during preexposure-conditioning training and testing with a LI procedure in CFP using oral simultaneous presentation of almond and sucrose with previous flavour-alone presentation. Experiment 9A revealed an effect on LI only in animals trained and tested hungry. The absence of LI in thirsty animals was not due to a deficit in CFP acquisition (Exp. 9B). Experiments 10 and 11 showed that training animals hungry was not a sufficient (Exp. 10) nor a necessary (Exp. 11) condition to observe LI. Animals trained hungry but tested thirsty did not show LI in the former, whereas animals trained thirsty but tested hungry did in the latter. Therefore, whichever the motivational state during training (provided, as is the case in all each experiment, the motivational state is the same during both preexposure and conditioning), LI is observed only when animals are tested hungry. This pattern of results is consistent with the proposal defended in this dissertation; animals thirsty or thirsty and hungry

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[6] Although animals trained thirsty may suffer from latent hunger and this might favour the formation of flavour-no nutrient during preexposure, the data of Killcross and Balleine (1996) does not correspond with this interpretation (see explanation in section 3.4.1. of the present manuscript).

acquire both flavour-taste and flavour-nutrient learning during conditioning, the motivational state at the time of testing selecting which one controls performance. Flavour-nutrient, but not flavour-taste, is affected by non-reinforced presentations of the CS previous to conditioning. Therefore, only animals tested hungry showed a LI in the present set of experiments.

The results showed that the preference for flavour in animals tested hungry or thirsty was due to conditioning, and this preference was impaired by previous exposure to the CS only when animals were tested hungry, independently of the motivational state during training (Exps. 9A, 10, and 11). By contrast, there was never a LI effect when animals were tested thirsty (Exps. 9A, 9B, 10 and 11).

The failure in getting LI in thirsty-tested animals is surprising given that preexposure to a stimulus has been shown to affect learning using a variety of conditioning paradigms, such as conditioned suppression (e.g., Kasprow, Catterson, Schachtman, & Miller, 1984), conditioned avoidance (e.g., Feldman, 1977), conditioned taste aversion (e.g., Kraemer & Roberts, 1984), conditioned eyeblink (e.g., Allen, Chelius, Masand, Gluck, Myers, & Schnirman, 2002), or appetitive conditioning (e.g., Channel & Hall, 1983).

Regarding theories of LI based on association-deficit models, the results in thirsty animals on test are not easy to fit into. They predict the failure of the flavour to enter into an association with the sucrose after exposure, due to a general (e.g., Pearce & Hall, 1980; Hall & Rodriguez, 2010) or a context-specific (e.g., Wagner, 1981) loss of associability during preexposure. These models also predict that LI should not be affected by manipulation conducted after the conditioning stage. Therefore, neither of them can explain the absence of differences in conditioned flavour preference between the preexposed and the non-preexposed groups when animals were tested thirsty, whichever the motivational state during training. For instance, the Wagner's (1981) model presents difficulties in explaining the absence of LI when the animals are tested thirsty because it does not consider the motivation in learning (like, e.g., Mackintosh, 1975; Pearce & Hall, 1980). In fact, the association between flavour and context during preexposure should impair the formation of the flavour-sucrose association during conditioning whatsoever the motivational state during training. The present findings are not considered by Killcross & Balleine's (1996) motivational model either. This model assumes that the retardation in conditioning following preexposure is totally controlled by the animal's motivational state, but it does not consider the motivational state on test as a relevant factor in the LI nor the irrelevance of the motivational state during preexposure-conditioning training for the expression of LI when rats are hungry on test.

Regarding theories of LI based on retrieval-interference models (e.g., Bouton, 1993, 1997; Kraemer & Spear, 1992), Bouton's (1993) model, for instance, also fails to explain the present results. In this model, the important factor is the sensitivity to context and time of each association, postulating that the learning about "nothing" (or "insignificance") is more context-dependent for retrieval. Thus, if rats are preexposed and conditioned with thirst, they acquire two associations: a context-dependent association during preexposure, the flavour-nothing association, and a context-independent association during conditioning, flavour-nutrient association. When the animals are tested in the same motivational context as that of preexposure (i.e., thirst), LI should be observed because the context-dependent flavour-nothing association should be retrieved. On the other hand, if rats trained thirsty are tested hungry, the LI should not be observed because the change of the motivational context between training and testing should retrieve the context-independent flavour-nutrient association. A revision of this model (Bouton, 1997) however does not account for the present results either. In this revision, Bouton claimed that it is not in the nature of the association, but the order in which they are learned that determines which association becomes context-dependent for retrieval. Specifically, it is the association learned in second place that depends on the context for its retrieval. Therefore, the flavour-nothing association should be relatively context-independent and stronger LI should be observed when the flavour is tested in a new context, but LI effect does not appear, for instance, when the animals are trained hungry and tested thirsty.

Taken together, what the animals learned about the flavour during the training phase may be inferred from the results. Given that animals are motivated to actively identify and sample the available food items in order to assess whether they are nutritionally beneficial or harmful, storing information concerning the nutritional properties of these food items (Day, Kyriazakis & Rogers, 1998), animals might learn about the absence of nutritive properties of flavour during the preexposure. They might form the flavour-no nutrient association. Then, animals received the flavour mixed with the sucrose during conditioning, giving rise to flavour-nutrient association. However, the acquisition of this second association might be impaired due to prior experience of the flavour in the absence of the sucrose, producing an associative interference. Simultaneously, animals might also learn that the flavour paired with sucrose tastes better because of the sweet taste of sucrose, i.e., flavour-hedonic taste of sucrose. This learning, however, did not interfere with the first association acquired during preexposure, flavour-no nutrient association, because they did not contain conflicting information. Finally, given that the motivational state during the test selected the learning mechanism, when animals were hungry on test the predictive mechanism based on expectancy of nutrient was selected, showing the impaired acquisition of flavour-nutrient association (i.e., LI effect). By contrast, when animas were thirsty on test, hedonic non-predictive flavour-taste learning was selected, showing a similar level of acquisition of the flavour-hedonic taste of sucrose association (i.e., non-LI effect).

The data from the present set of experiments support the differential sensitivity of contingency manipulations in flavour-nutrient and flavour-taste learning mechanisms respectively. It is clear that flavour CS exposure produced a LI effect on preference based on post-ingestive effects of nutrient, i.e. when animals are hungry. By contrast, when the animals are thirsty, there is no LI effect. Thus, the effect of non-reinforcement presentation of the flavour observed in the extinction of CFP seems to extend to other procedures that also involve exposing the rats to contingent CS-US pairings as well as to presentation of the CS alone. In fact, the parallel with the motivational regulation of the expression of learned flavour preference proposed by Harris et al. (2000), in which rats with a level of hunger (by water or food deprivation) form flavour-taste and flavour-nutrient associations, and their motivational state on test determining which of these associations controls the preference should be noted; likewise should the parallel with persistence of flavour preference after post-training flavour exposure in nutrient-based conditioned flavour preference (Harris et al., 2004). Therefore, the present data add more evidence to the dual mechanism account that operate in CFP, although further empirical data is needed to assess the relative contribution of these associations in this LI procedure.

Part 3

CONCLUSIONS

Chapter 8

CONCLUSIONS

8.1. CONCLUSIONS

This dissertation has provided a series of examinations of the effectiveness of non-reinforced trials in CFP through the procedure of extinction and LI using an orally consumed nutrient (sucrose) as US. The first goal was to examine the decrement in nutrient-based conditioned flavour preference found in hungry rats exposed to a flavour following simultaneous flavour-sucrose conditioning whilst thirsty (under similar conditions as for the procedure used by Harris et al., 2004, Exp. 2B).

In addition, given that the change of CS-US contingency after conditioning affects CFP based on nutrient, but not CFP based on taste (e.g., Harris et al., 2004, Exp. 2B), the second objective of this dissertation examined whether the effect of non-reinforced trials can extend to LI. Concretely, different motivational states of hunger or thirst present during both training (preexposure-conditioning) and testing were used to assess the effectiveness of non-reinforcement flavour presentations before flavour-sucrose pairing.

The findings presented in this dissertation provided answers to these questions. Firstly, the results confirmed the dissociation between flavour-taste learning and flavour-nutrient learning (Exps. 0A and 0B) in CFP. Animals trained thirsty by giving them simultaneous pairings of a non-preferred flavour and a palatable nutrient showed a corresponding preference when rats were tested hungry, but not when the same rats were tested thirsty (Exp. 0A). This dissociation was not due to a deficit in the acquisition of a flavour-taste association, as the US devaluation effect emerged in thirsty animals when the taste of sucrose was paired with i.p. injections of LiCl (Exp. 0B).

Secondly, Experiment 1 showed that simultaneous training was more effective than a serial procedure in CFP, and that the latter could be used as a control condition. Preference for the flavour in group simultaneous declined to the same level as that of the controls when the flavour was repeatedly experienced in the absence of sucrose under both hunger and thirst. The serial flavour-sucrose presentations to thirsty rats demonstrated to be an appropriate control condition because it did not produce evidence of a preference over plain water when tested hungry, eliminating the putative flavour avoidance problems arising from both the differential and the unpaired training procedures.

Thirdly, the results supported that the decrease in preference observed in hungry rats after flavour-exposure following simultaneous conditioning with sucrose might not be due to extinction. A number of findings allowed us to reach this conclusion. Experiments checked for the recovery of the putative extinguished conditioned preference through the phenomena of spontaneous recovery, reinstatement, and motivational renewal. They demonstrated that, although in each experiment a strong conditioned preference and a posterior decrease during post-training CS exposure were clearly found in group simultaneous, none of the extinction-related phenomena were found. In fact, no evidence of spontaneous recovery was found when the flavour preference was tested 2 weeks after the flavour-exposure phase (Exp. 2). No evidence of reinstatement was found either when the US was re-exposed after the flavour-exposure phase in the absence of presentation of the almond (Exp. 3). There was an effect in the opposite direction to contextual renewal using motivational states as contexts, in which the preference was indeed lower in the simultaneous group when tested in the motivational context of thirst if post-training flavour exposure was conducted under thirst and hunger (Exp. 4). In addition, similarly to other studies (Harris et al., 2004; Delamater, 2007), the failure to find a US devaluation effect after post-training CS exposure was also observed (Exp. 5), albeit the dominant view of extinction is that it has no impact on the strength of the CS-US association (Delamater, 1996; Rescorla, 1996). Taken together, this set of findings was congruent with the testable implication 1 for *Hypothesis 1*: the flavour did not show extinction-related phenomena of recovery of the extinguished response, neither did it show the US-devaluation effect.

Fourthly, Experiment 6 showed evidence of conditioned inhibition in CFP based on nutrient through conditioned Pavlovian inhibition procedure using the summation test. Subsequent results demonstrated that the decrease in conditioned flavour preference in hungry rats observed following presentations of the flavour alone after simultaneous flavour-nutrient training was due to net inhibitory properties acquired by the flavour. This was supported by empirical evidence of inhibitory learning using retardation (Exp. 7) and summation (Exp. 8) tests. In each experiment, rats trained thirsty with flavour + nutrient pairing and then exposed to the flavour while hungry showed a decrease in conditioned preference. None of these effects were found in the control group which had received serial flavour→nutrient presentations during training. Taken together, this set of findings was congruent with testable implication 2 for *Hypothesis 1*: the decrement in nutrient-based conditioned flavour preference found in hungry rats exposed to a flavour following simultaneous flavour-sucrose conditioning whilst thirsty was due to conditioned inhibition, showing retardation when retrained after the exposure phase (retardation test, Exp. 7), and a decrease in the preference for a second flavour previously paired simultaneously with sucrose when both flavours are presented forming an unreinforced compound (summation test, Exp. 8).

Fifthly, the results demonstrated that the LI effect depended on the animal's motivational state on test. A set of experiments used different motivational states of hunger or hunger and thirst during preexposure-conditioning training and testing to assess the effectiveness of non-reinforcement flavour presentations before flavour-sucrose pairing. In fact, the results were consistent in showing that the preference for a flavour in hungry or thirsty animals on test was due to the formation of a flavour-sucrose association (Exps. 9A and 9B), thought only hungry-tested animals exhibited a lesser almond preference when they were preexposed to flavour before conditioning, independently of the motivational state during training (Exps. 9A, 10, and 11). By contrast, there was never a LI effect when animals were tested thirsty (Exps. 9A, 9B, 10 and 11). Explanations in terms of either the relevance of motivational state during preexposure-conditioning training to the current motivational state on test, or in terms of state dependency were rejected, given that animals trained both thirsty or thirsty and hungry showed LI effect when tested hungry (Exps.10 and 11). Taken together, this set of findings was congruent with testable implication 3 for *Hypothesis 2*: the attenuation of flavour preference appeared in flavour-preexposed animals compared with flavour-non-preexposed animals whenever the animals were hungry on test, independently of whether the animals were preexposed and conditioned under water or both water and food deprivation; and no effect of LI was found in animals tested just thirsty.

Conditioned inhibition but not extinction

The examination of the inhibitory properties of a flavour after repeated flavour exposure in hungry animals following flavour-sucrose conditioning whilst thirsty confirmed that such a flavour passed both retardation and summation tests for conditioned inhibition. It seems reasonable to label the flavour in these experiments as a conditioned inhibitor (Rescorla, 1996). Given that the present research is the first demonstration that a flavour alone repeatedly exposed in hungry animals after flavour-sucrose conditioning passes the tests for conditioned inhibition, these results have implications for the study of CFP and for the mechanisms supporting flavour preference learning.

These inhibitory properties allow us to explain the conflicting conclusions found in the studies of “extinction” of conditioned flavour preference. For instance, Harris et al. (2004) and Delamater (2007) found that the extinction procedure in CFP weakens the flavour-sucrose association: a conclusion however that is at odds with the studies of extinction that have used more conventional learning paradigms (Delamater, 2007). By contrast, if an inhibitory flavour CS-sucrose association which endows the flavour with the properties of a net inhibitor develops after post-training flavour-exposure, the absence of the US-devaluation effect and the absence of phenomena of recovery of the conditioned preference after flavour-exposure should be expected, as the CS should not activate the representation of the US.

Given that numerous researchers have supposed that extinction is the result of an inhibitory process that reduces the excitation established during conditioning (e.g., Bouton & Nelson, 1994) and the examination of the inhibitory properties of a flavour exposed after training occurs in the context of what has been considered extinction, it should be noted that this dissertation does not propose that the post-training flavour-exposure in CFP converts an “extinguished” flavour CS into a net inhibitor. In fact, we suggest a possibility, following the suggestion of Higgins and Rescorla (2004), compatible with the net inhibitory properties of flavour and the apparent procedure of extinction. This explanation proposes that rats exposed to flavour-sucrose compound may not separately identify the flavour and the taste of sucrose as elements, rather that they may form a representation of a unitary flavour-taste of sucrose compound. Owing to the fact that sucrose has post-ingestive actions, the unitary representation of this compound is also linked to nutritive properties of sucrose. Then, what appears to be extinction of flavour preference by repeated presentation of flavour alone after conditioning may be the formation of the representation of the flavour by means of perceptual differentiation. In addition to the exposure of the element by itself without sucrose, the presentation of the flavour alone during testing under hunger could activate the representations of both US properties (the taste and the nutrient), which would be absent at that moment, especially the post-ingestive actions of sucrose. This activation can be explained, for example, by Wagner’s standard operating procedure (SOP; Wagner, 1981). Assuming that there is some generalization of the excitatory strength from the compound to the flavour in the first stages of the flavour alone exposure, the net inhibitory learning for the flavour can be explained. In fact, Wagner argued that an inhibitory association is formed when the CS representation in the A1 state is associated with the US representation in the A2 state. Therefore, if the presentation of the flavour alone activates its representation to the A1 and via generalization with the compound the flavour produces the associative activation of the representation of post-ingestive actions of sucrose into the A2 state, the inhibitory properties for the CS flavour may be observed. However, it should be noted that, although the present dissertation was specifically designed to examine the inhibitory properties for the flavour, it was not designed to assess the perceptual differentiation mechanism between compound and flavour suggested by Higgins and Rescorla (2004). This is an interesting and promising account which would need to be further investigated.

Although this process is merely speculative at this moment, it represents an explanation about the acquisition of net inhibitory properties by the flavour without entering in contradiction with the current accounts of extinction in Pavlovian conditioning. In agreement with Bouton (2007), we suppose that a CS does not become a net conditioned inhibitor after extinction and that an extinguished CS looks like an excitor rather than an inhibitor. This is also congruent with the models of extinction that involve some form of inhibitory learning process (e.g., Bouton, 1993; Delamater, 1996; Konorsky, 1948), which do not assume that an extinguished stimulus may become a net inhibitor.

Finally, another factor which merits some consideration is the change of motivational state between thirsty-trained and hungry-tested animals. Although these changes could make the analysis of the effectiveness of non-reinforced trials more difficult because of the introduction of additional cues, the impact of the motivational change seems to be mainly limited to effect of interoceptive hunger cues as Pavlovian occasion setter (e.g., Davidson, 1998) in CFP. For instance, Experiments 0A and 0B showed that animals learned both flavour-taste and flavour-nutrient associations under water deprivation, and that they may be dissociated by manipulating the motivational state at time of testing. In fact, it is well established that the motivational states affect the expression but not acquisition of flavour conditioning (e.g., Yiin et al., 2005a, 2005b). From this perspective, interoceptive cues produced by food deprivation may promote the expression of associations between flavour and sucrose by making it easier for CSs to excite the memorial representation of post-ingestive USs (Davidson, 1998). This account for the role of hunger in terms of expression of learning is consistent with the results presented in LI of this dissertation (Exp. 6 and Exps. 9A, 10 and 11).

The observation of Latent inhibition in CFP depends on the animal's motivational state on test

The motivational-dependent failure of LI effect in thirsty-tested animals (Exps. 9A, 9B, 10 and 11) is surprising and it is not easily accommodated into the classic theories of LI based on attention/associability (e.g., Lubow et al., 1981; McLaren & Mackintosh, 2000; Pearce & Hall, 1980), the contextual association of Wager model (1981), the associative interference (e.g., Hall & Rodriguez, 2010), or the performance-based models (e.g., Bouton, 1993; Kraemer & Spear, 1992).

We have considered this failure as an acquisition-deficit case based on associative interference, but not on attention/associability because the processing to flavour CS is not depressed during preexposure. In fact, thirsty or thirsty and hungry animals learned to associate the flavour and sucrose after flavour preexposure, demonstrated by the acquired flavour preference when animals were tested thirsty regardless of whether flavour CS was preexposed or not. One possibility is that the exposure to the flavour in the absence of the sucrose resulted in thirsty or hungry and thirsty rats encoding the flavour-no nutrient association, due to the tendency of animals to monitor the nutritional quality of the edible substance present in the environment. Then, this prior learning might interfere with subsequent formation of flavour-sucrose associations when the flavour and sucrose compound was presented. Concretely, the flavour-no nutrient association during preexposure may impair the subsequent formation of flavour-nutrient association during conditioning. Finally, when the animals were hungry on test, the predictive learning based on expectative of nutrient was selected, reflecting the flavour preexposure effect (LI). On the other hand, given that sucrose has two reinforcing properties, palatability and nutrient, the animals also learned the flavour-hedonic taste of sucrose during conditioning. However, this association was not impaired by the flavour-no nutrient association because of the independence of their contents. Finally, when the rats were thirsty on test, the non-predictive learning based on palatability was selected, reflecting the failure of flavour preexposure effect (no LI).

The major implications of the view presented here are that the learning that underlies LI depends on the class of reinforcer and that the expression of LI -or the absence of LI- depends on the animal's motivational state on test.

Mechanisms on conditioned flavour preference

The findings of this research about the effect of non-reinforced trials on conditioned flavour preference based on nutrient support the dual mechanism account in the CFP paradigm when a palatable nutrient is used as US. According to the sensitivity to non-reinforced trials, it seems clear that the flavour-taste learning mechanism is not affected by the flavour CS alone preexposure before flavour-sucrose pairings (suppression of latent inhibition effect). By contrast, flavour-nutrient learning mechanism is affected by flavour CS-alone presentation before or after contingent flavour-sucrose pairings (reduction of conditioned preference when the CS exposure is pre- or post-training).

This conclusion in terms of sensitivity to manipulation of CS-US contingency is congruent with the results about the decrement in conditioned preference observed in hungry animals after CS exposure following conditioning, and the persistence in preference observed when animals are tested thirsty (Harris et al., 2004). Therefore, the conclusions observed in the post-training CS flavour exposure procedure may extend to LI procedure.

One reason that might explain the difference between flavour-nutrient and flavour-taste mechanisms proposes that these mechanisms, from a survival perspective, have evolved to deal with specific problems that animals face in the wild. That is, given that the preference shown by animals can result in the predictive flavour-nutrient learning mechanism or the non-predictive flavour-taste learning mechanism, the motivational state of hunger on test is crucial to select the former. In fact, when the animals are tested hungry, the predictive flavour-nutrient mechanism operates according to the knowledge of anticipated consequences of prior experiences and expectations about food properties. It should be noted that animals are constantly motivated to identify and sample the available substances in order to assess whether they are nutritionally beneficial or harmful with independence of motivational state, due to pressure in terms of fitness and survival (Day, Kyriazakis, & Rogers, 1998). Therefore, they are continually learning about nutritional properties of food and they are sensitive to any modification of CS-nutrient contingency. By contrast, when the animals are thirsty on test, the non-predictive flavour-taste mechanism operates. Unlike the flavour-nutrient mechanism, this one is not adapted to solving nutritional needs, but it provides affective information about the stimulus. The result is a transfer of any emotional tone of one of the flavours to the other flavour by the contiguity between CS and US.

In summary, the results presented in this dissertation pointed out that manipulations of the CS-US contingency through non-reinforced presentations of the CS either before or after conditioning are effective only for the flavour-nutrient mechanism, and further suggest that this kind of learning is predictive. Flavour-taste learning, which is not based on the expectancy of the US occurrence (i.e., is not predictive) seems not to be affected by these same manipulations.

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Effectiveness of Nonreinforced Trias on
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Extinction and Latent Inhibition

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