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# ANÁLISIS DEL EFECTO DE PREEXPOSICIÓN AL EI CON UN PROCEDIMIENTO DE CONDICIONAMIENTO APETITIVO

# ANALYSIS OF THE US PREEXPOSURE EFFECT IN AN APPETITIVE CONDITIONING PROCEDURE

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Dña. ISABEL DE BRUGADA SAURAS, Titular de Psicología Básica de la Universidad de Granada

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Que el trabajo de investigación titulado "ANÁLISIS DEL EFECTO DE PREEXPOSICIÓN AL EI CON UN PROCEDIMIENTO DE CONDICIONAMIETNO APETITIVO" ha sido realizado por Dña. Marta Gil Nájera 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 la dirección del Profesor Geoffrey Hall (Universidad de York, UK). Reuniendo dicho trabajo los requisitos académicos, formales y de calidad necesarios para que pueda ser defendida públicamente.

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The work entitled "ANÁLISIS DEL EFECTO DE PREEXPOSICIÓN AL EI CON UN PROCEDIMIENTO DE CONDICIONAMIENTO APETITIVO / ANALYSIS OF THE US-PREEXPOSURE EFFECT IN AN APPETITIVE CONDITIONING PROCEDURE" was completed by Ms. Marta Gil Nájera to obtain the degree of European Doctor of Philosophy in the Department of Experimental Psychology and Physiology of Behavior, Faculty of Psychology, University of Granada. I can also confirm that this work has been carried out under my supervision and the supervisión of Dr Isabel de Brugada (University of Granada, Spain), and it fulfills all of the requirements for it to be publicly defended.

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## DECLARATION:

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“Science is a wonderful thing if one does not have to earn one´s living at it”.

Albert Einstein, 1951.

# CONTENTS

## CHAPTER III

### EXPERIMENTS 3-4: A ROLE FOR BLOCKING BY CONTEXT IN THE US-PREEXPOSURE EFFECT?



## CHAPTER IV

### EXPERIMENTS 5-8: THE ROLE OF TASTE CUES

## CHAPTER V

### Experiments 9-10: The role of motivational state



## CHAPTER VI

### Experiments 11-12: Studies of a non-nutritive US

## CHAPTER VII

### CONCLUSIONS

# ABSTRACT

## Abstract

This thesis made use of the conditioned flavour preference (CFP) paradigm, with rats subjects, in order to investigate the effect of prior exposure to an appetitive unconditioned stimulus (US) on a conditioning treatment (the US-preexposure effect). Previous work, mainly using aversive paradigms, has focused on two main explanations for this effect - associative blocking and in terms of a non-associative habituation process. In this thesis, the nature of the mechanisms operating in the appetitive version of the US-preexposure effect was explored.

The experiments reported in Chapter 2 first established the basic US-preexposure effect using the CFP procedure by demonstrating that preexposing rats to sucrose (US), will produce a reduced conditioned response (CR) to the conditioned stimulus (CS) when it is presented alone in a test. Chapter 3 then explored the role blocking by contextual cues might play in this demonstration of the US-preexposure effect. The experiments reported in Chapter 3 produced results that did not support a blocking-by-context explanation. Accordingly an alternative explanation was suggested that explains these results in terms of a modified version of the blocking-by-context hypothesis, in which not the context, but the taste of the US serves to block the acquisition to the CS during conditioning.

Chapter 4 explored this alternative account for the US-preexposure effect by using a different substance as the US. Results from the experiments reported in Chapter 4 showed that using maltodextrin as the US (a substance with similar nutritive consequences to sucrose but a less salient taste), produces only a weak

US-preexposure effect; this does not always occur and it depends on various procedural parameters.

In Chapter 5 a comparison was made between animals trained in different motivational states. Varying the motivational state of animals, allowed the experiments in Chapter 5 to test the blocking-by-taste hypothesis of the US-preexposure effect. If the mechanism underlying the effect relies on blocking by taste, then the US-preexposure effect should be demonstrated more readily in hungry animals than in those animals that are sated. This result is found in Experiment 9.

The experiments in Chapter 6 demonstrated, however, that the effect could be found both when the US is sucrose (providing both a sweet taste and motivational post-oral consequences) and when it is saccharin (a substance that lacks any caloric properties). This latter result does not support the blocking-by-taste account previously offered. A different mechanism is offered in terms other than blocking-by-taste for the saccharin case. The implications of these results for interpretations of the US-preexposure effect are considered in Chapter 7.

# CAPÍTULO I

## INTRODUCCIÓN TEÓRICA

# CAPÍTULO I: INTRODUCCIÓN TEÓRICA

Las teorías modernas del aprendizaje asociativo se han centrado fundamentalmente en explicar los mecanismos responsables de la formación de asociaciones entre dos estímulos que se presentan juntos (o temporalmente cercanos). Se asume que las representaciones de los elementos específicos de un par de estímulos se conectarán después de que éstos se hayan presentado al mismo tiempo y, como resultado, cuando uno de ellos se presente por sí solo, la representación del otro estímulo se activará asociativamente (Rescorla, 1988).

Sin embargo, existen ciertos fenómenos de aprendizaje que ocurren cuando un estimulo se presenta en solitario previamente a su emparejamiento con otro estímulo. Está ampliamente demostrado que la exposición repetida a un estímulo puede retrasar la adquisición o la manifestación de una respuesta condicionada (RC) durante un condicionamiento posterior. Un ejemplo bien conocido es el fenómeno de Inhibición Latente. El concepto de Inhibición Latente lo presentaron por primera vez (Lubow & Moore, 1959) definiéndolo como un aprendizaje latente que ocurre durante la exposición a un estímulo neutro y que, en una fase posterior de condicionamiento retrasa la formación de una asociación entre el estímulo neutro (EC) previamente expuesto y el estímulo incondicionado (EI) cuando se presenten juntos (ver Lubow & Weiner, 2010 para una revisión).

Si en cambio, el estímulo expuesto antes del condicionamiento es un estímulo con propiedades motivacionales o EI, el fenómeno es conocido como Efecto de Preexposición al EI. Se observa un Efecto de Preexposición al EI cuando a

un grupo de sujetos se le expone a un estímulo incondicionado (EI) por sí solo de forma previa al emparejamiento con un EC (fase de condicionamiento). Ésta preexposición al EI dará lugar a un retraso en el condicionamiento posterior, de forma que el estímulo condicionado elicitará una respuesta condicionada menor en una fase posterior de prueba en comparación con un grupo control que no ha recibido exposiciones previas al EI (Randich & Lolordo, 1979a; Riley & Simpson, 2001).

El procedimiento estándar del Efecto de preexposición al EI consiste en la presentación del EI en solitario con anterioridad a la fase de condicionamiento en la que se presentan emparejados un EC y ese mismo EI. Una de las primeras demostraciones de este efecto fue llevada a cabo por (Kamin, 1961). En éste estudio se mostró cómo la preexposición a una descarga eléctrica durante un periodo de 10 días, retrasó la adquisición de una respuesta emocional condicionada (REC) durante el condicionamiento con respecto a los animales que no habían sido preexpuestos a la descarga eléctrica.

Este Efecto de preexposición al EI se ha demostrado en una extensa variedad de especies animales así como con diversos paradigmas de condicionamiento. Los procedimientos mas comunes utilizadas en el estudio de este fenómeno han sido preparaciones de condicionamiento aversivo, tales como la de respuesta emocional condicionada (REC) utilizando descarga como EI (por ej. Baker & Mackintosh, 1979; Baker, Mercier, Gabel, & Baker, 1981; Kamin, 1961; Randich, 1981; Randich & Lolordo, 1979b; Rescorla, 1973) y la aversión condicionada al sabor con cloruro de litio (LiCl) como EI (Braveman, 1975;

Cannon, Berman, Baker, & Atkins, 1975; de Brugada, Hall, & Symonds, 2004; Domjan & Best, 1977; Gamzu, 1977; ver Hall, 2009 para una revisión); y con otras sustancias que producen aversión condicionada (ver Riley & Simpson, 2001).Otra preparación de condicionamiento aversivo en la que también se ha demostrado el Efecto de preexposición al EI es el condicionamiento parpebral, bien con humanos (Taylor, 1956) o conejos (Hinson, 1982; Mis & Moore, 1973) como sujetos experimentales.

Aunque la mayoría de las demostraciones del Efecto de preexposición al EI utilizan procedimientos de condicionamiento aversivo, el efecto también se ha obtenido cuando se ha utilizado un procedimiento de condicionamiento apetitivo. En este caso las demostraciones existentes han hecho uso del automoldeamiento (Brown & Jenkins, 1968) utilizando tanto ratas como palomas como sujetos (Balsam & Schwartz, 1981; Costa & Boakes, 2009; Engberg, Welker, Thomas, & Hansen, 1972; Timberlake, 1986; Tomie, 1976a, 1976b; Tomie, Murphy, Fath, & Jackson, 1980; Van Hest, Van Haaren, & Van De Poll, 1989).

## Mecanismos explicativos del Efecto de preexposición al EI

Se han ofrecido diferentes mecanismos tanto asociativos como no asociativos para explicar el efecto de preexposición al EI. Una explicación no asociativa del efecto sería en términos de un proceso de habituación, normalmente definida como una reducción en la capacidad del estímulo para evocar una respuesta incondicionada (Thomson & Spencer, 1966). Este proceso de habituación podría resultar también en una reducción en la efectividad del estímulo incondicionado (EI) lo que podría dificultar su capacidad para funcionar como un reforzador durante el condicionamiento posterior. Por otra parte, la explicación asociativa del efecto de preexposición al EI se da en términos de bloqueo (Kamin, 1969). Según esta hipótesis, la exposición al estímulo incondicionado durante la preexposición permitiría la formación de una asociación entre las claves contextuales y el EI. Éstas claves asociadas al EI durante la preexposición y presentes en el condicionamiento, bloquearían la adquisición de la asociación EC-EI durante la fase de condicionamiento. Éstos mecanismos, asociativos y no asociativos, propuestos para explicar el efecto de preexposición al EI no tienen porqué ser excluyentes entre sí y ambos podrían jugar un papel a la hora de explicar el efecto (ver Randich & Lolordo, 1979a para una revisión).

**Mecanismos no asociativos**

Diversos procesos no asociativos han sido propuestos para explicar el Efecto de preexposición al EI. Aunque distintos, todos los procesos propuestos (habituación, dependencia, tolerancia, procesos oponentes, etc.) asumen que la exposición repetida al EI reduce su efectividad para funcionar como un reforzador, lo que provoca un retraso en el condicionamiento posterior cuando ese mismo estímulo es usado como EI (Riley & Simpson, 2001).

Dentro de las diferentes explicaciones no asociativas propuestas para explicar el Efecto de preexposición al EI, una de las que ha recibido mayor atención es aquella que explica el efecto en términos de un proceso de habituación (Thomson & Spencer, 1966). Se define la habituación como un decremento en la magnitud de la RI como consecuencia de la exposición repetida al EI. De acuerdo con esto, las presentaciones repetidas del estímulo incondicionado (EI) provocarían una reducción en la respuesta del organismo hacia éste estímulo (reducción en la RI que se manifiesta en presentaciones futuras del estímulo) (Kamin, 1961; Mis & Moore, 1973; Taylor, 1956). Este proceso de habituación además de disminuir la capacidad del EI para evocar RI podría resultar en una disminución de su efectividad para actuar como reforzador en el condicionamiento posterior.

Esta hipótesis puede en principio explicar el retraso habitual en el condicionamiento que se observa tras la preexposición al EI, así como algunos de los resultados obtenidos en estudios que utilizan distintos paradigmas aversivos

de condicionamiento dirigidos a manipular parámetros que afectan al proceso de habituación. Por ejemplo, utilizando una preparación de aversión condicionada al sabor con LiCl como EI, Aguado, de Brugada, & Hall (1997) mostraron como un intervalo temporal entre la preexposición y el condicionamiento resultó en una atenuación del Efecto de preexposición al EI (ver también Cannon, et al., 1975). De acuerdo con estos resultados, un EI habituado podría presentar una recuperación espontánea de la RI cuando se presenta junto al EC tras el intervalo temporal, siendo su efectividad como reforzador similar a la de un EI que no ha sido preexpuesto. Aunque hay que señalar que éstos resultados también son susceptibles de una explicación en términos asociativos (Aguado, et al., 1997; Cannon, et al., 1975). Sin embargo, utilizando esta misma preparación, se ha mostrado que la manipulación de otros factores que afectan al desarrollo de la habituación, como la administración masiva o distribuida de las exposiciones al EI, no tiene ninguna repercusión sobre el tamaño del efecto de preexposición al EI (Cappell & LeBlanc, 1977; Riley & Diamond, 1998; Riley & Simpson, 1999).

Asimismo, es bien conocido que si un EI es precedido por una señal discreta durante la preexposición el Efecto de preexposición al EI se atenúa. De acuerdo con una hipótesis en términos de habituación, una señal presente durante la fase de preexposición y ausente durante el condicionamiento debería interrumpir el proceso de habituación al EI y por lo tanto éste debería ser tan efectivo en el condicionamiento como un EI no preexpuesto. Sin embargo, hay evidencia de que el Efecto de preexposición al EI no siempre se ve atenuado cuando se señala el EI

en la preexposición (Baker & Mackintosh, 1979; Baker, et al., 1981; Cannon, et al., 1975; Furedy & Doobs, 1972; Randich, 1981; Riley, Jacobs, & LoLordo, 1976).

En esta misma línea, se ha demostrado que cuando la habituación a un cierto estímulo se lleva a cabo en un contexto específico, si ese estímulo habituado se presenta en un contexto novedoso la respuesta hacia este se deshabitúa (Hall, 1991). De acuerdo con esta explicación, el Efecto de preexposición al EI debería atenuarse si las fases de preexposición y condicionamiento se llevan a cabo en contextos diferentes; sin embargo se ha visto que este cambio contextual no siempre supone una disminución del efecto (Baker, et al., 1981; Cannon, et al., 1975; Dacanay & Riley, 1982; de Brugada, González, & Candido, 2003a; Domjan & Siegel, 1983; Ford & Riley, 1984; Rudy, Owens, & Best, 1977; Stewart & Eukelboom, 1978; aunque ver, Best & Domjan, 1979; Braveveman, 1979; Cole, et al., 1996; Krane, 1980; Westbrook & Brookes, 1988).

Por otra parte, los estudios dirigidos a evaluar de manera directa la habituación a los efectos aversivos de los EIs utilizados y su efecto en el condicionamiento posterior ofrecen resultados contradictorios en función del paradigma empleado. Randich & Lolordo (1979a) con un procedimiento de supresión condicionada, utilizaron una medida directa de la habituación de la respuesta incondicionada hacia el EI (shock) durante la fase de condicionamiento. Esta medida de RI compara las repuestas del animal durante un periodo de tiempo inmediatamente posterior a la presentación del EI en el condicionamiento con las respuestas dadas en un intervalo similar previo a la presentación del EC. Los resultados mostraron que esta medida de razón de supresión (medida de RI)

estaba inversamente relacionada con la cantidad de preexposición recibida, es decir, un mayor número de presentaciones del EI durante la preexposición dio lugar a una menor supresión post-shock. Estos autores sugieren que el retraso observado durante el condicionamiento posterior fue debido a un proceso no asociativo. Asimismo, estudios recientes han mostrado que exposiciones repetidas a una débil descarga eléctrica puede reducir su capacidad para evocar una RI (por ej. Hall & Rodriguez, 2010) Dados estos resultados, parece viable la propuesta de que la reducción en la saliencia efectiva del EI inducida por un proceso de habituación pueda contribuir al Efecto de preexposición al EI observado en un paradigma de REC.

En el caso de la aversión al sabor los resultados son diferentes. Aunque la habituación (o el desarrollo de tolerancia) puede jugar un papel en el Efecto de preexposición al EI observado con otros agentes farmacológicos (por ejemplo ver Dacanay & Riley, 1982; Davis, de Brugada, & Riley, 2010, p. para una discusión en el caso de la morfina) esto no parece ser así en el caso del LiCl. Un estudio realizado por Batson (1983) mostró que la disminución de algunas RRII al LiCl (como temperatura rectal y actividad motora) no se reducían después de ocho preexposiciones; aunque este fue un numero suficiente de preexposiciones para observar un efecto de preexposición al EI. Asimismo, otros estudios (de Brugada, et al., 2003a; de Brugada, González, & Cándido, 2003b; de Brugada, et al., 2004) utilizando una RI diferente, el incremento transitorio de neofobia gustativa que se produce tras la administración de LiCl (Domjan & Best, 1977; Symonds & Hall, 2002), no encontraron signos de habituación. Esta RI fue similar tanto en ratas

preexpuestas al LiCl como en ratas que lo probaron por primera vez. Este tratamiento sin embargo, fue suficiente para producir un Efecto de preexposición al EI. Aunque estos resultados sugieren que el Efecto de preexposición puede ser obtenido en ausencia de habituación, no necesariamente implican que la habituación no juegue ningún rol en el Efecto de preexposición al EI. Posteriormente, de Brugada et al. (2005) mostraron como tras 6 inyecciones de LiCl (en lugar de 3), los animales mostraron una reducción de la respuesta de neofobia hacia un sabor nuevo tanto si la administración del EI en el test de RI se produjo de forma oral o mediante inyección (con las mismas claves presentes durante la preexposición), en comparación con los animales que fueron preexpuestos a inyecciones de salino. A pesar de la habituación mostrada en el test tras la preexposición, no se observó una reducción del Efecto de preexposición al EI cuando el LiCl fue administrado en ausencia de la claves de inyección. Estos resultados demuestran como el Efecto de preexposición al EI puede mostrarse en ausencia de habituación y e incluso como cuando se observa una respuesta de habituación el Efecto de preexposición al EI puede no obtenerse.

Los estudios descritos anteriormente parecen sugerir que el proceso de habituación puede jugar un papel en el Efecto de preexposición al EI cuando se utiliza un procedimiento de respuesta emocional condicionada (REC), sin embargo no parece plausible que esté implicada en el efecto en un procedimiento de aversión condicionada al sabor cuando el Ei es LiCl.

## Mecanismos asociativos: la Hipótesis del bloqueo

Entre las explicaciones que se han ofrecido para el Efecto de preexposición al EI, una de las que ha recibido mayor atención es la que explica el efecto en términos de bloqueo asociativo (ver Randich & Lolordo, 1979a para una revisión) y es esta hipótesis una de las que mas evidencia empírica ha aportado (Baker & Mackintosh, 1979; Baker & Mercier, 1982; Baker, et al., 1981; de Brugada, et al., 2004; Randich, 1981; Randich & Lolordo, 1979b; Tomie, et al., 1980). El efecto de bloqueo (Kamin, 1969) demuestra cómo el incremento en la fuerza asociativa de un EC durante el condicionamiento se ve reducido si éste se presenta en compuesto con un segundo EC que ha sido previamente emparejado con ese EI. De acuerdo con ésta hipótesis, la exposición repetida al EI dará lugar a la formación de una asociación entre las claves contextuales presentes y el EI, y es esta asociación la que interfiere con la adquisición de la asociación entre el EC-EI cuando el condicionamiento se lleva a cabo en ese mismo contexto.

Aunque los principales modelos asociativos explican el Efecto de preexposición al EI en términos de un déficit de adquisición (Mackintosh, 1975; Pearce & Hall, 1980; Rescorla & Wagner, 1972; Wagner, 1981) también han sido sugeridas explicaciones en términos de déficit de recuperación (Bouton, 1993; Miller & Matzel, 1988). Esta última interpretación supone que la asociación contexto-EI no interfiere con la adquisición de la asociación entre el EC y el EI en el condicionamiento; sino que la interferencia se produce en su manifestación en una prueba posterior cuando ésta se realiza en el mismo contexto.

De acuerdo con el modelo de Rescorla y Wagner (1972) se asume que durante la fase de preexposición se produce un condicionamiento excitatorio entre el contexto y el EI de forma que en una fase posterior de condicionamiento, al estar el EI perfectamente predicho por el contexto, el EC no adquiere fuerza asociativa, es decir, no se aprenderá nada acerca del EC. Si durante la preexposición, el condicionamiento excitatorio entre el EI y el contexto no alcanza la asíntota de aprendizaje, en el condicionamiento el Ei no será completamente predicho por el contexto, y el EC podrá adquirir fuerza asociativa cuando sea emparejado con el EI (Rescorla & Wagner, 1972).

Los modelos atencionales explican el Efecto de preexposición al EI en términos de cambios en la atención y por tanto en la asociabilidad de los estímulos en función de la experiencia previa. Según el modelo de Mackintosh (1975) no se aprenderá la asociación EC-EI tras la preexposición del EC por no ser éste EC el mejor predictor de las consecuencias de EI (al estar ya predichas por el contexto). De acuerdo con el modelo de Pearce y Hall (1980) el retraso en el condicionamiento se debe a una disminución a la atención prestada al EC debido a que sus propias consecuencias ya están perfectamente predichas por el contexto. Una característica importante de éstos modelos atencionales es su predicción sobre el fenómeno de Inhibición Latente. Esto resulta relevante para el Efecto de preexposición al EI puesto que predice que si la preexposición se lleva a cabo en un contexto familiar, la adquisición de una asociación entre el contexto y el EI se verá dificultada.

Finalmente, de acuerdo con Wagner (1981) la asociación formada durante la preexposición entre el contexto y el EI, dificultará el aprendizaje posterior entre el EC y el EI puesto que éste se encontrará activado asociativamente por el contexto en un estado A2 y por tanto se procesará con mayor dificultad.

Una predicción común a los modelos de déficit en la adquisición es que el Efecto de preexposición al EI se observará cuando el condicionamiento se lleve a cabo en presencia de las misma claves contextuales presentes durante a preexposición, la ausencia de estas claves durante el condicionamiento impediría el bloqueo de la asociación EC-EI y por tanto el condicionamiento se adquiriría normalmente.

Los modelos de déficit en la recuperación difieren únicamente de la explicación en términos de bloqueo por el contexto en suponer que las señales contextuales ejercen su efecto en la fase de prueba mas que durante el condicionamiento (Bouton, 1993; Miller & Matzel, 1988).

De acuerdo con esto, la fuerza del Efecto de preexposición al EI dependerá de la fuerza de la asociación entre el contexto y el EI por lo que una e las implicaciones de la hipótesis del bloqueo por el contexto es que debería ser sensible a las manipulaciones que afectan al bloqueo asociativo.

Se ha demostrado que las claves contextuales pueden asociarse con un EI del mismo modo que cualquier estímulo nominal (Colwill, Absher, & Roberts, 1988; Domjan, Greene, & North, 1989; Sheafor, 1975; Symonds & Hall, 1997). La hipótesis del bloqueo por el contexto predice que si estas claves contextuales presentes durante la preexposición no se encuentran presentes en la fase de

condicionamiento, la asociación EC-EI debería formarse con la misma intensidad que si el EI no hubiese sido preexpuesto. Braveman (1979) encontró que el efecto de preexposición al EI solo se mostraba cuando tanto la preexposición como el condicionamiento se llevaban a cabo en el mismo contexto. Estos resultados son consistentes con la hipótesis del bloqueo por el contexto a la hora de explicar el efecto de preexposición al EI (Baker, et al., 1981; Cole, et al., 1996; Dacanay & Riley, 1982; Domjan & Best, 1977; Krane, 1980; Westbrook & Brookes, 1988) Sin embargo existen estudios que no se muestran consistentes con la hipótesis del bloqueo contextual arriba expuesta y que muestran como un cambio de contexto no siempre atenúa el Efecto de preexposición al EI (Cannon, et al., 1975; Dacanay & Riley, 1982; de Brugada, et al., 2003b; Domjan & Best, 1980; Domjan & Siegel, 1983; Ford & Riley, 1984; Rudy, et al., 1977; Stewart & Eukelboom, 1978)

Una implicación de la hipótesis de bloqueo contextual es que las manipulaciones que afectan al bloqueo asociativo, como la familiaridad con el contexto de preexposición, la extinción de la asociación contexto-EI, señalar el Ei durante la preexposición, el desbloqueo y la introducción de intervalos temporales entre las fases del procedimiento deberían atenuar el Efecto de preexposición al EI.

Si el contexto de preexposición es familiar, la asociación entre éste y el EI durante la preexposición debería verse atenuada puesto que se encuentra inhibida latentemente y por tanto su asociabilidad es menor. Esta menor asociación entre el contexto y el EI permitirá que en el condicionamiento la EC-EI se adquiera mejor, por lo que el Efecto de preexposición al EI se verá atenuado (Hinson, 1982). Aunque algunos estudios sugieren que un contexto familiar atenúa el Efecto de

preexposición al EI en diversas preparaciones como por ejemplo, condicionamiento parpebral (Hinson, 1982), respuesta emocional condicionada –(REC) o condicionamiento de aversión al sabor (Batson & Best, 1979; Cole, et al., 1996; Klein, Mikulka, & Lucci, 1986; Miller, Jagielo, & Spear, 1993) otros estudios han mostrado un efecto de preexposición al EI cuando todas las fases del entrenamiento se han realizado en un contexto familiar (Aguado, et al., 1997; Cannon, et al., 1975; de Brugada, et al., 2003a; de Brugada, et al., 2003b; de Brugada, et al., 2004; Gil, Symonds, Hall, & de Brugada, 2011; Willner, 1978); .

Los resultados previos que demuestran que, cuando se utiliza una preparación de aversión condicionada al sabor, el Efecto de preexposición al EI no siempre es específico al contexto y que un robusto efecto puede ser obtenido cuando el contexto de preexposición es altamente familiar llevaron a la consideración de una hipótesis modificada del bloqueo en este caso (de Brugada, et al., 2004). Esta hipótesis inicialmente propuesta por by Rudy, Owens, & Best (1977) pone el énfasis en el papel de las claves relacionadas con la administración de la inyección. Aunque el contexto pueda haber sufrido inhibición latente estas claves de inyección son nuevas y únicamente predicen la náusea que sigue a la inyección. Se puede por tanto esperar que estas claves adquieran fuerza asociativa y bloqueen la posterior adquisición de la asociación EC-EI. Evidencia para esta interpretación del bloqueo viene dada en una serie de experimentos realizados por de Brugada et al. (2004) en los cuales el EI consistió en el consumo oral del LiCl. La aversión producida por este procedimiento no fue atenuada por la preexposición de inyecciones de LiCl, sugiriendo que las claves de inyección pueden ser las

responsables del bloqueo cuando el EI fue administrado vía inyección. En la ausencia de tales claves la aversión procedió normalmente. Apoyo para este análisis viene de la observación de que introducir tales claves (mediante inyecciones de salino) previamente al consumo oral del LiCl restaura el Efecto de preexposición al EI. Estos resultados permiten asegurar que cuando la preexposición al EI consiste en inyecciones de LiCl dadas en un contexto altamente familiar como las jaulas dormitorio, no existe evidencia ni de que la habituación ni el condicionamiento contextual jueguen ningún papel en producir el efecto de preexposición y que éste se debe exclusivamente al bloqueo por las claves de inyección.

Otra de las manipulaciones que afectan al Efecto de preexposición al EI sería mediante la reducción de la fuerza asociativa entre las claves contextuales presentes durante la preexposición y el EI, extinguiendo éstas de forma previa al condicionamiento. Randich (1981), utilizando un procedimiento de respuesta emocional condicionada (REC), obtuvo una atenuación del efecto de preexposición al EI extinguiendo las claves contextuales tras la preexposición. Los animales recibieron un periodo de extinción después de la preexposición en el que eran expuestos al contexto en el que esta tuvo lugar. Los resultados mostraron una razón de supresión en estos animales similar a los animales que no habían recibido preexposición a la descarga eléctrica, mientras que aquellos para los que las claves contextuales no se extinguieron mostraron un efecto de preexposición al EI (Baker & Mercier, 1982; de Brugada & Aguado, 2000; Hinson, 1982; Tomie, 1976b).

Asimismo, se ha demostrado que si un EI es precedido por una señal discreta durante la preexposición el Efecto de preexposición al EI se atenúa (Baker & Mackintosh, 1979; Baker, et al., 1981; Randich, 1981). De acuerdo con una hipótesis en términos de bloqueo, una señal presente durante la fase de preexposición y ausente durante el condicionamiento debería anular el bloqueo de la asociación EC-EI. Al no estar presente la señal asociada al EI durante la preexposición en la fase posterior de condicionamiento, la asociación EC-EI debería adquirirse como en una situación en la que el EI no ha sido preexpuesto. Sin embargo, hay evidencia de que el Efecto de preexposición al EI no siempre se ve atenuado cuando se señala el EI en la preexposición (Cannon, et al., 1975; Furedy & Doobs, 1972; Riley, et al., 1976).

Por otra parte, el efecto de preexposición al EI puede atenuarse mediante un proceso de desbloqueo. La hipótesis del bloqueo por el contexto asume que la asociación previa entre el contexto y el EI (en la fase de preexposición) bloquea la adquisición de la asociación entre el EC y el EI durante el condicionamiento. Sin embargo, si entre las fases de preexposición y condicionamiento se cambia la intensidad del EI, el Efecto de preexposición al EI se verá atenuado (ver también (Mis & Moore, 1973; Randich & Lolordo, 1979b; Taylor, 1956).

De igual forma, otra de las manipulaciones que afecta al Efecto de preexposición al EI supone introducir un intervalo de retención entre las fases de preexposición y prueba. Por ejemplo, utilizando una preparación de aversión condicionada al sabor con LiCl como EI, Aguado, de Brugada & Hall (1997) mostraron como un intervalo temporal entre la preexposición y el

condicionamiento resultó en una atenuación del Efecto de preexposición al EI (ver también Cannon, et al., 1975). Aunque, como ya se ha explicado anteriormente, éstos resultados pueden ser explicados en términos no asociativos, también son susceptibles de una explicación en términos asociativos. De acuerdo ésta última hipótesis, un intervalo de retención entre la preexposición y el condicionamiento podría resultar en una debilitación de la asociación contexto-EI, esta asociación contexto-EI debilitada tendrá menos fuerza para bloquear la asociación EC-EI (Aguado, et al., 1997; Cannon, et al., 1975), ésta predicción es apoyada tanto por los modelos que se basan en un déficit de adquisición como por aquello que se centran en un déficit de recuperación. Sin embargo, si el intervalo temporal se ubica entre las fases de condicionamiento y prueba, los distintos modelos tienen predicciones distintas. Un intervalo de retención entre el condicionamiento y la prueba no debería afectar al Efecto de preexposición al EI de acuerdo con los modelos basados en un déficit de adquisición (Aguado & de Brugada, 1997; Aguado, et al., 1997), pero sí atenuaría el efecto de acuerdo con modelos basados en déficit de recuperación (Miller, et al., 1993).

De las diferentes teorías que se han ofrecido para explicar el Efecto de preexposición al EI, la mayor parte de los estudios se han centrado en dos; la hipótesis del bloqueo asociativo y la hipótesis en términos no asociativos (habituación). Éstas dos explicaciones del efecto no tiene porqué ser excluyentes entre sí y pueden estar actuando al mismo tiempo (ver Hall, 2009; Randich & Lolordo, 1979a; Riley & Simpson, 2001). Se ha demostrado que las presentaciones repetidas a un EI (LiCL) pueden reducir la respuesta incondicionada (de Brugada,

et al., 2005), aunque el rol que la habituación juega en el efecto aun está por determinar. La hipótesis del bloqueo asociativo ha recibido una mayor atención y su papel en el Efecto de preexposición al EI ampliamente demostrado (Randich, 1981; Randich & Lolordo, 1979a) y existe evidencia de que una asociación contexto-EI puede bloquear el aprendizaje entre el EC y el EI en el condicionamiento. Las diferencias entre los distintos estudios y la falta de demostraciones explícitas del papel de la habituación en algunas procedimientos, dejan abierta la investigación acerca de la posible concurrencia de ambos procesos en el Efecto de preexposición al EI.

### Preexposición al EI con condicionamiento apetitivo

Aunque, como se ha expuesto anteriormente, la mayor parte de la investigación acerca del efecto del preexposición al EI se ha realizado con procedimientos aversivos como la respuesta emocional condicionada -REC- (ver Randich & Lolordo, 1979a) y con condicionamiento de aversión al sabor (ver Hall, 2009 para una revisión), existen menos demostraciones del efecto utilizando el procedimiento apetitivo de automoldeamiento (ver por ejemplo Balsam & Schwartz, 1981; Costa & Boakes, 2009; Timberlake, 1986). Al igual que en caso del condicionamiento aversivo, el efecto de preexposición al EI con un procedimiento apetitivo se ha explicado en términos de bloqueo por claves contextuales (ver Tomie, 1976a; Tomie, 1976b). Aunque parece poco plausible que un proceso de habituación pueda reducir la capacidad reforzadora de la comida en un animal

hambriento, no se descartar esta posibilidad. Se ha demostrado que, al menos con sujetos humanos, la preferencia por un tipo determinado de comida decae cuando se presenta de forma repetida (ver Hetherinton, Pirie, & Nabb, 2002; Meiselman, de Graaf, & Lesher, 2000). Parece por tanto plausible (aunque no se ha estudiado directamente) que el poder reforzante de una cierta comida pueda sufrir un decaimiento. Sin embargo, en el caso de el Efecto de preexposición al EI cuando se utiliza un procedimiento de condicionamiento apetitivo como el automoldeamiento se ha ofrecido una explicación mas sencilla que puede explicar los resultados en términos de competición de respuestas a nivel periférico (Costa & Boakes, 2009; Van Hest, et al., 1989). El procedimiento de preexposición que se utiliza en el automoldeamiento (Brown & Jenkins, 1968) puede establecer una respuesta de acercamiento al comedero que en una posterior fase de automoldeamiento podría interferir con la adquisición de la respuesta de seguimiento de señal. Sin embargo, la explicación del Efecto de preexposición al EI en términos de competición de respuestas a nivel periférico se encuentra limitada al procedimiento experimental de automoldeamiento y por tanto no puede ofrecer un explicación general del efecto. Uno de los objetivos del este trabajo se centra en demostrar el efecto de preexposición al Ei utilizando un procedimiento de condicionamiento apetitivo que no sea susceptible de explicarse en términos de competición de respuestas a nivel periférico como en el caso del automoldeamiento. Para ello, se va a utilizar un paradigma de preferencia condicionada al sabor (PCS). La ventaja de éste tipo de condicionamiento apetitivo permite demostrar el efecto de preexposición al EI con un procedimiento de

condicionamiento apetitivo que no puede ser explicado en términos de competición de respuestas.

## El paradigma de Preferencia condicionada al sabor (PCS)

Este paradigma de PCS se ha utilizado para explorar cuales son los mecanismos por los cuales se rigen las preferencias condicionadas. Se ha demostrado que existen preferencias innatas hacia ciertos sabores como el sabor dulce o salado (Hall & Bryan, 1981), mientras que otras sustancias con sabores amargo o ácido tienden a producir un rechazo innato (Hall & Bryan, 1981).

Mediante el procedimiento de PCS, la preferencia inicial por un sabor neutro (o incluso inicialmente aversivo) se incrementa mediante la presentación de éste en compuesto con un estímulo incondicionalmente preferido (EI). Los mecanismos para explicar la adquisición de estas preferencias se han ofrecido en términos de condicionamiento clásico.

Dos de los procedimientos mas usados dentro del paradigma de PCS incluyen la utilización de EEII que tiene tanto un sabor preferido (palatable) como propiedades motivaciones de signo positivo como por ejemplo azúcar (Boakes & Lubart, 1988; Capaldi, Hunter, & Lyn, 1997) o chocolate (Owens, Capaldi, & Sheffer, 1993). Se han propuesto dos formas de aprendizaje en las PCS (Ackroff, 2008; Fedorchak, 1997). Cuando se utiliza un EI palatable que además tiene propiedades motivacionales (consecuencias post-ingesta), como por ejemplo azúcar, el preferencia hacia el EC se verá reforzada por un lado por la palatabilidad del EI

(sabor dulce) y por otro por las consecuencias post-ingesta (nutrientes) que también se asociarán con el EC. Para este tipo de EEII, el mecanismo subyacente que se ha propuesto se basa en un aprendizaje predictivo sabor-calorías. Éste tipo de aprendizaje se ha demostrado mas claramente cuando los sujetos se encuentran en un estado de privación de comida durante la prueba de RC (Bolles, 1961; Fedorchack & Bolles, 1987; Harris, Shand, Carroll, & Westbrook, 2004).

Sin embargo, también se puede obtener PCS utilizando EEII que únicamente tienen la palatabilidad como valor reforzante (Diaz, de la Casa, & Beayens, 2004; Holman, 1975, 1980). En este último caso, el mecanismo responsable que se ha propuesto para explicar este tipo de aprendizaje se basa en un cambio condicionado en la palatabilidad del EC (aprendizaje sabor-sabor).

Los experimentos incluidos en esta tesis se basan en preparaciones del paradigma de PCS utilizando tanto EEII palatables con consecuencias post-ingesta como EEII palatables sin consecuencias motivacionales. Por un lado se examina si el efecto de preexposición al EI es susceptible de ser obtenido con este paradigma, y por el otro se examinan cuáles son los mecanismos responsables del efecto cuando se utiliza una preparación de PCS.

La ventaja del paradigma de PCS en este caso reside en la posibilidad de preexponer el EI directamente mediante una solución oral antes del condicionamiento, éste procedimiento excluye la posibilidad de la formación e una respuesta periférica (competición de respuestas) durante la preexposición, que pueda interferir en el condicionamiento. De este modo, la obtención del Efecto de preexposición al EI con un procedimiento de PCS proporciona un una preparación

paralela al caso aversivo, que puede ser susceptible de explicarse en los mismos términos (bloqueo contextual o habituación), proporciona además un método alternativo para explorar la validez de éstas dos explicaciones principales.

El primer objetivo de éste trabajo de tesis es encontrar una demostración del efecto de preexposición al EI utilizando un procedimiento apetitivo que no sea susceptible de ser explicado en términos de competición de respuesta como en el caso del automoldeamiento. Un vez demostrado este efecto básico, se estudiará cual es el papel de los procesos no asociativos (habituación) y asociativos (bloqueo contextual) en efecto de preexposición a EI utilizando el procedimiento de PCS.

# CHAPTER II

## EXPERIMENTS 1-2:

## DEMONSTRATION OF THE US-PREEXPOSURE FFECT

# CHAPTER II: DEMONSTRATION OF THE US-PREEXPOSURE EFFECT

## USING AN APPETITIVE PROCEDURE

### Introduction

As mentioned in the introduction, previous demonstrations of the US-preexposure effect using an appetitive stimulus as the US have come primarily from experiments using autoshaping procedures. The results of these experiments are open to an explanation in terms of competing responses (Timberlake, 1986). The US-preexposure procedure involves repeated presentations of the unconditioned stimulus prior to conditioning and as it has been suggested (Costa & Boakes, 2009; Van Hest, et al., 1989) that this preexposure phase can establish persistent food-tray directed behaviour which could interfere with the acquisition and performance of signal-directed responding. The effect seen in these experiments could thus be a consequence of response competition at a peripheral level.

It seems unnecessary, therefore, to explain the appetitive US-preexposure effect in terms other than the competing response mechanism (e.g., in terms of blocking by the context or of habituation) when the effect has been demonstrated using the autoshaping procedures. However, a different version of the appetitive US-preexposure effect has been provided by Harris et al. (2000), using sucrose as the unconditioned stimulus (US) and the conditioned flavour preference procedure. In this study, Harris et al. gave rats exposure to sucrose before training with an odour-sucrose compound. They found a reduced preference for the odour,

when it was presented in a preference test, in animals preexposed to sucrose prior to the conditioning phase. There is no obvious competing response explanation for this result. Furthermore, given that all the phases in this study were carried out in an experimental context (i.e., not the home cage), the findings can be readily explained in terms of blocking by contextual cues. However, these results do not offer a complete demonstration of the US-preexposure effect due, for instance, to the lack of a control group that received the same training with the odour-sucrose compound but were given preexposure to mere water and therefore it this demonstration of the US-preexposure effect does not allow the possibility to directly study the different mechanism responsible for the effect. The aim of the experimental work presented in this chapter was to provide a demonstration of an appetitive US-preexposure effect, based on the flavour preference procedure used by Harris et al., that would not be susceptible to explanation in terms of response competition.

In the experiments included in this chapter we made use of a conditioned flavour preference (CFP) procedure was used in which consumption of a neutral flavour is enhanced by prior experience of that flavour presented in compound with a sucrose solution. CFP procedures have been mainly explored using USs with a palatable flavour that also have motivational consequences such as sucrose (Boakes & Lubart, 1988; Capaldi, et al., 1997) or chocolate (Owens, et al., 1993). It has been widely proposed that two forms of learning occur with this type of conditioning (Ackroff, 2008; Fedorchak, 1997). When using a palatable US with nutritional consequences (such as sucrose) in a CFP procedure, the conditioned

stimulus (CS) will be reinforced at oral and post-oral levels. On the one hand, the palatability of the US will enhance the preference for the CS, and on the other, the nutrients obtained post-orally will become associated with the CS. CFP can also be obtained when the US is a non-nutritive but palatable substance such as saccharin (Diaz, et al., 2004; Holman, 1975, 1980) and in this case the mechamism can only be that involving a conditioned change in palatability (i.e., flavour-flavour association rather than flavour-nutrient learning).

The effect of the flavour-nutrient form of learning is most clearly seen when the animals are hungry during the test (Bolles, 1961; Fedorchack & Bolles, 1987; Harris, et al., 2000), and this procedure was adopted in the experiments included here. But if either of the forms of learning responsible for CFP is susceptible to the US-preexposure effect, prior exposure to sucrose should restrict the development of a conditioned preference.

The advantage of using a conditioned flavour preference procedure in this case is that it is possible to give prior exposure to the US simply by giving sucrose in the animal's drinking water before the start of conditioning, a procedure that precludes the formation of any obvious potentially competing response. A further advantage is that the rat's initial reaction to a strong sucrose solution is to show neophobia; monitoring consumption thus allows the possibility of assessing the degree to which this aspect of responding to the US shows habituation.

Experiment 1 sought to confirm that animals receiving a compound of a neutral flavour (CS) paired with sucrose (US) will show a higher preference for the CS in a further two-bottle choice test than rats that had experienced the stimuli on

separate trials (Boakes & Lubart, 1988; Capaldi, et al., 1997). The aim of Experiment 2 was to then ascertain whether such learning would be restricted by giving prior exposure to the sucrose before the conditioning phase, that is, whether a US-preexposure effect could be obtained using the conditioning parameters employed in Experiment 1.

## Experiment 1: Basic flavour-preference conditioning using sucrose as the unconditioned stimulus (US)

Experiment 1 was carried out in collaboration with M. Symonds in the Behavioural Neuroscience Laboratory, University of York. Half of the animals received trials in which a neutral flavour (CS) was simultaneously paired with sucrose (US) - Group Simultaneous - and the other half of the animals experienced alternate trials of either the CS (neutral flavour) or the US (sucrose) - Group UNP. In a subsequent test phase, all animals were given a preference test in which the CS was presented in conjunction with water (see Table 1). If the CS acquires excitatory properties during the conditioning trials, then a higher preference for the CS will be expected for the simultaneous group than for the unpaired group (given that the CS had never been presented in compound with the US for this group).

**Table 1**

**Experimental designs**

| EXPERIMENT 1 | | | | |
|---|---|---|---|---|
| **Group** | **Conditioning** | | **CR Test** | |
| **SIM** | 4 M + SUC | | M vs. W | |
| **UNP** | 4 M / SUC | | | |
| **EXPERIMENT 2** | | | | |
| **Group** | **Preexposure** | **UR Test** | **Conditioning** | **CR Test** |
| **PRE** | 8 SUC | SUC | 4 M + SUC | M |
| **CNT** | 8 W | | | |

***Note*.** SUC refers to a 20% sucrose solution; M refers to a 2% mint solution; W refers to tap Water. SIM: Group Simultaneous; UNP: Group Unpaired; PRE: preexposed; CNT: control. All trials in Experiment 2 occurred in an experimental context.

**Method**

*Subjects and apparatus.* The subjects were 16, experimentally naïve, male hooded Lister rats, obtained from Charles River Laboratories. They were housed individually in home cages measuring 35 x 22 x 19 cm, and made of translucent white plastic with wood shavings as bedding. The rats were maintained on a 12h light / 12h dark cycle (lights on at 8:00 a.m.). All the experimental procedures were conducted in the home-cages and during the light phase of the cycle. The US was a 20% (w/v) sucrose solution and the CS was a 2% (v/v) solution of mint (peppermint flavouring supplied by Supercook; Leeds, UK). The compound of sucrose and mint presented during conditioning was made up so as to preserve these concentrations. All the solutions were made with tap water and given to the animals in 50-ml graduated tubes fitted with a rubber stopper and a stainless steel ball-bearing tipped spout. Fluid intake was measured by weighing tubes before and after sessions.

*Procedure.* The rats were assigned to two equal-sized groups at the beginning of the experiment. To initiate a schedule of water deprivation, the standard water bottles were removed overnight; over the next two days access to water was restricted to two 30-min sessions per day (starting at 10:30 a.m. and 4:30 p.m.). During the conditioning phase the presentations of the stimulus in the Unpaired Group were counterbalanced. Thus, half of the animals in this group received 10 ml of the US solution during the morning session and 10 ml of the CS solution in the afternoon session, the pattern for the other half of the animals was

reversed. Group Simultaneous had all the compound (10 ml) trials in the morning sessions. In the afternoon of the last conditioning trial, food was removed for all animals. On the morning of the following day after the last conditioning trial, the rats were given access to the mint solution for 30-min as well as to the water, the conditioned response test (CR). The position of the bottles during the CR test was counterbalanced.

**Results & Discussion**

During the training period all animals drank all the solution that it was available, with the exception of the first trial in which rats had shown a slight neophobic response. Figure 1 shows the group means for consumption of the mint solution during the final test. A basic conditioning effect is evident with Group Simultaneous showing a markedly higher preference for the mint than Group Unpaired. An analysis of variance (ANOVA) with groups as factor conducted on the data displayed in Figure 1 showed a significant effect of group $F$ (1,14) = 4.34. The higher consumption of the CS solution for animals in the simultaneous group has two possible sources. Pairing a neutral odour with a substance that has post-oral positive motivational consequences (e.g. sucrose has nutrients) allows the formation of an association between the CS and the nutrients from sucrose. The substance used in this experiment as the US (sucrose) also has a palatable taste (sweet). Pairing this particular US with a CS (Group SIM) allows the occurrence of

flavour-flavour learning between the CS and the palatable taste of the sucrose. This type of learning too will increase the consumption on test for Group SIM.

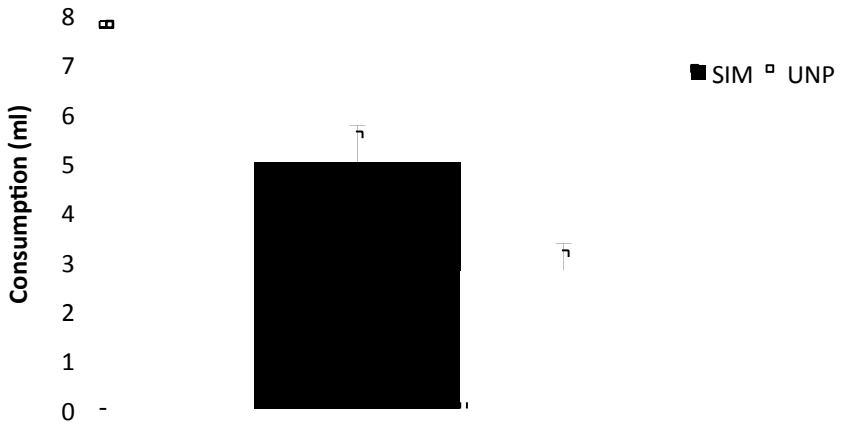


**Figure 1. Experiment 1:** Group means score for both simultaneous (SIM) and unpaired (UNP) groups on the test with the conditioned stimulus (CS). Vertical bars represent SEMs.

## Experiment 2: Demonstration of the US-preexposure effect with sucrose as the unconditioned stimulus (US)

Experiment 1 established that animals receiving conditioning trials in which a neutral odour was paired with a high concentration of sucrose in a compound showed a higher preference for the CS than animals given the CS and US in alternate unpaired trials.

The aim of Experiment 2 was to determine whether a US-preexposure effect could be obtained with the conditioning procedure established in Experiment 1. Two groups of rats received flavour-preference conditioning in which the novel flavour of mint was paired with a sucrose solution (US). This was followed by a test

in which the mint was presented alone - although I have referred to this procedure as flavour-preference conditioning, the term conditioning flavour acceptance is sometimes used for the behaviour shown on this form of test in which absolute consumption is measured, rather than a choice between alternative flavours. Rats in the preexposed group experienced presentations of the sucrose solution on eight occasions before the start of conditioning; those in the control group were given equivalent access to water. Lesser consumption of mint on the CR test in the preexposed group compared to the control group would indicate the occurrence of the US-preexposure effect. Experimental treatments were given in a novel and distinctive context to maximise the likelihood that effects depending on context conditioning would be obtained. In order to assess the extent to which preexposure reduced the neophobic response to sucrose, all subjects were given a single trial prior to conditioning in which consumption of sucrose was measured in an unconditioned response test (UR test).

**Method**

*Subjects and apparatus.* The subjects were 16, experimentally naïve, male hooded Lister rats, obtained from Charles River Laboratories (mean weight of 345 g) and maintained in the same conditions as those in Experiment 1. Experimental procedures were conducted in a distinctive experimental context. This context was a room located in a separate part of the laboratory, dimly lit by a 30-W red lamp; a background of continuous white noise (70dB) was provided by a speaker close to a

rack of cages measuring 33 x 20 x 19 cm. They differed from the homecages in that the walls were made of clear plastic and the floor was covered in commercially obtained cat litter. The substances and apparatus used during this experiment were the same as those used in Experiment 1.

*Procedure.* The rats were assigned to two equal-sized groups at the beginning of the experiment. To initiate a schedule of water deprivation, the standard water bottles were removed overnight; over the next two days access to water was restricted to two 30-min sessions per day (starting at 10:30 a.m. and 4:30 p.m.). Water continued to be made available in the home cage during the afternoon drinking session throughout the experiment. The next eight days constituted the preexposure phase. All rats were transferred to the experimental context for the morning session where they were given access for 30 min to 15 ml of the sucrose solution (the preexposed group) or 15 ml of water (the control group). On the next day all animals received access to 30 ml of sucrose for 30 min (the UR test). The next four days constituted the conditioning phase, in which all subjects were given 10 ml of the mint -sucrose compound in each morning session. After the last conditioning session, the rats were deprived of food; on the next day they received access to water for 30 min in the home cage in both morning and afternoon drinking sessions, and also had access to 10 g of food during the afternoon session. On the morning of the following day, the rats were given access to the mint solution for 30-min in the training context (the CR test).

## Results and discussion

Consumption of fluid during the preexposure phase (of sucrose for the preexposed group; of water for the control group) is shown in Figure 2. The amount consumed increased gradually over trials in both groups, perhaps as a consequence of habituation of an exploratory response to the context (a response that could interfere with drinking). In addition, consumption was particularly suppressed on Trial 1 in the group given sucrose, indicating a neophobic response to this substance. An analysis of variance (ANOVA) was conducted on the data summarised in the figure, with group and trial as the variables. There was a significant main effect of trial, $F$ (7, 98) = 19.77, but not of group, $F < 1$. The interaction between the variables was significant, $F$ (7, 98) = 2.72. Analysis of this interaction showed the difference between groups to be significant only on Trial 1, $F$ (1, 14) = 8.30; the next biggest difference, on Trial 3, yielded $F$ (1, 14) = 2.34. p = 15).

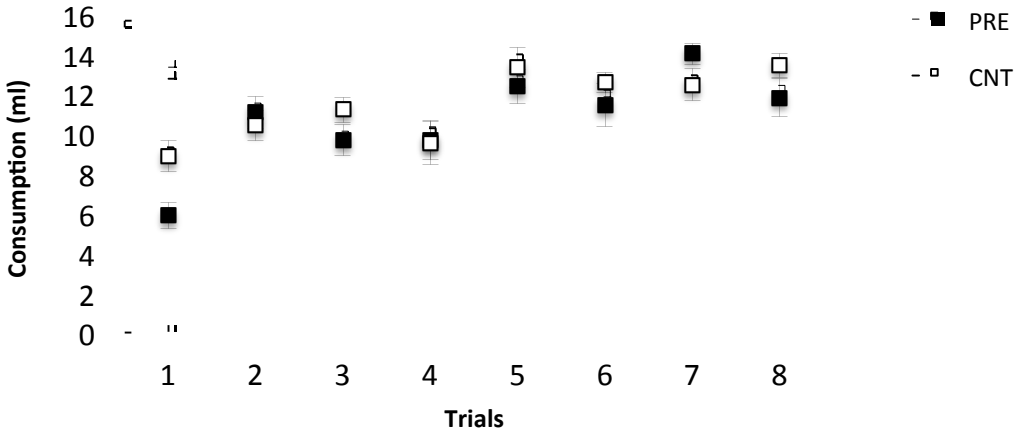


**Figure 2. Experiment 2:** Mean consumption scores during preexposure for the preexposed (PRE) and control (CNT) groups. Animals in Group PRE received sucrose; those in Group CNT received access to water. Vertical bars represent SEMs.

Figure 3 shows a difference between the groups on the UR test in that the control subjects drank marginally less sucrose than the preexposed subjects; this difference, however, fell short of statistical significance, however, $F$ (1, 14) = 1.74.

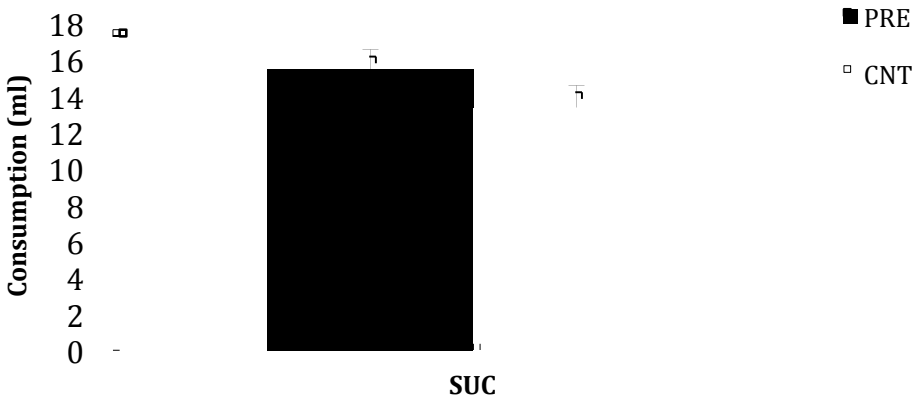


**Figure 3. Experiment 2:** Group mean scores on the unconditioned response (UR) test in which all animals received a one-bottle test with free access to a sucrose solution (US). Vertical bars represent SEMs.

The groups differed in their consumption of the mint-sucrose compound when it was first presented. Figure 4 shows that on the first conditioning trial, rats in the control group drank significantly less of the compound than those in Group Preexposed; thereafter, they drank all that was available. An ANOVA conducted on the last trial of conditioning did not show any differences between the groups, $F$ < 1, But the difference between the groups on the first trial was statistically reliable, $F$ (1, 14) = 17.94. The source of this effect is not clear – possibly a neophobic reaction to mint interacted with a similar response to the, still novel, sucrose to produce particularly marked suppression of drinking in the control group.

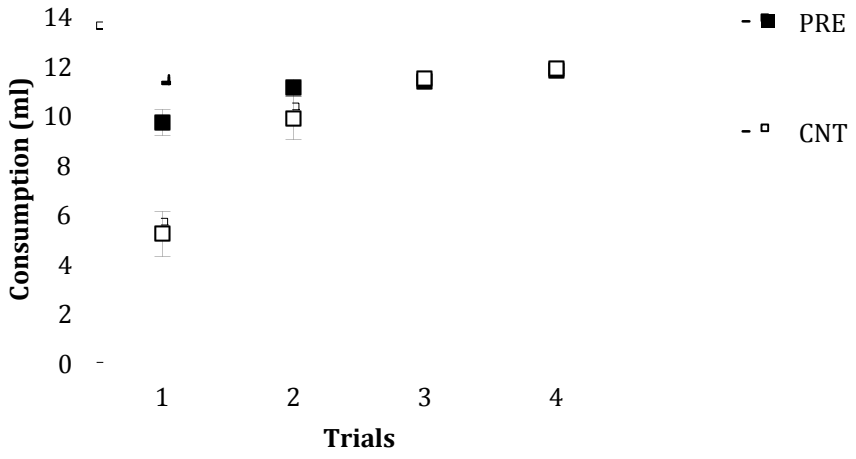


**Figure 4. Experiment 2:** Group means for consumption of the mint-sucrose compound solution during the conditioning phase for animals in the preexposed (PRE) and the control (CNT) groups. Vertical bars represent SEMs.

The critical results for the final CR test with mint are shown in Figure 5. In spite of the fact that the control subjects consumed somewhat less of the mint-sucrose compound during conditioning, these subjects drank more of the mint solution on test than did the preexposed subjects, $F$ (1, 14) = 30.65. That is, conditioning proceeded less readily in subjects given preexposure to sucrose, a demonstration of the US-preexposure effect.

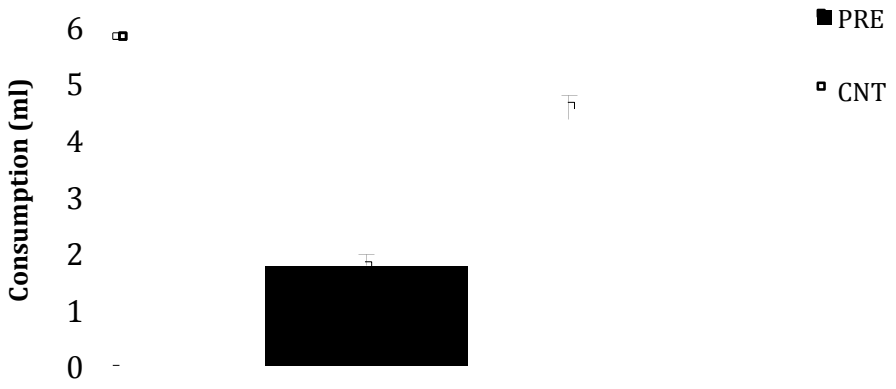


**Figure 5. Experiment 2:** Group means for consumption of the mint solution during CR test for animals in the preexposed (PRE) and the control (CNT) groups. Vertical bars represent SEMs.

## General Discussion

The experiments reported in this chapter show that rats can develop an increased acceptance of a novel flavour as a consequence of its pairing with a strong sucrose solution (Experiment 1). The strength of this conditioning effect, is, however, reduced when the subjects have previously been given exposure to the sucrose prior to conditioning – a US preexposure effect (Experiment 2). Demonstrating the effect with this particular version of appetitive conditioning is of interest since it rules out explanations based upon competing responses that were presented previously.

The next step is to determine what the source of this effect might be. There is evidence from Experiment 2 that the initial response to sucrose habituates with repeated presentation and it is possible (but by no means necessary) that the

reinforcing power of this substance changes along with the observed UR. An explanation of this sort, based on the change in effectiveness of the preexposed stimulus, cannot be ruled out on the basis of the present data. A second, and perhaps more obvious mechanism, however, could be one based on the contextual cues which are present during the preexposure and conditioning phases. In particular, blocking by context seems a real possibility, given that the use of a novel context for the preexposure phase will presumably increase the likelihood that contextual cues will form an association with events that occur in their presence. This issue is taken up in Chapter 3.

# CHAPTER III

## EXPERIMENTS 3-4: A ROLE FOR BLOCKING BY CONTEXT IN THE US-PREEXPOSURE EFFECT?

# CHAPTER III: EXPERIMENTS 3-4: A ROLE FOR BLOCKING BY CONTEXT IN THE US-PREEXPOSURE EFFECT?

## Introduction

As described previously, one mechanism that has been suggested to account for the US-preexposure effect is blocking (Kamin, 1969) by contextual cues. In particular, preexposure to the US will allow the formation of a context-US association which could interfere with the formation of a CS-US association during a subsequent phase of conditioning (Randich & Lolordo, 1979a). Although there is some evidence to support the possible role of blocking by context in other instances of the US-preexposure effect, for example using a conditioned taste aversion procedure (Baker, et al., 1981; Cole, et al., 1996; de Brugada, Candido, & Gonzalez, 2000; de Brugada, et al., 2004) or a conditioned fear procedure (Randich & Lolordo, 1979b), its role in the effect produced using an appetitive US is yet to be determined.

The blocking-by-context hypothesis generates two clear predictions regarding the conditions under which the US preexposure effect should most readily emerge. In particular, the US preexposure effect found in Experiment 2 could be particularly susceptible to an explanation in terms of blocking by context, since all phases of the experiment were conducted in a novel set of cages that were distinct from the usual home cage. These novel contextual cues can be expected to more readily enter into an association with the US than familiar (i.e. latently

inhibited) cues provided by the home cage. The blocking account therefore suggests that the US preexposure effect should be stronger when the procedure is carried out in a novel context, compared to the case in which the experiment is conducted in a familiar home cage environment. A further implication of this account is that the effect should be attenuated by a change of context between the preexposure and conditioning phases – such a context shift would cause the context-US associations formed during preexposure to be powerless in producing a blocking effect since a different set of contextual cues would be present during conditioning.

The aim of the experiments reported in this chapter was to directly test these predictions, by comparing the effectiveness of using the home cage and novel context environments in generating the US preexposure effect (Experiment 3), and by examining the effect of a context shift between the preexposure and conditioning phases (Experiment 4).

## Experiment 3: US preexposure effect in a familiar versus novel context

The aim of this experiment was to confirm the reliability of the US-preexposure effect obtained in Experiment 2, and to begin an analysis of the possible role of contextual cues. There were four groups of subjects (see Table 1). Two, the preexposed-context and control-context groups matched the groups of Experiment 1. The other two groups, preexposed-home and control-home, were treated identically except that they remained in their home cages throughout the

experiment, and all experimental treatments were given there. Given the well-established latent inhibition effect, we assume that the contextual cues of the very familiar home environment will be less likely to form associations with events that occur in their presence than will the cues provided by a novel context. If the US-preexposure effect depends on blocking by context, the effect should be attenuated or abolished in the groups trained in the home cage.

**Table 2**

**Experimental designs**

| EXPERIMENT 3 | | | | |
|---|---|---|---|---|
| **Group** | **Preexposure** | **UR Test** | **Conditioning** | **CR Test** |
| **PRE HOME** | 8 SUC | SUC | 4 M + SUC | M |
| **CNT HOME** | 8 W | | | |
| **PRE CONTEXT** | 8 SUC | | | |
| **CNT CONTEXT** | 8 W | | | |
| **EXPERIMENT 4** | | | | |
| **Group** | **Preexposure** | **UR Test** | **Conditioning** | **CR Test** |
| **PRE SAME** | 8 SUC (A) | SUC (B) | 4 M + SUC (A) | M (A) |
| **CNT SAME** | 8 W (A) | | 4 M + SUC (A) | M (A) |
| **PRE DIFF** | 8 SUC (A) | | 4 M + SUC (B) | M (B) |
| **CNT DIFF** | 8 W (A) | | 4 M + SUC (B) | M (B) |

***Note.*** SUC refers to a 20% sucrose solution; M refers to a 2% mint solution; Pre: preexposed. Both groups in Experiment 1 and the context groups of Experiment 2 experienced experimental treatments in a novel context. A and B (Experiment 3) refer to different experimental contexts.

## Method

*Subjects and apparatus.* The subjects were 32 experimentally naïve male Wistar rats (from Harlan Laboratories, Italy), with a mean weight of 299 g at the start of the experiment. They were housed and maintained under the same conditions as those described for Experiment 1, with the following exceptions. The home cages measured 50 x 56 x 14.5 cm and were made of transparent plastic. The cages used in the experimental context measured 32 x 21 x 12 cm and were made of translucent plastic.

*Procedure.* The rats were assigned to one of four equal-sized groups, group preexposed in the home cages (Group PRE HOME), group control in the home-cages (Group CNT HOME), group receiving preexposure in a novel context (Group PRE CONTEXT) and group control in a novel context (Group CNT CONTEXT). The treatment given to the preexposed-context and control-context groups exactly matched that given to the preexposed and control groups of Experiment 2. The preexposed-home and control-home groups differed only in that they remained in their home cages throughout the experiment. Prior to a one-bottle acceptance test, food was removed for all animals. Any other procedural details not specified were identical to those described for Experiment 2.

## Results and Discussion

As in Experiment 2, rats given preexposure to sucrose drank less on the first trial than subsequently (Figure 6). Water consumption remained stable for the

control groups. An ANOVA conducted on the scores shown in Figure 6 with preexposure condition, context, and trial as the variables yielded significant main effects of trial, $F$ (1, 28) = 15.67, and of preexposure condition, $F$ (1, 28) = 10.91, and a significant interaction between these variables, $F$ (1, 28) = 8.84 (all other $F$s < 1). Analysis of simple effects showed there to be a difference between Trials 1 and 8 in the preexposed subjects, $F$ (1, 15) = 22.32, but not in the control subjects, $F$ < 1.

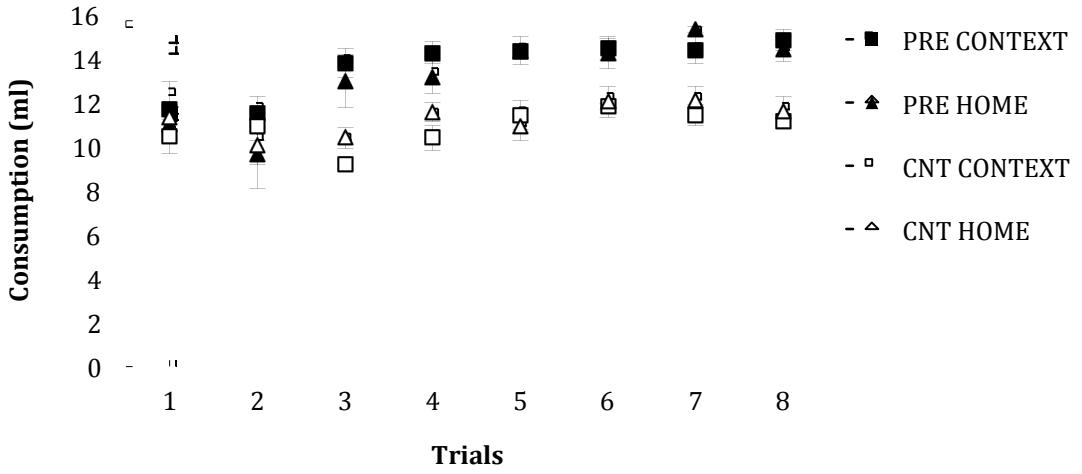


**Figure 6. Experiment 3:** Mean consumption scores during preexposure for the preexposed (PRE) and control (CNT) groups, both in the Home-cage and in the Context. Animals in Group PRE HOME and PRE CONTEXT received sucrose; those in Group CNT HOME and CNT CONTEXT received access to water. Vertical bars represent SEMs.

There was a clear effect of preexposure on the UR test (Figure 7), with rats in the preexposed groups drinking more than those in the control groups, who experienced sucrose for the first time on this test. An ANOVA conducted on the data presented in the figure, with preexposure condition and home vs. context as

the variables revealed a significant effect of preexposure, $F$ (1, 28) = 32.31; there was no effect of the type of context and no significant interaction between these variables, $F$s < 1.

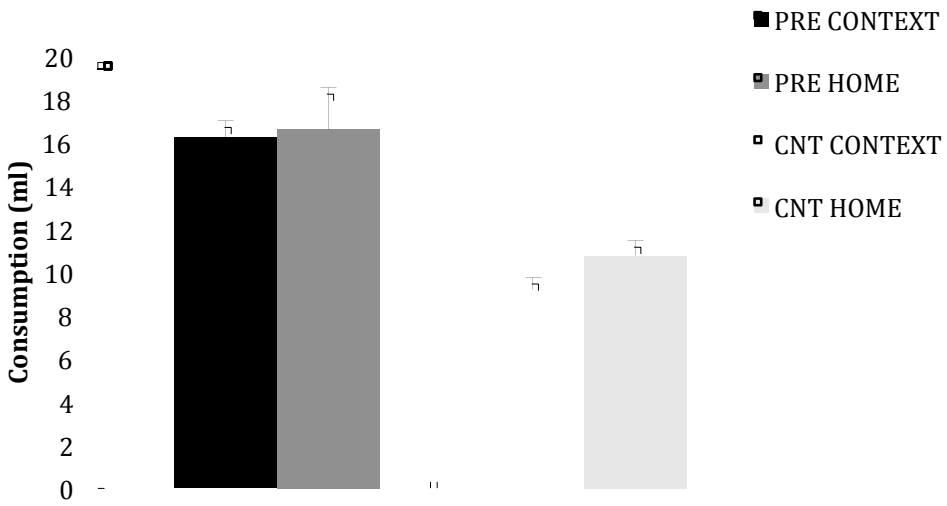


**Figure 7. Experiment 3:** Group means for consumption of sucrose on the UR test for groups given preexposure (PRE) or not (CNT), in the home cage (HOME) or in the experimental context (CONTEXT). Vertical bars represent SEMs.

There were no differences among the groups in the amount of the mint-sucrose compound they consumed during conditioning (Figure 8). An ANOVA with preexposure condition and context condition as the variables revealed a significant effect of trial, $F$ (3,84) = 24,73 but no significant effects for the preexposure variable, $F$ (1, 28) = 1.38; other $F$s < 1, nor the context variable, $F < 1$ and no interaction between these two variables, $F$ < 1.

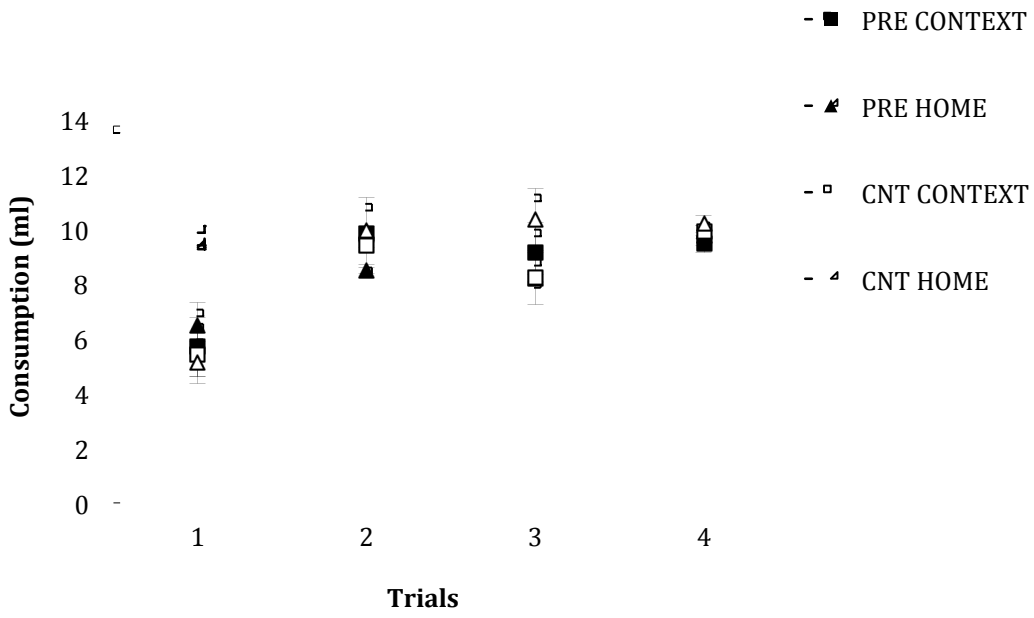


**Figure 8. Experiment 3:** Group mean scores during the conditioning trials. All animals received 10 ml of a mint-sucrose compound in each trial. Vertical bars represent SEMs.

The results of the CR test are shown in Figure 9. A US-preexposure effect was evident in that preexposed subjects drank less than control subjects. This was true both for the groups trained in the context and those trained in the home cage. There was no sign that the size of the effect was reduced in the home cage groups (in fact, the effect was numerically larger in the latter groups). An ANOVA showed there to be a significant effect of the preexposure variable, $F$ (1, 28) = 5.21; there was no significant effect of the home cage vs. context variable, $F < 1$, and no significant interaction between these variables, $F$ (1, 28) = 1.41.

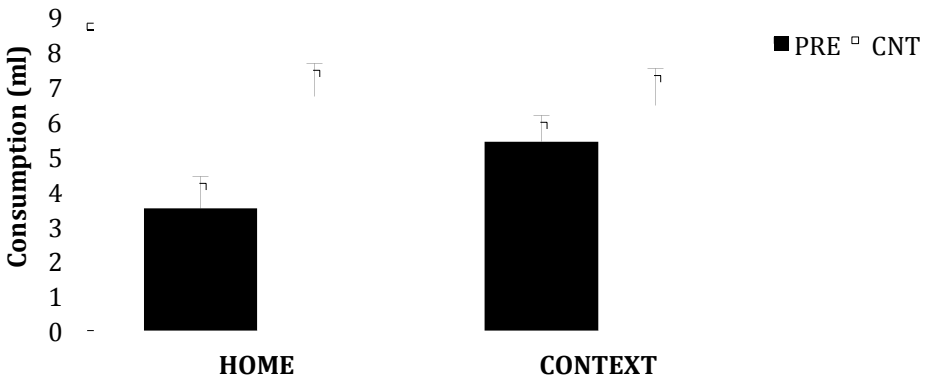


**Figure 9. Experiment 3:** Group means for consumption of mint on the CR test for groups given preexposure to sucrose (PRE) or not (CNT), in the home cage (HOME) or in the experimental context (CONTEXT). Vertical bars represent SEMs.

These results confirm the reliability of the effects demonstrated in Experiment 2. The results of the UR test were consistent with the proposal that exposure to sucrose results in habituation of neophobia; and the CR test produces a clear US-preexposure effect. They lend no support, however, to the hypothesis that the US-preexposure effect depends on blocking by contextual cues, given that it was observed as readily when these cues were familiar as when they were novel. But this result cannot be taken as decisive evidence against the context-blocking interpretation of the effect – the notion that latent inhibition will restrict acquisition of associative strength by home-cage cues, however plausible, is no more than an assumption for which there is no direct evidence. In Experiment 4, therefore, we adopted a different procedure for assessing the role of context.

## Experiment 4: Context change

Evidence that blocking by context plays a role in generating the US-preexposure effect obtained in the CER procedure has come from experiments in which exposure to the shock is given in one context and conditioning is given in a different context. In these circumstances, the effect is attenuated (Randich & Ross, 1984). This strategy was adopted in the present experiment. Two experimental contexts were used, both of which were different from the home cage. All subjects experienced sessions in both contexts during the preexposure phase, but, for the preexposed groups, sucrose was presented in just one of them. Rats in the preexposed-same group then received conditioning trials in the context in which the sucrose had previously been presented; rats in the preexposed different group received conditioning in the other context. If the US-preexposure effect depends on blocking by contextual cues, the effect might be expected to be attenuated in the latter group.

The contexts used were the same as those described by (Symonds & Hall, 1997) in a study of context-aversion conditioning, in which rats received a lithium injection in one context but not in the other. This experiment showed that an aversion was established just to the context associated with injection, demonstrating that the rats could discriminate between these contexts, that the contextual cues are capable of supporting conditioning, and that the CR established to one context does not generalize substantially to the other. In the experiment by Symonds and Hall, the strength of the context aversion was assessed by means of a

blocking test, acquisition of a nausea-based aversion to a novel flavour being blocked when conditioning was given in the context in which the US had previously been presented. Their result thus constitutes, for the aversive case, a demonstration of the US-preexposure effect, and provides evidence that blocking by contextual cues is (at least in part) responsible for that effect. The present experiment allows a parallel investigation using of the appetitive case.

**Method**

*Subjects and apparatus.* The subjects were 32 male hooded Lister rats (from Charles River Laboratories) with a mean-feeding weight of 457 g (380-500g) at the start of the experiment. They were assigned to one of four equal-sized groups (see Table 2). Two sets of cages, both distinct from the home cage, served as the experimental contexts. One set consisted of the small dark cages used as the experimental context in Experiment 2. Those in the second set were larger, measuring 42 x 35 x 16 cm, and were located in a fully lit colony room situated in a separate part of the laboratory. The walls and floor of the cage were made of translucent white plastic and the wire mesh roof included a section through which a drinking spout could be inserted. There was no bedding in this cage.

*Procedure.* Throughout the experiment all subjects received two 30-min sessions per day, one in each context. For half the rats in each group the small dark cage was experienced in the morning session during preexposure, and the large bright cage in the afternoon session; for the remainder the arrangement was

reversed. Rats in the preexposed groups received access to 15 ml of the sucrose solution in the morning sessions of each of the 8 days of the preexposure phase; in the afternoon sessions 15 ml of water was made available. Control subjects received equivalent treatment except that water was presented in both sessions. The UR test was conducted on the day following the end of the preexposure phase. On this session all animal received access in the morning session to 30 ml of sucrose for 30 min in the context that they had previously experienced in the afternoon sessions; this allowed the response to sucrose to be assessed for all subjects in a context in which it had not previously been experienced. Water was given in the other context in the afternoon session. Over the next four days (the conditioning phase), all animals received the mint-sucrose compound in the morning session with water being presented in the afternoon session. This was followed by a test trial on which mint was presented in the morning session. For rats in the preexposed-same and control-same groups the contexts were arranged as during preexposure. For rats in the preexposed-different and control-different groups the context experienced in the afternoon session was now presented in the morning session, and vice versa. In details not specified here, the procedure followed that described for Experiment 3.

**Results and Discussion**

Group mean scores for the amount of fluid consumed during the preexposure phase are presented in Figure 10, which collapses the same and

different groups in each preexposure condition (these groups receiving identical experience in this phase of training). Consumption increased over trials in both groups, but much more markedly in the subjects preexposed to sucrose than in the control subjects Initial neophobia meant that the former drank rather less than the latter group on the first trial; thereafter the rats given sucrose drank more than those given water. An ANOVA conducted on the data summarised in the figure showed there to be a significant effect of preexposure condition, *F* (1, 28) = 35.77, a significant effect of trial, F (7, 196) = 21.59, and a significant interaction between these variables, F (7, 196) = 11.47. Analysis of simple effects showed that the groups differed on each trial; for the smallest difference (on Trial 7), F (1,28) = 7.83.

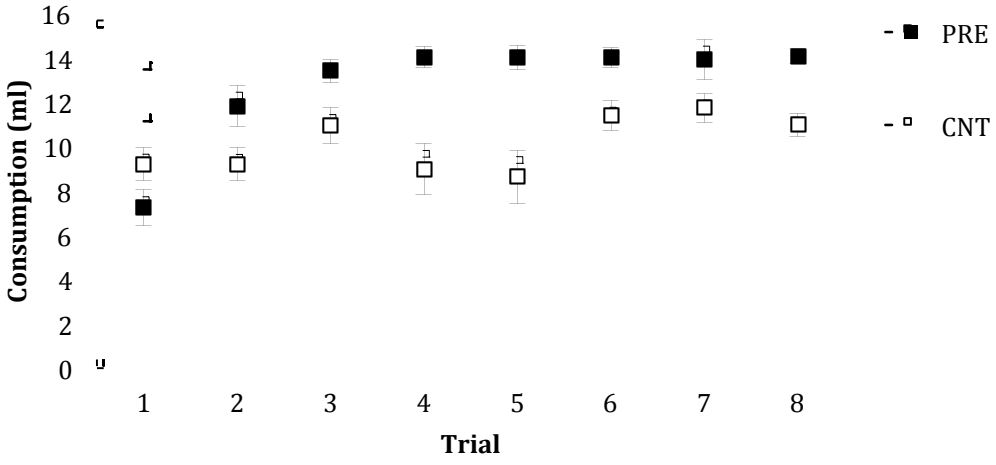


**Figure 10. Experiment 4:** Mean consumption scores during preexposure for the preexposed (PRE) and control (CNT) groups. Animals in Group PRE received sucrose; those in Group CNT received access to water. Vertical bars represent SEMs.

The results of the UR test are presented in Figure 11. As in previous experiments, there was evidence of neophobia in the control subjects who drank less on this test than did the preexposed subjects. An ANOVA with preexposure condition and context condition as the variables revealed a significant effect of preexposure, $F$ (1, 28) = 17.54. The different context groups drank somewhat more on this test than did the same context groups, but the difference was not statistically significant, $F$ (1, 28) = 3.34. The interaction between the variables was not significant ($F < 1$).

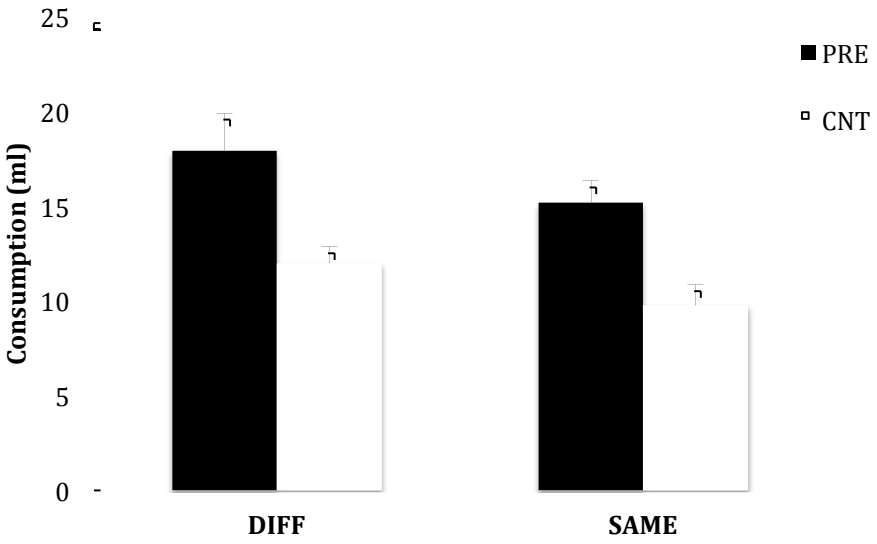


**Figure 11. Experiment 4:** Group means for consumption of sucrose on the UR test for groups given preexposure (PRE) or not (CNT), in the same context (SAME) or in a different context (CONTEXT). Vertical bars represent SEMs.

Neophobia was also evident on the first conditioning trial. On this trial, subjects in the control groups drank rather less of the mint-sucrose compound than did subjects in the preexposed groups; thereafter, all drank the full amount made available. An ANOVA, with preexposure condition and context condition as

the variables, revealed a significant effect of trial, $F$ (3,84) = 24,73, and a significant interaction between trial and preexposure conditions, $F$ (3,84) = 5,84. Analysis of the simple effect revealed there to be significant differences between groups preexposed and control on trial 1 of conditioning, $F$ (1,28) = 8,35, but no differences on the last trial, $F < 1$.

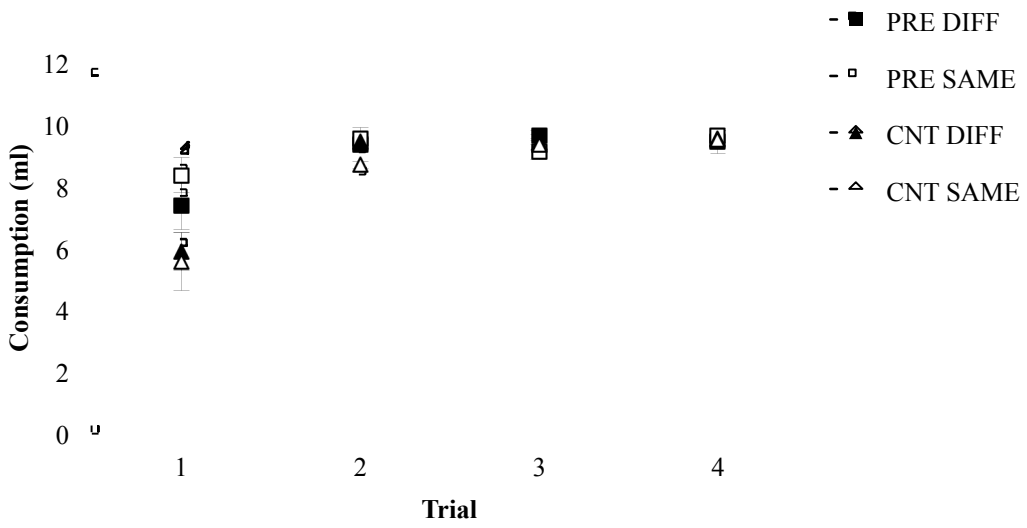


**Figure 12. Experiment 4:** Group mean scores during the conditioning trials. All animals received 10 ml of a mint-sucrose compound in each trial. Groups PRE SAME and CNT SAME received all conditioning trials in a different context; those animals in groups PRE DIFF and CNT DIFF experienced conditioning trial in the same context where they had preexposure. Vertical bars represent SEMs.

The results of the final, CR test are shown in Figure 13. It is evident that, as in the previous experiments, the preexposed subjects consumed less than the control subjects. This effect was not influenced by the change of context. An ANOVA with preexposure condition and context condition as the variables showed there to be a significant effect of preexposure, $F$ (1, 28) = 8.68; there was no significant effect of context, $F$ (1, 28) = 1.31, and no significant interaction ($F < 1$).

It is therefore concluded that, in this preparation, the US-preexposure effect is quite immune to a change in context between preexposure and conditioning.

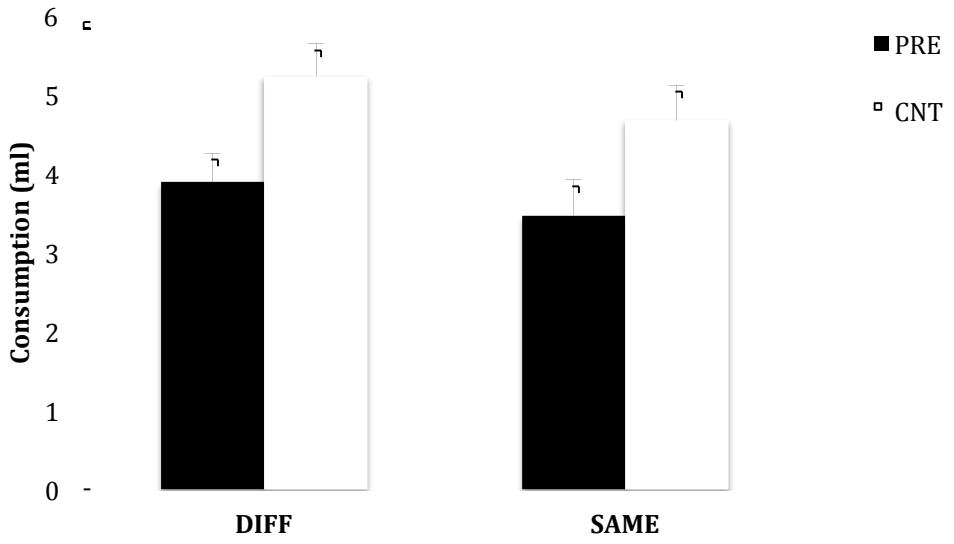


**Figure 13. Experiment 4:** Group means for consumption of mint on the CR test for groups given preexposure to sucrose (PRE) or not (CNT), in same context (SAME) or in a different context (CONTEXT). Vertical bars represent SEMs.

## General Discussion

The experiments reported in this chapter showed that the US-preexposure effect can be obtained both when the experiment is conducted in a novel context and when all the experimental phases occur in the home-cages. Moreover, a contextual change between preexposure and conditioning did not have any impact on this effect (Randich & Lolordo, 1979b). A widely accepted hypothesis to explain the US-preexposure effect relies on blocking by the context. According to this account, the context will become associated with the US during the preexposure phase and will block conditioning to the CS introduced in the conditioning phase.

This was the explanation favoured by Harris et al. (2000) for their demonstration of the appetitive US-preexposure effect. The experiments reported here, however, provide no support for the view that blocking by the context plays any significant role in this version of the US-preexposure effect.

Habituation has been defined as a decline in the magnitude of the UR as a result of repeated presentation of the US. The results obtained in this chapter provided evidence of this phenomenon, with consumption of the sucrose solution being less on early trials than on later ones. It is not obvious, however, why a substance that appears to become increasingly acceptable with experience, should then function less well as a reinforcer, but perhaps the apparent increase in palatability is accompanied by a decline in the effective salience of the stimulus. Such a reduction in salience would reduce the effectiveness of sucrose as a reinforcer. If this latter possibility is accepted, there is no reason why it should be applied just to appetitive examples of the US-preexposure effect. Admittedly, the evidence that the effect obtained in flavour-aversion learning depends on blocking by contextual cues is strong (de Brugada, et al., 2004) and there is little sign of habituation when nausea is used as the US (de Brugada, et al., 2005); but things may be different for the CER procedure. Blocking by contextual cues may play some role in this procedure, but there is evidence that an effect can be found even when contextual blocking appears to be ineffective (Baker, et al., 1981). Given the evidence that repeated exposure to a shock can reduce ability of the shock to evoke its UR (Hall & Rodriguez, 2010) the proposal that habituation-induced reduction in

the effective salience of the US contributes to the US-preexposure effect in CER seems viable.

Although it has been said that the blocking-by-the-context does not support the US-preexposure effect found in this chapter, an alternative explanation for these results can be seen as being a revised version of the blocking by the context account. If experience of sucrose allows the formation of an association between its flavour and its nutritional consequences, then the current preexposure procedure should ensure that this association is well established in the preexposed subjects prior to the conditioning phase. The presence of this association might be expected to block the formation of a direct association between the flavour of the CS and the consequences of ingesting sucrose. If the CR to the CS on test is a reflection of the strength of this direct association, then it is to be expected that prior exposure to sucrose will attenuate the CR.

As a next step, Chapter 4 considers the implications of the view that the taste of the sucrose might by crucial in producing a blocking effect by exploring the possibility of obtaining a US-preexposure effect with a substance that provides similar nutritive post-oral consequences to the sucrose solution but which seems less likely to provide a salient taste that could associate with them and therefore block a further association between the CS and US during conditioning.

# CHAPTER IV

## EXPERIMENTS 5-8:

## THE ROLE OF TASTE CUES

# CHAPTER IV: EXPERIMENTS 5-8: THE ROLE OF TASTE CUES

## Introduction

The results of the experiments reported in Chapter 3 (Experiments 3 and 4) showed no role for the context in the present version of the US-preexposure effect using an appetitive conditioning procedure. When a context change occurred between the preexposure phase and the conditioning and test phases, animals receiving preexposure to sucrose showed retardation during the following conditioning phase compared to animals in a control group (that had no previous experience with the US). Although these results argue against the blocking-by-context hypothesis, it is possible that other cues present during preexposure could serve to produce blocking in the way that injection cues have been shown to do in the aversion learning procedure (de Brugada, et al., 2003a). This notion is examined in this chapter and in Chapter 5.

An important feature of the experimental paradigm used in this thesis (the flavour preference procedure) is that animals can learn about different elements of the appetitive US (e.g. sucrose). For instance, animals having experience with sucrose could learn both about its sensory properties and about its motivational consequences (that it contains calories). A revised version of the blocking by context account could be applied for this case. If the exposure to sucrose allows the formation of an association between its sensory (taste) and its motivational properties (post-oral consequences), it might be expected that this association

would block the acquisition of a CR to the CS present during conditioning and therefore produce a US-preexposure effect.

One way of testing this hypothesis is to use an appetitive reinforcer with the same postingestive consequences as sucrose but which lacks a strong taste. For such a substance, preexposure would not produce a strong association between taste and nutritional consequences; accordingly the taste would not be able to block learning about the CS when it is added in the conditioning phase. Previous research has shown that maltodextrin (MD) can support strong conditioned flavour preferences (Sclafani, Thompson, & Smith, 1998)

Various studies have shown that rats find MD as palatable as sucrose (Bonacchi, Ackroff, & Sclafani, 2008; Sclafani & Clyne, 1987; Sclafani, et al., 1998) but have also shown that maltodextrin and sucrose have qualitatively different tastes. Further studies have demonstrated that glucose (as the reinforcing component of maltodextrin and sucrose) infusions after a series of CS-flavour pairing trials, enhanced the palatability of the CS-flavour compound (Azzara & Sclafani, 1998; Bonacchi, et al., 2008; Sclafani, Fanizza, & Azzara, 1999). But although in the case of sucrose the shift in preference can be supported by both its sensory properties - flavour-taste learning – and by its motivational post-oral properties - flavour-nutrient learning – (Bonacchi, et al., 2008), maltodextrin fails to support flavour preferences based only on its sensory properties (Bonacchi, et al., 2008) Based on these studies, MD could be used as the US to provide a demonstration of the US-preexposure effect in which a taste-flavour association formed during conditioning would not be relevant.

In the experiments reported in this chapter, maltodextrin will be used as the US. Before looking for the US-preexposure effect with maltodextrin as the US, it seems necessary first to demonstrate (with the basic procedure previously used in this thesis – Experiment 1) that it can indeed support the acquisition of a conditioned preference. This issue is addressed in Experiment 5 using the basic conditioning procedure that was used to demonstrate preference conditioning with sucrose in Experiment 1 (Chapter 2).

**Experiment 5: Demonstration of the basic conditioning effect with MD**

For Experiment 5, an appetitive conditioning procedure was employed with maltodextrin (MD) as the unconditioned stimulus. There were two groups of subjects, both of whom received trials with a neutral flavour as the CS and maltodextrin as the US. As is detailed in Table 3, the schedule used to present these stimuli differed between the two groups. Group Simultaneous received trials in which a neutral flavour was simultaneously paired with maltodextrin and Group Unpaired experienced alternate trials of either the CS or the US. In a subsequent one-bottle test all animals had access to the CS (as in the previous demonstration of conditioning effect using sucrose as the US). Given that the US used for this experiment (maltodextrin) has postingestive nutritive consequences likely to support the development of a preference, then it is expected that, as a consequence of the conditioning trials, the CS would acquire excitatory properties. It is therefore

anticipated that animals from Group SIM would show a higher consumption of the taste used as the CS than those in Group UNP.

**Table 3**
**Experimental designs**

| **EXPERIMENT 5** | | |
|---|---|---|
| **Group** | **Conditioning** | **CR Test** |
| **SIM** | 4 M + MD | M |
| **UNP** | 4 M / MD | |

| **EXPERIMENT 6** | | | | |
|---|---|---|---|---|
| **Group** | **Preexposure** | **UR Test** | **Conditioning** | **CR Test** |
| **PRE** | 8 MD | MD | 4 M + MD | M |
| **CNT** | 8 W | | | |

| **EXPERIMENT 7** | | |
|---|---|---|
| **Group** | **Conditioning** | **CR Test** |
| **SIM** | 4 A + MD | A vs. W |
| **UNP** | 4 A / MD | |

| **EXPERIMENT 8** | | | |
|---|---|---|---|
| **Group** | **Preexposure** | **Conditioning** | **CR Test** |
| **PRE** | 8 MD | 4 A + MD | A vs. W |
| **CNT** | 8 W | | |

***Note.*** MD refers to a 21,6% maltodextrin solution; M refers to a 2% mint solution; A refers to a 1% almond solution; W refers to tap Water. SIM: Group Simultaneous; UNP: Group Unpaired; PRE: preexposed; CNT: control. Animals in Experiments 5 and 6 were water deprived during all the experiment and food and water deprived for test. Animals in Experiments 7 and 8 were food and water deprived throughout the experiment.

**Method**

*Subjects and apparatus.* The subjects were 16 male naïve Lister hooded rats (Charles River Laboratories) with a mean free-feeding weight of 498 g (range: 483-513 g). They were maintained in the same conditions as in previous experiments. As in a previous experiment (Chapter 2, Experiment 1), all experimental procedures were conducted in the home cages. The US was a 21.6% (w/v) MD solution and the CS was a 2% (v/v) solution of mint (peppermint flavouring supplied by Supercook; Leeds, UK). The compound used for the Group Simultaneous (Group SIM) was made up so as to preserve these concentrations. All the solutions were made with tap water and given to the animals in a 50-ml graduated tubes fitted with a rubber stopper and a stainless steel ball-bearing tipped spout. Fluid intake was measured, as in previous experiments, by weighing tubes before and after sessions.

*Procedure.* A schedule of water and food deprivation was established, as in the previous experiments (e.g., Chapter 2, Experiment 1). Over the next four days all animals received exposure to mint (CS) and maltodextrin (US). Animals in Group SIM received both the CS and US simultaneously, whereas those in Group Unpaired (Group UNP) experienced separate exposure to these stimuli, in alternating trials. During the conditioning trials the presentations of the stimuli in the Group UNP were counterbalanced, that is, half of the animals received the US (10 ml) during the morning session and the CS (10 ml) in the afternoon session, whilst this pattern was reversed for the other half of the animals. Animals in Group

SIM had the compound trials during the morning sessions (10 ml) and water in the afternoon sessions (10 ml). After the last conditioning trial, food was removed for all animals. On the morning session of the day following the last conditioning trial, animals had access to a one-bottle test with the mint solution for 30 minutes.

**Results & Discussion**

Neophobia was evident on the first conditioning trial. On this trial, subjects in Group SIM drank rather less of the mint-MD compound than on the later trials of conditioning. Animals in Group UNP showed an even greater neophobic response to mint over the four conditioning trials but drank all that was available of the MD solution. Figure 14 shows the mean amount of the mint solution consumed by each group during the test. A conditioning effect was evident in Group SIM. Animals receiving the compound solution showed higher consumption than those in Group UNP. An ANOVA with group as factor was conducted in order to show the significance of the data. This analysis revealed a significant effect of group, $F$ (1,14) = 50,07. The higher consumption of the CS solution for the animals in Group SIM shows that pairing a neutral odour (in this case mint) with a substance with positive motivational consequences (maltodextrin) allows the formation of an association between these two stimuli and therefore animals drink more of the CS than those that experienced the stimuli separately.

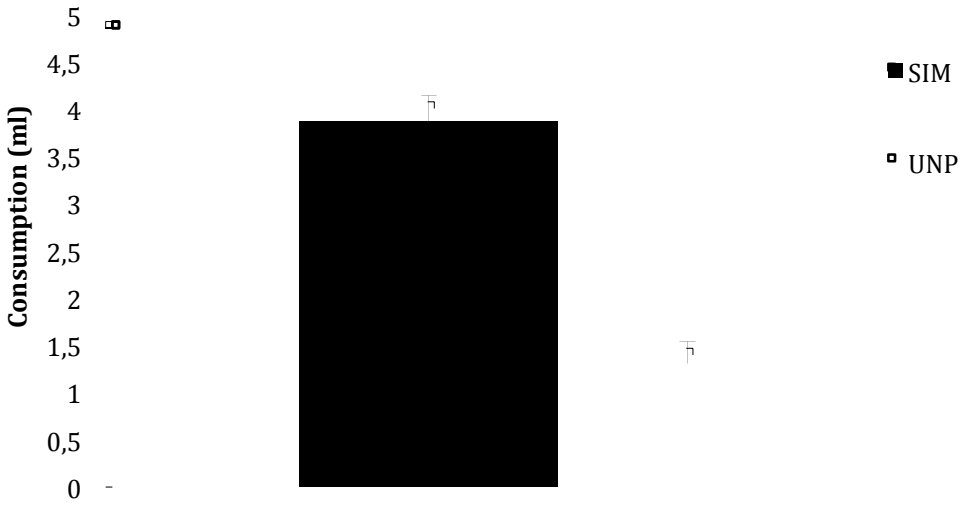


**Figure 14. Experiment 5.** Group means for consumption of mint on the CR test for groups given paired trials of the maltodextrin-mint compound (SIM) or unpaired trials of maltodextrin/mint (UNP). Vertical bars represent SEMs.

## Experiment 6: Demonstration of the US-preexposure effect using maltodextrin (MD) as the unconditioned stimulus

Experiment 5 has demonstrated that MD can produce a clear conditioning effect when it is paired simultaneously with a neutral odour. The next step was to look for a US-preexposure with this substance using the flavour acceptance paradigm. To do this, a CS and procedure similar to those used in the previous demonstration of the effect (Chapter 3, Experiment 3) was employed. As previously, half of the animals received preexposure to MD alone whereas the other half had access to water during this phase. In the subsequent conditioning procedure, all animals received trials with the US and CS paired before a one-bottle test (the CR test) with access to the CS. Between the preexposure and conditioning phases, all animals were given a single trial in which consumption of maltodextrin

was measured; this UR test was intended to assess the existence of any neophobic response to MD. If maltodextrin has a salient taste similar to that of sucrose, an initial neophobic response would be expected at the beginning of the preexposure phase followed by an increase in consumption over the preexposure trials. Animals in Group Preexposed (PRE) consuming significantly less mint than those in Group Control (CNT) during the acceptance test (CS alone) will reveal a US-preexposure effect.

**Method**

*Subjects and apparatus*. The subjects were 16 male hooded Lister rats with a free-feeding weight of 442 g (range 427-457 g). They had previously served as subjects in a Conditional Emotional Response (CER) experiment but were naïve to the flavours and procedures used in the present experiment. All experimental procedures were conducted in the home cages. The solutions used in this experiment were the same as in Experiment 5.

*Procedure.* The rats were assigned to two equally-sized groups at the beginning of the experiment. The initial two days schedule of water deprivation and maintenance conditions were established as in all the previous experiments, so that the animals had two 30-min sessions per day (10:30 a.m. and 4:30 p.m.). Water continued to be made available during the afternoon drinking session throughout the experiment. During the following eight days the preexposure phase occurred. The subjects in Group PRE had access for 30 minutes to 15 ml of the

maltodextrin solution and those in Group CNT to 15 ml of water. On the next morning session all animals received access to 30 ml of maltodextrin for 30 min, this constituted the UR test. During the conditioning phase (4 days) a compound made with mint and maltodextrin was given to all the animals. After the last conditioning session, the rats were food deprived and during the next three days they received access to water in both morning and afternoon session, and access to 10 g of food only during the afternoon session. The morning of the following day constituted the CR test, during which all the animals received a one-bottle test with access to the CS solution for 30 min (see Table 3).

**Results & Discussion**

Group means from the preexposure phase are presented in Figure 15. Animals given preexposure to maltodextrin drank less on the first trial and then subsequently showed an increased intake during the course of preexposure. An ANOVA with group and trial as the variables revealed that there was a significant effect of trial, $F$ (7,98) = 9,65, no significant effect of group, $F < 1$, and a significant interaction between these two variables, $F$ (7,98) = 7,68. A simple main effects analysis showed there to be a significant difference among trials in Group PRE, $F$ (7,49) = 15,91, but no trial difference in Group CNT, $F$ (7,49) = 2,70. The fact that animals in Group PRE showed less consumption in the first Preexposure trial than those in Group CNT (ANOVA showed a significant difference between groups, $F$ (1,14) = 79,14) means that MD is likely to have produced a neophobic response

when it was first encountered on Trial 1, confirming that the concentration of MD has a taste that the rats can detect.

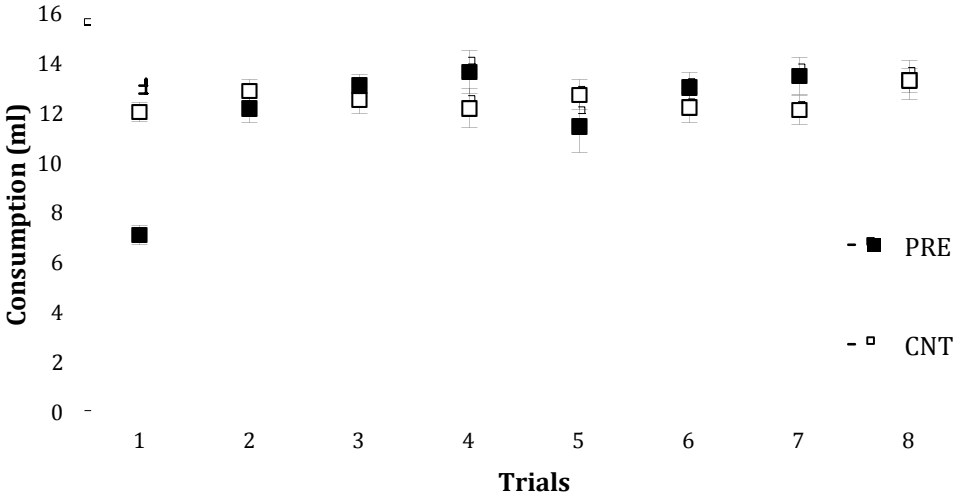


**Figure 15. Experiment 6.** Mean consumption scores during preexposure for the preexposed (PRE) and control (CNT) groups. Animals in Group PRE received maltodextrin trials; those in Group CNT received access to water. Vertical bars represent SEMs.

Performance on the UR test trial with MD is shown in Figure 16. Although animals in group PRE showed a neophobic response to MD on the first trial (Figure 15), the UR test failed to show any differences between Groups PRE and CNT (Figure 14). An ANOVA conducted on the data summarised in Figure 16 revealed no significant differences between the groups, $F$ (1,14) = 1,27. This absence of a significant difference between Group PRE and Group CNT constitutes a difference in the UR test results between sucrose (Chapter 3, Experiment 3) and maltodextrin. It perhaps supports the notion that the taste of MD is less salient than that of sucrose.

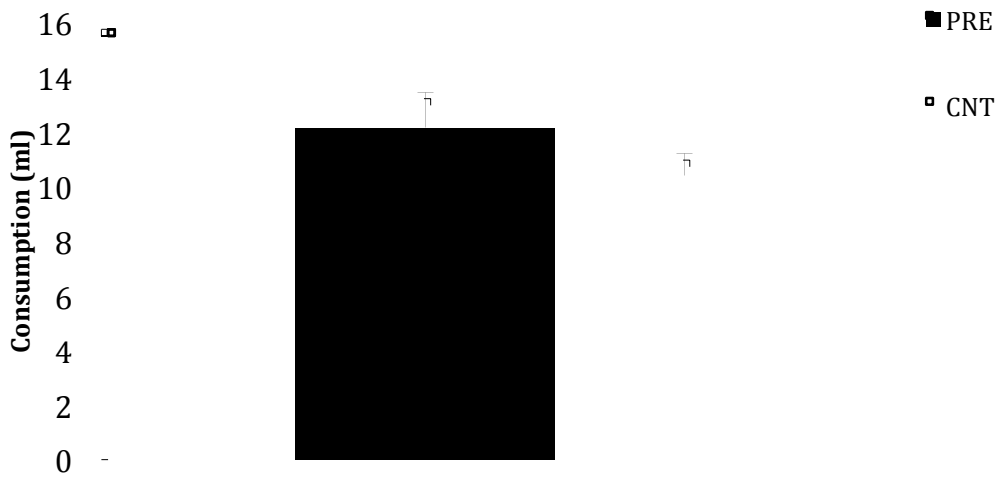


**Figure 16. Experiment 6.** Group means for consumption of MD on the UR test for groups given preexposure (PRE) or not (CNT). Vertical bars represent SEMs.

The mean amount consumed by the groups during the conditioning phase is summarized in Figure 17. It shows a similar consumption of the CS-US compound for both groups PRE and CNT. A statistical analysis of the group means during the conditioning phase revealed no differences between groups PRE and CNT, $F < 1$, or among the conditioning trials, $F$ (3,42) = 3,96; and no significant interaction was found between these two variables, $F$ (3,42) = 2,10.

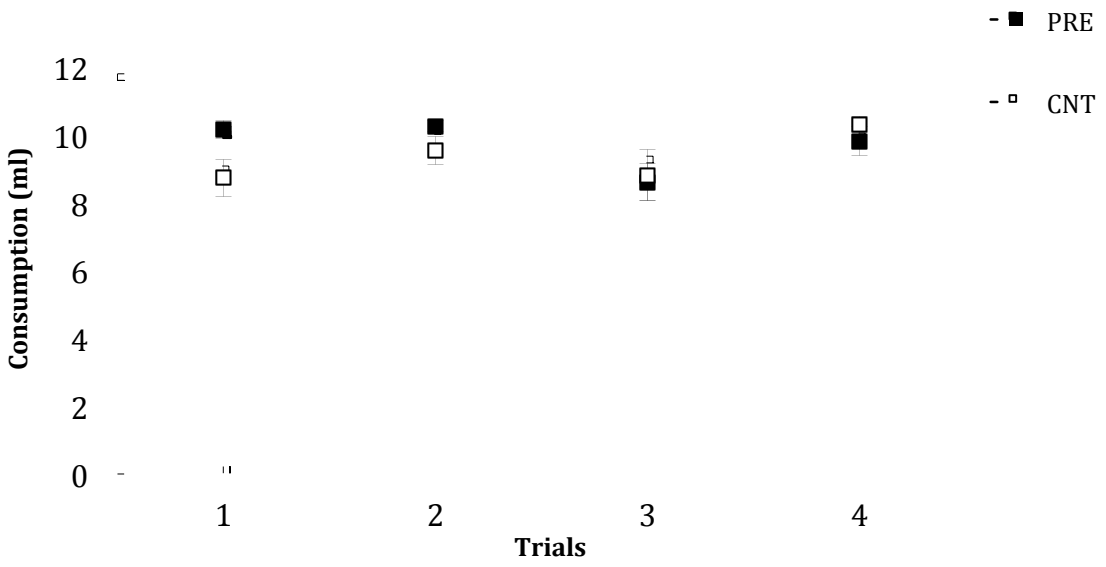


**Figure 17. Experiment 6.** Group mean scores during the conditioning trials. All animals received 10 ml of a mint-maltodextrin compound in each trial. Vertical bars represent SEMs.

The results of the CR test are shown in Figure 18. No evidence of a US preexposure effect was found, in that preexposed animals drank almost the same amount of the test solution as those in Group CNT. An ANOVA with preexposure and group as variables revealed no significant effects, $F < 1$. It seems that a procedure that is effective in demonstrating the US-preexposure effect using sucrose as the unconditioned stimulus (US), fails to generate an effect with maltodextrin. The result of this experiment could be taken to support the proposed hypothesis of blocking by taste; but it would be premature to place too much weight on a single null result. It is possible that the conditioning parameters employed in this experiment were not sensitive enough to obtain a result with a substance such as MD. This matter is taken up in the next experiment.

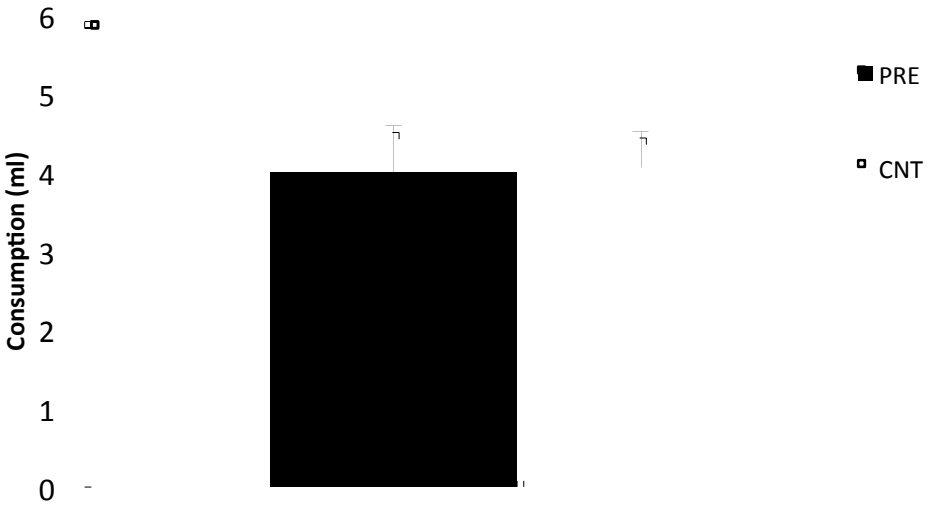


**Figure 18. Experiment 6.** Group means for consumption of mint on the CR test for groups given preexposure to maltodextrin (PRE) or not (CNT). Vertical bars represent SEMs.

## Experiment 7: Replication of the basic conditioning effect with MD as the US using different parameters

The results of Experiment 5 have shown how, after a exposure to a neutral odour and maltodextrin, animals experiencing these stimuli in a simultaneous compound (Group SIM) drank significantly more than those experiencing both stimuli in separate trials. Having demonstrated that a conditioned flavour preference can be acquired with MD as the US, a further experiment looking at a version of the US-preexposure effect using MD as the unconditioned stimulus was carried out (Experiment 6). This latter experiment failed to obtain the US-preexposure effect. One interpretation of this null result is that it supports the blocking by taste hypothesis of the US-preexposure effect. Although the results of Experiment 6 seem to support this explanation, one null result might not be enough to demonstrate this. It has been found in this laboratory that the use of mint as the CS

in a 2% (v/v) solution can result in some neophobic responses when it is presented alone. It is possible then, that some tendency to shun this flavour on the test in both preexposed and control groups might obscure the US preexposure effect by masking any differences in consumption between the two groups on the CR test. It seems sensible therefore to try to use a different CS that results in a less neophobic response when presented alone. In order to test this possibility the next experiments attempted to replicate the basic conditioning effect and the US-preexposure effect with MD as the unconditioned stimulus with a different CS and after procedural changes that would perhaps increase the sensitivity of the test (Table 3). In order to maximize the likelihood of obtaining an effect, food deprivation was used throughout training, rather than just on test; and to increase the sensitivity of the test, a two-bottle preference test was used in the test phase.

**Method**

*Subjects and apparatus.* The subjects were 16 male-hooded Lister naïve rats (obtained from Charles River Laboratories) with a mean free-feeding weight of 323 g (range: 319-328 g). Animals were water and food deprived prior the beginning of the experiment and maintained this way throughout the experiment (with access to food and water in the afternoon). All the experimental procedures were conducted in the home-cages throughout the experiment. The US was, as in Experiment 5, a 21,6% (w/v) maltodextrin solution (to match the amount of calories obtained from a 20% (w/v) solution of sucrose) and the conditioned

stimulus was a 1% (v/v) solution of almond (almond flavouring supplied by Supercook, Leeds, UK). The compound of maltodextrin and almond was made up so as to preserve these concentrations. All the solutions were made with tap water and given to the animals in 50-ml graduated tubes fitted with a rubber stopper and a stainless steel ball-bearing tipped spout. Fluid intake was measured by weighing tubes before and after the sessions.

*Procedure.* The rats were assigned to two equal-sized groups at the beginning of the experiment. The schedule of water deprivation and maintenance conditions were established so that the animals had 30-min access to water in the morning session (10:30 am) and had access to water and food during the afternoon session for 90 minutes (from 4:30 pm). All the experimental sessions were conducted during the morning sessions. The presentation of the stimuli over conditioning sessions was counterbalanced, with half of the subjects in each group receiving the following pattern of presentations, X-Y-Y-X; and the other half of the animals receiving Y-X-X-Y; where X means A+MD (Group SIM) or MD (Group UNP) and Y means Water (Group SIM) or Almond (Group IMP). After the conditioning phase subjects received a two-bottle preference test for 30-min, with one tube containing the CS (almond) and the other containing water. The left-right position of the tubes was counterbalanced within each group to control for side preferences; and also the positions for each rat were swapped after the first 15 minutes of the test.

**Results & Discussion**

On the first trial of conditioning, subjects in Group SIM drank slightly less of the almond-MD compound than on later trials; animals receiving MD alone drank almost all the solution available over the four trials of conditioning. Animals in Group UNP drank less of the almond solution than the MD solution, following a similar pattern than animals in Group SIM consumption of water.

The intakes of almond during the CR test differed substantially between the groups SIM and UNP. Those animals that had experienced simultaneous pairings of almond and maltodextrin drank more of the almond on test than those that had experienced almond and maltodextrin on separate trials. Preference ratios (volume of Almond / volume of Almond + volume of water) were also calculated for the two-bottle test. The mean intake of the almond solution and water on test by rats in each of the group in Experiment 6 is shown in Figure 19a. Group means of preference ratio data from the test trial are presented in Figure 19b. It is clear from Figures 19a and 19b that the subjects exposed to the almond in Group SIM consumed substantially more of this flavour than those in Group UNP. An analysis of the direct intake of almond for both groups showed a significant difference between these two groups, $F$ (1,14) = 44,70. A single factor ANOVA conducted on the preference ratio data indeed confirmed this difference to be statistically significant, $F$ (1,14) = 23,05.

The results from Experiment 7 were clearcut. As in Experiment 5, animals receiving simultaneous pairings of the compound of almond and MD (Group SIM)

showed significantly higher consumption of the CS when presented alone compared to those animals receiving both stimuli in alternate presentations (Group UNP). This conditioning effect was also obtained with a preference score. These results, along with those from Experiment 5, confirm that flavour preference can be obtained using MD as the US, using either an acceptance test or a two-bottle preference test. Experiment 8 will therefore explore whether these changes to the conditioning parameters will be able to generate the US-preexposure effect.

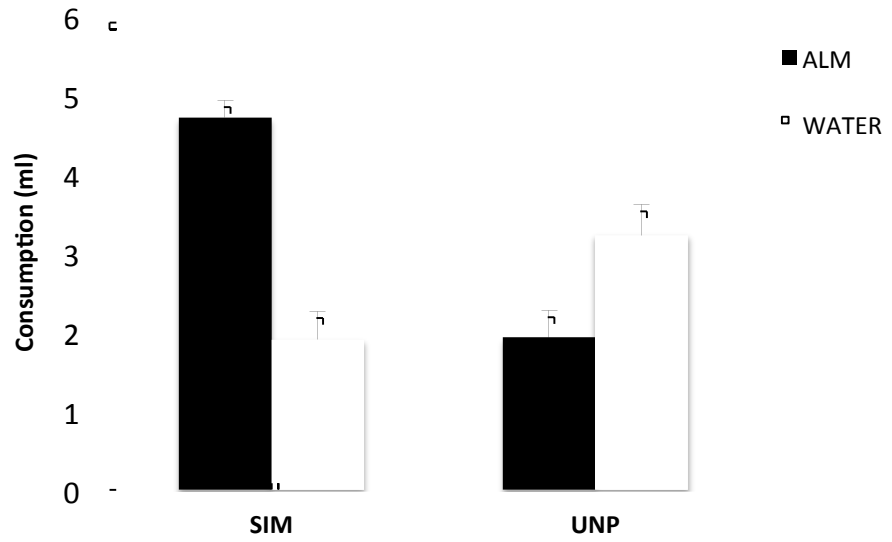


**Figure 19a**

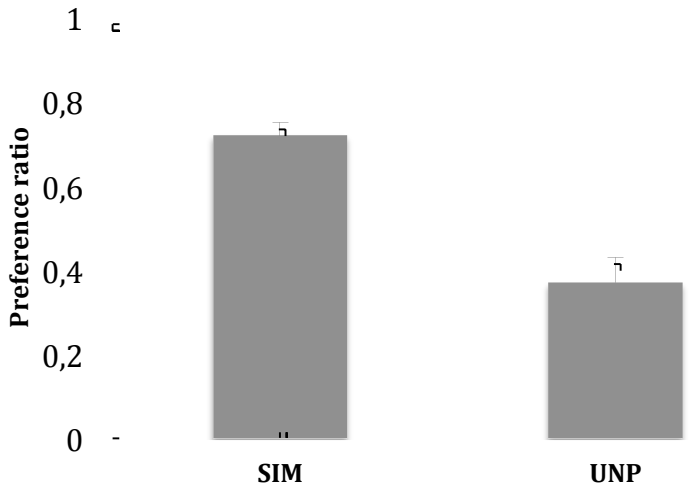


**Figure 19b**

**Figure 19. Experiment 7.** Mean intake on test of almond and water (Figure 19a) and the means of almond intake over total intake (Figure 19b) for groups given paired trials of the MD-almond compound (Group SIM) or unpaired trials of MD / almond (Group Unpaired). Vertical bars represent SEMs.

## Experiment 8: Replication of the US-preexposure effect with MD as the unconditioned stimulus

In response to the null result of Experiment 6, some procedural changes were made in Experiment 7 in order to obtain a perhaps more sensitive way of testing the US-preexposure effect with maltodextrin as the US. Using the same procedure as in the previous one, this experiment attempted to explore whether the US-preexposure effect could be found with maltodextrin as the US and almond as the CS. Two groups of animals received a flavour preference conditioning procedure in which the novel flavour of almond (CS) was paired with a maltodextrin solution (US). This was followed by a preference test in which the CS was presented in a choice test along with water. Half of the animals (Group PRE) experienced eight presentations of the maltodextrin solution alone before the start of the conditioning phase; those in Group CNT were given access to water during this phase. A lower preference for the CS during the test in the preexposed animals compared to those in Group CNT would indicate the occurrence of the US-preexposure effect.

### Method

*Subjects and apparatus.* The subjects were 16 experimentally naïve male hooded (Lister) rats (obtained from Charles River Laboratories) with a mean free-feeding weigh of 334 g (range: 327-340 g). The animals were water and food deprived and then randomly assigned to two groups, matched for baseline levels of

water consumption. All the animals were maintained under food and water deprivation - as described for Experiment 7 in this chapter -. The CS consisted of a 1% almond (v/v) solution and the US was a 21,6% (w/v) MDsolution, as used in Experiment 7. All the experimental sessions occurred in the morning.

*Procedure.* Before the start of the experiment, all the animals were food-deprived and kept under these conditions throughout the experiment (with access to food and water in the afternoon sessions during a 90 min session). The next eight days constituted the preexposure phase. One each of these days half of the animals received 15-ml of a MD solution during 30-min (Group PRE) whereas animals in Group CNT received access to water. Over the following four days all the animals received a compound with both the CS (almond) and the US for 30 min. For the CR test the subjects received a two-bottle test with one tube containing almond and the other water (with the same parameters as in Experiment 7).

**Results & Discussion**

Consumption of fluid during the preexposure phase (of maltodextrin for the preexposed group, or water for the control group) is shown in Figure 20. Unlike Experiment 6, animals receiving maltodextrin (Group Pre) for the first time (i.e. on the first trial of the preexposure phase) drank more than those in Group CNT who were given water. One explanation for this difference between Experiments 6 and 8 is that the lower consumption of water in the latter experiment during preexposure could be caused by the motivational state of the rats (they were

maintained on water and food deprivation from the beginning of the experiment). The amount consumed increased gradually over trials in Group PRE but not in Group CNT. An ANOVA conducted on these data confirmed that there was an effect of trial, $F$ (7,98) = 8,13; of group, $F$ (1,14) = 96,636 and a significant interaction between these two factors, $F$ (7,98) = 10,42. A simple main effects analysis showed there to be a significant difference over trials in Group PRE, $F$ (7,49) = 125,01, but no differences over the trials in Group CNT, $F < 1$.

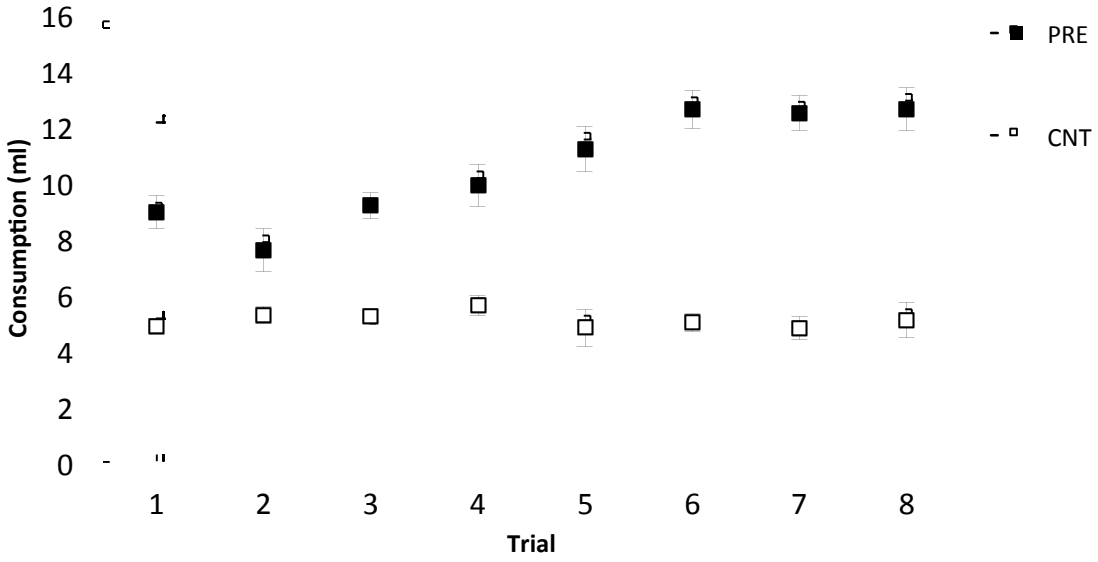


**Figure 20. Experiment 8.** Mean consumption scores during preexposure for the preexposed (PRE) and control (CNT) groups. Animals in Group PRE received MD; those in Group CNT received access to water. Vertical bars represent SEMs.

Consumption of fluid during the conditioning phase is shown in Figure 21. An ANOVA showed that there were significant differences over the trials, $F$ (3,42) = 3,05, a significant difference between the two groups, $F$ (1,14) = 6,01 and a significant interaction between these variables, $F$ (3,42) = 3,77. An analysis of the

simple effects showed a difference between groups PRE and CNT in trial 1, $F$ (1,14) = 4,76, but not in the last trial of the conditioning phase, $F$ (1,14) = 3,36.

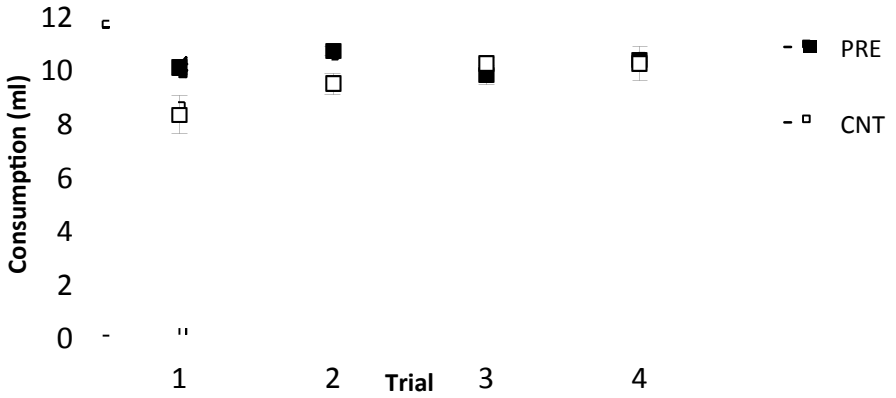


**Figure 21. Experiment 8.** Group mean scores during the conditioning trials. All animals received 10 ml of an almond-maltodextrin compound in each trial. Vertical bars represent SEMs.

Group mean scores for the CR test are shown in Figure 22. The mean intake of the almond solution and water on test by rats in each of the groups are shown in Figure 22a, whereas Figure 22b shows the preference ratios based on these intakes. There is evidence of a US-preexposure effect, in that the PRE group had a lower consumption level and a lower ratio score than group CNT. Statistical analysis partly confirmed this impression. An ANOVA conducted on the almond consumption data summarized in Figure 22a failed to show a significant effect of Group, $F$ (1,14) = 3,07, but analysis of the preference ratios summarised in Figure 22b revealed a clear effect of group, $F$ (1,14) = 17,80. These results differ from those in Experiment 6 in that a clear US-preexposure effect was obtained at least when preference ratios were used as the measure of the strength of the CR.

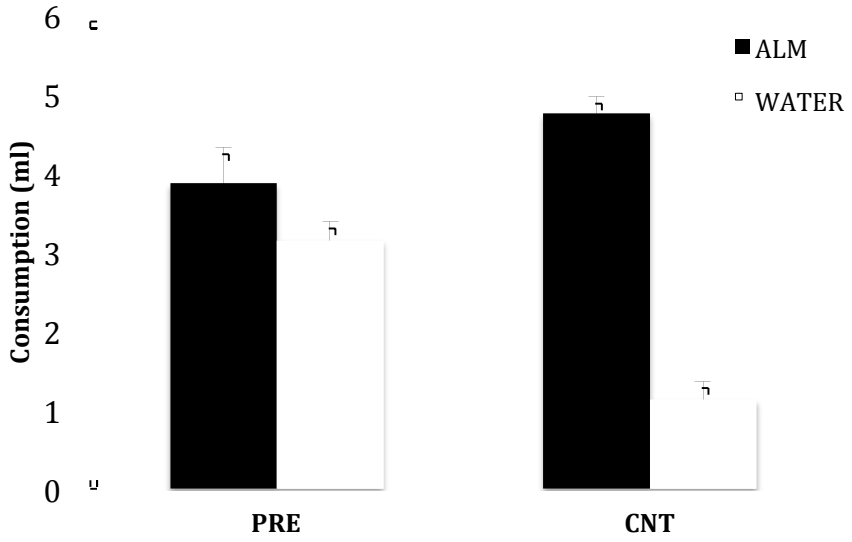


**Figure 22a**

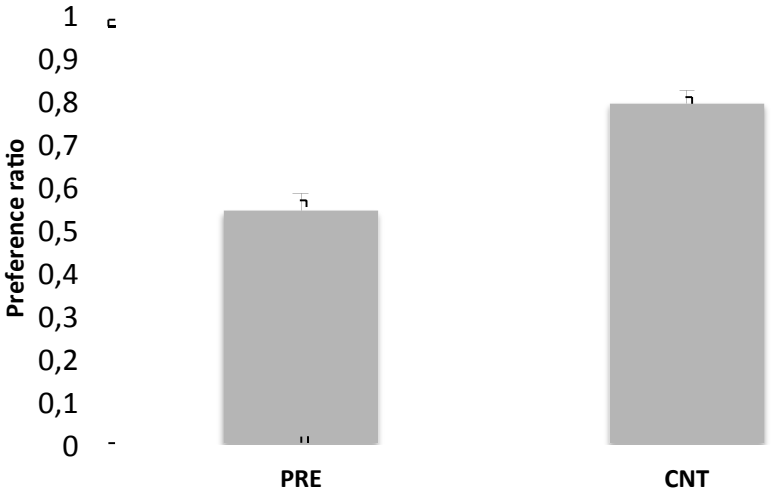


**Figure 22b**

**Figure 22. Experiment 8.** Mean intake on test of almond and water (Figure 22a) and the mean ratio of almond intake overt total intake (Figure 22b) for groups given preexposure to maltodextrin (group PRE) or not (group CNT). Vertical bars represent SEMs.

The discrepancies between the results obtained in Experiments 6 and 8 must be due to some feature of the procedural differences between them. The UR test in Experiment 6 could have obscured the US-preexposure effect because even animals in Group CNT did have some, albeit slight, experience with the US. It is therefore possible that this might have been sufficient to produce some decrement in conditioning in this group. Perhaps a more obvious possibility, however, concerns the motivational state of the subjects in these experiments. Animals in Experiment 6 were made hungry prior to the CR test, whereas those in Experiment 8 were maintained hungry throughout all phases of the experiment. The enhanced motivational state of hunger in the latter experiment could perhaps render the US with its post-oral consequences more salient for these animals; for hungry animals, the impact of the preexposure trials may therefore be greater. Another procedural difference between the Experiments (6 and 8) was the stimulus used as the CS. In Experiment 6, a 2% mint solution was used as the CS whereas in Experiment 8 the CS was a 1% almond solution. Previous unpublished data (from this laboratory) have revealed there to be stronger neophobic responses to mint than to almond. The results reported in Experiments 6 and 8 seem to confirm this impression since different responses to these CSs were observed. One final and possibly important procedural change between experiments 6 and 8 is in the type of test. For Experiment 6 a one-bottle acceptance test was used whereas a two-bottle preference test was used in Experiment 8, and it is commonly accepted that a preference test is more sensitive than an acceptance test (Elizalde & Sclafani, 1990). Any or all of these factors could have contributed to the appearance of the

US-preexposure effect in Experiment 8. These implications are taken up in the general discussion.

## General Discussion

The aim of the experiments reported in this chapter was to examine the role played by the taste of the US in the US-preexposure effect using maltodextrin as the unconditioned stimulus (US). Previous results in this thesis constitute a demonstration of the US-preexposure effect in an appetitive conditioning paradigm (Experiment 2, Chapter 2). Chapter 3 examined the role played by the context in order to offer an explanation for the US-preexposure effect, and the findings from these experiments lead to the conclusion that the effect is unlikely to be explained in terms of this mechanism (Harris, et al., 2000; Randich & Lolordo, 1979b). An alternative explanation has been suggested in previous chapters. This hypothesis constitutes a revision of the blocking by the context account. During the preexposure phase, experience of sucrose will allow the formation of an association between its flavour and its nutritional consequences. During the following conditioning phase, this well-formed association will block the formation of the association between the CS present during the conditioning trials and the nutritional properties of sucrose. Therefore, a preexposure phase prior to conditioning will attenuate the conditioned response to the CS in the test.

In order to test this revised version of the blocking account, Experiments 5 and 7 reported a demonstration of basic flavour preference conditioning using

mint or almond as the CS and maltodextrin as the US. Maltodextrin is a polysaccharide with postingestive nutrients (like sucrose) but is a substance that has been assumed to lack a strong and salient taste (in contrast to sucrose). And although MD has a detectable and palatable taste to rats, it is said to support conditioning by an association between the CS and its nutrients (Bonacchi, et al., 2008; Dwyer, 2008; Elizalde & Sclafani, 1988). Certainly any taste that MD possesses is not enough in itself to support conditioned preferences; it has been shown that when its postingestive consequences are eliminated, maltodextrin does not support flavour preference conditioning (Bonacchi, et al., 2008). In the experiments reported in this chapter, we made use of this substance as the US. We assume that the increase in consumption of the CS produced by experience of an odour-maltodextrin compound depends on an association between the CS and the nutrients from maltodextrin. If this is true, finding a US-preexposure with maltodextrin as the US will offer some information about the role played by the taste in the sucrose case.

Experiment 6 was unsuccessful in generating the US-preexposure effect. However, a US-preexposure effect was found in Experiment 8 (which used different parameters). The apparent failure to obtain a US-preexposure effect in Experiment 6 in which maltodextrin is used seems to provide support for the blocking by taste hypothesis, as this is consistent with the idea that the critical factor in previous experiments using sucrose was the presence of a strong taste which can form an association with its nutritive consequences. No firm conclusions, however, can be drawn on the basis of one null result. Indeed, it was

found that changing aspects of the experimental procedure (the nature of the CS, the use of a UR test, the different type of test, and the motivational state of the animals) produced a very different outcome in Experiment 8.

Experiment 8 shows that it is clearly possible, under certain conditions, to produce a US preexposure effect when MD is the US. What is rather less clear, however, is the interpretation of this finding. If, as already suggested, the post-oral consequences of MD (and not its palatable taste) are the features that support conditioning (Bonacchi, et al., 2008), this finding appears to rule out a role for the blocking by taste hypothesis described earlier in this chapter. On the face of things, the weak taste of MD could not form a strong association with its nutritive consequences during the preexposure trials, and might thus be unable to block the formation of a further association between the CS and the US in the conditioning phase. It is possible, however, that the failure of the taste of MD to support basic conditioning is due to its lack of palatability rather than a lack of salience. And even if the taste of MD is not palatable enough to support basic conditioning, remains possible that it could be salient enough to form an association with calories during preexposure, and that such an association would be sufficient to retard subsequent conditioning. On the basis of the current results, it can only be suggested that blocking by taste is a doubtful candidate for explaining the US preexposure effect obtained with MD, but one that cannot be completely ruled out.

The results of the experiments in this chapter, therefore, do not allow any firm conclusions to be drawn in relation to the blocking-by-taste hypothesis. They

do, however, strongly suggest that motivational factors may play a critical role in determining whether an effect can be found at all. Before speculating any further about the importance of the modified blocking account, it seems necessary to clarify the role of the motivational properties of the US in generating the US-preexposure effect. This issue is taken up in Chapter 5.

# CHAPTER V:

## EXPERIMENTS 9-10:

## THE ROLE OF MOTIVATIONAL STATE

# CHAPTER 5: EXPERIMENTS 9-10: THE ROLE OF MOTIVATIONAL STATE

## Introduction

The experiments reported in the preceding chapters have examined the nature of the US-preexposure effect when using an appetitive flavour-preference conditioning procedure. This procedure will establish a preference for a neutral odour paired with a US such as sucrose. It is assumed to do so by way of two types of association, one of which is a link between the CS and the taste or sensory properties of the US, and the other a link between the CS and the nutritive or calorific qualities of the US (Fedorchack & Bolles, 1987; Fedorchak, 1997; Harris, et al., 2000). According to Harris et al., when animals are hungry during the test, as they were in the present experiments, it is the latter association that controls performance.

A modified blocking mechanism has been considered as a possible account of the instances of the US preexposure generated by this conditioning procedure. According to this explanation, an association will be formed during the preexposure phase between the taste of the sucrose and its post-oral or nutritive consequences, and this taste-nutrient association will block the formation of an association between the flavour used as the CS and the US during the conditioning trials.

When the animals are non-hungry, the preference for an odour previously paired with a nutritional substance has been explained in terms of the formation of

an association between the CS and the positive properties of the sucrose taste (Fedorchak, 1997); that is the effective properties of the US related to its sensory, rather than its motivational properties. In such circumstances the blocking mechanism just described would not be operative. If these assumptions are true, then the modified blocking account predicts that the strength of the US preexposure effect should critically depend on the motivational state of the subjects. Specifically, this hypothesized mechanism will be relevant only if the animals are hungry so that their preference is controlled by the CS-nutrient association. For non-hungry animals, a blocking mechanism based on the formation of taste-nutrient associations during preexposure would be ineffective in interfering with the CS-taste association and no US-preexposure effect should be observed in satiated animals. Animals that are not hungry may still show a preference on test (because of the flavour-flavour association), but if the blocking by the taste of sucrose mechanism is the sole source of this US-preexposure effect in flavour preference conditioning when using sucrose as the US, then no effect should be obtained in this case.

The aim of the experiments reported in the present chapter, therefore, was to explicitly test this suggestion by directly comparing the magnitude of the US preexposure effect obtained between hungry and non-hungry animals using appetitive flavour conditioning. If the US preexposure effect is less marked in sated animals than in hungry animals, then this would have clear implications for the role played by taste-nutrient associations in the modified blocking account offered previously.

## Experiment 9: US-preexposure effect and motivational state

In this experiment half of the subjects were maintained hungry throughout whereas the other half of subjects were only water deprived. Half of the subjects received preexposure to a strong sucrose solution and control animals had access to water. This phase was then followed by a conditioning phase in which all subjects received presentations of a CS-US compound. All animals then received a CR test (Table 4). As in the experiments reported in Chapters 2 and 3, mint was used as the CS, but to increase its sensitivity, a two-bottle preference procedure was used in the test phase (as in Experiment 8, Chapter 4). In addition, in order to maximize the likelihood of obtaining an effect of motivational state, food deprivation was used throughout training, rather than just on test. On the basis of previous results, it was expected that hungry animals that had been given preexposure to the US would show less preference for the CS on the test than those subjects that were hungry but had access to only water during preexposure – the standard US preexposure effect. If the previous blocking by taste account does have a role in the US-preexposure effect, those animals that were only water deprived and received preexposure to the US will fail to show such effect.

**Table 4**

**Experimental designs**

| EXPERIMENT 9 | | | |
|---|---|---|---|
| **Group** | **Preexposure** | **Conditioning** | **CR Test** |
| **PRE TH** | 8 SUC | 4 M + SUC | M vs. W |
| **CNT TH** | 8 W | | |
| **PRE HUN** | 8 SUC | | |
| **CNT HUN** | 8 W | | |
| **EXPERIMENT 10** | | | |
| **Group** | **Preexposure** | **Conditioning** | **CR Test** |
| **PRE TH** | 8 SUC | 4 A + SUC | A vs. W |
| **CNT TH** | 8 W | | |
| **PRE HUN** | 8 SUC | | |
| **CNT HUN** | 8 W | | |

***Note*.**
SUC refers to a 20% sucrose solution; M refers to a 2% mint solution; A: refers to a 1% almond solution; W refers to tap Water. PRE TH: preexposed and thirsty; CNT TH: control and thirsty; PRE HUN: preexposed and hungry; CNT HUN: control and hungry.

**Method**

*Subjects and apparatus.* The subjects were 32 male hooded (Lister) naïve rats with a mean free-feeding weight of 479 g (range: 467-490 g). They were housed individually in cages (35 x 22 x 19 cm) of transparent polycarbonate plastic. These cages were located in the main colony room that was brightly lit from 08:00 to 20:00 h each day. The animals were randomly assigned to one of four groups (Groups preexposed and thirsty – PRE TH -, preexposed and hungry – PRE HUN -, control and thirsty – CNT TH – and control and hungry – CNT HUN). Half of the animals were permitted continuous access to laboratory chow throughout the experiment (Groups PRE TH and CNT TH) whereas the other half were food deprived (Groups PRE HUN and CNT HUN) throughout the experiment (being given access to food for 90 min each afternoon). All the solutions were presented in 50-ml centrifuge plastic tubes equipped with stainless-steel ball-bearing tipped spouts. The solutions used for the experiment were a 20% (w/v) sucrose solution, 2% (v/v) mint solution and a compound of the sucrose and mint (w/v) solution. The solutions were mixed so as to maintain their individual concentrations. Consumption was measured by weighing the tubes before and after all the experimental sessions.

*Procedure.* The experimental design is detailed in Table 4. At the beginning of the experiment the standard water bottles were removed for all the subjects; for half of the subjects the food was also removed. The next two days established a schedule of water deprivation in which all subjects had access to water twice per day in a 30 min session in the morning (from 11 am) and 90 min during in the

afternoon (from 4:30 pm). During the latter period the food-deprived animals also had access to food. The next eight days constituted the preexposure phase. On each of the eight days, animals in Group PRE TH and PRE HUN received access to 15 ml of sucrose solution and those in Groups CNT TH and CNT HUN had access to 15 ml of water in the morning sessions. In conditioning phase that followed, all the subjects received 4 trials in which they had access to a 10-ml CS-US compound (mint-sucrose) solution. On the day following the final conditioning trial each subject was given a two-bottle preference test (mint vs. water). Procedural details for CR test were as in previous experiment 8 (Chapter 4).

**Results & Discussion**

Figure 23 shows the mean amount consumed by each group over the eight preexposure days (of sucrose for the preexposed groups; of water for the control groups). The amount consumed increased from the first to latter trials in both preexposed groups, but not in control groups. An analysis of variance (ANOVA) conducted on the data summarised in the figure, with preexposure condition, motivational state and trial as the variables yielded a significant main effects of trial, $F$ (7,196) = 29,61; of motivational state, $F$ (1, 28) = 69,28, and of preexposure condition, $F$ (1,28) = 27,39, and significant interactions between trial and preexposure condition, $F$ (7,96) 27,99, trial and motivational state, $F$ (7,196) = 2,69, and between preexposure condition and motivational state $F$ (1,28) = 52,16. Analysis of simple effects showed there to be a difference between Trials 1 and 8 in

the preexposed subjects, $F$ (7,98) = 43,85, but not in the control subjects, $F < 1$. Statistical analysis on the first and last trial of preexposure revealed a significant difference between groups preexposed and control when animals were water deprived, $F$ (1,14) = 51,14, but not when animals were water and food deprived, $F$ (1,14) = 1,84. On the last trial of preexposure an analysis of ANOVA revealed statistical differences between groups preexposed and control when animals were water and food deprived, $F$ (1,14) = 173,56, but not when animals were only water deprived, $F$ (1,14) = 1,12.

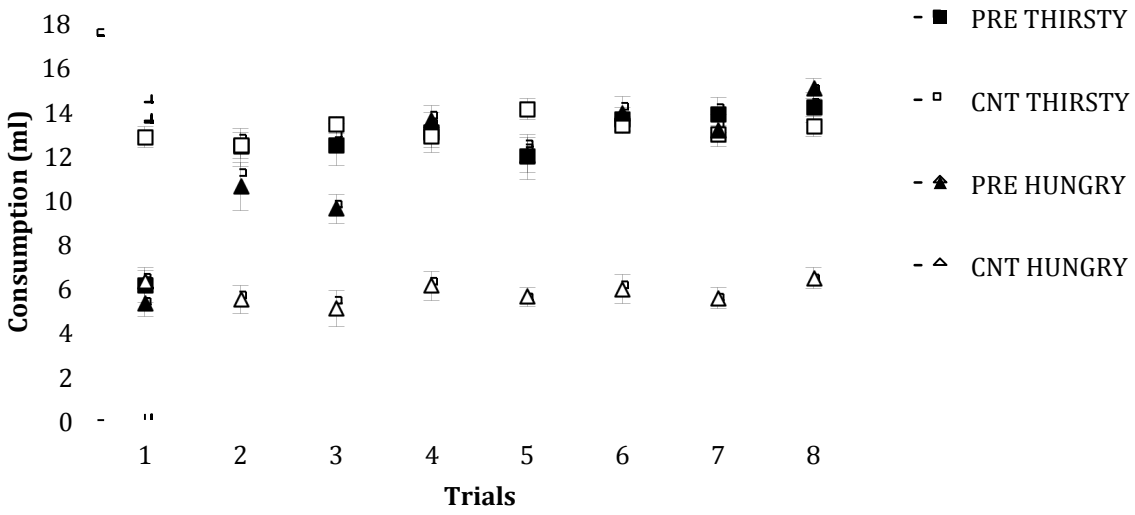


**Figure 23. Experiment 9.** Mean consumption scores during preexposure for the preexposed (PRE) and control (CNT) groups, both thirsty and hungry. Animals in Group PRE TH and PRE HUN received sucrose; those in Group CNT TH and CNT HUN received access to water. Vertical bars represent SEMs.

Figure 24 shows the course of conditioning with the compound solution for all the groups. There were no obvious differences between groups over the trials of conditioning, and ANOVA analysis confirmed this. No differences were found between groups Thirsty and Hungry $F$ (1,28) = 1,28, Groups Preexposed and Control $F < 1$ and no significant interaction between these two variables $F < 1$.

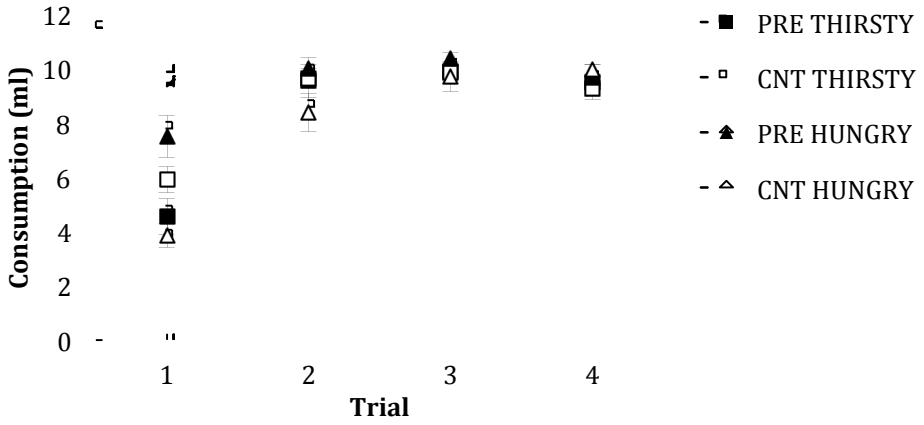


**Figure 24. Experiment 9:** Mean consumption scores during conditioning for the preexposed (PRE) and control (CNT) groups, both thirsty and hungry. Vertical bars represent SEMs.

The results for the final CR test are shown in Figure 25 (25a and 25b). The mean intake of the mint solution and water on test by the rats in each group are shown in Figure 25a, whereas Figure 25b shows the preference ratios based on these intakes. No evidence of a US-preexposure effect was found, in that preexposed and nonpreexposed animals drank similar amounts of the CS solution during the CR (Figure 25a), and this was true for both thirsty and hungry animals. An ANOVA on the data summarised in Figure 25a with preexposure and motivational state as variables revealed no significant preexposure effect, $F$ (1,28) = 1,15; no effect of motivational state condition, $F < 1$, and no interaction between these two variables, $F < 1$. The preference ratios (volume of almond / volume of almond + volume of water) that were calculated for rats in each of the groups are shown in Figure 25b. An analysis of the data summarised in Figure 25b revealed significant differences between thirsty and hungry animals, $F$ (1,28) = 17, 90, but no effect of preexposure condition or an interaction between these two variables, $F$s < 1.

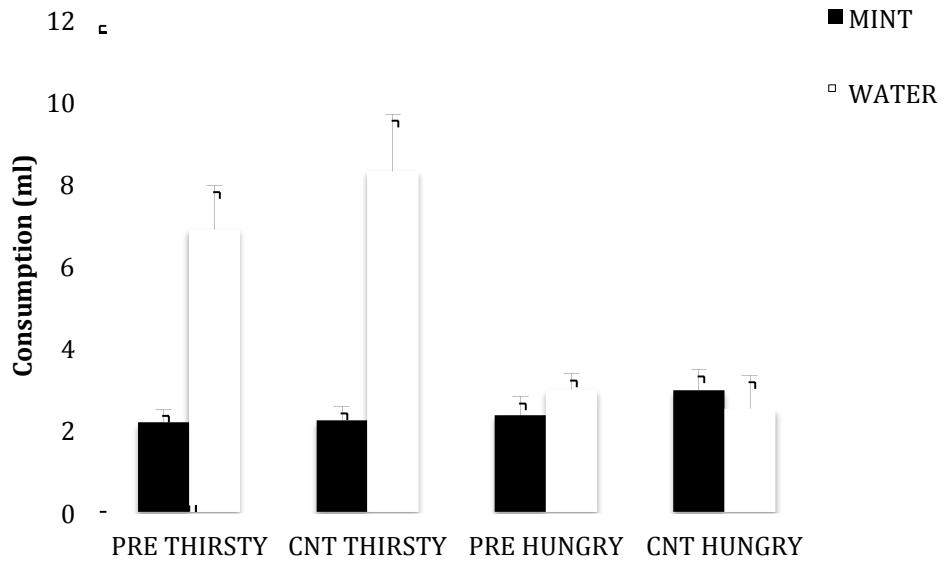


**Figure 25a**

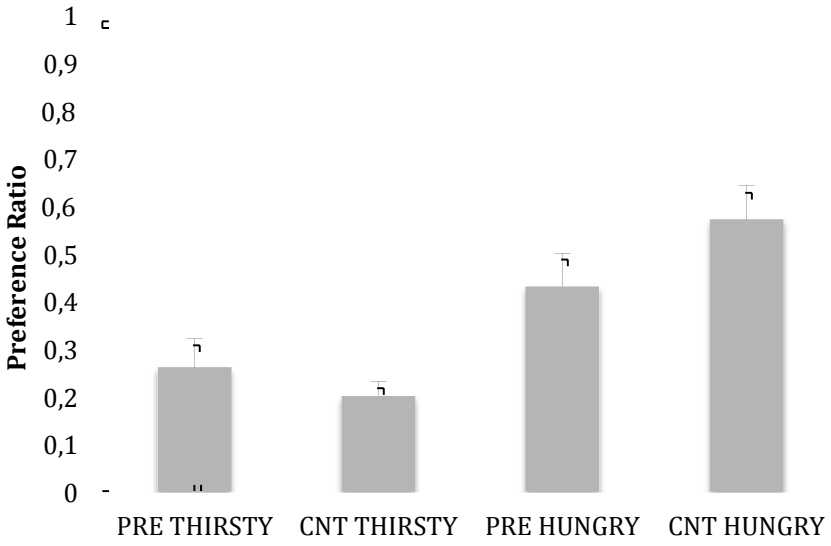


**Figure 25b**

**Figure 25. Experiment 9:** Mean intake on test of mint and water (Figure 25a) and the mean ratio of mint intake over total intake (Figure 25b) for groups preexposed (PRE) and control (CNT), both thirsty and hungry. Vertical bars represent SEMs.

This experiment failed to find the US-preexposure effect with non-hungry animals, but it also failed to replicate the effect when animals were hungry (compared to previous successful results using sucrose as the US in Chapters 2 and 3). The difference in results between previous demonstrations of the US-preexposure effect using sucrose as the US and the results found in this experiment must be due to some procedural difference among the experiments. There were several differences in procedure between this and previous experiments, including the type of test and the motivational state of the animals, but perhaps the use of the two-bottle test was critical. Rats have no liking for mint and will tend to choose water over a mint solution when they are given the choice. With a one-bottle test the effect of conditioning might overcome this; but on a two-bottle test the preference for water could mean that conditioning differences between groups in their response to mint would be obscured. Experiment 10 therefore replicated the US-preexposure effect using sucrose as the US and comparing different motivational state of the rats but made use of a CS (almond) that is not so disliked by rats.

**Experiment 10: US-preexposure effect and motivational state (almond)**

As in Experiment 10, half of the animals received a series of preexposure trials in which they had access to a strong sucrose solution and the other half had access to water, followed by conditioning with a CS-US compound. Experiment 10 was a replication of the previous Experiment 9 except that almond was used CS

was used in this case. In order to avoid any neophobic response that could obscure the results on test, an almond solution was used as the CS (Table 4).

**Method**

*Subjects and apparatus.* The subjects were 32 male hooded (Lister) naïve rats (obtained from Charles River Laboratories) with a mean free-feeding weigh of 330 g (range: 322-337 g). They were housed individually in cages (35 x 22 x 19 cm) of transparent polycarbonate plastic. Inverted 50-ml centrifuge tubes equipped with stainless steel, ball-bearing-tipped spouts were used to present all the fluids. The solutions used for this experiment were a 20% (w/v) sucrose solution, 1% (v/v) almond or a compound of the almond and sucrose (w/v) solution. The solutions were mixed as to maintain the concentrations. Fluid consumption was measured by weighing the tubes before and after fluid presentation.

*Procedure.* Before the start of the experiment, all the animals were randomly assigned to one of four groups (Groups PRE TH, PRE HUN, CNT TH and CNT HUN), half of the animals were food and water deprived (groups PRE HUN and CNT HUN), whereas the other half was only water deprived (groups PRE TH and CNT TH). The next two days established the schedule of water deprivation in which all subjects had access to water twice per day in a 30 min session in the morning (from 11 a.m.) and 90 min during in the afternoon (from 4:30 p.m.) - during which food-deprived animals also had access to food. Animals were

maintained in the same conditions throughout the experiment. All the procedural details for all the phases in the experiment were as described for Experiment 9, except that the CS used for this experiment was an almond solution.

**Results & Discussion**

The group mean quantities of fluid consumed on each of the 8 preexposure days are shown in Figure 26. It is evident that both preexposed groups showed neophobia on the first preexposure trial and the consumption of sucrose increased gradually over the trials. Consumption of water in control groups remained steady throughout, although (as is commonly found) levels were reduced in the hungry rats (Bolles, 1961). An ANOVA with preexposure condition, motivational state, and trial as the variables yielded significant main effects of trial, $F$ (7,196) = 22,90, of preexposure condition, $F$ (1,28) = 15,61, and of motivational state, $F$ (1,28) 91,91; and a significant interaction between trial and preexposure condition, $F$ (7,196) = 25,13, and between preexposure condition and motivational state, $F$ (1,28) = 40,85. Statistical analysis on the first and last trial of preexposure revealed a significant difference between groups preexposed and control when animals were water deprived, $F$ (1,14) = 94,91, but not when animals were water and food deprived, $F < 1$. Analysis of the simple effects was carried out to explore the interaction. On the first trial of preexposure and analysis of ANOVA revealed statistical differences between groups preexposed and control when animals were water deprived, $F$ (1,14) = 94,91, but not when animals were food and water

deprived, $F < 1$. The opposite pattern of results was obtained when analysing the last trial of preexposure. Preexposed and control groups differed only when animals were hungry, $F(1,14) = 107,08$, but not when they were thirsty, $F < 1$.

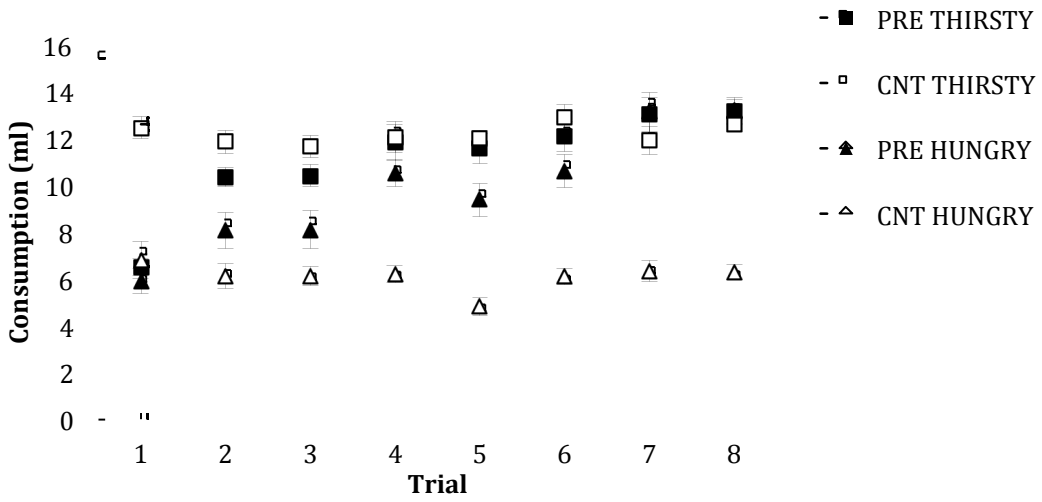


**Figure 26. Experiment 10.** Mean consumption scores during preexposure for the preexposed (PRE) and control (CNT) groups, both thirsty and hungry. Animals in Group PRE TH and PRE HUN received sucrose; those in Group CNT TH and CNT HUN received access to water. Vertical bars represent SEMs.

Group mean scores for the conditioning phase are shown in Figure 27. Animals in the preexposed groups drank almost as all the solution that was available from trial throughout the four trials of conditioning, but, on Trial 1, animals in the control group drank slightly less than those in the preexposed groups. An ANOVA with preexposure condition, motivational state and trial as the variables revealed significant main effects of trial, $F(3,84) = 6,35$, and preexposure condition, $F(1,28) = 10,26$, and an interaction between these two factors, $F(3,84) = 5,59$. An analysis of simple effects showed significant differences between groups preexposed and control in the first conditioning trial, $F(1,28) = 10,40$, but not in the last trial of conditioning, $F(1,28) = 1,34$.

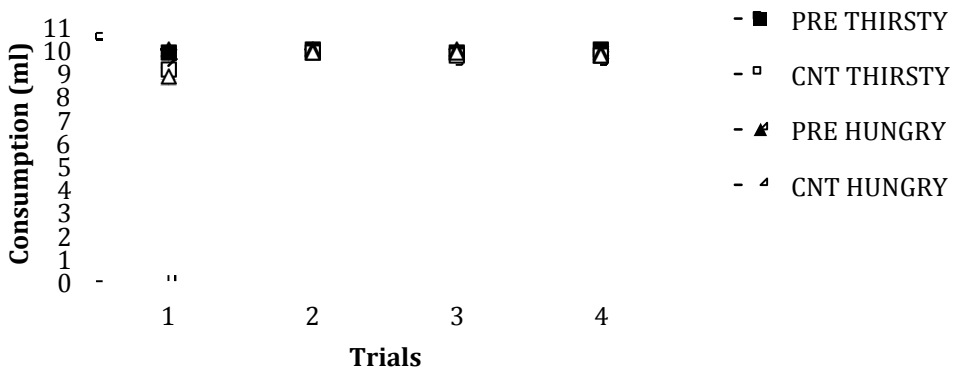


**Figure 27. Experiment 10:** Mean consumption scores during conditioning (sucrose and almond compound) for the preexposed (PRE) and control (CNT) groups, both thirsty and hungry. Vertical bars represent SEMs.

The mean intake of the almond solution and water on test by the rats in each group is shown in Figure 28a, whereas Figure 28b shows the preference ratios (volume of almond / volume of almond + volume of water) based on these intakes. Animals in the preexposed groups drank less of the CS (almond) than those in control groups, a difference that appeared to be substantial only in the hungry animals.. An ANOVA analysis of the direct consumption data in Figure 28a (almond) showed an effect of preexposure condition, $F$ (1,28) = 8,73, and effect of motivational state, $F$ (1,28) = 13,13, but no significant interaction, $F < 1$. An ANOVA conducted on the preference ratios revealed no effect of preexposure condition, $F$ (1,28) = 2,69, no effect of motivational state, $F < 1$, but a significant interaction between these two variables, $F$ (1,28) = 6,55. This interaction was explored using an analysis of simple main effects. This showed there to be a significant difference between groups preexposed and control when animals were hungry, $F$ (1, 14) = 20,37, but no effect was found among thirsty animals, $F < 1$.

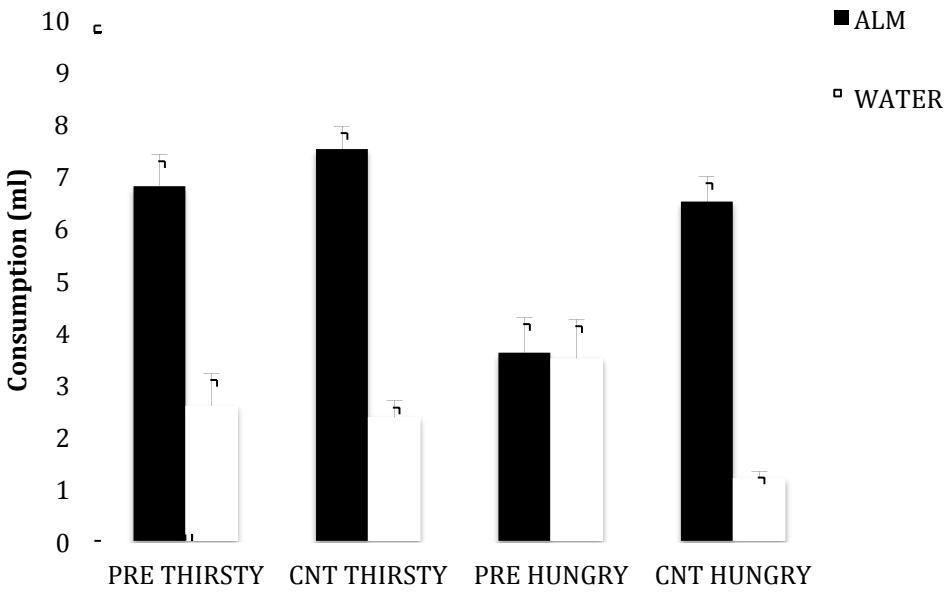


**Figure 28a**

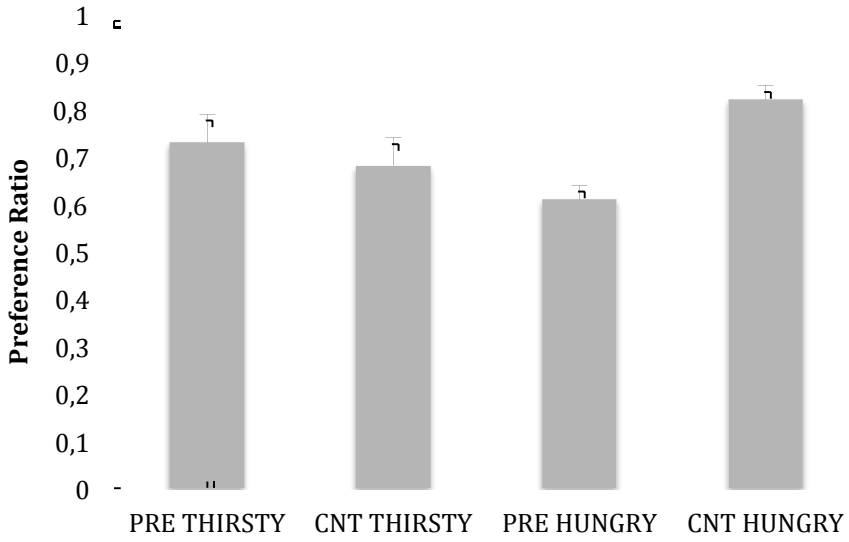


**Figure 28b**

**Figure 28. Experiment 10:** Mean intake on test of almond and water (Figure 28a) and the mean ratio of almond intake over total intake (Figure 28b) for groups preexposed (PRE) and control (CNT), both thirsty and hungry. Vertical bars represent SEMs.

## General Discussion

The aim of the experiments reported in this chapter was to examine the role played by motivational state in producing a US-preexposure effect when using an appetitive conditioning procedure. More specifically, the aim was to directly compare the ease with which the US-preexposure effect can be obtained in hungry and in non-hungry animals. When animals are in a motivational state of hunger the main mechanism supporting appetitive conditioning when using a US such a a sucrose solution (which has both a palatable taste and positive motivational post-oral consequences) is thought to be based on the post-oral consequences of the US (Capaldi & Myers, 1994; Fedorchack & Bolles, 1987). When animals are not hungry, the main mechanism supporting this type of conditioning will be based on the palatable taste of the US (Diaz, et al., 2004; Fedorchak, 1997; Holman, 1980). Manipulating the motivational state should therefore be informative in terms of testing the plausibility of the blocking account proposed in the previous chapter. In hungry animals, it has been suggested that the US preexposure effect could be generated by a blocking mechanism that depends on a taste-calories association; this would not operate in non-hungry animals.

Experiment 9 failed to obtain the US-preexposure effect with non-hungry animals, but it also failed to replicate the effect (well established in previous experiments - Chapters 2 and 3) when animals were hungry. The failure to obtain any US-preexposure effect in this experiment appears to have been due to the type of CS used (mint). Accordingly, for Experiment 10, a different (less neophobic) CS

was used. In this latter experiment, the effect was replicated for hungry but not for non-hungry animals. This latter result seems to be contradicted by an experiment reported by Harris et al. (2000), in which evidence for a US-preexposure effect was obtained in rats that, nominally at least, were give full access to food. It is possible, however, that these rats were, in fact, in a state of hunger throughout the experiment. Harris et al. used a schedule of water deprivation much more severe than that used in the present experiments. Rats that are water deprived will reduce their food intake making it possible that those used in Harris et al's experiment were in a state of "latent hunger". This hypothesis needs to be tested experimentally.

The chief finding to emerge from this chapter is that, at least under certain circumstances, the US preexposure effect can be found when animals are hungry, but might fail to appear when they are non-hungry. This finding seems to depend on employing a particularly non-aversive odour as the CS, and also on using preference ratios as the measure of conditioning. The reason why using preference ratios may be important is unclear, though it may be possible to argue that this may be a more sensitive measure of conditioning than using the levels of consumption from a single bottle. Whatever the reasons for the effectiveness of these procedural changes, a clear US preexposure effect is demonstrated in hungry animals. It must be noted, however, that although no US preexposure effect was evident in non-hungry animals, the control (non-preexposed) animals did show a clear preference for the odour used as the CS on test, indicating that some degree of conditioning, presumably based on flavour-flavour learning, had occurred for

these animals. Although based on a null result, the tentative suggestion is that this form of learning is not susceptible to the effect of US preexposure, and that in cases when the US preexposure effect is found, it depends on blocking of CS-nutrient learning by the taste of sucrose.

One way to examine the susceptibility of flavour-flavour learning to the US-preexposure effect would be to use a US that has sensory properties similar to those of sucrose but that lacks the motivational consequences of sucrose. An artificial sweetener that lacks nutritive properties would be suitable. Accordingly, Chapter 6 examines whether the US-preexposure effect can be obtained with saccharin as the US.

# CHAPTER VI

## EXPERIMENTS 11-12:

## STUDIES OF A NON-NUTRITIVE US

# CHAPTER VI: EXPERIMENTS 11-12: STUDIES OF A NON-NUTRITIVE US

## Introduction

The evidence presented in preceding the chapters raises some alternative possible explanations for the US-preexposure effect generated in the conditioned flavour preference procedure. The majority of the studies of conditioned flavour preferences (CFP) have used a US that has a positive taste and also produces positive post-oral consequences (Boakes & Lubart, 1988; Capaldi, et al., 1997; Capaldi & Myers, 1994; Capaldi, Myers, Campbell, & Sheffer, 1983; Fedorchack & Bolles, 1987; Owens, et al., 1993). Evidence for a conditioned flavour preference using a US (such as saccharin) that has a sweet taste but does not have post-oral consequences is less secure (Capaldi, et al., 1997). But when a preference is obtained (Diaz, et al., 2004; Fedorchak, 1997; Holman, 1975, 1980) this must be produced solely by the hedonic property of that US. Unlike the conditioned preferences acquired to a nutritional US (flavour-nutrient learning), the preference acquired to a US without calories will be based only on its taste (commonly described as flavour-flavour learning).

The experiments reported in the present chapter will make use of the CFP paradigm with saccharin as the US. The experiments reported in previous chapters have found a US-preexposure effect with sucrose as the US, but the mechanisms underlying this effect still remain unclear. Previous chapters have examined the hypothesis that the effect depends on the formation during preexposure of an association between the sweet taste of the sucrose and its post-oral consequences,

an association that will block the formation of any other association between the CS and US during the conditioning phase. One possible test of that proposal is to look for a US-preexposure effect using a US (such as saccharin) that supports conditioned preferences only based on its hedonic properties (its taste). According to this hypothesis, since the US lacks calorific consequences, the association formed during preexposure in the sucrose case could not be formed when using saccharin, and therefore there should be no US-preexposure effect.

This chapter attempts to explicitly examine the role played by taste in generating the US-preexposure effect. Experiment 12 used the same procedure as that of the previous chapters, in that subjects received exposure to either a US or water prior to conditioning with CS-US pairings. It differed only in that saccharin rather than sucrose was used as the US. But before looking for the US-preexposure effect with saccharin as the US, it is necessary first to demonstrate that saccharin is able to serve as a US in the basic acquisition of a conditioned flavour preference (using a procedure similar to that used in Expriment 7). Therefore Experiment 11 attempted to demonstrate basic appetitive conditioning with saccharin as the US (using the standard procedure already employed in preceding experiments in this thesis – see Chapters 2, 5 and 7).

## Experiment 11: Basic conditioning effect using saccharin as the unconditioned stimulus (US)

There were two groups in this experiment. Half of the subjects (Group SIM) were given trials in which they received a compound solution (US-CS), and the other half (Group UNP) received the US and the CS in on unpaired trials (CS / US). For the test all the subjects from both groups were given a two-bottle test with one tube containing the CS and the other tap water (Table 5). Given the possible problems associated with mint as the CS (see Chapter 5), almond was used in this study.

### Method

*Subjects and apparatus.* The subjects were 16 male hooded Lister rats (Charles River Laboratories) with a mean free-feeding weight of 482 g (range: 473-492 g). They had previously served as subjects in an experiment using the CER paradigm but were naïve to all aspects of the current stimuli and procedures. They were housed individually in home cages measuring 35 x 22 x 18 cm, and made of translucent white plastic with wood shavings as bedding. The rats were maintained on a 12h light / 12h dark cycle (lights on at 8:00 a.m.). All experimental procedures were conducted in the home cages throughout the experiment. The stimuli used for this experiment were a 1% (v/v) almond solution (CS) – almond flavouring supplied by Supercook; Leeds, UK), a 0.40% (w/v) saccharin solution

(US), and a compound solution of 1% (v/v) almond and 0.4% (w/v) saccharin solution. The compound used for Group SIM was made up so to preserve these concentrations. All solutions were made with tap water and given to the animals in inverted 50-ml centrifuge tubes equipped with stainless steel, ball-bearing-tipped spouts in the home cages. Fluid consumption was measured by weighing the tubes before and after fluid presentations.

*Procedure.* The rats were assigned to two equal-sized groups at the beginning of the experiment. To initiate a schedule of water deprivation, the standard water bottles were removed overnight; over the next two days access to water was restricted to two 30-min sessions per day (starting at 11 a.m. and 4:30 p.m.). The presentation of the stimuli over conditioning sessions was counterbalanced, with half of the subjects in each group receiving the following pattern of presentations, X-Y-Y-X; and the other half of the animals receiving Y-X-X-Y; where X means A+Sacc (Group SIM) or Sacc (Group UNP) and Y means Water (Group SIM) or Almond (Group UNP). All the trial presentations were counterbalanced over the a.m. and p.m. sessions. After this cycle was completed, all subjects received a two-bottle test (30 ml) with almond (CS) and water (see Table 5). The test was carried out during the morning session. Animals in Group SIM showing a significant greater preference score for almond than those in Group UNP will reveal a conditioned flavour preference.

**Table 5**

**Experimental designs**

| **EXPERIMENT 11** | | |
|---|---|---|
| **Group** | **Conditioning** | **CR Test** |
| **SIM** | 4 A + SACC | A vs. W |
| **UNP** | 4 A / SACC | |

| **EXPERIMENT 12** | | | |
|---|---|---|---|
| **Group** | **Preexposure** | **Conditioning** | **CR Test** |
| **PRE_SUC** | 8 SUC | A + SUC | A vs. W |
| **CNT_SUC** | 8 W | | |
| **PRE_SACC** | 8 SACC | A + SACC | |
| **CNT_SACC** | 8 W | | |

***Note*.** SUC refers to a 20% sucrose solution; SACC refers to a 0,4% saccharin solution; A refers to a 1% almond solution; W refers to tap Water. SIM: Group Simultaneous; UNP: Group Unpaired; PRE: preexposed; CNT: control. All animals in Experiment 12 were maintained hungry and thirsty throughout the experiment.

**Results & Discussion**

The intakes of almond during the CR test differed substantially between the groups SIM and UNP. Those animals that had experienced simultaneous pairings of almond and saccharin drank more of the almond on test than those that had experienced almond and saccharin on separate trials. Preference ratios (volume of almond / volume of almond + volume of water) were also calculated for the two-bottle test. The mean intake of the almond solution and water solution on test by rats in each of the groups in Experiment 11 is shown in Figure 29a. Group means for preference ratio are presented in Figure 29b. It is clear from Figures 29a and 29b that the subjects exposed to the almond in simultaneous compound (Group SIM) consumed substantially more of this flavour than those in Group UNP. An analysis of the direct intake of almond for both groups showed a significant difference between these two groups, $F$ (1,14) = 12,45. A single factor ANOVA conducted on the preference ratio data confirmed this difference to be statistically significant, $F$ (1,14) = 11.24.

The results from Experiment 1 were clearcut. As in previous experiments using a basic conditioning procedure, with sucrose as the US (Experiment 1) or with MD as the US (Experiments 5 and 7), animals receiving pairings of the compound (Group SIM) CS (almond in this case) and US (saccharin in this case) showed significantly higher consumption of the CS when it was presented alone than did animals that had received the stimuli in alternate presentations (Group UNP). These results confirm that a flavour preference can be obtained using

saccharin as the unconditioned stimulus (US). Experiment 12 will therefore explore whether if, using saccharin as the US, animals will show a US-preexposure effect.

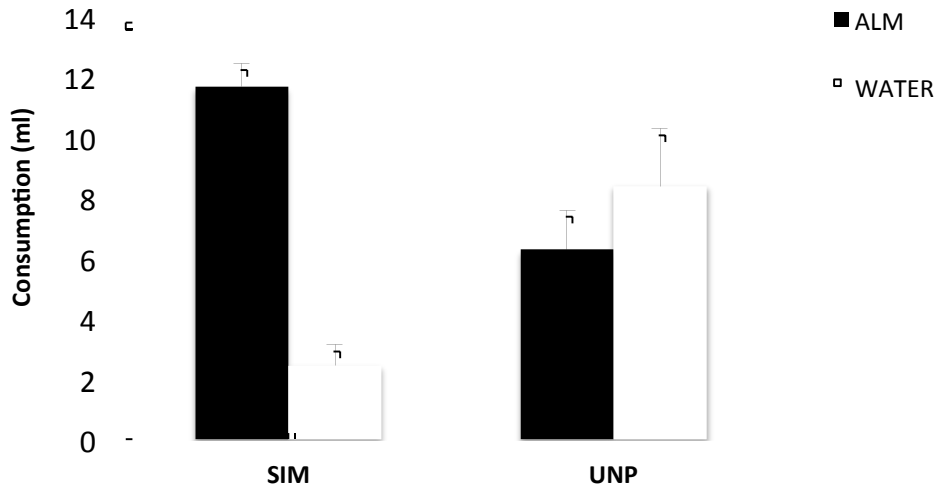


**Figure 29a**

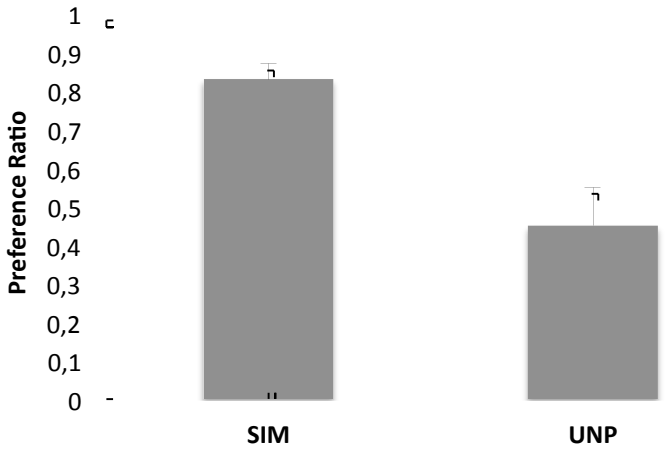


**Figure 29b**

**Figure 29 (29a & 29b): Experiment 11:** Group mean scores for both simultaneous (SIM) and unpaired (UNP) groups on the test with the conditioned stimulus (CS) and water. Figure 29a reflects group means for consumption of the almond and water, and Figure 29b represents the mean ratio of almond intake over total intake. Vertical bars represent SEMs.

## Experiment 12: US-preexposure using saccharin as the unconditioned stimulus (almond)

The results of Experiment 11 have shown that exposing animals to a series of pairings of an almond-saccharin compound simultaneously (Group SIM) will enhance the consumption of the almond (and increase the preference when presented along with water in a preference test), compared with animals exposed to the stimuli on separate trials (Group UNP). Now that a basic conditioned flavour preference has been demonstrated using saccharin as the US, a further experiment looking for the US-preexposure effect with saccharin as the US can be carried out. The design of the experiment is shown in Table 5. Two groups of rats received either preexposure to saccharin (Group PRE) or preexposure to water (Group CNT). Two extra groups were included in this experiment using sucrose as the US in order to allow a direct comparison between a nutritive and non-nutritive US. These animals received exactly the same treatment except that the US was the sucrose solution used in previous demonstrations of the effect (Chapters 2, 3 and 5). Subjects were maintained food and water deprived throughout the experiment (as in previous demonstrations of the US-preexposure effect using sucrose as the US – Chapter 5) and, to maximize the comparability of the results animals with saccharin as the US were also maintained under the same conditions.

**Method**

*Subjects and apparatus.* The subjects were 32 male-hooded Lister naïve rats (obtained from Charles River Laboratories) with a mean free-feeding weight of 427 g (range: 416-348 g). Animals were water and food deprived prior to the beginning of the experiment and maintained this way throughout the experiment (with access to food and water in the afternoon). All the experimental procedures were conducted in the home-cages throughout the experiment. Animals were divided into four groups, group preexposed-sucrose (Group PRE SUC), group preexposed-saccharin (Group PRE SACC), group control-sucrose (Group CNT SUC) and group control-saccharin (Group CNT SACC). Two different USs were used for this experiment, a 20% sucrose solution was used for groups PRE SUC and CNT SUC, and a 0,40% saccharin solution was used for groups PRE SACC and CNT SACC. The conditioned stimulus for all the subjects was a 1% almond solution (almond flavouring supplied by Supercook, Leeds, UK). The compound of sucrose-almond and saccharin-almond used for the conditioning trials was made up as to preserve these concentrations. All the solutions were made with tap water and given to the animals in a 50-ml graduated tubes fitted with a rubber stopper and a stainless steel ball-bearing tipped spout. Fluid intake was measured by weighing tubes before and after the sessions.

*Procedure.* The animals were assigned to four equally-sized groups and were water and food deprived at the beginning of the experiment. The schedules of water and food deprivation and maintenance conditions were established so that

the animals had 30-min access to water in the morning session (from 11 a.m.) and had access to water and food during the afternoon session for 90 min (from 4:30 p.m.). All the experimental sessions were conducted during the morning sessions. The next eight days constituted the preexposure phase. One each of these days animals in groups preexposed received either 15 ml of sucrose solution (Group PRE SUC) or a 15 ml of saccharin solution (Group PRE SACC), whereas animals in the control groups received the equivalent amount water (groups CNT SUC and CNT SACC). Over the following four days all the animals in groups PRE SUC and CNT SUC received 15 ml of a compound with the CS (almond) and sucrose (US), and those animals in groups PRE-SACC and CNT-SACC received 15 ml of a compound with the CS (almond) and saccharin as the US for 30 min. For the CR test all the subjects received a two-bottle test with one tube containing 30 ml of almond and the other containing 30 ml of water.

**Results & Discussion**

Figure 30 shows consumption of fluid during the preexposure phase for all the subjects. Rats given preexposure to sucrose and saccharin drank less on the first trial than on subsequent drinking sessions. Animals with access to sucrose drank consistently more over the preexposure trials than those that had exposure to saccharin. Water consumption remained stable for the control groups although levels were substantially lower than for sucrose or saccharin. An ANOVA conducted on these scores with preexposure condition, CS condition, and trial as

the variables yielded significant main effects of trial, $F$ (7,189) = 22,92, significant effect of preexposure condition, $F$ (1,28) = 64,43, a significant effect of CS condition, $F$ (1,28) = 15,85, and a significant interaction between preexposure condition and CS condition, $F$ (1,28) = 14,09, trial and preexposure condition, $F$ (7,189) = 30,46 and between trial and CS condition, $F$ (7,189) = 2,36. Analysis of simple effects showed there to be a difference between trials 1 and 8 in groups preexposed, $F$ (7,98) = 42,48, but not between trials 1 and 8 in groups control, $F$ (7,91) = 1,10. There were also significant effect of CS condition on trial 1 for preexposed animals, $F$ (1,27) = 125,46 but not for control animals, $F < 1$, and significant differences between preexposed and control animals in the last trial of the preexposure phase, $F$ (1,27) = 119,34.

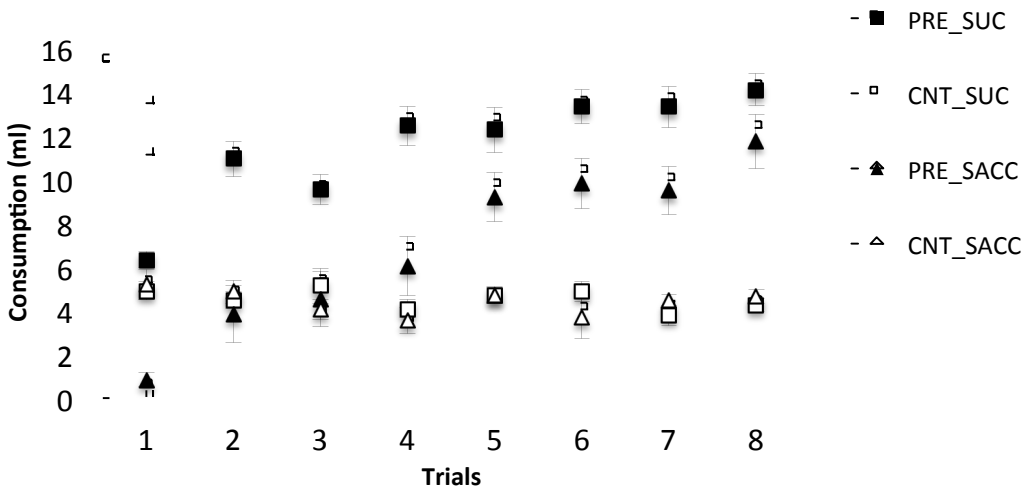


**Figure 30: Experiment 12:** Mean consumption scores during preexposure for the preexposed (PRE) and control (CNT) groups. Animals in Group PRE SUC and PRE SACC received either sucrose or saccharin; those in Groups CNT SUC and CNT SACC received access to water. Vertical bars represent SEMs.

Figure 31 shows consumption of the CS-US compound over conditioning trials. Animals in the control groups (particulary those given saccharin) drank less that those in the preexposed groups on the first trial but there were no differences on the last trial between preexposed and control animals. An ANOVA with preexposure condition, CS condition, and trial as the variables revealed a significant effect of trial, $F$ (3,81) = 39,31, a significant effect of CS condition, $F$ (1,27) = 108,74, a significant effect of preexposure condition, $F$ (1,27) = 70,52, and significant interactions between preexposure condition and CS condition, $F$ (1,27) = 20,94, between trial and preexposure condition, $F$ (3,81) = 23,87, and between trial and CS condition, $F$ (3,81) = 23,54. Analysis of simple effects showed there to be a significant effect of CS condition, $F$ (1,27) = 4,31, but no differences between preexposed and control animals, $F$ <1.

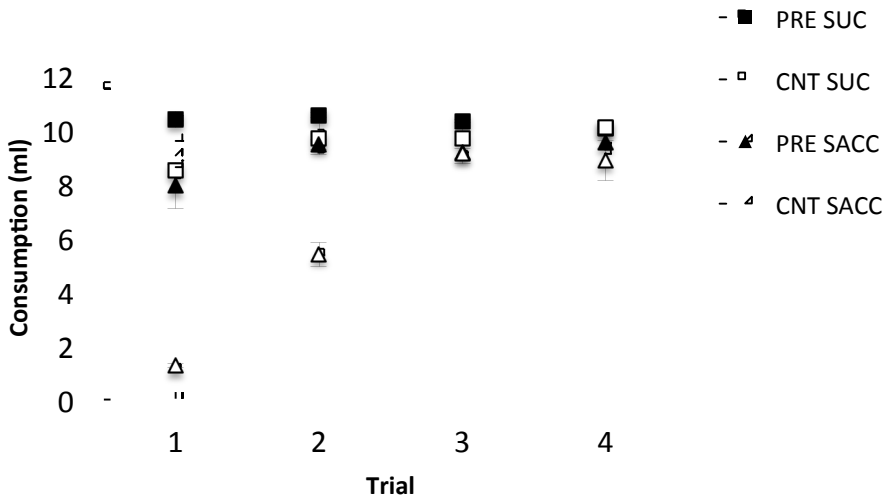


**Figure 31. Experiment 12:** Group means for consumption of the almond-sucrose compound solution during the conditioning phase for animals in the preexposed (PRE) and the control (CNT) groups. Vertical bars represent SEMs.

The results of the CR test are shown in Figure 32. The mean intake of the almond solution and water on test by rats in each of the groups is shown in Figure 32a, whereas Figure 32b shows the preference ratios based on these intakes. All animals drank more of the CS solution that water, but Figure 32b showed a lower preference ratio for animals in groups preexposed that animals in groups control. An ANOVA with preexposed condition and US substance used as the variables conducted on the almond consumption data summarize in Figure 32a failed to show a significant effect of Group, $F < 1$, but there was an effect of US substance, $F$ (1,27) = 12,38, no interaction was found, $F < 1$. Analysis of the preference ratios summarized in Figure 32b revealed a significant difference between preexposed and control animals, $F$ (1,27) = 4,34, a significant difference between animals receiving sucrose or saccharin as the US, $F$ (1,27) = 5,38, but no significant interaction between there two variables, $F < 1$. These results succeeded in showing a US-preexposure effect both when sucrose and saccharin solution were used as the US when preference ratios were used as the measure of the strength of the CR.

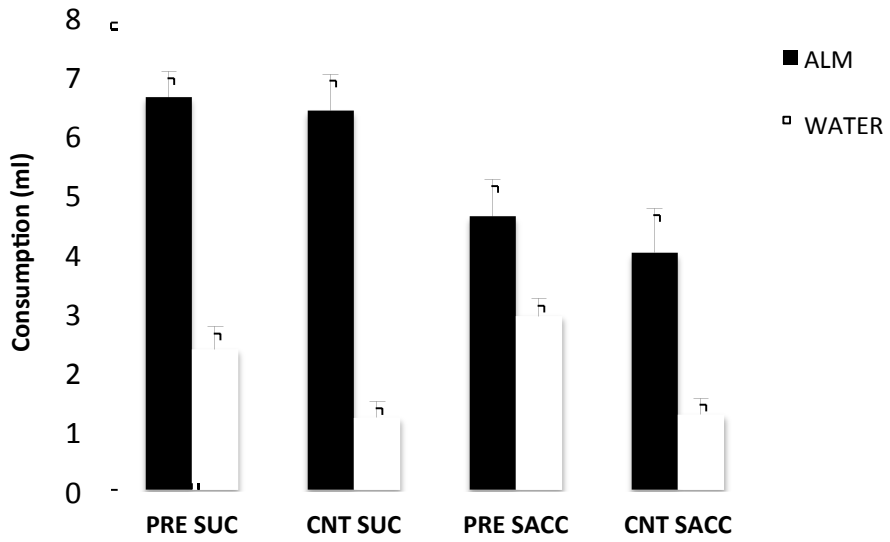


**Figure 32a**

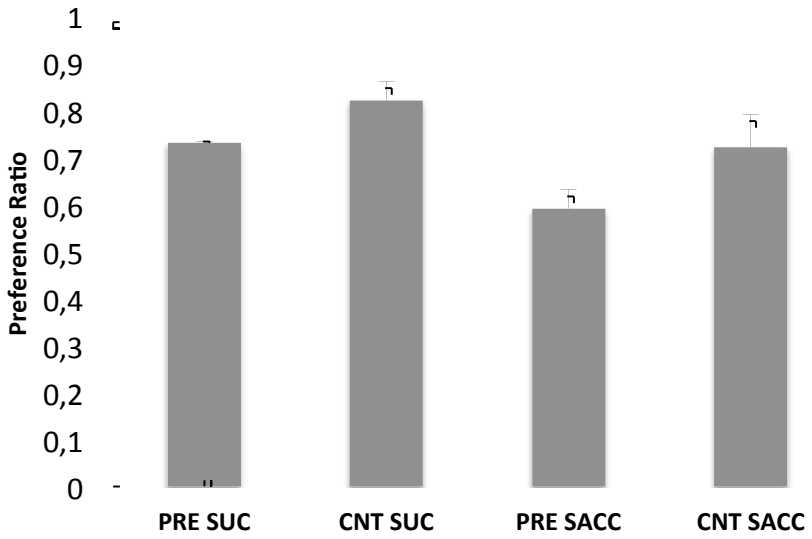


**Figure 32b**

**Figure 32. Experiment 12:** Group means for consumption of the almond and water during the CR test for animals in the preexposed (PRE) and the control (CNT) groups (Figure 32a), and the mean ratio of almond intake over total intake (Figure 32b) for groups given sucrose (Groups PRE SUC and CNT SUC) and saccharin (Groups (PRE SACC and CNT SACC) as the US. Vertical bars represent SEMs.

**General Discussion**

Experiment 11 provided a clear demonstration of conditioned flavour preference using saccharin as the US. Not many studies have succeeded in demonstrating acquired preferences to a flavour without post-oral consequences (Holman, 1975, 1980) and others have failed to show this preference at all (Capaldi, et al., 1997). Although this result constitutes a clear demonstration of conditioned flavour preference based on flavour-flavour learning, this was not the central aim of the experiments reported in this chapter. Rather, Experiment 11 was carried out in order to demonstrate a basic conditioning effect using saccharin as the US in order to allow investigation of the US-preexposure effect using the same US. Experiment 12 was carried out to examine this.

The results of Experiment 12 showed that preexposure to either sucrose or saccharin prior to conditioning trials with that US resulted in a reduced CR; that is, the US-preexposure effect was found with both US´s. Demonstrating the US-preexposure effect using saccharin as the US is of particular interest as this example of the effect is difficult to explain in terms of the blocking-by-taste account previously suggested as an explanation for the effect obtained with sucrose. Since saccharin lacks any post-oral nutritive consequences, no association between the taste and the nutritional properties of the US during preexposure can be formed. Although this form of blocking remains possible for the sucrose case, it seems that cannot be the whole story and that a different explanation for the saccharin case is needed.

But before pursuing this, it is worth considering weather it might be possible to modify the blocking by taste mechanism so that it could also operate with a substance such as saccharin. Perhaps it can be argued that preexposure could establish an association between the taste and its property of palatability, this taste-palatability association might then block the formation of the CS flavour-palatability association during conditioning. Although this explanation is possible, other results from previous experiments (Chapter 5) do not seem to support it. A US-preexposure effect using CFP based on flavour-flavour learning should also have been found in the experiments described in Chapter 5. According to the blocking hypothesis,, a reduction in the CR to the CS should also have been found in animals receiving exposure to sugar prior to conditioning where animals were only water deprived (Experiments 9 and 10) and therefore may be assumed to have a acquired a preference based only on flavour-flavour learning. This suggests that although the blocking by taste mechanism may provide a satisfactory account of the US-preexposure effect when sucrose is used, it is likely that an alternative mechanism will be needed to explain the case in which saccharin is used. The experiments in this chapter therefore generate some interesting results and imply the need for new research on the US-preexposure effect using a palatable non-nutrient US.

# CHAPTER VII

## CONCLUSIONS

# CHAPTER VII: CONCLUSIONS

## Summary of the new findings

The experiments reported in Chapters 2 to 6 have investigated the US-preexposure effect using an appetitive paradigm (the CFP procedure). The results from this experimental work have yielded the following main findings:

1) Rats given preexposure to sucrose (as the US) showed a reduction in the strength of subsequent conditioning when a CS was simultaneously paired with the US, as measured by the subjects' willingness to consume the flavour used as the CS when presented alone on an acceptance test (Chapter 1 – Experiment 2). This result provides a basic demonstration of the US-preexposure effect with an appetitive conditioning procedure.

2) A second consequence of giving preexposure to a highly concentrated sucrose solution was that subjects were found to consume it increasingly more readily during the course of preexposure. This can be taken to reflect the tendency for an initial neophobic response to the sucrose to decline during preexposure and can be regarded as an instance of the well-established phenomenon of *habituation*.

3) The third finding was that the US-preexposure effect found in the CFP paradigm was insensitive to manipulation of the context. Preexposing

animals to the US in the home-cages produced an effect as substantial as that found when the animals experienced the US preexposure treatment in a salient and novel context. Moreover, a contextual change between the preexposure and test phases did not have any impact on the size of the effect.

4) It proved possible to find a US-preexposure effect when the US was a substance with broadly similar nutritional consequences to those of sucrose, but lacking its sensory (taste) properties. In particular, although an initial attempt to find a US preexposure effect using maltodextrin (MD) failed (Experiment 6), a number of procedural modifications allowed this effect to be found in Experiment 8. In addition, there was some indication of habituation of neophobia to MD over the course of preexposure, although no differences were observed on a final UR test (Experiment 6).

5) The US preexposure effect with sucrose as the US was found to be dependent on the motivational state of the subjects. An initial study (Experiment 9) found no US-preexposure effect when the animals were non-hungry, but this experiment also failed to generate the basic effect with hungry animals. Modifying the parameters of the experimental procedure resolved this issue; when the experiment was replicated using a less neophobic CS (Experiment 10), animals that were hungry throughout the experiment and preexposed to the US, showed a decrement in the CR on the

test; but when the animals were not hungry, no US-preexposure effect was found.

6) Finally, the US-preexposure effect was found with a substance that has sensory properties that are comparable with those of sucrose, but which lacks its motivational properties (saccharin). Subjects trained with saccharin showed an effect as readily as those that had been preexposed to sucrose (Experiment 12).

## Implications of these findings

### Reality of the effect in appetitive conditioning

It has been widely demonstrated that the conditioned response shown to a CS as a result of CS-US pairings, otherwise strongly acquired during conditioning, can be reduced by prior exposure to that particular US, a phenomenon known as the US-preexposure effect. The effect has been examined in a variety of paradigms such as CER (i.e., (i.e., Baker & Mackintosh, 1979; Baker, et al., 1981; Kamin, 1961; Randich, 1981; Randich & Lolordo, 1979b), CTA (i.e., Braveman, 1975; de Brugada, et al., 2004; Domjan & Best, 1977; Gamzu, 1977), eyeblink responding (i.e., Hinson, 1982; Taylor, 1956), and appetitive autoshaping (i.e, Engberg, et al., 1972; Tomie, 1976a, 1976b). Work based primarily on the aversive version of the US-preexposure effect has generated two main proposals as to the mechanisms responsible for the effect: an associative (blocking) account, and a nonassociative (habituation) account.

The associative hypothesis has been the one most often commonly offered to explain the US-preexposure effect and it has generated a considerable amount of research. The main explanation offered according to this account is in terms of blocking by context, in which an association between the experimental context and the US formed during preexposure is thought to block the acquisition of a CR to the CS during the conditioning phase (e.g., Baker & Mackintosh, 1979; Randich & Lolordo, 1979b). A modified version of this account has also been offered for the

effect obtained in the CTA paradigm (de Brugada, et al., 2004). According to this account, the critical association responsible for blocking is one involving the cues arising from the injection used to administer the US during preexposure.

The second main account offered for the US-preexposure effect is in terms of habituation (Randich, 1981; Randich & Lolordo, 1979a). According to this, the repeated presentation of the US during preexposure will produce habituation so that the US´s ability to evoke a UR will be affected and its effectiveness to function as a reinforcer will be reduced. Consequently, conditioning with this less effective US will be slower than with a nonhabituated US.

These notions have been derived from and have been applied to aversive versions of the US-preexposure effect. The appetitive case has received much less attention and whether it is susceptible to processes of this sort remains to be determined. Studies that make use of the autoshaping procedure are open to alternative explanations. In particular, preexposure to an appetitive US is likely to establish patterns of behaviour that will compete with the acquisition of the CR during conditioning (see, e.g., Timberlake, 1986). The new results obtained from the experiments in this thesis provide a demonstration of the US-preeexposure effect using an appetitive procedure that cannot be explained by competing responses. Moreover, they provide a parallel procedure to the aversive case and could be susceptible to an explanation in the same terms (i.e, in terms of blocking by context or habituation). They provide an alternative method for testing the validity of these accounts.

**Mechanisms responsible for the appetitive effect: Blocking**

As has already been noted, blocking by contextual cues has been widely advanced as a mechanism for the US-preexposure effect. The work presented in Chapter 3 of this thesis, however, appears to rule out a role for blocking by the context, at least for the version in which the context is defined by the particular place in which preexposure is given.

In Experiment 3, animals were preexposed to the US either in a novel context or in their home cages. A strong US-preexposure effect was obtained when animals were trained and tested in the novel context, but this effect was also evident when training and test occurred in the home cage. The blocking-by-context account will predict that the context supplied by the home cages will not be associated with the US as readily as will a novel context. Extensive previous experience of the home cage should render that context latently inhibited. But it was found that animals trained and tested in the home cages produced an effect as readily as those trained and tested in a novel context.

Experiment 4 further investigated the possible role played by the context in the US-preexposure effect using in our CFP paradigm, by changing the experimental context between the preexposure and test phases. In this case, the blocking-by-context account would predict that the US preexposure effect should be attenuated when there is a shift of context between the preexposure and conditioning phases, since the context-US association formed during preexposure will no longer be present during the conditioning phase, and thus will be

ineffective in blocking the formation if the CS-US association. Although results obtained using the CTA paradigm have shown such an attenuation of the effect using a context shift, such an effect was not found with the present experimental procedure. Animals tested in a context different from the one in which they were preexposed to the US, showed a diminished CR on test as readily as those preexposed and tested in the same context. The results from the latter experiment confirmed that, at least using this particular appetitive procedure using a palatable US with post-oral consequences, the US-preexposure effect cannot be interpreted in terms of blocking by the context.

However, an alternative associative hypothesis has been proposed (Chapter 3) to explain the results obtained in Experiments 3 and 4. This hypothesis is specific to the CFP paradigm used in this version of the US-preexposure effect (i.e. the use of a palatable US with motivational consequences) and the type of learning that occurs during the conditioning training using such a US. This modified version of the blocking-by-context account assumes that with such a US, that has both a strong palatable taste and motivational consequences, an association between these two properties could be formed during the preexposure phase. This association might then be expected to block the formation of an association between the taste of the CS and the consequences of sucrose during subsequent conditioning. This revised version of the blocking account is suggested to operate in much the same way as the blocking by context mechanism, except that the critical cue in this case is the taste of the US that forms an association with

its nutritional consequences. The experiments presented in Chapter 4 were a preliminary attempt at testing this new blocking account.

Experiments 6 and 8 (Chapter 4) used an appetitive reinforcer (maltodextrin, MD) thought to have similar postingestive consequences as sucrose but which lacks a strong taste. Given this presumed lack of a strong taste, it was suggested that no association could be formed between the motivational properties of MD and its taste during preexposure. Hence, according to the revised blocking account, there should be no US preexposure effect when MD is used as the target US. In accord with this prediction, Experiment 6 failed to produce an effect and therefore supported this blocking-by-taste hypothesis. Experiment 8, however, succeeded in producing a US-preexposure effect. The discrepancy between the results of Experiments 6 and 8 is likely to be due to the use of a more sensitive test procedure in the latter. However, the fact that the effect was obtained in Experiment 8 does not completely rule out the blocking-by-taste account. MD is not without some taste and, accordingly, an effect of blocking by its sensory properties might still be obtained – albeit a less powerful one than that generated by sucrose.

Given the ambiguous nature of these findings, the experiments in Chapter 5 adopted a different strategy to further investigate the possible role of blocking by taste. In particular, the blocking-by-taste hypothesis assumes that, in order to form the association that will block the acquisition of a CR during conditioning, some predictive learning occurs during preexposure, that is, the taste of the US will become associated with its motivational properties and therefore predict its

occurrence. It is widely assumed that in the CFP paradigm, when animals are hungry, the conditioned preference for flavour will be based on flavour-nutrient learning, but when animals are non-hungry that preference will be based on flavour-flavour learning, that is, on an association between the taste of the CS and the palatability of the US. The experiments in Chapter 5 directly compared the role that the motivational state of the animals might play in the US-preexposure effect with the CFP paradigm. According to the blocking-by-taste account, if animals are non-hungry, the mechanism involved in conditioning will be based on flavour-flavour learning and therefore the US-preexposure effect will not be expected to occur. In general, the results found in experiments 9 and 10 (Chapter 5) seem to support this theory. At least under certain experimental conditions, the US-preexposure effect was found when animals were maintained hungry throughout the experiment but not when they were non-hungry.

The results obtained in this thesis so far seem to support the blocking-by-taste account suggested in Chapter 3. It seemed worthwhile, however, to test a further implication of this account, and this was the purpose of the experiments presented in Chapter 6. In particular, in its present form, the blocking by taste account should play no role in generating a US preexposure effect when the target US is a sweet taste, such as saccharin, that lacks the motivational properties of sucrose, In this case, although saccharin might be expected to support basic CFP learning (by virtue of a flavour-flavour association), it is not clear how the blocking by taste account could predict that preexposure to the US should retard such conditioning. If saccharin only has taste properties and no nutritional

consequences, then any learning that it supports should not be susceptible to the effects of US preexposure if the critical association is one formed between the taste of the US and its nutritive consequences.

The results of Experiment 12 (Chapter 6) did not support this analysis. When animals were preexposed to saccharin, a US-preexposure effect was found as readily as when animals were exposed to sucrose. This result is, at first sight, problematic for the blocking-by-taste explanation for the US-preexposure effect using a CFP paradigm, and an alternative hypothesis will be needed to explain this result.

**Mechanisms responsible for the appetitive effect: Habituation**

Repeated presentations of a stimulus will produce habituation, evident as an attenuation of the evoked UR (Thomson & Spencer, 1966). If this effect is taken to reflect a loss of effective salience by the stimulus (Hall, 2003), then it is possible that repeated presentations of a US will result in a reduced ability of that stimulus to function as a reinforcer. It has been demonstrated, in the aversive case, that habituation can occur alongside the US-preexposure effect (de Brugada, et al., 2005; Randich & Lolordo, 1979b). There is little direct evidence, however, even for the aversive case, to show that this habituation process is critical in producing the US preexposure effect; and it is particularly unclear as to how such a process could contribute to the US preexposure effect found with an appetitive conditioning procedure. It does not seem plausible, for instance, to assume that habituation will

occur to the postingestive properties of a nutritive stimulus. Although it has been shown that repeated exposure to foods can generate a form of habituation, this seems to be specific to the particular taste of the food. Human subjects given a monotonous diet tend to avoid the preexposed food, but readily turn to other foods (Hetherinton, et al., 2002; Meiselman, et al., 2000).

There is some evidence supporting the occurrence of this form of habituation in the results reported in this thesis. When animals were preexposed to sucrose, a subsequent UR test showed that such animals drank more than those that had experienced only water (Experiments 3 and 4). There was, in addition, a evidence of habituation over the preexposure trials, both with sucrose and with saccharin (in Experiment12). The animals showed a neophobic response when they first experienced the US but this UR decreased over the preexposure trials. Habituation to the taste of the US during preexposure can supply an explanation for the US–preexposure effect obtained with saccharin. If the CR depends on the CS-taste association and the latter is of reduced salience, then conditioned responding might be expected to be weak. It is less clear, however, that this hypothesis can explain the result obtained with sucrose. The chief reason for doubting the importance of this process when sucrose serves as the US is that the US-preexposure effect does not appear when the animals are non-hungry; that is, it fails to appear in a situation in which (as was the case for saccharin) the CR is thought to depend on the association between the CS and the taste of the US. More generally, there is no obvious reason to suppose that motivational state should

play a role in the extent to which a UR is evoked by a novel taste, or in the subsequent decline in this response during preexposure.

## Conclusions

The results reported in this thesis do not fully resolve the puzzles noted above, but they suggest some hypotheses for further study

The results obtained when sucrose is used as the US support the blocking-by-taste account; in particular the lack of an effect when animals are non-hungry lends strong support to this analysis. The results obtained using MD are ambiguous -- although MD may lack a taste strong enough to block the formation of an association of the CR during conditioning, some other sensory properties of such substance, perhaps in conjunction with its taste, might be able to do so. If this is true, the blocking-by-taste hypothesis previously offered could still play a role in producing the US- preexposure effect when this substance is used as the US.

However, the results reported when saccharin is used as the US, seem particularly problematic for this interpretation of the US-preexposure effect. If the CR shown by saccharin is supported solely by the CS-flavour association then blocking (which is taken to act on the CS-nutritional consequence association) could play no role. One might speculate that, during preexposure to saccharin, an association might be established between the sweet taste of saccharin and the palatable properties related to it. This taste-palatability association could be the one blocking the acquisition of a CS-palatability association during conditioning.

The problem for this notion is that it must also predict that a similar process would operate with sucrose; but we have seen that the US-preexposure effect is not obtained with sucrose in non-hungry animals, Perhaps, however, the general notion can be saved if we take note of the fact that saccharin is a complex substance combining both bitter and sweet tastes.. This might allow preexposure to establish an association between the two components of the taste; bitter signalling sweet might then act to block the CS-sweet association during conditioning, the association on which the CR may be assumed to depend. This analysis is rather more complex than the simple notion (discussed above) that the effect obtained with saccharin is a direct effect of habituation. It has the advantage, however, that it is consistent with the explanation favoured for the results of the experiments with other USs

Future research should therefore be directed at testing the hypothesis just outlined. The obvious implication is that the US-preexposure effect should not appear if the US is simply sweet but non-nutritive (i.e., is like saccharin but lacks the bitter taste that characterises that substance). This is just what is claimed for a range of modern artificial “sweeteners”, and research with these should help settle the issues not resolved by the experiments reported here.

# ÍNDICE DE FIGURAS Y TABLAS

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## CHAPTER II



## CHAPTER III

**CHAPTER IV**

**CHAPTER VI**

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