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The Neurobiology of Latent Learning in the Rat using Salt Appetite and Its
Dissociation from Conditioning

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June, 2006

A thesis submitted to McGill University in partial fulfillment of the requirements
of the degree of Doctor of Philosophy

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ABSTRACT

The brain areas required for latent learning in the rat are not currently understood. Previous tasks used to assess latent learning, defined as the acquisition of neutral information that does not immediately influence behavior, have shared characteristics that prevented their use to determine the neurobiology of latent learning. This thesis describes a new task called the Latent Cue Preference (LCP) task, derived from the Conditioned Cue Preference (CCP) task that has been successfully used to determine the brain areas required for conditioning in the rat and other animals. In the LCP task, water deprived rats alternately drink a salt solution in one distinctive compartment of a CCP box apparatus and water in the other compartment over 8 days (training trials). They are then given a choice between the two compartments with no solutions present (preference test). The results of the behavioral experiments showed that this training results in two parallel forms of learning: (1) latent learning of an association between salt and salt-paired compartment cues, and (2) conditioning to water-paired compartment cues. Latent learning itself involved two components: (1) the latent association between salt and salt-paired cues, and (2) motivational information about salt deprivation used to retrieve the latent association, and used to compete with the conditioning to water-paired cues. In addition, the findings showed that latent learning and conditioning involve different neural circuits. Latent learning required an intact cortical-to-hippocampus circuit via the entorhinal cortex, while conditioning required an intact subcortical-to-hippocampus circuit via the fimbria-fornix. The acquisition and storage of the latent association depended on an intact entorhinal cortex/dorsal hippocampus circuit, while the use of motivational information to retrieve the association recruited the ventral hippocampus. Conditioning, on the other hand, required an intact fimbria-fornix, lateral amygdala, and hippocampus. These findings provide new knowledge to the field of learning and memory research, and allowed an update of the current Multiple Memory Systems model.

RÉSUMÉ

Les régions de cerveau nécessaires à l'apprentissage latente chez le rat ne sont pas actuellement comprises. Les tâches précédemment utilisées pour évaluer l'apprentissage latent, défini comme l'acquisition d'information neutre qui n'influence pas immédiatement le comportement, ont partagées des caractéristiques qui ont empêché leur usage pour déterminer la neurobiologie de l'apprentissage latente. Cette thèse décrit une nouvelle tâche appelé la Préférence d'Indicateur Latente (PIL), dérivée de la Préférence d'Indicateur Conditionnée (PIC) qui a été utilisée pour déterminer avec succès les régions du cerveau nécessaires au conditionnement chez le rat et autres animaux. Dans la tâche PIL, des rats privés d'eau boivent alternativement une solution de sel dans un compartiment distinctif d'un appareil et de l'eau dans un autre compartiment pendant 8 jours (entraînement). Ils sont alors donnés un choix entre les deux compartiments avec aucunes solutions présentes (le test de préférence). Les résultats des expériences comportementales ont démontré que cet entraînement a pour résultat deux formes parallèles d'apprentissage: (1) l'apprentissage latente d'une association entre le sel et les caractères indicateurs du compartiment approprié et (2) le conditionnement des caractères indicateurs du compartiment associé à de l'eau. L'apprentissage latente lui-même contient deux composantes: (1) l'association latente entre le sel et les caractères indicateurs présents dans le compartiment contenant le sel et (2) l'information motivationnelle produite par la déficience en sel utilisé pour activer l'expression de l'association latente, et aussi utilisé pour faire la concurrence aux caractères indicateurs conditionnés à l'eau. De plus, les conclusions ont démontrés que l'apprentissage latente et le conditionnement impliquent différents circuits neuronaux. L'apprentissage latente requiert un circuit intact du cortex à l'hippocampe par le cortex entorhinal. Cependant, le conditionnement requiert un circuit intact entre des régions sous-cortical et l'hippocampe par le fornix. L'acquisition et l'emmagasinage de l'association latente ont dépendu d'un circuit intact entre le cortex entorhinal et l'hippocampe dorsal. Cependant, c'est l'hippocampe ventral qui a servi de lien entre l'information motivationnelle et cette association latente. Le

conditionnement, d'autre part, a requiert le fornix, la amygdale latérale et le hippocampe intact. Ces conclusions fournissent de nouvelles connaissances au champ de recherche d'apprentissage et de la mémoire, et a permis une mise à jour du model des Systèmes de Mémoire Multiples.

PREFACE

A common activity among the internet-savvy youth of today is to perform a Google search of your own name, in the hopes of elevating yourself above your usual anonymity to find yourself in the global stream of consciousness. However if Hugh Blodgett, the innovative originator of the concept of latent learning, were alive today he would be disappointed to discover that a Google search of his own name would return as its top match an announcement for the Crenshaw-Kleve – Blodgett wedding planned for June 10, 2006, in Jamaica. Nice folks, I'm sure, but hardly pioneers in the field of learning theory. The match for Hugh Blodgett occurs in the third Google item, which turns out to be a short reference in a biography of Edward Tolman, one of the more recognized originators of Cognitive learning theory.

It is therefore not surprising to learn that Blodgett's concept of latent learning, the unintentional acquisition of neutral information that does not affect immediate behavior but can be recalled and used when it becomes relevant, has been largely overlooked. This is despite the fact that many of our biographical memories rely on latent learning. We do not set out to intentionally remember many things that happen in our everyday lives, and many of these events involve neutral stimuli. For example, imagine you are walking down a street and notice a Peruvian restaurant. You have never had Peruvian food before, but you do not stop for a bite to eat and you make no attempt to remember its location. Several weeks later a friend suggests going out for Peruvian food and you are able to recall the location of the restaurant. This is latent learning. However, latent learning is not included in the current Multiple Memory Systems (MMS) model that is widely accepted by modern learning researchers, despite the fact that it was the original demonstration of latent learning that gave credence to Cognitive learning theories as an alternative to Behaviorist theories at a time when Behaviorism had a stronghold on psychology. The MMS model includes Cognitive and Behaviorist ideas, but not latent learning.

One reason for this exclusion is that the brain areas that are necessary for latent learning are not as well established as they are for the other three types of

learning (cognitive learning, habit learning, and conditioning) included in the MMS model. This is primarily due to the fact that the latent learning tasks that have been developed thus far have used reinforced spatial learning trials to illustrate latent learning that occurred during unreinforced pre-exposure to the maze, or have used an increase in bar pressing rates to illustrate a latent learning effect. However, these tasks had one major flaw: when brain lesions were made to examine their effect on latent learning they also produced side effects that produced a change in the behaviors (reinforced maze trials or bar-pressing) that were used to illustrate latent learning. This obscured the effect of the brain lesion. Therefore it was difficult to determine if the disruption in latent learning was due to a true learning deficit produced by the brain lesion, or if it was due to a side effect independent of learning. My main purpose for conducting this thesis research was to introduce a new behavioral task that could be used to demonstrate latent learning but that would also be immune to potential side effects of brain lesions on other learning and memory systems in the brain. I would then use this new task to determine some of the brain areas involved in latent learning and contrast them to the brain areas involved in conditioning, which involves rewarded rather than neutral learning during training. This information would then be used to update the current MMS model.

I developed the Latent Cue Preference (LCP) task as an adaptation of the Conditioned Cue Preference (CCP) task to assess latent learning in rats. The CCP task has been used successfully to determine the brain areas involved in conditioning. The standard CCP apparatus consists of two main compartments that are joined together by a smaller third compartment. Each of the two main compartments has distinct visual, tactile, and olfactory cues. Training in the LCP task involved placing water deprived rats in one compartment of the apparatus in which a salt solution was available to drink on one day, then placing it in the opposite compartment with water available to drink on the next day. During these training trials the rats were not salt deprived, making the salt solution a neutral stimulus. The neutral salt stimulus was associated with the cues in the compartment in which salt was available. Following several of these training trials

rats were given a compartment preference test in which they were allowed free access to both compartments with the solutions removed. Some of the rats were kept water-only deprived, while others were salt+water deprived. The latter rats spent more time in the salt-paired than in the water-paired compartment during the test. This is a demonstration of latent learning. If a rat was salt+water deprived during the test, the latent association between salt and compartment cues associated with salt was retrieved, providing the rat with information required to find salt and leading to the observed preference.

In contrast to the LCP task, training in the CCP task resulted in conditioning. Salt+water deprived rats were placed into one compartment in which a salt solution was available to drink on one day, then were placed into the opposite compartment with water available on the next day. During these training trials the rats were salt deprived, making the salt solution a rewarding unconditioned stimulus. This unconditioned stimulus was associated with the cues in the compartment in which salt was available, making them conditioned stimuli. Following several training trials rats were given a compartment preference test in which they were allowed free access to both compartments with the solutions removed. During this preference test, the salt-paired compartment cues elicited conditioned responses which resulted in the rats spending more time in the salt-paired than water-paired compartment. This is a demonstration of conditioning.

I used the LCP and CCP tasks to conduct four sets of experiments that examined the differences between latent learning and conditioning. These experiments yielded several important findings, which represent original contributions to the field of learning research. First, although both latent learning and conditioning result in rats preferring their salt-paired rather than water-paired compartments, there is a behavioral dissociation between these preferences. The acquisition of latent learning during training trials involves no salt deprivation state, making the salt solution a neutral stimulus. The expression of latent learning requires the presence of motivational information about salt deprivation during the preference test. If this motivational information is not present during

the preference test, the latent association between salt and salt-paired compartment cues is not retrieved and no preference for the salt-paired compartment is expressed. In contrast, conditioning involves a salt or water deprivation state during training trials, making the salt or water a rewarding stimulus. However, conditioning does not require the presence of the motivational information about a deprivation state during testing to be expressed. The salt-paired compartment cues can elicit conditioned responses in the absence of a deprivation state.

Second, the LCP training procedure results in the simultaneous acquisition of latent learning and conditioning. During training rats are water-only deprived, making the water that is available in one compartment an unconditioned stimulus. The water-paired compartment cues become conditioned stimuli and elicit conditioned responses that result in a preference for the water-paired compartment if a rat *is not salt deprived* during the preference test. If a rat *is salt deprived* during the preference test, latent learning is expressed. The salt deprivation results in motivational information that serves two functions: (1) it initiates the retrieval of the latent association between salt and salt-paired compartment cues, and (2) it results in salt-seeking behaviors that compete with and nullify the conditioned responses to the water-paired compartment cues.

Third, there is a neural dissociation between latent learning and conditioning. The hippocampus has two major input/output pathways: (1) a subcortical-to-hippocampus pathway that involves the fimbria-fornix, and (2) a cortical-to-hippocampus pathway that involves the entorhinal cortex. Results of lesion experiments demonstrated that damage to the subcortical-to-hippocampus pathway impaired conditioning, while damage to the cortical-to-hippocampus pathway impaired latent learning.

Fourth, if the ventral hippocampus is inactivated during the expression of latent learning, rats behave as though they have no motivational information about salt deprivation and show a preference for the water-paired compartment. These temporary inactivation results led me to conclude that the ventral hippocampus is necessary for processing motivational information about deprivation states, and

utilizing that information to retrieve the latent association between salt and salt-paired compartment cues that is processed by the entorhinal cortex/dorsal hippocampus circuit, as well as produce salt-seeking behaviors that compete with and nullify the conditioned responses to water-paired compartment cues.

The results of these experiments provide information about the neural substrates involved in one type of latent learning. In addition, the results provide insight into a functional dissociation between the dorsal and ventral hippocampus: the dorsal hippocampus appears to be involved in the formation of associations among external stimuli, while the ventral hippocampus appears to be involved in use of internal stimuli (motivational information) to retrieve associations formed by the entorhinal cortex/dorsal hippocampus circuit in the production of appropriate behaviors. These findings constitute original contributions to the field of learning and memory research, and allow me to update the current MMS model to include at least one type of latent learning, the LCP. In addition, these findings provide insight into the formation of biographical memories that rely on latent learning. Acquisition and retrieval of these biographical memories may depend on a functional entorhinal cortex/dorsal hippocampus circuit, and amnesia of these biographical memories may represent a dysfunction in this circuit.

CHAPTER ONE: DEFINITIONS OF CONCEPTS AND SALT APPETITE IN THE RAT

Definitions of Concepts

A number of concepts and terms that are unique to the experiments described in this thesis, as well as to the fields of learning and memory research, are used in this thesis. Therefore, it is necessary to provide operational definitions for these concepts and terms.

Salt appetite occurs when a rat becomes sodium deprived, resulting in the production of two neurohormones (angiotensin and aldosterone) which lead to behaviors that result in the increased consumption of sodium to relieve the deficiency. This should be differentiated from salt preference, which is the differential consumption of sodium-salt solutions depending on their concentrations, and which occurs during a state of sodium satiety. Lower concentrations of salt solutions are more palatable than higher concentrations and are therefore consumed more. Salt preference does not involve angiotensin or aldosterone.

Latent learning, a term first coined by Blodgett (1929), is the incidental, unreinforced and unrewarded acquisition of “neutral” information with no immediate implication for behavior. The existence of the learned information becomes apparent when it later influences the acquisition or expression of some behavior.

An incentive is any stimulus that is biologically relevant to the animal and is able to trigger behaviors that result in the animal approaching or spending time with the incentive stimulus. This incentive stimulus may be food, water, salt, drugs, or even novelty. The desire or “wanting” to approach, spend time with, and/or consume the incentive stimulus after experiencing the incentive is called motivation. This desire or wanting may be based on the pure hedonic qualities of the incentive (e.g. a sucrose solution has pure hedonic qualities) or may be produced by an internal state in which the animal is deprived of the incentive (e.g. water deprivation or salt deprivation produce motivation to approach and consume water and salt, respectively).

Although spatial learning and contextual learning both involve the integration of several distinct stimuli into one representation, several features distinguish the two types of learning. Spatial learning incorporates various visual or other stimuli present in an environment. The formation of the spatial representation of the environment requires movement throughout the environment (White, 2004), which results in encountering the separate visual cues at different points in time. Contextual learning, on the other hand, incorporates multiple (often multimodal) cues that are experienced simultaneously in a smaller environment into one contextual representation.

Throughout this thesis, the term reward will be mentioned frequently. This term should be differentiated from the term reinforcement. Although both reward and reinforcement result in a change in behavior, they act through different mechanisms. In a distinction similar to one made previously by White (1989), reward will be referred to as the positive affect or hedonic state that is produced by encountering or consuming an incentive stimulus that results in the animal wanting to maintain contact with that incentive. This experience produces conditioned responses toward that incentive and any stimuli associated with the incentive. This positive affect or hedonic state may be the result of drive reduction (e.g. giving water to a water deprived rat can be rewarding) or the result of the incentive having some elementary hedonic value (e.g. a sucrose solution given to a satiated rat still has hedonic value and can be rewarding). The term reinforcement will be used to refer to the process of strengthening stimulus-response (S-R) associations. This is done by making the availability of a reinforcing stimulus contingent upon the performance of the response element of the S-R association. After repeated (S-R)-reinforcer pairings, the stimulus is able to elicit the response even in the absence of the reinforcer.

Salt Appetite in the Rat

The experiments described in this thesis use procedures to induce salt appetite in the rat in order to initiate the retrieval of a latently learned association between compartment cues and salt. The purpose of this chapter is to describe the mechanisms of action of salt appetite in the rat. Much like salt preference, salt

appetite appears to be dependent on an increased palatability of salt solutions during a state of salt deprivation. The mechanisms that underlie this increased palatability are discussed in a later section.

The rat's apparently innate ability to recognize sodium deficiency and engage specifically in the consumption of sodium salts to relieve that deficiency arises at a very young age. Lashem et al. (1994) showed that the rat's ability to differentiate between a sodium-salt solution and other salt solutions (potassium, ammonium, lithium, and calcium chlorides) during a state of sodium deprivation emerges between the ages of 3 and 18 days. In addition, Stricker and Wilson (1970) demonstrated that the sodium appetite drive is so strong that a sodium deprived rat will consume a salt solution in amounts equal to control rats despite the fact that it had a previous conditioned aversion to the salt solution. Evidence presented in this chapter also suggests that the increased consumption of sodium salts during sodium deprivation is not a learned behavior, as rats increase consumption of sodium during their first experience with sodium deprivation.

Methods of Inducing Salt Appetite

Various methods have been used to induce salt appetite in rats, involving different mechanisms of action. One of the earliest methods of inducing salt appetite involved the surgical removal or ablation of the adrenal glands, called adrenalectomy. This results in the uncontrolled loss of sodium in excreted urine (Richter, 1939). However, adrenalectomy also produces long term cell death in the dentate gyrus of the hippocampus (Sloviter et al., 1989).

A less invasive method of inducing salt appetite through the uncontrolled loss of sodium in urine results from an injection of the diuretic drug furosemide. Furosemide is a sodium ion inhibitor that prevents the utilization of sodium present in the body and results in the excretion of this excess sodium in the urine (Jalowiec, 1974). Inducing hypovolemia, a decrease in body fluid levels, also produces salt appetite (Stricker, 1966) by increasing the production of renin by the kidneys, which leads to increased production of the neurohormones angiotensin and aldosterone. A subcutaneous injection of formalin was one early method used by researchers to induce hypovolemia in rats. Formalin produces

tissue damage that results in the redistribution of plasma into extravascular spaces, which results in hypovolemia and salt appetite (Wolf and Steinbaum, 1965).

The least invasive method for inducing salt appetite is to feed a rat a low-sodium diet (< 0.01% sodium content) for a period of at least one week. The decreased consumption of dietary sodium results in the increased production of renin, angiotensin, and aldosterone, which promote salt appetite (Fregley and Rowland, 1985). Renin administration in the absence of actual sodium loss is sufficient to produce salt appetite (Bryant et al., 1980) Likewise, administration of aldosterone (Wolf and Handal, 1966) or its synthetic precursor deoxycorticosterone acetate (DOCA) (Wolf and Quartermain, 1966) also results in an increase in salt appetite without inducing sodium loss in the body.

Salt Depletion Changes Taste Properties of Salt

In early research on salt appetite in rats, Handal (1965) showed that rats made sodium deprived with a subcutaneous injection of formalin immediately accepted a salt solution at a concentration that was rejected when they were sodium replete. In addition, Handal demonstrated that rats also preferred sodium salts to non-sodium salts, and the rapidity of the preference suggested it was mediated by taste rather than by post-ingestion factors. This was the first evidence that salt appetite in rats is mediated by taste rather than by post-ingestion factors. Epstein (1991) postulated that both need-free and need-induced salt intakes are based upon the taste properties of salt. Epstein hypothesized that need-free salt intake (salt preference) is based upon the hedonic properties of the taste of salt at specific low concentrations, while need-induced salt intake (salt appetite) is based upon internal events that are related to sodium deficiency that increased hedonic value of the salt taste.

Berridge et al. (1984) provided evidence that increased salt consumption during salt deprivation may be based upon altered taste properties of salt. These researchers demonstrated that rats shifted their reflexive oral responses to salt stimuli from a mixture of both ingestive (tongue protrusions and mouth licking) and aversive (gaping mouth) responses while sodium replete to exclusively

ingestive responses while sodium deprived. This shift in oral responses occurred the first time the rats became sodium deprived, and was specific to the sodium chloride solution. This shift in salt taste sensitivity seems to operate at the neural level rather than the taste receptor level. The chorda tympani fibers relay taste information about salt from facial nerves to the nucleus of the solitary tract (NST). Induction of sodium appetite through either diet (Contreras, 1977) or adrenalectomy (Contreras and Frank, 1979) results in decreased responses of the chorda tympani fibers to the taste of sodium salts but not to sucrose, hydrochloric acid, or quinine. Daniels and Fluharty (2004) proposed that decreased response of chorda tympani fibers during sodium deprivation may be due to activation of intrinsic GABAergic neurons in the chorda tympani pathway that receive projections from angiotensin-sensitive brain regions, such as the circumventricular organs.

Tamura and Norgren (2003) examined the responses of taste neurons in the NST to various stimuli following intracranial administration of renin or vehicle and found that these neurons showed decreased responding to saline solutions of 0.3 and 1.0 M following renin administration compared to vehicle administration. No other taste stimuli used produced this difference in neural responding. McCaughey and Scott (2000) induced sodium appetite with administration of DOCA and renin, and then monitored the activity of single taste neurons in the NST as the rats went from a normal state to a state of enhanced salt appetite. These researchers found decreased neural responding to high concentration salt solutions across all neurons during enhanced sodium appetite, and specifically in cells most sensitive to salt taste. In addition, there was increased neural responding to high concentration salt solutions in most sugar sensitive neurons during enhanced salt appetite. The researchers hypothesized that these changes indicate not only a decrease in the aversiveness of high concentration salt solutions because of the decreased responding of salt sensitive neurons, but also an increase in their perceived reward value during states of increased salt appetite because of the increased responsiveness of sugar sensitive neurons.

Neurons in the NST send projections to the parabrachial nucleus (PBN), which in turn projects to the parvocellular ventral posteromedial thalamic nucleus (Daniels and Fluharty, 2004). Interestingly, Shimura et al. (1997) demonstrated that salt deprivation is associated with an increase in the responsiveness of sugar-oriented taste neurons to salt in the PBN. The general profile of neural activation evoked by salt was similar to sugar response profiles in the NST demonstrated by McCaughey and Scott (2000). In support of this idea of increased reward value for salt during salt depletion, Lucas et al. (2000) provide evidence that the dopaminergic mesolimbic system, specifically the shell of the nucleus accumbens, a brain region associated with motivation and reward, is involved in the generation of salt appetite in rats given DOCA. Furthermore, Roitman et al. (2002) showed that rats that had undergone sodium depletion and increased salt appetite had an increase in dendritic branches and spines on medium spiny neurons in the shell of the nucleus accumbens. These rats with a history of sodium depletions showed sensitization to the effects of amphetamine.

However, Curtis et al. (2001) found that an altered taste value of salt is not solely responsible for the increased ingestion of salt during salt deprivation. Without water depriving rats, Curtis et al. trained rats to drink a salt solution by mixing it with a dilute sucrose solution. After two days of dietary salt deprivation, the licking rate for the salt solution increased, and increased further after five days of sodium deprivation. After ten days on the low sodium diet, the licking rate for the salt solution was not different than after two days. So, rats showed an increased licking response to the salt solution after only two days on the low sodium diet. However, these rats only showed a significant increase in 24-hr ingestion of the salt solution after one week of the low sodium diet. The fact that an altered taste response to the salt solution preceded the actual increase in salt consumption by several days led to the conclusion that the increase in salt consumption cannot only be due to altered taste responses to salt.

Post-ingestion factors may play a role in increased salt consumption during sodium deprivation, but there is conflicting evidence for this. Levy and McCutcheon (1974) demonstrated that post-ingestion factors do act to regulate

salt appetite in sodium depleted rats. These researchers showed that rats given intragastric infusions of saline ten hours before testing consumed less salt solution than rats that had intragastric infusions of sucrose or deionized water. A second experiment demonstrated that the amount of salt solution consumed after intragastric infusion was negatively related with the amount of saline infused. In other words, less intragastrically infused saline resulted in more salt solution consumed afterwards. However, Mook (1969) provided evidence that post-ingestion factors do not play a role in mediating salt appetite. Mook implanted adrenalectomized rats with an esophageal fistula that prevented salt solutions from being absorbed, and with a gastric cannula that delivered water into their stomachs while they were drinking the salt solutions. Mook showed that despite the lack of absorption of salt, the adrenalectomized rats expressed a robust salt appetite.

These studies demonstrate that increased ingestion of high concentration salt solutions during times of salt deprivation appear to be primarily due to altered processing of the taste of these salt solutions. When a rat is salt replete, the same high concentration solutions are aversive. However, when a rat is salt deprived, the tastes of high concentration solutions shift from aversive to appetitive. The shift in taste processing appears to take place at the level of neurons in the nucleus of the solitary tract and the parabrachial nucleus. This results in rats consuming more salt and reducing their need or drive for salt. The neurochemical mechanisms responsible to producing this drive to ingest higher levels of salt are discussed in the next section.

Neurochemical Mechanisms of Salt Appetite

Increasing Salt Appetite

Numerous studies have demonstrated the role of angiotensin and aldosterone in the development of salt appetite in rats. Angiotensinogen is released by the liver and is converted to angiotensin I by renin produced in the kidneys. Angiotensin I is then converted in the brain to angiotensin II by the angiotensin-converting enzyme (ACE). Angiotensin II binds to AT1 receptors in several circumventricular organs to induce salt appetite, and also acts on the

adrenal cortex resulting in the production of aldosterone. Aldosterone appears to have its main effect on salt appetite by binding with mineralocorticoid receptors that are widespread in the brain (Fluharty and Sakai, 1995). Activation of mineralocorticoid receptors induces the synthesis of proteins that up-regulate angiotensin II action and down-regulate oxytocin action. This increases the excitation of salt appetite while simultaneously decreasing the inhibition of salt appetite.

Manipulation of angiotensin and aldosterone levels has been shown to alter salt appetite. For example, Bryant et al. (1980) showed that repeated administration of renin, which increases circulating angiotensin levels, produced an increase in the ingestion of a 7% sodium solution. In addition, continuous infusions of angiotensin II directly into the brain produced a marked increase in the consumption of a 3% sodium solution. This infusion of angiotensin II also increased the consumption of water, but had no effect on the ingestion of potassium chloride or ammonium chloride. Dalhouse et al. (1986) showed that peripheral administration of angiotensin produced significant increases in sodium consumption compared to controls. Fluharty and Manaker (1983) and Fluharty and Epstein (1983) demonstrated that the increased salt appetite demonstrated after intracerebroventricular administration of angiotensin II is not obviously due to a dramatic loss of body sodium, indicating it may simply be due to increased activation of angiotensin receptors.

Paradoxically, Evered and Robinson (1983) showed that oral administration of captopril, an inhibitor of an angiotensin converting enzyme that converts angiotensin I to angiotensin II, produced increases in the consumption of a sodium solution but had no effect on the consumption of potassium chloride or sucrose solutions. The researchers explained this finding by proposing that the inhibition of the renin-angiotensin system may have caused a paradoxical increase in the synthesis of angiotensin II in the brain. In support of this proposal, Buggy and Jonklaas (1984) demonstrated that the potentiating effect of captopril on salt appetite is reversed by concurrent blockade of angiotensin receptors. Moe et al. (1984) further demonstrated that high doses of captopril given peripherally did

produce a decrease in salt appetite, and low doses of captopril delivered centrally to the brain produced decreases in salt appetite. Moe et al. suggest that the increase in salt appetite produced by low peripheral doses of captopril was probably due to an increase in peripheral angiotensin I, which is then delivered to the brain and converted to angiotensin II.

The most common method used by researchers to induce salt appetite, an injection of furosemide, also acts on the angiotensin/aldosterone system. Rowland and Morian (1999) examined the time course effects of a single furosemide treatment (10 mg/kg) on aldosterone and angiotensin II plasma levels in male and female rats. Both aldosterone and angiotensin II were significantly elevated above control levels 3 hours after the injection, with the greatest levels of aldosterone occurring 12-24 hours after the injection while the greatest levels of angiotensin II occurred 3 hours after injection. The greatest amount of consumption of a sodium solution occurred 24 hours after injection. In addition, the furosemide treatment induced c-fos expression in the subfornical organ (SFO) and organum vasculosum laminae terminalis (OVLT), with maximum expression occurring 3 and 24 hours after injection in both regions.

Epstein (1991) proposed a model in which angiotensin II and aldosterone act synergistically in the brain to produce increased salt intake when an intact animal is made sodium deficient. The model proposes that each hormone can act alone to increase salt intake, but it also suggests the two hormones can act together. In this model, sodium depletion acts as an initiator of the increases in angiotensin II and aldosterone in the brain, which in turn, act synergistically in the brain to promote the search for and intake of salt substances. Specifically, peripherally circulating angiotensin II stimulates the secretion of aldosterone following sodium depletion. The aldosterone then enters the brain and synergistically interacts with central angiotensin II to produce salt appetite. Evidence for this synergistic action is provided by Fluharty and Epstein (1983) and Sakai (1986), who demonstrated that when angiotensin II and aldosterone were given together at doses that do not elicit salt appetite when given alone, they act together to produce a robust salt appetite greater than the additive effect of

each substance. In addition, both aldosterone and angiotensin II are elevated concurrently during sodium appetite, allowing for interaction (Stricker et al., 1979).

Epstein (1991) also showed that rats will demonstrate salt appetite through exposure to angiotensin and aldosterone alone in the brain, even in the absence of a loss of body sodium. Furthermore, Epstein showed that it is not necessary for rats to consume salt after they become sodium deficient for the first time in order for their salt appetite to be enhanced during subsequent episodes of sodium depletion. Thunhorst (1996) also provides evidence that the hormone angiotensin II that is produced peripherally interacts with circumventricular organs of the brain directly to produce salt appetite during episodes of sodium depletion, and that this hormone is different from angiotensin produced inside the brain and acts as a neurotransmitter in central pathways involved in sodium appetite.

Inhibition of Salt Appetite

Although it is relatively clear that angiotensin II and aldosterone play an important role in increasing salt appetite in rats, the mechanisms involved in inhibiting salt appetite are not clearly established. Several candidate mechanisms have been proposed. Stricker and Verbalis (1996) proposed that inhibition of salt intake in rats is regulated by oxytocin secretion from a subset of hypothalamic neurons, and that salt appetite is in part caused by an inhibition of central oxytocin secretion. Blackburn et al. (1992b) showed that systemic injections of naloxone, which increases oxytocin secretion in the brain, inhibited salt appetite. Blackburn et al. (1992a) demonstrated that destruction of oxytocin receptors resulted in enhanced salt appetite.

Another potential inhibitor is the tachykininergic system. Lucas et al. (2003) showed that repeated administration of DOCA was associated with a down-regulation of tachykinin receptors in various brain regions associated with salt and fluid balance. The researchers suggest this is consistent with the idea that salt appetite is, in part, due to suppression of the inhibition of salt appetite mediated through the tachykininergic system. In support of this idea, Flynn et al. (1999) showed that the administration of an NK3 receptor agonist produced a

decrease in salt intake during a state of sodium deprivation, while an NK1 receptor agonist had no effect on salt intake behavior.

There is also evidence that suggests the serotonergic system modulates salt appetite. Menani et al. (1998) showed that bilateral injection of the serotonin receptor antagonist methysergide into the lateral parabrachial nucleus (LPBN) increased sodium and water intake after sodium depletion while having no effect on the intake of a sucrose solution. In an early study involving the same research group, Colombari et al. (1996), showed that serotonergic mechanisms in the LPBN act to inhibit the increased salt appetite induced by angiotensin II injection into the SFO.

Brain Regions Involved in Salt Appetite

Several brain regions are involved in mediating the development and expression of salt appetite in the rat. These brain areas include the subfornical organ (SFO), the gustatory thalamus (GT), the parabrachial nuclei (PBN), and the central nucleus of the amygdala (CeA).

Subfornical Organ

The SFO appears to play an important role in both the regulation of sodium levels in the body and also hydration levels. The SFO itself contains receptors for angiotensin II (Simpson, et al., 1978), and therefore a lesion of this area should reduce salt appetite induced by increased angiotensin expression. However, early experiments by Schulkin et al. (1983) did not demonstrate a disruption in sodium appetite following SFO lesions. Schulkin et al. used knife cut transactions of the SFO ventral stalk efferents, and induced sodium deficiency via injection of 5 mg of DOCA and 10 mg of furosemide. Sodium solution and water consumption were measured over the next two days. SFO and control animals had similar increases in sodium consumption following the acute sodium deprivation treatment. In another experiment, Schulkin et al. placed other SFO cut animals and controls on a low-sodium diet for 4 days to induce sodium appetite. Again, sodium and water consumption were measured over the next 2 days, and again SFO and control animals had similar increases in sodium consumption following the chronic sodium deprivation treatment.

Other researchers have demonstrated a disruptive effect of SFO lesions on sodium appetite when acute sodium deprivation treatments are used, although hydration status seems to play a mediating role. Thunhorst et al. (1990) measured water and sodium consumption over a 45-hour period during a regimen that combined furosemide diuresis and access to a low-sodium diet. Only water was available for 23 hours after diuresis, and water and sodium solution were available by choice for the next 22 hours. After diuresis, rats with SFO lesions drank significantly less water in the first 2 hours than controls but achieved equivalent water and sodium balances before access to salt 20 hours later. After salt access, rats with SFO lesions drank significantly less sodium and water than controls in the first 2 hours but had similar sodium and water intakes over the next 20 hours. Thus, SFO lesions reduced acute but not chronic sodium and water intakes in response to sodium deprivation, and the reduced intakes were not explainable by hydration status. Thunhorst et al. (1999) further demonstrated the role of the SFO in acute sodium appetite expression by showing that electrolytic lesions of the SFO greatly reduced water intake, and nearly abolished the sodium intake produced by injections of furosemide and captopril. Ruff et al. (2001) also demonstrated that SFO lesions significantly reduced acute salt appetite in response to furosemide treatment, but did not affect long-term sodium appetite 24-26 hours after furosemide treatment.

However, Starbuck et al. (1997) provide evidence that hydration status mediates the effects of SFO lesions on acute salt appetite. These authors examined whether the level of hydration after furosemide diuresis and 22 hours of sodium deprivation affected the amount of water or sodium solution consumed by rats with intact brains or with lesions of the SFO. Rats received 2 (underhydrated) or 10 (hydrated) ml/kg water by gavage as the only fluid input 2, 4, and 20 hours after a 10 mg/kg furosemide injection. These hydration treatments had little or no effect on the amount of sodium consumed in the first 2 hours by intact rats. SFO lesions reduced water intake regardless of hydration condition. Hydrated, SFO-lesioned rats drank a normal amount of sodium, but underhydrated SFO-lesioned rats drank less sodium than any other group. Starbuck et al. stated that hydration

may facilitate salt appetite in SFO-lesioned rats, and the deficits in salt appetite noted in SFO-lesioned rats may result from deficits in water ingestion rather than from a destruction of angiotensin II receptor sites that directly provoke salt appetite.

Gustatory Thalamus and Parabrachial Nuclei

Several lesion studies are consistent with the idea that increased salt appetite appears to be dependent upon intact taste processing. Reilly (1998) reviewed experiments examining the role of the GT in salt appetite. Large electrolytic lesions of the medial thalamus produced complete impairment of salt appetite produced by formalin or DOCA. However, these lesions reduced salt appetite in only 70% of rats. Other experiments showed that electrolytic lesions of the ventral thalamus diminished the increase of salt intake following sodium deprivation via formalin injection. However, lesions that destroyed cell bodies of the GT but spared fibers of passage did not disrupt salt appetite produced by sodium deprivation, indicating that the GT itself may not be responsible for producing salt appetite.

Spector (1995) reviewed the lesion literature concerning the PBN, in which third order gustatory neurons reside. Lesions of this brain area severely impair some, but not all, taste-guided behaviors, such as conditioned taste aversion and salt appetite. For example, Spector et al. (1993) examined whether rats with PBN lesions could show normal concentration-dependent changes in licking behavior to very small volumes of sodium and sucrose. Water deprived control rats progressively decreased their licking responses to sodium compared with water as the concentration of sodium was raised. In contrast, water deprived PBN-lesioned rats that showed normal licking responses to sucrose concentrations did not decrease their licking responses to sodium relative to water until the concentration reached 1.0 M. In a water satiated state, however, both control and PBN-lesioned rats decreased their responsiveness as a function of sodium concentration.

Scalera et al. (1995) examined salt appetite and a conditioned taste aversion to sodium using rats with ibotenic acid lesions of the PBN. Following

furosemide treatment, PBN-lesioned rats drank significantly less sodium solution than controls at 2 and 24 hours after the injection. In addition, PBN-lesioned rats also failed to acquire a conditioned taste aversion to sodium, which lead the authors to conclude that the PBN lesion may have disrupted the taste properties of sodium. However, Spector et al. (1995) provide contradictory evidence. Rats with either electrolytic or excitotoxic lesions of the gustatory zone of the PBN were tested for sucrose and sodium taste detection thresholds in a conditioned avoidance task. The PBN-lesioned rats did demonstrate severe deficits in acquiring a lithium chloride based conditioned taste aversion to sucrose, sodium, or alanine. The excitotoxic-lesioned rats also had failed to express a deprivation-induced salt appetite. However, the PBN-lesioned rats exhibited varied performance in thresholds for detecting sodium, with approximately one-third of rats showing normal taste thresholds. These findings demonstrate that the elimination of the conditioned taste aversion following PBN lesions was not necessarily linked to impairment in sodium taste detection.

Central Nucleus of the Amygdala

Zardetto-Smith et al. (1994) tested sodium solution consumption both before and after electrolytic lesions of the CeA. Before surgery, to-be-lesioned rats and control rats drank equivalent amounts of sodium in response to furosemide treatment. After surgery, rats with CeA lesions showed significant decreases in their intake of the salt solution after furosemide treatment, while intakes of the sham lesioned groups remained unchanged. This finding suggests that the CeA may be involved in salt preference rather than salt appetite, as the furosemide injection was not able to induce salt appetite in the control group but still reduced salt intake in the lesioned group. In fact, lesions of the CeA virtually eliminated 24-hour need-free salt intake. Galaverna et al. (1992) also showed that bilateral lesions of the CeA abolished need-free salt intake, suggesting that CeA lesions cause a disruption in salt preference-based sodium intake behaviors.

Seeley et al. (1993) found that bilateral lesions of the CeA disrupted both need-free and need-induced sodium intake. Seeley et al. proposed that this was due to interference with the consumption phase of sodium intake behaviors. In

this study, a sodium solution was delivered directly into the mouths of rats rather than through a normal drinking behavior. Control rats enhanced their intraoral intake of the sodium solution in response to sodium deprivation while CeA-lesioned rats did not. CeA-lesioned rats, however, still were able to demonstrate discriminative intake responses to different tastes. Like controls, CeA-lesioned rats promptly rejected quinine infusions and ingested sucrose for prolonged periods. Galaverna et al. (1993) also showed that the impairment in need-induced and need-free sodium intake resulting from a CeA lesion is not due to general impaired taste function. Using intraoral administration, Galaverna et al. found that rats with CeA lesions demonstrate normal reflexive oral responses to sodium, sucrose, and citric acid compared to controls, and were able to acquire a conditioned taste aversion.

Other Areas

Several other brain regions have also been implicated in salt appetite behaviors through lesion experiments. The ventral ventral median preoptic nucleus (VVMnPN) contains angiotensin receptors and it would therefore be predicted that a lesion of this area would disrupt salt appetite. However, Fitts et al. (1990) and Fitts (1991) found that a lesion of the VVMnPO actually enhanced salt appetite in response to DOCA treatment without sodium deprivation. However, a lesion of the VVMnPO did reduce salt appetite in response to a furosemide injection that induced sodium deprivation.

Zardetto-Smith et al. (1994) found that a lesion of the bed nucleus of the stria terminalis (BNST) impaired salt appetite in response to furosemide treatment. However, lesions of the BST also reduced need-free salt intake, indicating that it may be involved in global salt ingestion behaviors rather than salt appetite specifically. In support of this, Reilly et al. (1994) also found that need free salt intake was reduced by BNST lesions.

Summary

The research findings described above can be summarized in Figure 1. Low levels of sodium in the body result in increased production of angiotensinogen that is released by the liver. Angiotensinogen is then converted

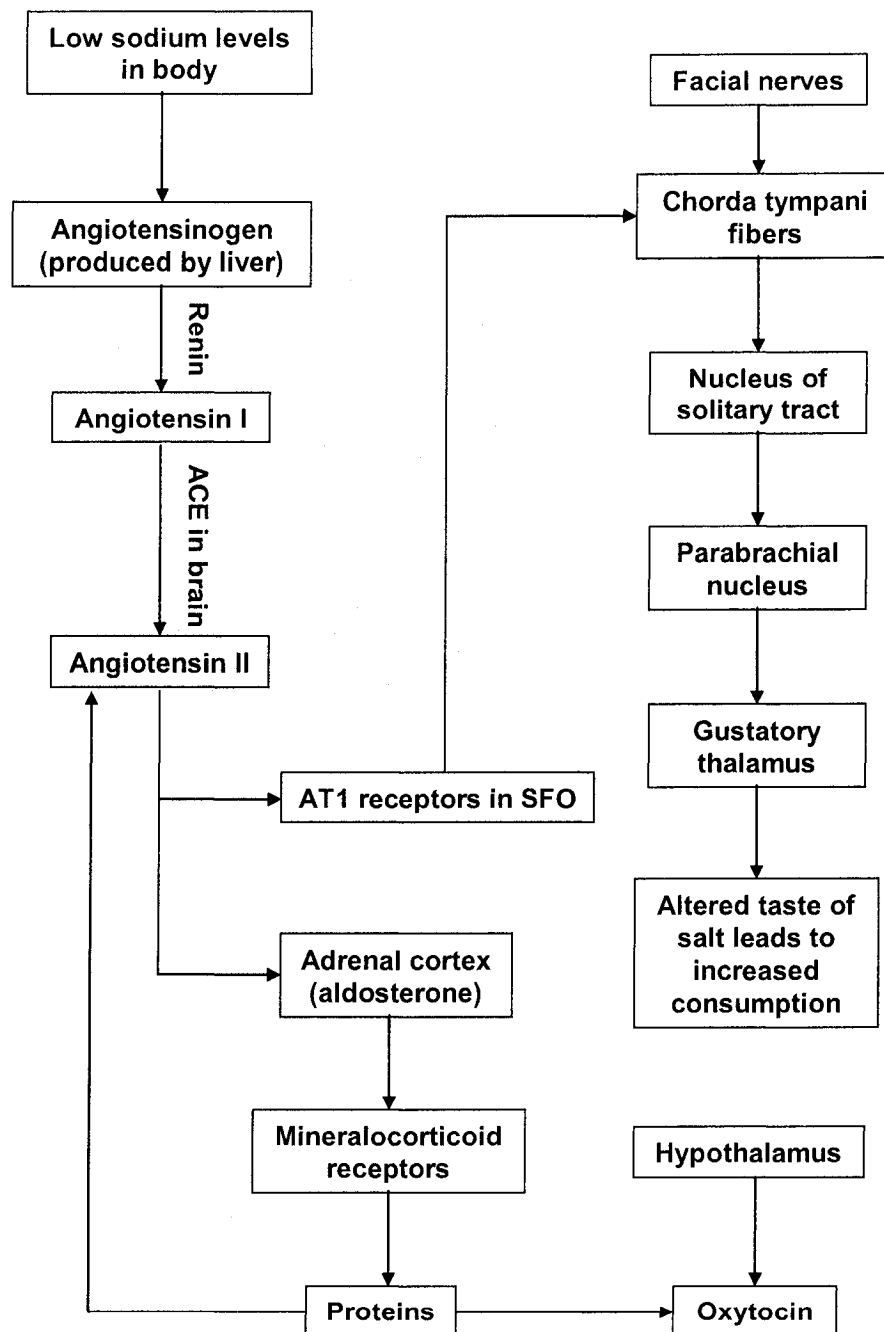


Figure 1. Diagram of the neural pathway responsible for salt appetite in the rat.

by angiotensin I by renin that is produced by the kidneys. Angiotensin I is carried to the brain where it is converted to angiotensin II by the angiotensin-converting enzyme (ACE). Angiotensin II has two actions in the brain. One, angiotensin II binds to AT1 receptors located in the circumventricular organs, such as the subfornical organ (SFO). These circumventricular organs send projections to the chorda tympani fibers that receive projections from facial nerves carrying taste information about salt. The input from the circumventricular organs alters this salt taste information, which is subsequently sent to the nucleus of the solitary tract (NST), the parabrachial nucleus (PBN), and the gustatory nuclei of the thalamus. Two, angiotensin II also binds to receptors in the adrenal cortex, which results in the production of aldosterone. Aldosterone binds to mineralocorticoid receptors that are widespread in the brain, activating proteins that regulate angiotensin II activity, as well as the activity of oxytocin that is released by the hypothalamus and normally inhibits salt appetite.

CHAPTER TWO: LATENT LEARNING AND MULTIPLE MEMORY SYSTEMS

Latent Learning

Thistlethwaite (1951) reviewed and classified early latent learning experiments into two major categories. In one category are experiments involving unrewarded pre-exposure to mazes followed by the introduction of a rewarding incentive at some location in the maze. The unrewarded pre-exposure to the maze results in spatial latent learning. In the second category are experiments in which rats are pre-exposed to an incentive in a satiated state. When later deprived of that incentive they acquire a new behavior that allows them to obtain it. Thistlethwaite called this second category irrelevant-incentive learning. The experiments described in this thesis were aimed at determining the neural system involved in irrelevant-incentive latent learning in the rat.

Spatial Latent Learning

The initial demonstrations of spatial latent learning refuted two basic assertions of Behaviorist learning theory (Thorndike, 1932; Skinner, 1938; Hull, 1943): 1) that reinforcement is necessary for learning to occur and 2) that learning cannot occur without performing the behaviors that ultimately reveal its existence (Thistlethwaite, 1951). Blodgett (1929), as well as Tolman and Honzik (1930), provided the initial experiments demonstrating spatial latent learning in rats. In these seminal maze learning experiments, one group of food+water deprived rats were given several trials of free exploration of a maze but were not given a wet food mash reward once they reached the goal box. Another group of food+water deprived rats were given standard rewarded trials in which the wet food mash was present in the goal box from the beginning of training. This reinforced group showed a gradual decrease in the number of errors made in reaching the goal box over the trials. However, the unreinforced group showed almost equal performance to the reinforced group after one trial once the wet food mash reward was placed in the goal box. Blodgett stated that the evidence for latent learning came from the fact that the pre-exposed rats had a greater rate of learning after the food reward was introduced into the maze than did control rats

that were not pre-exposed to the maze. This finding supported the idea that the rats acquired some information during unrewarded exploration of the foodless maze, which was later used to locate the food in the maze once it was introduced.

These initial demonstrations of latent learning were soon supported by numerous other demonstrations. Herb (1940) used a procedure similar to Blodgett except that when she introduced the food reward she placed it at the entry of the food-paired alley rather than at the end of the alley. Herb demonstrated that rats given unrewarded pre-exposure trials in the maze showed a sharper increase in the number of food-paired alley entries after the introduction of the food reward than did control rats given no pre-exposure to the maze. Haney (1931) used a slightly different procedure for pre-exposing rats to the experimental maze. Rather than giving discrete unrewarded trials in the maze, Haney placed rats in the maze for 18 consecutive hours on four successive days. During this time the rats were free to explore the maze. A control group was given equal exploration periods in a different maze. When both groups of rats were given rewarded trials in the experimental maze, the pre-exposed rats made significantly fewer errors than control rats. Karn and Porter (1946) demonstrated that this pre-exposure period must include actual free exploration of the maze, rather than simple confinement in the maze, to facilitate subsequent performance on rewarded trials. Movement within the maze appears to be a requirement for rewarded spatial learning as well, as rats are unable to differentiate between two adjacent arms on an 8-arm radial arm maze unless they are allowed to move between them during training (McDonald and White, 1995; White and Ouellet, 1997).

Not all early attempts to demonstrate spatial latent learning were successful, however. Reynolds (1945), as well as Meehl and MacCorquodale (1949), failed to show the usual facilitating effect of maze pre-exposure on subsequent maze performance. However, this may have been due to motivational factors. Blodgett (1929) used rats that were both food and water deprived, and the eventual reward was a wet food mash, thus satisfying both food and water needs. The procedures used by Reynolds, as well as Meehl and MacCorquodale,

involved the use of food-only deprived rats and an eventual food-only reward. The increase in performance by Blodgett's rats may have been due to the interaction of their increased motivation to find the wet food mash in the maze after it was introduced, which facilitated the recall of the latently acquired spatial information (Thistlethwaite, 1951).

Interestingly, latent learning of spatial information does not appear to depend on an intact dorsal hippocampus, a structure implicated in various other forms of spatial learning (e.g. O'Keefe and Nadel, 1978; Olton and Papas, 1979; Moser and Moser 1998). In one series of experiments (Kimble and BreMiller, 1981; Kimble et al., 1982) rats with hippocampal lesions were either pre-exposed to a maze or not. Both groups were then given rewarded trials in the same maze. Control rats that were pre-exposed to the maze showed enhanced performance compared to control rats that were not pre-exposed to the maze, demonstrating the latent learning effect. Hippocampus-lesioned rats that were pre-exposed to the maze showed enhanced performance compared to hippocampus-lesioned rats that were not pre-exposed to the maze, indicating that the hippocampus was not necessary for spatial latent learning. However, it is important to note that both pre-exposed and non-pre-exposed groups with hippocampal lesions were impaired at finding the reinforcer in the maze on the rewarded trials than their respective non-lesioned controls.

The general finding that the hippocampus is not necessary for the acquisition of unreinforced or unrewarded spatial information was recently replicated by Gaskin et al. (2005). Normally, rats do not demonstrate a CCP in the radial arm maze when the paired and unpaired arms are adjacent to each other unless they are given unrewarded pre-exposure to the two arms. In this experiment, rats were given infusions of the GABA agonist muscimol into the dorsal hippocampus prior to the pre-exposure trials, making it inactive during these trials. However, the muscimol treatment had no effect on the acquisition of unreinforced spatial information.

Irrelevant Incentive Latent Learning

The experiments described in this thesis involved irrelevant-incentive learning of salt-cue associations. The ability of the rat to form associations between a neutral salt solution and specific environmental cues has been repeatedly demonstrated using irrelevant-incentive tasks. Krieckhaus and Wolf (1968) trained rats to press one bar for water or another bar for a salt solution, and were then given an extinction trial. When salt deprived, rats that were trained on the salt-paired bar showed a greater resistance to extinguish the bar-pressing response than rats that were trained on the water-paired bar. This experiment was later replicated and extended by Khavari and Eisman (1971) to show that the increased resistance to extinguish the bar-pressing response was not simply due to an enhanced reward value of the salt solution compared to the water during training. Khavari and Eisman showed that a rewarding sucrose solution did not produce any greater resistance to extinction than water or salt solutions when rats were not deprived of any of the incentives. The increased resistance to extinction occurred only when the rats were deprived of the specific incentive that they had been exposed to during training. In addition, Coldwell and Tordoff (1993a) demonstrated that the bar-pressing task could be used with calcium as the irrelevant-incentive instead of salt.

Wirsig and Grill (1982) demonstrated that latent learning in this bar-pressing task was not disrupted by either pre-training or post-training cortical lesions. The cortical lesions destroyed most of the neocortex, but spared the most posterior cortical areas that include the entorhinal and perirhinal cortex. Owen and Butler (1980) showed that lesions of the fimbria-fornix did disrupt latent learning on this task, eliminating the difference in bar-pressing rates between salt deprived and non-deprived groups. However, the conclusion that this was due to elimination of the latent learning effect was called into question by Owen and Butler, and later by Shull and Holloway (1985) because both salt deprived and non-deprived groups showed increased bar-pressing rates, which may have been due to the well-known disinhibitory effects of fimbria-fornix and hippocampal lesions on rates of responding (Douglas, 1967; Coutureau et al., 2000).

Other paradigms have also been used to show the rat's ability to latently learn associations involving salt. Colwell and Tordoff (1993b) gave salt replete rats exposure to flavors that were paired with either salted or unsalted foods. During training, the salt replete rats ate significantly more of the unsalted food. However, when these rats were then made salt deprived they demonstrated a preference for the flavor that was previously associated with the salted food. A similar experiment by Westbrook et al. (1995) demonstrated that exposure to the pairing of an almond flavor with salt early in training while rats were salt replete resulted in a preference for the almond flavor when the rats later became salt deprived. Berridge and Schulkin (1989) paired quinine and citric acid with salt during a training period when rats were salt replete. Rats showed preferences for these two tastes when subsequently salt deprived. Therefore, it appears that rats are able to latently associate different flavors with salt during a training period, and are able use that association to show a preference for the salt-paired flavor when they later become salt deprived.

The ability to latently associate salt with other stimuli is not limited to the sense of taste. Rats can also latently associate salt with a spatial location. Normally, rats with damage to the thalamic taste relay in the ventral posterior medial area of the thalamus do not show increased salt appetite when salt deprived (Wolf, 1968). However, Paulus et al. (1984) showed that when normal rats were allowed to drink a salt solution prior to surgery they exhibited an increased salt appetite when salt deprived following a thalamic lesion. But when the location of the salt was changed the rats did not increase their intake. This demonstrated that the apparent protective effect of salt pre-exposure was due to latent learning of the location of the salt. Hartzell et al. (1985) showed that this pre-operative experience with salt can be as brief as 30 seconds and still be sufficient to produce the location-specific protective effect against deficits in salt appetite that normally result from damage to the thalamic taste relay. These findings show that rats can acquire latent associations between salt and spatial locations and can express these associations when later salt deprived.

Historical Development of the Multiple Memory Systems Model

The initial demonstrations of latent learning by Blodgett (1929) and Tolman and Honzik (1930) changed the way mammalian learning was viewed and studied. These experiments provided the first evidence that mammals can learn information that will change subsequent behavior without that information being rewarding and without any particular behavior being reinforced. McNamara et al. (1956) further separated learning and performance by comparing one group of rats that performed a place learning task in a T-maze in the standard way, running down the arms for the food reward, to another group of rats that were placed in a basket and were transported down the arms by a pulley system to get the food reward. Despite the fact that the basket group did not perform the behavior during training, they performed as well as controls during extinction trials.

These kinds of observations broke the bonds that held together learning and reinforcement. In Behaviorist learning theories (Thorndike, 1932; Skinner, 1938; Hull, 1943), the operational definitions of learning and reinforcement go hand-in-hand: learning is defined as an observable increase in behavior due to the acquisition of some information, while reinforcement is defined as anything that produces an increase in the performance of a behavior. By these definitions, reinforcement produces learning, and learning is based on reinforcement. The discovery that mammals can learn without performance, and without reinforcement of that performance, led to a polarization in the field of learning research into two schools of thought: Behaviorism and Cognitivism. Tolman (1949) first proposed a resolution to the emerging polarization, claiming that there many different types of learning present in the mammal and no one theory could explain them all. As research continued on human subjects and with animal models it became clear that Tolman was correct, and that both types of learning represented by Behaviorism (response-reinforcement dependent) and Cognitivism (response-reinforcement independent) exist in the mammalian brain.

Evidence also became available showing multiple types of learning and memory occur in the human brain. Scoville and Milner (1957) described severe

memory deficits in patients that had undergone bilateral surgical removal of the medial temporal area, including the hippocampus, in treatment of schizophrenia, epileptic seizures, or other maladies. While general intelligence and personality were unchanged by the surgeries, these patients could no longer form new long-term memories and also showed retrograde amnesia for events that occurred several years prior to the surgery. However, continued work with these patients demonstrated that they did indeed have the capability to acquire and store new “skills” that could be similar to stimulus-response associations postulated by Behaviorist learning theory. Milner (1962) described how one particular patient, H.M., showed normal improvement in a mirror drawing task over a period of days despite not explicitly remembering ever having done the task before. Corkin (1968) further described that H.M. showed intact learning on three unique motor learning tasks despite having no memory of having performed them. Milner et al. (1968) demonstrated that H.M. had the ability to remember his path in a simple walking maze but only when his starting point was unchanged, indicating H.M. was remembering a series of turns rather than using a spatial map of his environment. In addition, Milner et al. (1968) showed that H.M. demonstrated some perceptual learning that was assessed using an incomplete-pictures test in which objects can be recognized in successively more incomplete drawings. These findings would lead to the distinction between learning based on conscious memories and motor learning, or stimulus-response learning. These two types of learning, often called declarative and nondeclarative learning (Squire et al., 1993), or explicit and implicit learning (Graf and Schacter, 1985; Schacter, 1987), were believed to be mediated by different neural structures.

Hirsh (1974, 1980) was the first theorist to explicitly propose the possibility of relatively independent multiple retention systems in the mammalian brain. Hirsh hypothesized that information can be stored along a “performance line” by the formation of a connection between neural elements sensitive to a stimulus and neural elements responsible for the production of a response (i.e. S-R learning). In addition, explicit mnemonic information can also be stored outside of this performance line. The hippocampus was theorized to be part of the

system that is involved in the explicit mnemonic storage of “contextual” information. This information involved stimuli that were associated with specific internal motivational states, or internal contexts. These associations could influence the production of behavior by being placed onto the performance line during retrieval when the internal context (motivational state) was present. If this stored information resulted in a ‘correct’ behavioral response then that information remained in control of behavior; if this stored information resulted in an ‘incorrect’ response then that information would be replaced by other stored information that influenced behavior in a different manner. This procedure would be conducted until a correct response or solution was produced. In the absence of this explicit memory retrieval system due to hippocampal ablation, the stimulus-response (S-R) associative retrieval of the performance line would operate to control behavior.

The presence of both systems was considered by Hirsh to have adaptive and developmental value. The use of explicit memory retrieval allowed behavior to be highly flexible, and to undergo radical shifts in direction as a consequence of relatively subtle conditional changes. However, this cognitive system required a certain amount of information to be present before learning can begin. The associative system, however, required no prior information to exist. The strength of S-R connections are influenced by reinforcement or non-reinforcement on every trial, with no memory of prior information needed (Hirsh, 1980).

Mishkin and Petri (1984; Petri and Mishkin, 1994) expanded on this theory of multiple retention systems by describing the anatomical substrates that underlie the two systems, based on research conducted on non-human primates by Mishkin and colleagues (e.g. Mishkin, 1978; 1982; Zola-Morgan et al., 1982; Parkinson et al., 1988; Wang et al., 1990). The temporal lobe memory system was proposed to be the basis of the explicit cognitive retention system, while the basal ganglia and cerebellum were proposed to be the anatomical basis of the S-R habit system. Mishkin and Petri proposed that information gained from experience is stored in both systems, yet they function relatively independent of one another.

In addition to the theory proposed by Hirsh and expanded by Mishkin and Petri, several other researchers have developed similar models of information retention. For example, Schneider and Shiffren (1977; Shiffren and Schneider, 1977) proposed a model of controlled and automatic processing. Automatic processes are characterized as fast, effortless, and proceduralized, and not easily altered by the individual's conscious control. This automatic processing allows for the parallel operation of multiple information processing components. Automatic processing develops through extensive practice only when the conditions of the task are consistent. Controlled processes are characterized as slow and effortful, allowing for quick alterations because the processing is under the individual's cognitive control. Controlled processes are utilized initially during all tasks, but if the characteristics of the task are consistent throughout practice, automatic processes will develop. However, if the characteristics of the task are inconsistent, so that the characteristics are constantly changing and novel, then controlled processing will continue to be utilized.

Toates (1998) proposed a model by which cognition and the S-R system interact in the production of behavior. The model proposes a constant interaction between S-R and cognitive factors, with S-R mechanisms and cognitive mechanisms being weighted differentially as the result of experience. Behavior in a new situation is mostly cognitively based because S-R links are weak and the cognitive influence over the weak S-R links is relatively strong. As S-R connections are strengthened with repeated exposure to stimuli and repeated responses, the S-R connections become stronger and the cognitive influence over behavior becomes weaker and weaker by comparison. When S-R connections are very strong, a certain amount of autonomy from cognition will be attained, and a response will be made even when it is at odds with goals and cognitions. This relative autonomy of the S-R system from the cognitive system in controlling behavior allows much of behavior to be organized at the unconscious level. Conscious cognitions can then be used for other things, needing to be alerted only when novelty is involved or when strong habitual responses need to be overcome.

In addition to these theoretical models of multiple memory systems, numerous researchers (for example, Cohen and Squire, 1980; Packard et al., 1989; McDonald and White, 1993; McDonald and White, 1994; Knowlton et al., 1996; Packard and Teather, 1997; Packard and Teather, 1998; Kleim et al., 1998a; Kleim et al., 1998b; Devan and White, 1999) have experimentally demonstrated the differential anatomy of the multiple memory systems. These experiments provide evidence for the differing neural substrates that underlie the different functions of these systems. The following section describes these types of learning and their neural substrates in detail.

Current Multiple Memory Systems (MMS) Model

Figure 2 illustrates the current MMS model, which includes three basic types of information storage: cognitive learning, habit learning, and conditioning (White and McDonald, 2002).

Cognitive Learning – Stimulus-Stimulus Associations

In general, it is believed that the cognitive system is required for the formation of stimulus-stimulus (S-S) associations, which are representations of the relations between various stimuli, and also for the flexible use of these representations in novel situations and contexts (Eichenbaum et al., 1988; Eichenbaum et al., 1989; Otto et al., 1991). In this type of learning, no specific behavior is performed and therefore the acquisition of the S-S association is independent from performance. In their seminal triple dissociation paper, McDonald and White (1993) used the win-shift task for the 8-arm radial maze to demonstrate this type of learning. In the win-shift task, food deprived rats must learn the spatial locations of food within the radial maze, as well as learn not to return to previously visited spatial locations. Particular locations of food are defined by specific S-S associations within the maze room.

In addition to the win-shift task, the water maze has also been utilized to demonstrate S-S learning in rats. Morris (1981; 1984) first described the use of the hidden-platform task for the water maze to assess spatial S-S learning in rats. In this water maze task rats are placed into a pool of opaque water and must swim around in the pool until they locate and climb onto a hidden platform. The rats

<i>Type of Learning</i>	<i>What is Learned?</i>	<i>What is Expressed?</i>	<i>Central Structure</i>
Cognitive (S-S)	Stimulus-stimulus (S-S) associations among environmental cues	The S-S associations are used to guide novel controlled behaviors	Hippocampal formation
Habit (S-R)	Stimulus-response (S-R) associations that are strengthened through repeated pairing with a reinforcer	Responses are elicited by presentation of the stimulus, even in the absence of the reinforcer	Dorsal Striatum (Cerebellum)
Conditioning (S-Af)	A neutral stimulus (CS) is associated with a stimulus (US) that produces an affective state and an unlearned behavioral response (UR)	When the CS is encountered it elicits a behavioral response (CR) similar to the one originally elicited by the US	Amygdala

Figure 2. The three basic types of stored information currently included in the MMS model.

must learn the location of this hidden platform by learning associations among various extra-maze cues in the experimental room. The particular spatial location of the hidden platform is defined by a particular S-S association, which is then associated with a reward because the rat is allowed to escape from the aversive experience of being in the water.

The formation of S-S associations may also take the form of pattern association and subsequent pattern completion. Gilbert and Kesner (2002) describe pattern association as the ability to associate various stimuli that are present in different spatial locations in the environment or are present at different times in the learning experience. The task used to assess pattern association and pattern completion involves a cheeseboard maze, a large circular board that has many holes drilled into the surface of the board which are covered by particular objects. Some of the holes contain a food reward. To solve the task, the rat must learn to associate a particular object with a particular spatial location on the maze, and learn to tip the object over to get the food when the object is in a rewarded location. The rat must also learn to not tip the object over when it is in a non-rewarded location. In the temporal version of the pattern association task (Kesner et al., 2002) rats are sequentially presented several odors that are all paired with a food reward. During the test phase, rats are presented with two of the odors and must remember which of the two odors was presented earlier in the sequence in order to get the food reward. Learning this task requires rats to form associations between the various stimuli that occur at different time points during the learning experience, and then use that temporal association during the test period.

Contextual learning is another form of S-S learning. Contextual learning differs from spatial learning in that contextual learning involves forming associations between various stimuli (usually multimodal) that are experienced simultaneously in a small environment, whereas spatial learning involves forming associations between various visual stimuli experienced at different time points in a large environment. Contextual learning usually takes place in a small box apparatus that has two compartments with distinct visual, tactile, and/or olfactory cues (Carr et al., 1989; Schechter and Calgagnetti, 1993; 1998; Tzschentke,

1998). One of these compartments contains some type of reward, usually food or drugs. Rats must learn to associate the particular cues present in each of the compartments in order to later discriminate between the two compartments during a test trial.

S-S learning is dependent on a neural system that includes the hippocampus as its central structure (discussed in detail in the next section). Damage to or disruption of this hippocampal system impairs various types of S-S learning, including the win-shift task for the radial arm maze (e.g. Walker and Olton, 1979; Packard et al., 1989; McDonald and White, 1993), the hidden-platform task for the water maze (e.g. Morris, 1981; Sutherland et al., 1982; Buresova et al., 1986; Eichenbaum et al., 1990; Gallagher and Holland, 1992; Skelton and McNamara, 1992), pattern association and completion (e.g. Gilbert and Kesner, 2002; Kesner et al., 2002; Gilbert and Kesner, 2004; Kesner et al., 2004), and contextual learning (e.g. Winocur and Olds, 1978; Nadel and Zola-Morgan, 1984; Winocur et al., 1987; Selden et al., 1991; Honey and Good, 1993; Myers and Gluck, 1994; Phillips and LeDoux, 1995; Maren and Fanselow, 1997).

Functional Anatomy of the Cognitive System

The cognitive system involves several cortical regions and subcortical structures that interact with each other to perform this memory function. Neocortical areas receive sensory information and create perceptual representations that can be sustained for a brief period of time. These neocortical areas project to the cortical areas of the parahippocampal and perirhinal regions where the distinct perceptual representations of the sensory event converge prior to any processing in the hippocampal formation. The perirhinal and parahippocampal cortices then send projections to the entorhinal cortex. Each of these three areas sends and receives projections to and from neocortical association areas and to and from the hippocampus (Eichenbaum, et al., 1994; Petri and Mishkin, 1994). The perirhinal, parahippocampal, and entorhinal cortices probably play an important role in selecting and compressing sensory information, processing contextual information, and the formation of stimulus-stimulus (S-S) associations (Bucci et al., 2000; Shohamy et al., 2000). However,

the entorhinal cortex may not be involved in all types of contextual learning (Good and Honey, 1997). Damage to these cortices in addition to the hippocampus produces more dramatic cognitive memory impairments than damage to the hippocampus alone (Zola-Morgan et al., 1994).

According to Amaral and Witter (1989), the entorhinal cortex sends a major projection to the dentate gyrus of the hippocampus. The fibers of this projection perforate the pyramidal layer of the subiculum and ultimately enter the dentate gyrus. The neurons within the dentate gyrus in turn send a prominent projection, called the mossy fibers, to the CA3 field of the hippocampus, as well as project to other neurons within the dentate gyrus. Pyramidal cells in the CA3 field of the hippocampus give rise to the Schaffer collateral projections to the CA1 field of the hippocampus. These pyramidal cells within the CA3 field also give rise to projections to other neurons within the CA3 field. The pyramidal cells in the CA1 field send projections to the subiculum and the deep layers of the entorhinal cortex. The subiculum also sends direct projections to the entorhinal cortex. Finally, there are projections within the entorhinal cortex that link various parts of the region and appear to link the deep layers with the more superficial layers. The interaction among these areas is thought to produce the formation and storage of cognitive memories.

The area of permanent storage of these cognitive neural representations has been the topic of some debate. Most researchers believe the hippocampus is only a temporary store of these representations, which are then stored long-term in the cortex (Mishkin and Appenzeller, 1987; Petri and Mishkin, 1994). The representations most likely take the form of interconnected neural assemblies. However, Nadel et al. (2000) proposed a new theory of cognitive memory storage. The 'multiple trace theory' of cognitive memory states that the hippocampus is involved in the permanent storage and retrieval of specific episodic memories, rather than being just a temporary store. The cortex is involved only in the storage and retrieval of non-specific or 'gist' information.

The retrieval of these cognitive representations into working memory is necessary in order for them to have an influence on behavioral output. Numerous

studies have demonstrated that neurons in the prefrontal cortex are involved in the functions of working memory (Wickelgren, 1997). In addition, a series of studies by Floresco et al. (1999) demonstrated the importance of the interaction between the prefrontal cortex and subcortical structures in the form of thalamic-cortical-striatal circuits during working memory performance that involved the retrieval of stored cognitive information. Furthermore, the prefrontal cortex seems to play an integral role in the cognitive control of behavior through the formation of plans, goals, associations, and rules that govern purposive behavior (Miller, 2000).

Habit Learning – Stimulus-Response Associations

A second type of learning involves the formation of stimulus-response (S-R) associations that are strengthened by the presentation of a reinforcer after the response has been performed. In this case, learning and performance are bound together because the animal must perform a specific behavior in order for the reinforcer to occur. Once the S-R association has been sufficiently strengthened through repeated training, when the animal encounters the stimulus again the behavioral response will be elicited automatically. McDonald and White (1993) used the win-stay task on the 8-arm radial maze to demonstrate this type of learning. In the win-stay task, a food deprived rat is trained to enter arms that are paired with a light. The behavior of approaching a lighted arm and running down to the end of the arm is reinforced by the consumption of food at the end of the arm. Therefore, the stimulus (light) becomes associated with a particular response (entering arm). Repeated reinforcement of that S-R association strengthens the association and the rat soon only enters lit arms.

There is also a task for the water maze that assesses S-R learning. Morris (1984) described a task for the water maze in which a visible escape platform is present within the water maze and extra-maze cues are obscured, forcing the rat to only use the visible cue associated with the escape platform. After repeated training, rats associate the visible platform cue with the behavior of swimming over to and climbing onto the platform. This S-R association is thought to be strengthened by the fact that the rat escapes from the aversive water, a reinforcing event.

Many operant or instrumental learning tasks also involve forming S-R associations. These tasks usually involve pressing a bar or lever, or pulling a chain to get a reinforcing stimulus. A stimulus such as a light or tone is presented and signals the rat to bar-press or chain-pull to obtain the reinforcer (e.g. Harrison and Tracy, 1955; Ridgley et al., 1957). Through repeated training, the stimulus (light or tone) is able to elicit the behavioral response without the reinforcer present.

This reinforced S-R learning is dependent on a neural system that includes the dorsal striatum/basal ganglia as its central structure (discussed in detail in the next section). Damage to or disruption of the dorsal striatum impairs various types of S-R learning, including win-stay learning in the radial arm maze (e.g. Colombo et al., 1989; McDonald and White, 1993; McDonald and Hong, 2004), the visible platform task for the water maze (McDonald and White, 1994; Devan et al., 1996; Packard and Teather, 1997; Devan et al., 1999), and operant or instrumental learning tasks (e.g. Hansing et al., 1968; Schmaltz and Isaacson, 1972; Olmstead et al., 1976; Featherstone and McDonald, 2004).

Functional Anatomy of the Habit System

Less is known about the anatomical substrates of habit formation than is known about the substrates of the cognitive memory system. However, habit formation appears to be dependent on the interconnections among the cortex, the basal ganglia, and the cerebellum.

Hauber (1998) provides a detailed description of the anatomy and neurophysiology of the basal ganglia and its connections with the cortex and other subcortical structures. The striatum is the main input structure of the basal ganglia, receiving afferent projections from the entire cerebral cortex. The incoming signals to the striatum are relayed via direct and indirect pathways to the entopeduncular nucleus in rats (or the medial pallidal segment in primates) and the substantia nigra pars reticulata, which represent the major output nuclei of the basal ganglia. Projections from the output structures lead to motor nuclei of the brainstem and to motor cortical areas via the thalamus. The signal transfer and processing within these basal ganglia loops and pathways is mediated by a

variety of neurotransmitters and neuromodulators, including dopamine, glutamate, GABA, serotonin, and acetylcholine.

The striatum itself is a functionally heterogeneous structure, with a patch-matrix compartmentalization. The two compartments not only have distinct neurochemical activity, but they also have distinct input and output connections. The patches receive input from the prelimbic cortex, a medial frontal cortical area that receives direct input from the amygdala and hippocampus. The patches send projections to the substantia nigra pars compacta, which is the source of nigrostriatal dopamine. The matrix, on the other hand, receives input from sensory and motor cortical areas, and sends projections to the substantia nigra pars reticulata, the source of the non-dopaminergic nigrothalamic and nigrotectal systems (Gerfen, 1984). White (1989) hypothesized that the patch compartment of the striatum mediates the reward function of behavior, while the matrix compartment mediates sensory-motor memory, or S-R associations.

Evidence for the neural mechanism of the formation of S-R associations may have been provided by Jog et al. (1999). In this study, Jog et al. demonstrated a reorganization of neural assembly activation during the process of habit formation. During the initial stages of habit learning, most of the assembly units recorded in the basal ganglia showed activity related to the intermediate turning behavior required for the T-maze task. A lower number of units demonstrated activity related to starting point and ending point activity. As learning progressed, a shift in this activation pattern took place as a higher number of assembly units demonstrated activity related to starting and ending point activity and the number of units demonstrating activity related to the intermediate turning behavior decreased. Jog et al. suggested that this shift in activity may represent a de-emphasizing of the specific behavioral activity required in the habit and an increasing emphasis on the relationship between the stimulus information at the beginning stage and the end stage response. Jog et al. also hypothesized that this shifting might also represent an increasing emphasis on the cues that can elicit the habit response.

Leiner et al. (1986, 1989) suggest that the cerebellum is an important structure in habit formation. They proposed that the cerebellum, through its interactions with the cortex and basal ganglia, is involved in the learning of response programs. Response programs can be thought of as the linking of S-R associations, in which an elicited response provides the stimulus for the next S-R pairing. This learning of response programs allows for the learning of complex motor, and possibly non-motor, tasks. Leiner and colleagues apply this cerebellar function to language functioning in humans. In complimentary research, Kleim and colleagues (Kleim et al., 1998a, Kleim et al., 1998b) demonstrated that the cerebellum played an important role in the learning of complex motor skills that required the formation of response programs.

Conditioning – Stimulus-Affect Associations

A third type of learning, termed classical or Pavlovian conditioning (Pavlov, 1927), involves the formation of stimulus-affect (S-Af) associations in which a neutral stimulus (conditioned stimulus; CS) is paired with an unconditioned stimulus (US) that, without any prior learning, produces some approach or avoidance behavioral response (unconditioned response, UR) from which either a positive (pleasure) or negative (fear) affect is inferred. After repeated pairings, the CS is able to elicit the same behavioral response as the US (a conditioned response, CR). If the US produces a positive affect the conditioning is said to be appetitive; in these cases the US is usually food (or sucrose), water, or drugs. If the US produces a negative affect the conditioning is said to be aversive; in these cases the US is usually foot-shock. Again, McDonald and White (1993) used the 8-arm radial maze, implementing the separated-arms Conditioned Cue Preference (CCP) task (also called Conditioned Place Preference, CPP) to assess appetitive S-Af learning. In this CCP task, a food deprived rat is confined to the end of one radial arm where it consumes food on one day, and then is confined to the end of an arm on the opposite side of the maze with no food present on the next day. During these training trials the rat associates specific extra-maze cues (CS) with the food present on the maze (US). After several of these two-day training trials, the rat is allowed to freely explore

both arms during a preference test trial. Encountering the CS during the test trial elicits a CR to the CS previously paired with food and positive affect, resulting in a preference for the food-paired arm over the unpaired arm.

The CCP task is also utilized in a box apparatus, consisting of two compartments (one paired with the US, and one unpaired) and a neutral area that connects the two compartments (Carr et al., 1989, Schechter and Calgagnetti, 1993; 1998; Tzschentke, 1998). During training rats are placed into one compartment of the box with the US (food, water, or drug) on one day and then are placed in the opposite compartment with no US on the next day. A stimulus (light, color of compartment) that is present in the compartment that contains the US becomes a CS and will elicit a CR during the preference test when rats are allowed to freely explore both compartments. The CR results in the rats spending more time in the compartment that contains the CS.

Aversive S-Af learning is most commonly evaluated using fear conditioning. Fear conditioning was made infamous by Watson and Raynor (1920) with their experiment on 11-month old Albert B. Watson and Raynor paired the presentation of a white rat (CS) with the loud striking of a steel bar (US). As Albert reached to pet the rat the steel bar was struck with a hammer behind his head, causing Albert to jump and whimper (UR). Subsequent presentations of the CS or similar stimuli resulted in whimpering and avoidance behaviors (CR). Fear conditioning in rats is usually done in a small conditioning chamber that contains an electrified floor. During training a rat is placed into the chamber and is presented either a tone or light (CS) followed by a foot-shock (US). The foot-shock elicits a freezing response (UR) and the rat remains motionless. During a testing period, the rat is placed back into the chamber and is presented the CS again, which elicits the freezing response (CR) even in the absence of foot-shock. Another version of the fear conditioning task, called avoidance learning, allows the rat to avoid the CS by moving to another chamber that does not contain the CS.

This S-Af learning is dependent on a neural system that involves the amygdala as its central structure (described in detail in a later section). Damage

to or disruption of the amygdala impairs appetitive S-Af CCP learning in the radial arm maze (White and McDonald, 1993; McDonald and White, 1993, McIntyre et al., 1998; Holahan, 2005) and box apparatus (Hiroi and White, 1991; Brown and Fibiger, 1993; Hsu et al., 2002; Schroeder and Packard, 2002), as well as aversive S-Af learning in fear conditioning tasks (Goldstein, 1965; Hitchcock and Davis, 1986; Hitchcock and Davis, 1987; Cahill and McGaugh, 1990; Selden et al., 1991; Helmstetter, 1992; Holahan and White, 2002).

Functional Anatomy of the Conditioning System

The amygdala is thought to be the central structure necessary for S-Af learning, or conditioning. Both the amygdala and hippocampus receive many projections from the same cortical and subcortical regions (Mishkin and Appenzeller, 1987). Price (2003) provides a detailed review of amygdala connectivity. The amygdala receives input from all major sensory areas. Olfactory input is sent directly from the olfactory bulb and accessory olfactory bulb to the cortical surface of the amygdala. Taste and visceral sensory input is sent to the nucleus of the solitary tract, which has direct projections to the central nucleus of the amygdala. The nucleus of the solitary tract also projects to the parabrachial nucleus which connects to the central nucleus of the amygdala. The parabrachial nucleus also projects to the thalamic taste relay, which in turn projects to the lateral nucleus of the amygdala and the basolateral nucleus of the amygdala. Visual, auditory, and somatosensory input is first directed through sensory cortical areas before terminating onto the lateral nucleus of the amygdala. Auditory information also reaches the amygdala through the posterior thalamus.

Output pathways from the amygdala mostly originate from the central nucleus of the amygdala, although some projections originate from the medial and basal nuclei as well. The amygdala is able to influence visceral and autonomic reactions through its output connections with the hypothalamus, midbrain, and brainstem. The central nucleus of the amygdala projects to the lateral hypothalamus, which projects indirectly to the periaqueductal gray area and the parabrachial nucleus. Some fibers of passage continue to the reticular formation,

medulla, and other brainstem regions that are involved in the regulation of various autonomic functions.

In a standard model of conditioning, US and CS information enter the lateral nucleus of the amygdala, where an association is made between the two stimuli. This association is sent to the central nucleus of the amygdala, where the CR output originates (Kim and Jung, 2006). However, Pare et al. (2004) propose a different model of conditioning in which the central nucleus of the amygdala may be an important site for making associations as well. Pare and colleagues point out that the lateral amygdala has no direct projections to the central amygdala that would allow for direct passage of the CS-US association to output neurons in the central amygdala. In addition, Pare and colleagues provide evidence that the central amygdala receives direct input of CS and US information from the thalamus, allowing for the central amygdala to be a site of association making as well.

In addition to the connections with areas responsible for processing visceral and autonomic reactions to stimuli, the amygdala is also interconnected with the hippocampus. The CA1 subfield in the ventral hippocampus has substantial reciprocal connections with the lateral and basal nuclei of the amygdala, and lighter reciprocal connections with the central and medial nuclei (Pitkanen et al., 2000). The amygdala is also part of a circuit that includes the orbital and medial prefrontal cortex, thalamus, and striatum that allows the frontal cortices to influence behavioral choice and reward assessment (Price, 2003; Gabbott et al., 2006). The primary reciprocal connections occur between the amygdala and the medial prefrontal cortex. Both the medial prefrontal cortex and the amygdala have direct connections with the nucleus accumbens and dorsal striatum, allowing the amygdala to influence reward assessment, decision making, and S-R behavioral output (Packard and Teather, 1998; Holland and Gallagher, 2004; Floresco and Ghods-Sharifi, 2006).

Aims of the Current Research

Despite the fact that the initial demonstrations of latent learning indirectly led to the development of the MMS hypothesis, the phenomenon of latent learning is not yet specifically included in the current MMS model (White and McDonald, 2002). This may be due to the lack of understanding of the neural structures that underlie it. Latent learning involves the formation of S-S associations that are not bound to the performance of a particular behavior, nor are they associated with a rewarding stimulus or reinforcement. As previously described, this can occur in situations when a rewarding incentive is simply not present during the learning period, as in the classic latent learning tasks involving unrewarded pre-exposure to mazes (Blodgett, 1929; Tolman and Honzik, 1930), or in situations when the animal is not deprived of the incentive about which it is learning (Thistlethwaite, 1951). In this situation, the incentive is not considered to be rewarding for two reasons: (1) it is not reducing a physiological drive, and (2) the incentive is not hedonically pleasing enough to produce a rewarding/positive affective state. In this sense, the components of the S-S association are neutral.

One reason that the neurobiological substrates underlying irrelevant-incentive latent learning have not been clearly determined is because of the lack of a reliable behavioral task that is not susceptible to the side effects of lesions of neural structures, specifically the fimbria-fornix and hippocampus (see Douglas, 1967; Coutureau et al., 2000; this matter is discussed in greater detail in a later section). Latent learning tasks that have been previously developed have used reinforced spatial learning trials to illustrate latent learning that occurred during unreinforced pre-exposure to the maze, or have used an increase in bar pressing rates to illustrate a latent learning effect. However, both of these behavioral measures were affected by side effects of brain lesions, which obscured the effect of the brain lesion. Therefore it was difficult to determine if the disruption in latent learning was due to a true learning deficit produced by the brain lesion, or if it was due to a side effect independent of learning. However, this thesis introduces a new behavioral task that can be reliably used to demonstrate

irrelevant-incentive latent learning in rats, and describes the use of this task in identifying several neural structures shown to be necessary for this type of latent learning.

Despite evidence that spatial latent learning does occur during unrewarded pre-exposure to mazes, a maze-based spatial latent learning task was not chosen for these experiments. These latent learning tasks involved the use of reinforced maze trials to illustrate latent learning that had taken place during previous unreinforced pre-exposure to the maze. However lesions of the hippocampal system affect the performance of the reinforced trials, which introduces ambiguity in the interpretation of the lesion effects. For example, in one study (Kimble et al., 1982), hippocampus-lesioned rats that were pre-exposed to the maze performed much poorer than control rats that were pre-exposed to the maze. This was because an intact hippocampus was necessary for spatial learning that occurred during the reinforced trials, and therefore the hippocampus-lesioned rats were impaired relative to controls. However, the researchers still claimed that the hippocampal rats demonstrated latent learning because they performed better than non-pre-exposed rats on the reinforced trials. This finding introduced ambiguity into the interpretation of the lesion effects on latent learning. Therefore, one goal of this thesis was to develop a new behavioral task to assess latent learning using a behavioral measure that would not be susceptible to these brain lesions.

The CCP paradigm has previously been used to determine the neural bases of conditioning for food and drug reinforcers (Schechter and Calgagnetti, 1993; 1998; Tzschentke, 1998). In addition, the CCP paradigm is insensitive to changes in rates of behavioral responding that may occur from hippocampal system brain lesions or drug administration (White and McDonald, 1993; Hiroi and White, 1991). Therefore, the standard CCP paradigm was adapted to assess latent learning.

The latent learning task described in this thesis used the CCP paradigm to demonstrate latent learning that does not rely primarily on spatial information. The standard CCP apparatus consists of two distinct compartments with unique cues that are joined by a tunnel or common area (Carr et al., 1989; Schechter and

Calgagnetti, 1993; 1998). Several measures were taken to reduce the possibility that rats could identify the locations of the salt and water solutions using spatial cues. First, the visibility of cues outside the apparatus was reduced by the height of its opaque walls (45 cm). Second, the entire apparatus was rotated 180 degrees between training trials so that any external cues that remained visible were not consistently associated with the stimuli in either compartment. Third, to make relational learning among the cues in the two compartments difficult, the apparatus was designed so that rats could see the cues in only one of the compartments from any location within either the salt- or water-paired compartments or from the connecting compartment. Fourth, given evidence that movement restriction prevents or greatly retards spatial learning (McDonald and White, 1995), the size of the compartments was reduced to restrict rats' movement within the apparatus during training.

In the present series of experiments, water deprived rats were exposed to water in one compartment and to an equally-preferred salt solution in the other compartment over several 2-day training cycles (trials). The rats were then tested when they were salt+water deprived or when they were water-only deprived. A preference for the salt-paired compartment by the salt+water deprived rats was taken as evidence that the rats had latently learned an association between the taste of the salt solution and the cues in the compartment where they consumed it. After the behavioral demonstration of the latent learning phenomenon, I wished to determine the neural structures necessary for this type of learning. Once these structures were determined, I could then determine their involvement in the acquisition and expression of this type of latent learning. These findings could then be used to update the current MMS model to include information about neutral S-S associations.

CHAPTER THREE: GENERAL METHODS

ANIMALS

Male Long-Evans rats (Charles River, St. Laurent, QC) weighing 275-325 grams at the start of the experiments were used (see Appendix C). Rats were housed individually in hanging wire-mesh cages in a temperature-controlled room maintained on a 12:12-hr light/dark cycle (lights on at 7:00). All training and testing procedures were conducted during the light phase, starting at approximately 13:00 each day. Ad lib access to rat chow was given throughout the experiment unless noted otherwise in the procedure.

APPARATUS

Figure 3 illustrates the apparatus used in the experiments. It was adapted from the standard CCP box (Carr, et al., 1989; Schechter and Calgagnetti, 1993; 1998). The outer walls and floor of the apparatus were constructed of plywood. The partitions that separated the three chambers of the box were made of opaque Plexiglas. Compartments 1 and 3 (28 cm x 8 cm x 45 cm) were positioned alongside each other, and shared one opaque partition as a common wall. These two compartments served as the salt-paired and water-paired compartments. The inside walls and floor of compartment 1 were painted with black and white vertical stripes, and the floor was covered with a 1-cm wire mesh. The inside walls and floor of compartment 3 were painted black and the floor was covered with wood-chip bedding. Compartment 2 (17 cm x 8 cm x 45 cm) was positioned across the ends of 1 and 3, and served as the start compartment from which rats had free access to 1 and 3 during the test trials. The inside walls and floor of compartment 2 were painted gray, and the floor was left bare. The partition that separated compartment 2 from 1 and 3 did not have door openings during training, but did have door openings (6 cm x 6 cm) during the test trial. Fifty-ml water tubes were mounted on the opposite ends of compartments 1 and 3 so that their spouts protruded into the compartments. The apparatus was placed on the floor of the experimental room.

An infrared motion detector (RadioShack model 49-208A; using wide-angle lens) was mounted on the wall of each compartment, facing down,

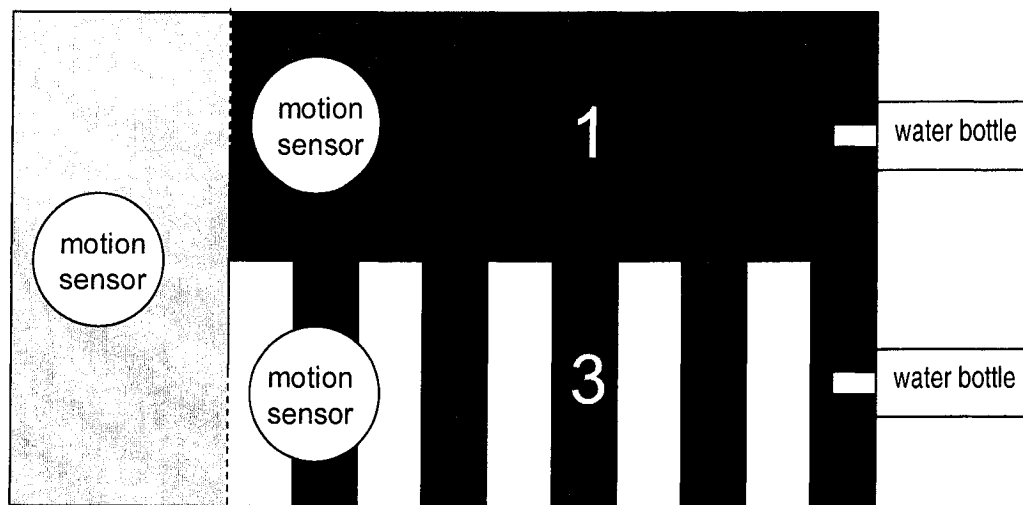


Figure 3. Top view of CCP apparatus used in all experiments. Compartments 1 and 3 served as the salt-paired and water-paired compartments, counterbalanced. Compartment 2 served as the neutral start compartment for the preference test trial (from Stouffer & White, 2005).

approximately 30 cm above the floor. A computer program written using SoftWIRE graphical program for Microsoft VisualBasic received information about the position of the rats in the apparatus from the infrared motion sensors and exported the compartment entrance and exit times into a Microsoft Excel worksheet. These entrance and exit times were used to calculate the total time spent in each of the compartments.

GENERAL PROCEDURES

Latent Cue Preference Task

Training Trials

Prior to training on the LCP task, all rats were placed on a water deprivation schedule that allowed them access to tap water for 30 minutes each day for five days. This was sufficient for rats to reach a plateau of daily water consumption (15-20 ml per day). The LCP procedure began on day six of the water deprivation schedule.

The LCP procedure involved three phases: training trials, salt deprivation, and a preference test (see Figure 4, top). On each training trial, rats were placed into one compartment with either the salt solution (12.5 mg/ml sodium chloride; pilot experiments determined this concentration to be preferred equally to tap water in a one-bottle test procedure; see Appendix A) or water in the drinking tube for 30 minutes on one day, then in the other compartment with the other substance on the next day. There were 4 training trials over 8 days. The compartments assigned as salt-paired and water-paired and the orders of salt and water presentation were counterbalanced within each group. The amounts of each solution consumed during the training trials were recorded. The entire apparatus was rotated 180 degrees between each training trial.

Salt Deprivation Procedures

On the day after the last training trial, experimental rats were given a salt deprivation treatment. Two salt deprivation procedures were used in the experiments. One, rats' normal rat chow was replaced with a low-salt diet (Harlan Teklad, < 0.01% sodium salt) for 14 days, a time previously demonstrated

Latent Cue Preference Task	Days 1-8				Day 9	Day 11
	Training Trials				Sodium Deprivation	Preference Test
	4 two-day pairings: salt solution in one compartment, water in other compartment				Furosemide injection Or PBS injection	20 mins, no solutions present
Conditioned Cue Preference Task	Day 1	Days 3-4	Day 5	Days 7-8	Day 9	Day 11
	Sodium Deprivation	Training Trial 1	Sodium Deprivation	Training Trial 2	Sodium Deprivation	Preference Test
	Furosemide injection	Two-day pairing: salt solution in one compartment, water in other compartment	Furosemide injection	Same as Training Trial 1	Furosemide injection	20 mins, no solutions present

Figure 4. Procedures for the Latent Cue Preference (LCP) and Conditioned Cue Preference (CCP) tasks. Gray shaded areas indicate the periods when rats were salt deprived. PBS = phosphate buffered saline.

to induce salt deprivation and a robust salt appetite (Stricker et al., 1991; Curtis et al., 2001). These rats were also given distilled water on the normal water deprivation schedule. Two, rats were given injections of furosemide (10 mg, pH 7.0, intraperitoneal), which produces a similar state of salt deprivation in less than 48 hours (Starbuck et al., 1997; Thunhorst et al., 1999). These rats were also given the low-salt diet until the preference test trial, and were given distilled water on the normal water deprivation schedule. Control rats underwent one of two corresponding control procedures. One, rats were maintained on the normal rat chow diet for a period of 14 days, and were given tap water on the normal water deprivation schedule. Two, rats were given a control injection (an equal volume of phosphate-buffered saline, pH 7.0, i.p.), and were maintained on the normal rat chow diet for two days after the last day of training, and were given tap water on the normal water deprivation schedule.

Preference Test Trial

The compartment preference test trial followed the salt deprivation and control procedures. If rats were given the low-salt diet procedure, the preference test trial was given on day 15 of the deprivation procedure. If rats were given the furosemide injection, the preference test was given 48 hours after the injection. At the start of the test trial, rats were placed into compartment 2 and were given free access to all compartments for 20 minutes with the solutions removed. A computer program written using SoftWIRE graphical program for Microsoft VisualBasic received information about the position of the rats in the apparatus from the infrared motion sensors and exported the compartment entrance and exit times into a Microsoft Excel worksheet. The total time spent in each chamber was then calculated (the automated compartment time recordings were shown to have correlation of 0.93 with hand-recorded compartment times in a pilot experiment; see Appendix B).

Verification of Salt Deprivation during Testing

Immediately following the preference test trial, rats were given 30 minutes access to the 12.5 mg/ml salt solution in their home cage. However, in Experiments One and Two (see Chapter 4) half of the rats were given 30 minutes

access to the salt solution in their home cage and the other half was given 30 minutes access to tap water. This was done to insure that the salt deprivation produced an increase in consumption specific to the salt solution rather than a general increase in fluid consumption. In all experiments, a rat was considered salt deprived if it consumed more than 2 standard deviations above the average amount of salt solution it consumed during the last three training trials. If a rat was given a salt deprivation treatment but did not meet this criterion, then it was not considered to be salt deprived and was not included in the compartment preference analyses.

Conditioned Cue Preference Task

The purpose of the CCP task was to provide a comparative task in which all sensory stimuli were held constant with the LCP task, with the only difference between the two tasks being the time period in which rats were salt deprived. In the LCP task, rats were only salt deprived during the preference test trial. A preference for the salt-paired compartment during the test trial would therefore be based upon a latently acquired (neutral) association between compartment cues and the presence of salt. In the CCP task, rats were salt deprived during training trials and testing. A preference for the salt-paired compartment during the test trial would therefore be based on a conditioned (rewarded) association between compartment cues and the presence of salt. The brain regions responsible for processing these different types of learning should therefore be differentiable with lesion studies, thus demonstrating the independence of the two learning systems.

Salt Deprivation Procedure

As with the LCP procedure, prior to training on the CCP task rats were placed on a water deprivation schedule that allowed them access to tap water for 30 minutes each day for five days. The CCP procedure began on day six of the water deprivation schedule. The CCP task consisted of three phases: a salt deprivation phase, a training phase, and a test phase (see Figure 4, bottom). During the salt deprivation phase, rats were given an injection of furosemide (10 mg; pH 7.0, i.p.) to induce salt deprivation and were given ad lib access to the low-salt diet (Harlan Teklad, < 0.01% sodium salt). They were also given

distilled water on the normal water deprivation schedule for the remainder of the experiment.

Training Trials

Two days after the furosemide injections, rats were given the first training trial. During each trial, rats were placed into one compartment with either the 12.5 mg/ml salt solution or distilled water in the drinking tube for 30 minutes on one day, then in the other compartment with the other solution on the next day. On the day after the first training trial, rats were again given a furosemide injection. Two days after the second injection, rats were given the second training trial. The compartments assigned as salt-paired and water-paired and the orders of salt and water presentation were counterbalanced. The amounts of each solution consumed were recorded, and the entire apparatus was rotated 180 degrees between each training trial.

Preference Test Trial

On the day after the second training trial, rats were again given the furosemide injection. A compartment preference test trial was given 48 hours later. During the preference test trial, rats were placed into compartment 2 and were given free access to all compartments for 20 minutes with the solutions removed. The total time spent in each compartment was recorded and calculated.

Verification of Salt Deprivation during Training

Immediately following the preference test trial, rats were given 30 minutes access to the salt solution in their home cage and the amount of solution consumed was recorded. A rat was considered to be salt deprived during training if the amount of salt solution it consumed was two or more standard deviations above the amount of water it consumed during the training. If a rat did not meet this criterion it was excluded from the compartment preference test analyses.

Statistical Analyses

Several types of statistical analyses were conducted in the experiments that follow. Consumption of salt solution and water during training trials and during the post-test consumption period was analyzed by multifactor Analysis of Variance (ANOVA), followed by either Tukey's HSD post-hoc tests to examine

unpredicted group differences or by planned comparisons to examine predicted group differences (Howell, 2002; Keppel, 1991; Tabachnick and Fidell, 2001). Compartment preference times were analyzed by multifactor ANOVA as well, followed by planned comparisons to examine predicted group differences.

CHAPTER FOUR: DEMONSTRATION OF THE LCP AND CCP TASKS

This chapter describes the development of the latent cue preference (LCP) and conditioned cue preference (CCP) tasks. The first series of experiments used a low sodium diet to produce salt deprivation. The second series used furosemide. These two tasks were used in subsequent experiments to compare the neural substrates of the two kinds of learning.

Experiment One: Demonstration of LCP Task

Methods

Forty-eight rats were divided into four groups prior to the start of the experiment. The first group was given the salt solution in one compartment and water in the other compartment during training while water-only deprived, and then was salt+water deprived during the preference test trial (SWDep; $n = 12$). The second group was given the salt solution in one compartment and water in the other compartment during training while water-only deprived and was water-only deprived during the preference test (SWND; $n = 12$). The third group was given water in both compartments during training while water-only deprived and was salt+water deprived during the preference test trial (WWDep; $n = 12$). The fourth group was given water in both compartments during training while water-only deprived and was water-only deprived during the preference test (WWND; $n = 12$).

Immediately following the preference test trial, all rats were returned to their home cages and were given the solution consumption test. Half of the rats each group were given the salt solution and the other half was given water. This procedure was conducted to make sure any increase in salt solution consumption produced by the low-salt diet was specific to the salt solution and was not simply the result of a general increase in fluid consumption.

Results and Discussion

Salt and Water Consumption during Training Trials

The mean amounts of salt solution consumed on each training trial ranged between 12.5 ml and 15.3 ml, while the mean water consumption ranged between 13.7 ml and 15.8 ml. A three-way mixed design ANOVA (Group x Solution x

Pairing) revealed no significant main effects or interactions. This indicates there were no significant differences in the mean amounts of salt solution and water consumed during the training trials.

Verification of Salt Deprivation during Testing

Solution consumption during the post-test consumption period is shown in Figure 5. A Two-way independent groups ANOVA (Diet x Solution) revealed a significant main effect of Diet [$F(1, 41) = 33.403, p < 0.01$], a significant main effect of Solution [$F(1, 41) = 16.405, p < 0.01$], and a significant Diet x Solution interaction [$F(1, 41) = 29.222, p < 0.01$]. Planned comparisons on the Diet x Solution interaction showed that the rats given the low salt diet drank significantly more salt solution than water [$F(1, 41) = 24.135, p < 0.01$], and the rats given the low salt diet drank significantly more salt solution than did the rats given the normal diet [$F(1, 41) = 27.534, p < 0.01$]. These findings confirm that the low salt diet increased salt appetite, and did not produce a general increase in fluid consumption.

Compartment Preference Test

A total of 3 rats was eliminated from the compartment preference analyses for failing to meet the salt deprivation criterion described in the General Methods chapter. Figure 6 shows the compartment preference times for the four groups during the preference test trial. The rats that drank the salt solution and water in different compartments during training and were then salt+water deprived during testing (SWDep) showed a preference for their salt-paired compartments. The rats that were trained in the same way but water-only deprived during testing (SWND) showed a preference for their water-paired compartments.

A two-way mixed design ANOVA (Group x Compartment) revealed no significant main effects, but did reveal a significant Group x Compartment interaction [$F(3, 39) = 4.352, p < 0.05$]. Planned comparisons showed that the rats in the SWDep group spent significantly more time in their salt-paired than in their water-paired compartments [$F(1, 39) = 6.24, p < 0.05$] and that the rats in

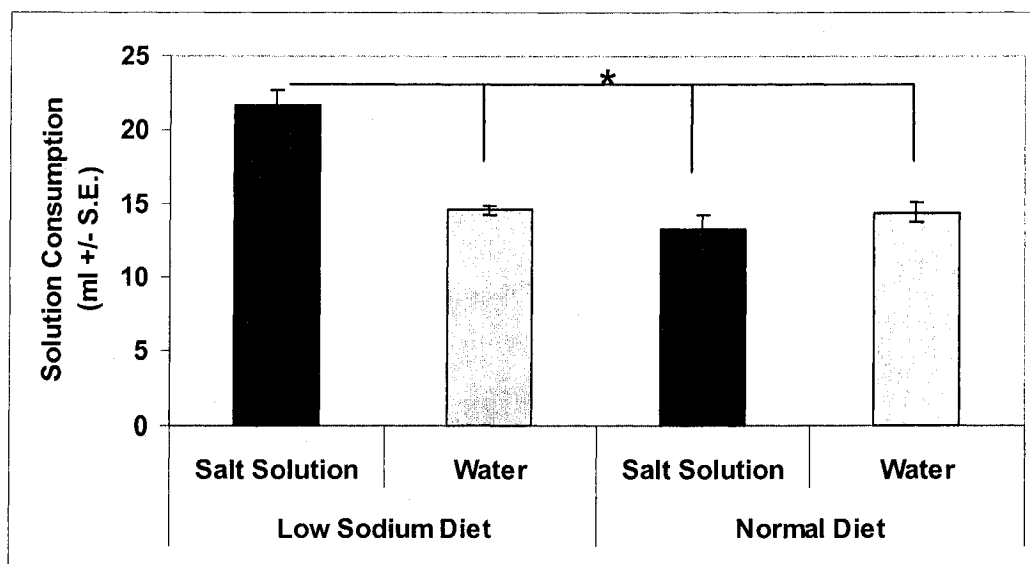


Figure 5. Experiment One post-test solution consumption results. Analyses of the data revealed that rats given the low sodium diet consumed significantly more of the salt solution compared to rats maintained on the normal diet, verifying sodium deprivation. * = $p < 0.01$.

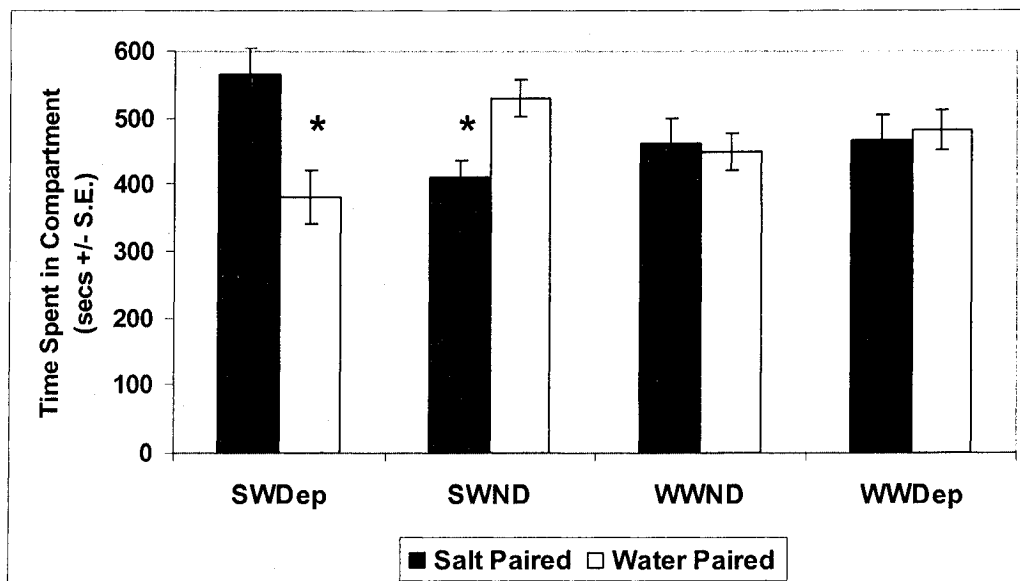


Figure 6. Experiment One compartment preference results. Analyses revealed that rats that were salt+water deprived during testing (SWDep) showed a significant preference for the salt-paired compartment, a demonstration of latent learning. Rats that were water-only deprived during testing (SWND) showed a significant preference for the water-paired compartment, a demonstration of conditioning. Rats that were given only water during training and then were salt+water deprived (WWDep) or water-only deprived (WWND) during the test showed no preferences, indicating that salt deprivation alone or compartment cues alone did not produce compartment preferences. * = $p < 0.05$.

the SWND group spent significantly more time in their water-paired than in their salt-paired compartments [$F(1, 39) = 6.73, p < 0.05$]. In addition, there was a strong positive curvilinear relationship ($R^2 = 0.46$) between the degree of salt deprivation during the preference test and compartment preference times, indicating that the more salt deprived a rat was the more time it spent in its salt-paired compartment (see Figure 7).

The two groups of rats that were exposed to water in both compartments during training (WWDep, WWND) showed no significant preference for either compartment. This indicates that the salt deprivation did not produce a compartment preference in the absence of any previous learning about salt, and that the compartment cues alone did not produce any compartment preferences. The preference for the salt-paired compartment by the salt+water deprived rats constitutes an LCP, or latent learning. The rats acquired an association between the taste of salt and the cues in the compartment where it was presented during the training trials. During testing this association and the salt appetite produced by salt deprivation interacted to produce behaviors that led to a preference for spending time in the salt-paired compartment.

The significant preference for the water-paired compartment by the rats that were water-only deprived during testing suggests that these rats may have developed a CCP for the water-paired cues. Because the rats were water deprived during training the water may have been rewarding to the rats, leading to this form of learning. The difference between the two forms of learning is that during training the water was rewarding for the water deprived rats, resulting in conditioning; however, the salt solution was not rewarding to rats because they were not salt deprived during training, resulting in latent learning.

Experiment Two: Replication and Reversal of LCP

The purpose of Experiment Two was to replicate the findings of Experiment One, to demonstrate that the information gained during training trials could be used flexibly, and to show that the expression of LCP learning is dependent on deprivation state. In other words, could the preferences

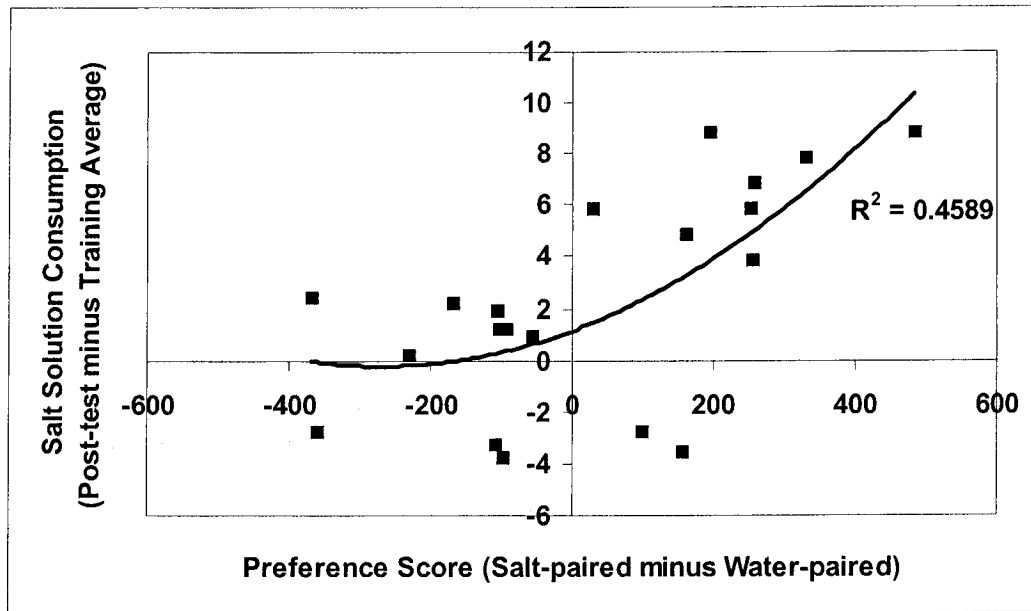


Figure 7. The relationship between the degree of salt deprivation during testing (defined as post-test consumption minus average training consumption) and compartment preference times. In general, the more salt deprived a rat was, the more time it spent in the salt-paired compartment.

demonstrated in one preference test trial be reversed in a second preference test trial simply by reversing the rats' deprivation states?

Methods

Thirty rats were divided into three groups. The first group was given the salt solution in one compartment and water in the other compartment during training while water-only deprived, and then was salt+water deprived during testing via an injection of furosemide (SWDep; $n = 8$). The second group was given the salt solution in one compartment and water in the other compartment during training while water-only deprived, and was also water-only deprived during testing (SWND; $n = 11$). The third group was given water in both compartments during training while water-only deprived, and then was salt+water deprived during testing (WWDep; $n = 9$). This group was included to determine if the furosemide-induced salt deprivation produced a compartment preference in the absence of any previous learning about salt.

Immediately following the first preference test trial, all rats were returned to their home cages and were given a consumption test. Half of the rats in each group were given the salt solution and the other half was given water. This procedure was conducted to make sure any increase in salt solution consumption produced by the furosemide injection was specific to the salt solution and was not simply the result of a general increase in fluid consumption.

Following the completion of the first preference test trial and post-test consumption period, the rats in the SWDep and SWND groups were given 30 minutes daily access to the salt solution in their home cage for 3 days, followed by 30 minutes daily access to water for 4 days. They also received the normal diet during this 7-day period. This was done to return all rats to a normal salt balance. All rats were then given an additional 2-day training trial, followed by the treatment opposite to the one they received prior to the first preference test. Rats that were salt+water deprived during the first preference test were water-only deprived during the second preference test (SWDep/ND; $n = 10$). Conversely, rats that were water-only deprived during the first preference test were salt+water deprived during the second preference test (SWND/Dep; $n = 10$). This procedure

was done to determine if latent learning involving salt could be used flexibly to produce preference behaviors.

Results and Discussion

Salt and Water Consumption during Training Trials

The mean amounts of the salt solution and water consumed during the training trials were again similar across groups. The mean salt consumption ranged between 17.0 ml and 17.3 ml, while the mean water consumption ranged between 16.3 ml and 19.0 ml. A three-way mixed design ANOVA (Group x Solution x Pairing) revealed no significant main effects or interactions.

Verification of Salt Deprivation during First Preference Test

The post-test consumption of the salt solution and water is illustrated in Figure 8. A two-way independent groups ANOVA (Treatment x Solution) revealed a significant main effect of Treatment [$F(1, 27) = 29.361, p < 0.01$], a significant main effect of Solution [$F(1, 27) = 23.752, p < 0.01$], and a significant Diet x Solution interaction [$F(3, 27) = 27.934, p < 0.01$]. Planned comparisons on the Treatment x Solution interaction showed that the rats given furosemide drank significantly more salt solution than water [$F(1, 27) = 19.575, p < 0.01$], and the furosemide treated rats drank significantly more salt solution than did PBS treated rats [$F(1, 27) = 16.263, p < 0.01$]. These findings confirm that the furosemide increased salt appetite, and did not produce a general increase in fluid consumption.

First Preference Test Trial

A total of 2 rats were eliminated from the compartment preference analyses for failing to meet the salt deprivation criterion described in the General Methods chapter. The compartment preference times for each group are illustrated in Figure 9. The rats that were given the salt solution in one compartment and water in the other compartment during training and then salt deprived during testing (SWDep) showed a preference for the salt-paired compartment. The rats that were trained the same way, but were water-only deprived during testing (SWND) showed a preference for the water-paired compartment. A two-way mixed design ANOVA (Group x Compartment)

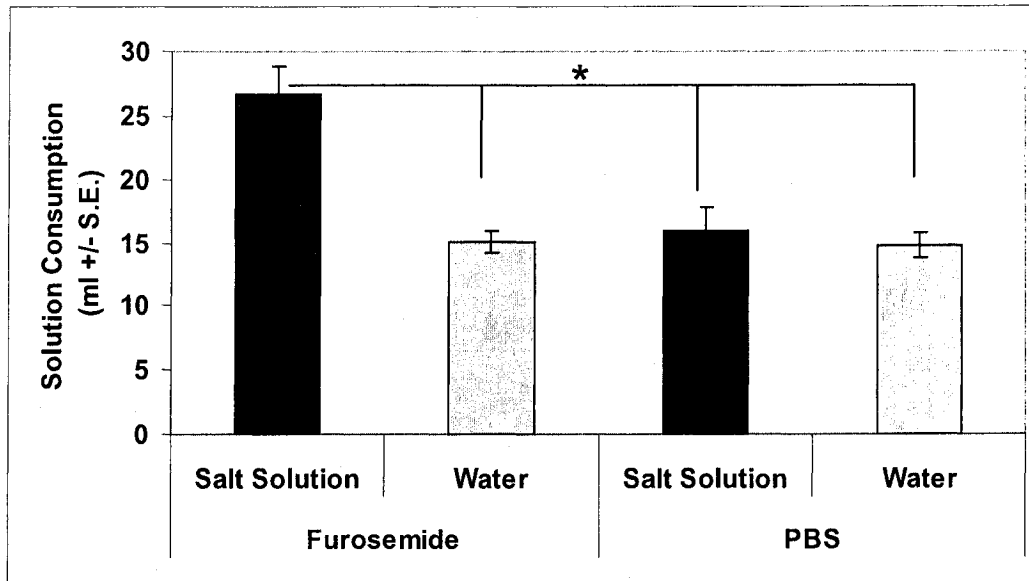


Figure 8. Experiment Two post-test solution consumption results. Analyses of the data revealed that rats given the furosemide injection consumed significantly more of the salt solution compared to rats given the PBS injection, verifying salt deprivation. * = $p < 0.01$.

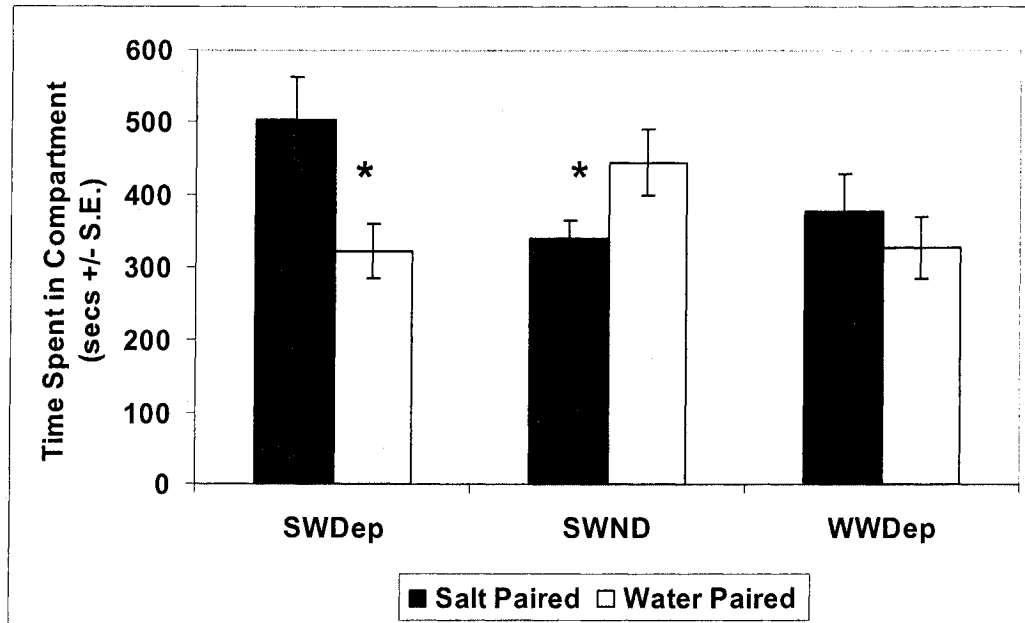


Figure 9. Experiment Two results of first compartment preference test. Analyses revealed that rats that were salt+water deprived during testing (SWDep) showed a significant preference for the salt-paired compartment, a demonstration of latent learning. Rats that were water-only deprived during testing (SWND) showed a significant preference for the water-paired compartment, a demonstration of conditioning. Rats that were given only water during training, and then were salt+water deprived during testing (WWDep) showed no preferences, verifying that salt deprivation alone did not produce a compartment preference.

* = $p < 0.05$.

indicated that there were no significant main effects, but did reveal a significant Group x Compartment interaction [$F(2, 27) = 4.823, p < 0.05$].

Planned comparisons of the compartment times showed that the rats in the SWDep group spent significantly more time in their salt-paired than in their water-paired compartments [$F(1, 27) = 5.23, p < 0.05$], while the rats in the SWND group showed the opposite preference [$F(1, 27) = 4.46, p < 0.05$]. In addition, there was a strong positive curvilinear relationship ($R^2 = 0.51$) between the degree of salt deprivation and compartment preferences, indicating that the more salt deprived a rat was the more time it spent in its salt-paired compartment (see Figure 10). Rats that drank water in both compartments during training and were then salt deprived via furosemide (WWDep) showed no significant preference for either compartment. This verifies that the salt deprivation procedure did not produce any compartment preferences in the absence of previous learning about salt.

These findings replicate those of Experiment 1, showing that the salt deprivation state produced by furosemide results in the expression of the latently acquired association between salt and compartment cues during the preference test trial. Also as in Experiment 1, the rats that were water-only deprived during training and testing preferred the water-paired compartment during the preference test trial.

Second Preference Test Trial

Only weak preferences were shown on the second preference test, but it was noted that these preferences were much larger during the early part of the test than during the later part. Accordingly, the 20 minute test trial was split into two 10-minute parts, and the data for these parts are shown in Figure 11. The rats that were now salt+water deprived (SWND/Dep) showed a preference for the salt-paired compartment, and the rats that were now water-only deprived (SWDep/ND) showed a preference for the water-paired compartment. A three-way mixed design ANOVA (Group x Time Period x Compartment) revealed no significant main effects, but did show a significant Group x Compartment x Time Period interaction [$F(1, 17) = 12.973, p < 0.05$]. Tukey's HSD post-hoc tests on

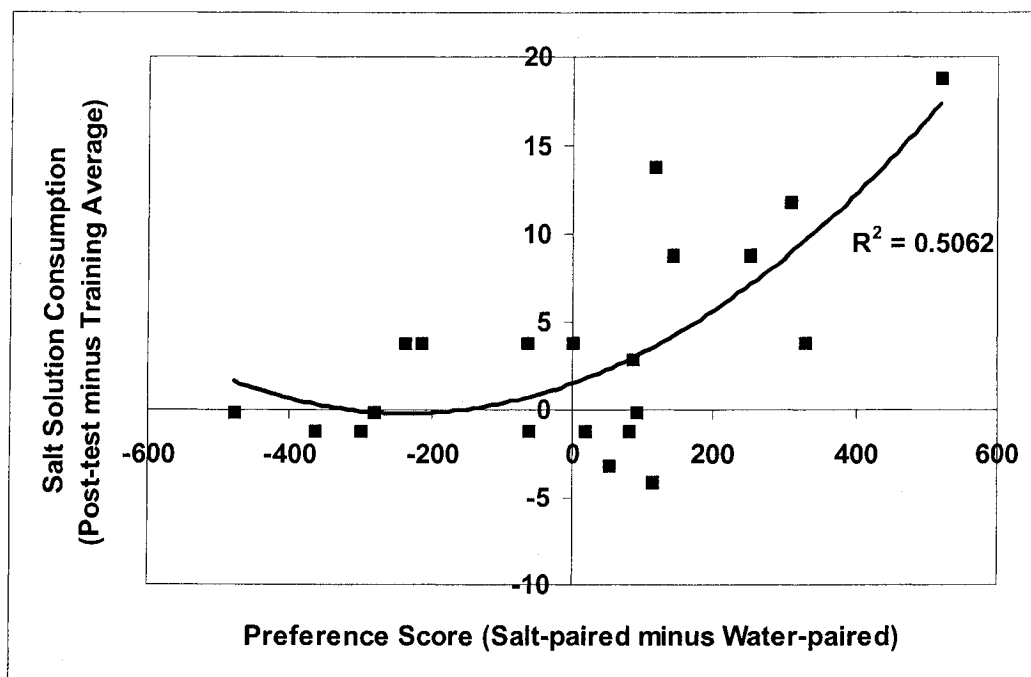


Figure 10. Experiment Two relationship between degree of salt deprivation during the first preference test (defined as post-test salt solution consumption minus average training consumption) and compartment preference times (defined as salt-paired time minus water-paired time). In general, the more salt deprived a rat was, the more time it spent in its salt-paired compartment.

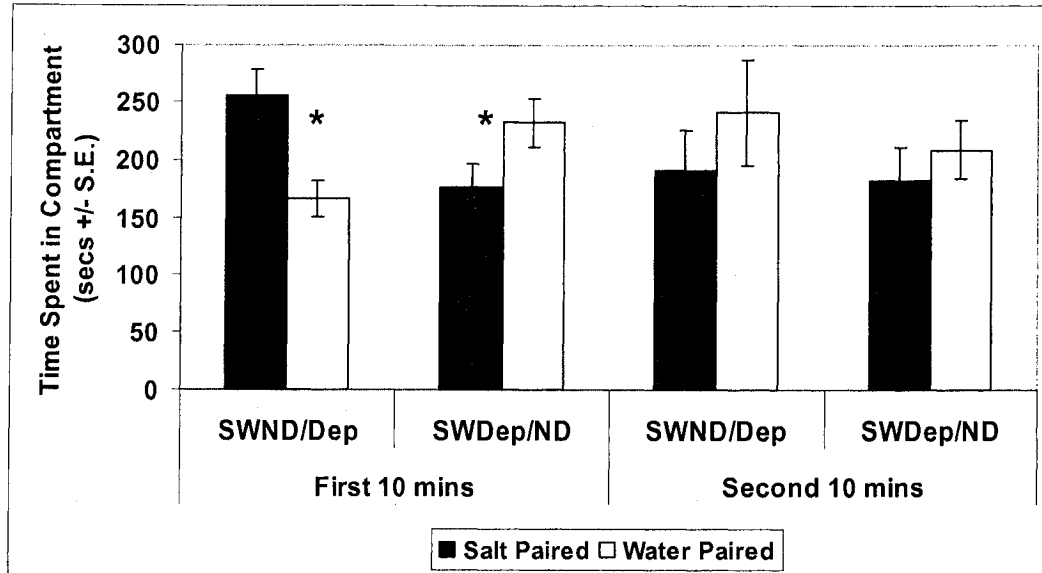


Figure 11. Experiment Two results for second compartment preference test. Analyses revealed that rats that were salt+water deprived during testing (SWND/Dep) showed a significant preference for the salt-paired compartment during the first 10 minutes of the test trial, a demonstration of latent learning. Rats that were water-only deprived during testing (SWDep/ND) showed a significant preference for the water-paired compartment during the first 10 minutes of the test trial, a demonstration of conditioning. These preferences extinguished during the second 10 minute period. These results demonstrate that the latent learning could be used flexibly depending on deprivation state.
 * = $p < 0.05$.

the first 10-minute period showed that the rats in the SWND/Dep group spent significantly more time in their salt-paired than in their water-paired compartments ($p < 0.05$), while the rats in the SWDep/ND group spent significantly more time in their water-paired than in their salt-paired compartments ($p < 0.05$). During the second 10-minute period neither group showed a significant preference for either compartment (p 's > 0.05).

Verification of Salt Deprivation during Second Preference Test

An independent groups t-test showed that the furosemide treated group (SWND/Dep) consumed significantly more salt solution than the SWDep/ND group during the second post-test consumption period, $t(18) = 5.631$, $p < 0.05$. These results confirm increased salt appetite in the rats that received furosemide. Additional analysis of the consumption data revealed no significant differences in the mean amount consumed during both post-test consumption periods by the rats that had received furosemide (28.4 ml following second preference test, 27.5 ml following first preference test). This suggests that the level of salt deprivation during the two preference tests was approximately equal, suggesting that differences in salt appetite cannot explain the relatively weak compartment preferences in the second preference test trial.

Another possible explanation for this weak preference could be extinction of the latently acquired association between the taste of salt and the compartment cues. Even though the rats were given an additional training trial, learning that salt and compartment cues were not always associated may have occurred after a total of 30 minutes (20 during first preference test, 10 during the second preference test) of exposure to the cues in the absence of the solution, resulting in the preference being extinguished during the second 10 minute period of the second preference test.

The results of the second preference test trial showed that the rats' compartment preferences were controlled by their salt deprivation states during the first 10 minutes of the test trial. Importantly, this shows that the latently acquired association between the salt solution and compartment cues could be

used flexibly, depending upon whether the rat was in a state of salt deprivation or was salt replete.

Experiment Three: Demonstration of Salt-CCP

Because the CCP task was used as a control for the LCP task, all stimuli were held constant across the two tasks, with the only difference being that during the CCP task rats were salt deprived during both training and testing. Salt deprivation during training resulted in the salt solution becoming a rewarding stimulus. The purpose of this experiment was to demonstrate a preference for the salt-paired compartment based on conditioning rather than latent learning.

Methods

Twenty-four rats were divided into two groups, both of which received two training trials as part of salt-CCP training. The first group was salt+water deprived during training trials, in which they were given the salt solution in one compartment and water in the other compartment (DepSW; $n = 12$). The second group was water-only deprived during training trials, in which they were given the salt solution in one compartment and water in the other compartment (NDSW; $n = 12$). All rats were salt+water deprived during the preference test.

Results and Discussion

Verification of Salt Deprivation during Training

The amount of the two solutions consumed during the training trials are illustrated in Figure 12. A three-way mixed design ANOVA (Group x Trial x Solution) revealed significant main effects for Trial [$F(1, 22) = 216.50, p < 0.01$] and Solution [$F(1, 22) = 4.689, p < 0.05$], as well as significant interactions for Trial x Group [$F(1, 22) = 5.204, p < 0.05$] and Solution x Group [$F(1, 22) = 22.010, p < 0.01$]. All other effects were non-significant. Planned comparisons on the significant Solution x Group interaction indicated that rats in the SWDep group consumed significantly more salt solution than water during training [$F(1, 22) = 13.738, p < 0.01$], while rats in the SWND group consumed equal amounts of the solutions during training [$F(1, 22) = 0.186, p = 0.67$]. These results verify that rats in the SWDep group were salt deprived during the training trials, therefore making the consumption of the salt solution rewarding.

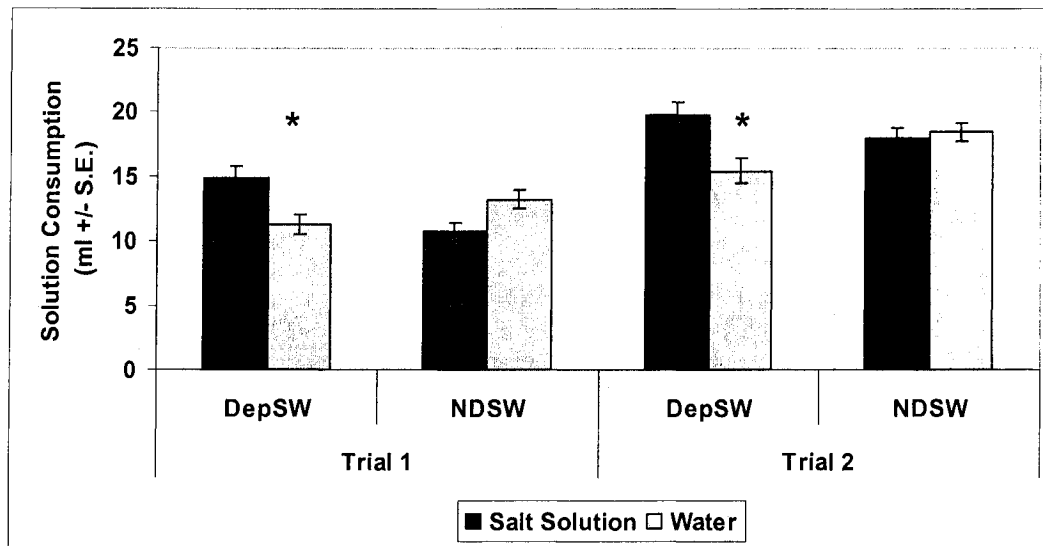


Figure 12. Experiment Three (CCP) training trial solution consumption results. Rats in the DepSW group consumed significantly more salt solution than water during the two training trials, verifying they were salt deprived during training. Rats in the NDSW group showed no preference for either solution. * = $p < 0.01$.

Compartment Preference Test

Figure 13 shows the compartment preference times for the two groups during the preference test trial. The rats that were salt deprived during both training and testing preferred their salt-paired compartments. The rats that were not salt deprived training trials but were salt deprived during testing (NDSW) showed no preferences for either compartment. A two-way mixed design ANOVA (Group x Compartment) revealed a significant main effect for Compartment [$F(1, 22) = 6.056, p < 0.05$] and a significant Group x Compartment interaction [$F(1, 22) = 4.136, p < 0.05$]. The main effect for Group was not significant. Planned comparisons on the significant interaction revealed that rats in the SWDep group spent significantly more time in their salt-paired versus water-paired compartments [$F(1, 22) = 8.862, p < 0.01$]. This is a demonstration of a salt-CCP.

In Experiments One and Two, rats tasted the salt during training and learned about its relationship to the external cues, but because they were not deprived of salt during training the ingestion of salt was without consequence. The learned information about the availability of salt interacted with the deprivation state during the preference test to produce behaviors that resulted in a preference for the salt-paired cues. This kind of learning is a latent cue preference for the salt-paired cues (salt-LCP). In contrast to this type of learning, the rats in Experiment Three learned about the availability of salt and because they were salt deprived the ingested salt was rewarding. The compartmental cues associated with the rewarding salt stimulus gained the ability to elicit conditioned approach behaviors. During testing, as rats explored the compartments of the CCP apparatus, the salt-paired cues produced these conditioned approach behaviors causing the rats to prefer the salt-paired compartment. This kind of learning is a conditioned cue preference for the salt-paired cues (salt-CCP).

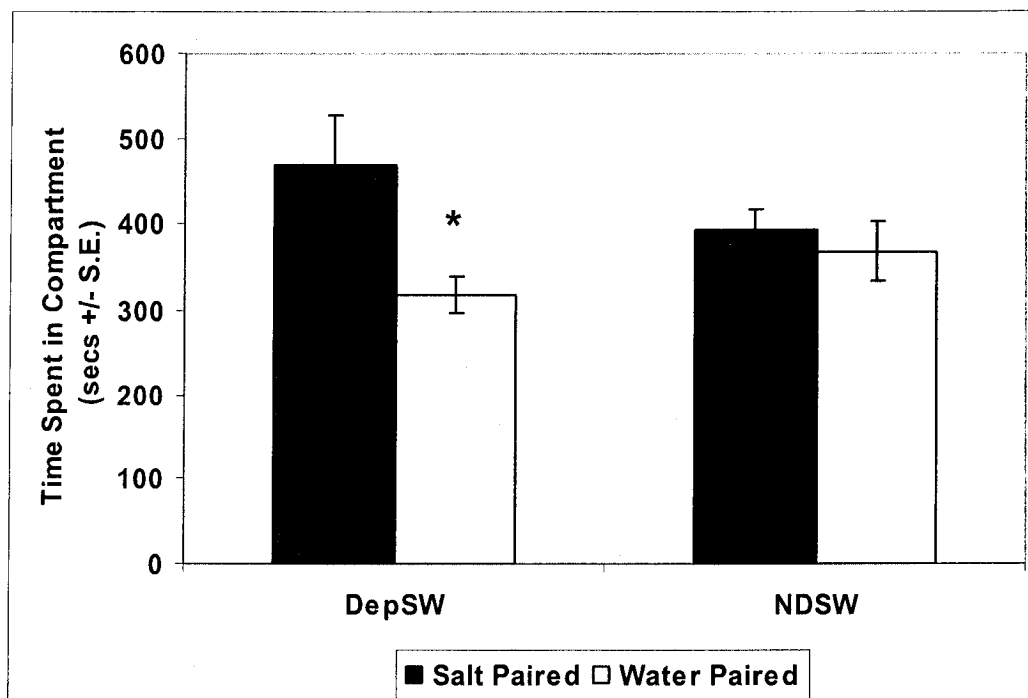


Figure 13. Experiment three (CCP) compartment preference results. Rats that were salt+water deprived during the two training trials and during testing (DepSW) spent significantly more time in their salt-paired than water-paired compartments, a demonstration of conditioning. Rats that were water-only deprived during the two training trials and then salt+water deprived during testing (NDSW) showed no compartment preferences, indicating that the salt solution did not produce a conditioned preference unless rats were salt deprived during training. * = $p < 0.05$.

Experiment Four: Role of Deprivation in Salt-CCP Expression

Experiments One and Two demonstrated that the preference for the salt-paired compartment in the LCP task (a salt-LCP) only occurred when rats were salt deprived during the preference test. If rats were not salt deprived during the preference test, they preferred their water-paired compartments (a water-CCP). It was hypothesized that the motivational information about salt deprivation during the preference test produced the salt-LCP. Therefore, the purpose of Experiment Four was to determine if this motivational information about salt deprivation during the preference test was required for the expression of a salt-CCP, or if the salt-CCP could be expressed in the absence of salt deprivation during the preference test.

Methods

Seventeen rats were divided into two groups: TestSaltDep rats ($n = 8$) were salt+water deprived during both training and testing (identical to the DepSW group in Experiment Three); TestNotDep rats ($n = 9$) were salt+water deprived during training, but then were water-only deprived during the preference test.

The CCP procedure described in the General Methods chapter was used, with one exception. Although both groups of rats in the experiment were given furosemide injections and the low salt diet prior to training trials, only rats in the TestSaltDep group were given a furosemide injection prior to the preference test. Rats in the TestNotDep group were maintained on the restricted-access water schedule, but were given ad lib access to the salt solution in their home cages following the last day of training until the preference test trial. This was done to ensure they were not salt deprived during the preference test.

Results and Discussion

Verification of Salt Deprivation during Training

The amount of the two solutions consumed during training trials are illustrated in Figure 14. A three-way mixed design ANOVA (Group x Trial x Solution) revealed a significant main effect for Solution [$F(1, 30) = 109.644, p < 0.001$] and a significant Solution x Trial interaction [$F(1, 30) = 4.744, p = 0.04$]. There were no other significant effects [Group, $F(1, 30) = 0.032, p = 0.86$; Trial,

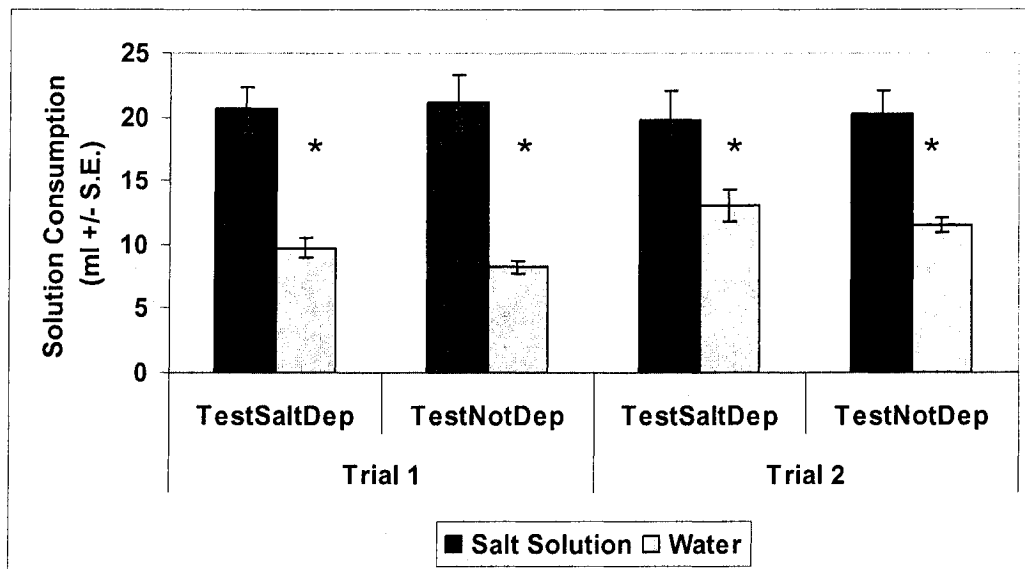


Figure 14. Experiment Four consumption of salt solution and water during training trials. Results demonstrated that rats in both groups consumed significantly more salt solution than water, verifying salt deprivation during training. * = $p < 0.05$.

$F(1, 30) = 1.217, p = 0.28$; Trial x Group, $F(1, 30) = 0.063, p = 0.80$; Solution x Group, $F(1, 30) = 2.717, p = 0.11$; Solution x Group x Trial, $F(1, 30) = 0.273, p = 0.61$]. The main effect for Solution indicates that rats in both groups consumed significantly more salt solution than water during training trials. A planned comparison revealed that rats in the TestSaltDep and TestNotDep groups consumed an equal amount of salt solution during training trials [$F(1, 30) = 0.518, p = 0.48$]. These results verify that both groups were salt deprived during training trials and there was no difference in the degree of salt deprivation between the two groups.

Compartment Preference Test

Figure 15 shows the compartment preference times for the two groups during the preference test trial. The rats that were salt+water deprived during training trials and the preference test (TestSaltDep) showed a preference for their salt-paired compartments. The rats that were salt+water deprived training trials but were then water-only deprived during the preference test (TestNotDep) also preferred their salt-paired compartments. A two-way mixed design ANOVA (Group x Compartment) revealed a significant main effect for Compartment [$F(1, 15) = 15.606, p < 0.01$]. No other effects were significant [Group, $F(1, 15) = 2.885, p = 0.11$; Group x Compartment, $F(1, 15) = 0.029, p = 0.87$] and a significant Group x Compartment interaction [$F(1, 22) = 4.136, p < 0.05$]. Planned comparisons revealed that rats in the TestSaltDep group spent significantly more time in their salt-paired versus water-paired compartments [$F(1, 15) = 9.023, p < 0.01$], and rats in the TestNotDep group spent significantly more time in their salt-paired versus water-paired compartments [$F(1, 15) = 6.746, p = 0.02$].

These results demonstrate that motivational information about salt deprivation during testing is not required for the expression of the salt-CCP. This contrasts with the results of the LCP task, in which motivation information about salt deprivation is required during testing for the expression of a salt-LCP. This finding demonstrates an important difference between LCP and CCP learning. While the expression of CCP learning involves conditioned approach responses

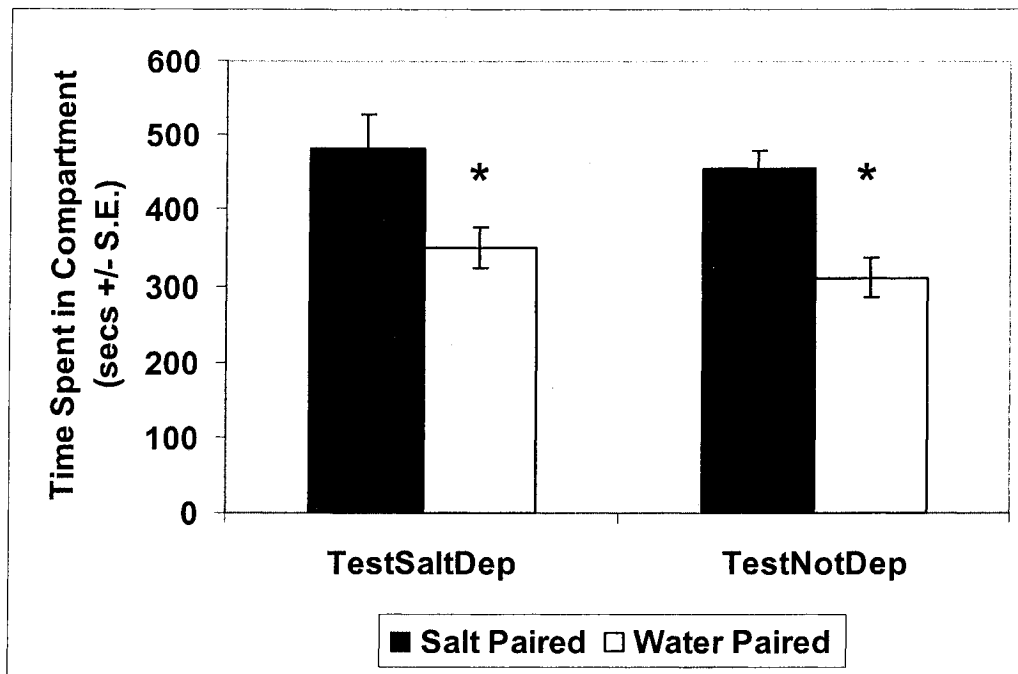


Figure 15. Experiment Four compartment preference times. Results demonstrated that rats in both groups spent significantly more time in their salt-paired compartments than water-paired compartments, indicating that salt deprivation during the preference test is not required for the expression of a salt-CCP. * $p < 0.05$.

that are elicited by previously rewarded stimuli, the expression of LCP learning requires the use of current internal cues about motivational state in order to initiate the recall of the neutral association between salt and the compartment cues paired with salt.

Experiment Five: Additional LCP Task Control Groups

Experiment Four demonstrated that the motivational information about salt deprivation during the preference test was not required for the expression of a salt-CCP. One purpose of Experiment Five was to determine if the water-CCP behavior in the LCP task showed the same pattern; i.e. is motivational information about water deprivation during the preference test necessary for the expression of a water-CCP?

Experiment Two demonstrated that rats could change their compartment preferences based on their motivational states. This flexibility of behavior does not necessarily imply it is based on latent learning. Previous experiments using T- or Y-mazes have demonstrated that changes in motivational state can induce behavioral changes during rewarded or reinforced learning as well (Hsiao and Isaacson, 1971; Hirsh et al., 1978; Hirsh et al., 1979). In these experiments, rats were trained to turn one direction in the maze to get food when food deprived and another direction to get water when water deprived. During testing rats made responses appropriate to their motivational state; if they were tested while food deprived they would turn in the direction of the food-paired maze cues, and if they were tested while water deprived they would turn in the direction of the water-paired maze cues. It could be argued that the preferences for the salt-paired and water-paired compartment cues in the LCP task are based on this type of behavior if the salt solution was rewarding to the rat.

Therefore, the second purpose of Experiment Five was to determine if the salt solution used in present experiments was rewarding to a water deprived rat, which would make the apparent latent learning, in fact, conditioning. However, if it was demonstrated that the salt solution did not have enough reward value to alter compartment preferences, then information about the salt solution could be

considered to be neutral and therefore any associations made involving that information could be considered latent learning.

Methods

Sixteen rats were divided into two groups: NoWDep/CCP rats ($n = 8$) were water-only deprived during training trials, but then were salt+water replete during the preference test; NoSDep/LCP rats ($n = 8$) were water-only deprived during training and testing.

Both groups were trained using the LCP task procedure described in the General Methods chapter, with two exceptions. One, NoWDep/CCP rats received the standard LCP training trial procedure, but following training were given ad lib access to water in their home cages until the preference test trial. This was done to insure that they were not water deprived during the preference test. Two, NoSDep/LCP rats had access to only the salt solution during training trials, and were water-only deprived during both training and testing. During training, NoSDep/LCP rats were given the salt solution in their salt-paired compartments, but were given nothing to drink in the normally water-paired compartment. On training days in which these rats received no fluids in the compartment, they were given 30 minutes access to water in their home cages a minimum of one hour following their training trial. NoSDep/LCP rats were then tested while water-only deprived.

Results and Discussion

Compartment Preference Test

Analyses on the compartment preference test data were conducted separately for the two groups because of the extreme differences in training procedures. Figure 16 illustrates the compartment preferences for both groups during the preference test trial. A paired samples t-test on the compartment times for the NoWDep/CCP group revealed a significant preference for the water-paired compartment [$t(7) = -6.482, p < 0.001$]. A paired samples t-test on the compartment times for the NoSDep/LCP group demonstrated no significant compartment preferences [$t(7) = -0.225, p = 0.829$].

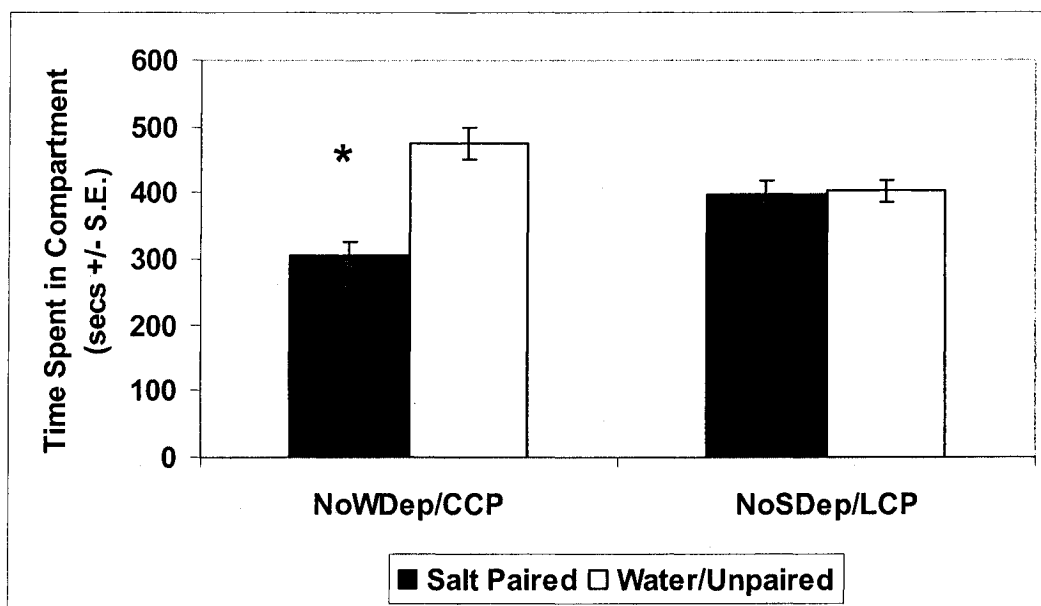


Figure 16. Experiment Five compartment preference times. Results showed that rats that were not water deprived during the preference test spent significantly more time in their water-paired compartments, demonstrating that water deprivation is not required for the expression of a water-CCP in the LCP task. In addition, rats that received only the salt solution during training while water-only deprived did not show a preference for the salt-paired compartment during the preference test, demonstrating that the salt solution is not rewarding to a water deprived rat. * $p < 0.05$.

The results of the NoWDep/CCP group demonstrate that water deprivation during the preference test trial is not required for the expression of a water-CCP. Therefore, the water-CCP in the LCP task and the salt-CCP in the CCP task are behaviorally identical in that a deprivation state is not required during testing for the CCP to be expressed. Again, this contrasts with salt-LCP learning, in which the state of salt deprivation is required during the preference test for the salt-LCP to be expressed. It is thought that the water- and salt-CCPs are based on conditioned responses to the water- and salt-paired compartment cues, which do not require deprivation information during testing to be expressed.

The results of the NoSDep/LCP group demonstrate that the consumption of the salt solution during training by a water deprived rat is not rewarding enough to produce conditioned responses to the salt-paired compartment cues. Therefore, consuming the salt solution does not alter compartment preference behavior unless the rat is salt deprived during the preference test. This result confirms that the salt solution is not producing conditioned responses to the salt-paired cues, and that the salt solution is not rewarding to a water-deprived rat. Therefore, information about the salt solution can be considered neutral, and any associations made with this neutral information can be considered latent learning.

Summary of Behavioral Results

The procedures and results of the unique behavioral groups described in this chapter are summarized in Table 1. Comparison of the groups reveals several important behavioral findings. One, when a rat is exposed to the salt solution and water during training trials while in a state of water-only deprivation it acquires two types of information: a salt-LCP and a water-CCP. If that rat is then salt+water deprived during the test trial (SWDep), it will express the salt-LCP in a demonstration of latent learning. If that rat is water-only deprived during the test trial (SWND), it will express the water-CCP in a demonstration of conditioning. Both types of learning occur in parallel within the same rat. Two, if a rat is given only two training trials in which it is exposed to the salt solution and water while in a state of water-only deprivation it does not acquire the salt-LCP. When that rat is subsequently salt+water deprived during the test trial (NDSW), it will show

Behavioral Group	Solutions given during Training	Training State	Testing State	Compartment Preference	Effect
SWDep	Salt and Water	Water deprived, Salt replete	Salt+water deprived	Salt-paired	Salt-LCP (latent learning)
SWND	Salt and Water	Water deprived, Salt replete	Water deprived, Salt replete	Water-paired	Water-CCP; (conditioning)
NDSW	Salt and Water	Water deprived, Salt replete	Salt+water deprived	None	Salt-LCP requires at least 3 training trials
DepSW	Salt and Water	Salt+water deprived	Salt+water deprived	Salt-paired	Salt-CCP; (conditioning)
TestNotDep	Salt and Water	Salt+water deprived	Water deprived, Salt replete	Salt-paired	Salt-CCP does not require deprivation to be expressed
NoWDep/CCP	Water	Water deprived, Salt replete	Salt+water replete	Water-paired	Water-CCP does not require deprivation to be expressed
NoSDep/LCP	Salt	Water deprived, Salt replete	Salt+water replete	None	Salt-LCP requires deprivation to be expressed; salt solution not rewarding

Table 1. Summary of procedures and findings of behavioral groups described in Chapter 4.

no preferences for either compartment, indicating that two training trials are not enough to result in latent learning.

Three, if a rat is exposed to the salt solution and water during training trials while in a state of salt+water deprivation it acquires a salt-CCP. This salt-CCP is expressed if the rat is salt+water deprived during the test trial (DepSW) or if the rat is water-only deprived (salt replete) during the test trial (TestNotDep). This indicates that the expression of the salt-CCP does not require a salt deprivation state during testing in order to be expressed. Similarly, the water-CCP also does not require water deprivation during testing in order to be expressed (NoWDep/CCP). Conversely, the salt-LCP does require a state of salt deprivation during testing in order to be expressed (NoSDep/LCP). This highlights an important difference between LCP and CCP learning: LCP learning is expressed only during a state of deprivation that is relevant to the incentive they were exposed to during training when they were not deprived of the incentive; CCP learning, however, is expressed whether or not the rat is deprived of the incentive they were exposed to during the training trials when they were deprived of the incentive.

Four, the NoSDep/LCP group also highlights the important finding that the consumption of the salt solution by a water deprived rat does not result in a preference for the salt-paired compartment in the absence of salt deprivation. This indicates that the salt solution is not rewarding to the water deprived rat and does not result in conditioned responses to the salt-paired cues. This finding verifies that the salt solution is in fact a neutral stimulus to a water deprived rat.

CHAPTER FIVE: NEURAL DISSOCIATION BETWEEN LCP AND CCP LEARNING

The previous experiments demonstrated a behavioral dissociation between LCP and CCP learning: CCP learning involves rewarded learning whose expression is not dependent on a deprivation state for expression, while LCP learning involves unrewarded learning that is dependent on a deprivation state for expression. The purpose of Experiment Six was to examine and compare the neural bases of LCP and CCP learning. The brain structures examined (dorsal and ventral hippocampus, fimbria-fornix, amygdala and entorhinal cortex) are known to be involved in spatial learning (e.g. Aggleton et al., 2000; Becker et al., 1980; Fyhn et al., 2004; Steffenach et al., 2005), spatial and contextual latent learning (e.g. Barrientos et al., 2002; Matus-Amat et al., 2004; Rudy and Matus-Amat, 2005; Gaskin and White, 2005), and Pavlovian conditioning in the CCP (Cador et al., 1989; White and McDonald, 1993; Everitt et al., 2003) and other paradigms (e.g. LeDoux et al., 1990; Maren, 1999; Sananes and Davis, 1992).

Once the neural structures necessary for LCP learning were determined, I wished to determine if these structures were necessary during the acquisition, expression, or both phases of LCP learning. These results are described in Experiment Seven. These results of these two experiments provide additional information concerning the MMS model, and allow that model to be updated to include the neural basis of latent learning.

Experiment Six: Effects of Pre-Training Lesions on the LCP and CCP Tasks

Experiment Six examined the effects of pre-training lesions of the fimbria-fornix, lateral region of the amygdala, dorsal hippocampus, ventral hippocampus, and entorhinal cortex on the salt-LCP and water-CCP learning that takes place in the LCP task, and on salt-CCP learning in the CCP task.

Methods

One hundred ninety-four rats were divided into six lesion groups: fimbria-fornix (FF; $n = 27$), lateral region of the amygdala including parts of the dorsolateral and basolateral nuclei (LA; $n = 28$), dorsal hippocampus (DH; $n = 32$), ventral hippocampus (VH; $n = 30$), entorhinal cortex (EC; $n = 30$), and sham

(SH; $n = 47$).

Prior to training on the LCP or CCP tasks, rats in each lesion group underwent lesion surgery. All rats were anesthetized using isoflurane (5% induction, 2.5% maintenance) and were given an intramuscular injection of Dipyrone (analgesic, 0.5 mg) approximately 30 minutes prior to recovery from anesthesia. Stereotaxic coordinates were based on the atlas of Paxinos and Watson (1998; 2005). Coordinates for each lesion type are shown in Table 2.

FF lesions were made in 27 rats using radiofrequency current (10 mA, 20 seconds) passed through a nichrome electrode with a 1.0 mm exposed tip. The ground was attached to the ear-bar. The electrode was left in place for an additional 60 seconds. The coordinates used for the FF lesion were chosen to avoid damaging the subfornical organ, which may be necessary for the expression of acute salt appetite in rats (Thunhorst et al., 1999; Starbuck et al., 1997). For the FF lesions the stereotaxic arm and electrode were angled at 10° toward the midline on both sides, so that both lesions were made at the same medial site.

LA lesions were made in 28 rats using electrolytic current (1.5 mA, 20 seconds) passed through a nichrome electrode with a 0.5 mm exposed tip and an ear-bar ground. The electrode was left in place for an additional 60 seconds. DH, VH, and EC lesions were made in 32, 30, and 30 rats, respectively, using N-methyl-d-aspartate (NMDA; 10 mg/ml, dissolved in normal saline) infused at 0.1 μ L/min with a pump (Cole Parmer 74900 series) and 10 μ L Hamilton 80000 syringes. For each site the infusion was started with the cannula tip 0.2 mm below the D/V coordinate; it was raised dorsally 0.4 mm halfway through the injection. The injection cannula was left in place for 2 minutes post-injection.

SH ($n = 47$) animals were anesthetized and placed into the stereotaxic instrument, their skulls were exposed and several holes were drilled. However, no electrode or injection cannula was lowered into the brain.

All rats were allowed to recover from surgery for 7 days and were then placed on the water deprivation schedule. Rats were trained using either the LCP procedure or the CCP procedure described in the General Methods chapter. The rats in each lesion group were run separately, together with a group of SH rats in

Lesion Type	A/P	M/L	D/V	Volume (μ L)
Fimbria-fornix	-0.25	\pm -1.0	-5.6	N/A
Lateral Amygdala	-2.5	\pm -5.2	-8.4	N/A
	-3.5	\pm -5.4	-8.2	N/A
Dorsal Hippocampus	-2.6	\pm -1.4	-3.6	0.200
	-3.2	\pm -1.4	-3.2	0.150
	-3.4	\pm -3.2	-3.6	0.200
	-4.4	\pm -3.4	-4.0	0.150
	-4.4	\pm -3.4	-3.0	0.150
Ventral Hippocampus	-4.4	\pm -4.5	-7.5	0.075
	-5.2	\pm -5.3	-7.5	0.200
	-5.2	\pm -5.3	-5.5	0.150
	-5.8	\pm -5.0	-7.5	0.200
	-5.8	\pm -5.0	-6.0	0.150
Entorhinal Cortex	-6.5	\pm -5.0	-8.5	0.200
	-7.5	\pm -5.0	-8.0	0.100
	-7.5	\pm -5.0	-7.0	0.100
	-8.3	\pm -5.0	-7.0	0.200

Table 2. Coordinates for the lesion sites for each of the lesion groups, relative to bregma. Also shown are the volumes of NMDA neurotoxin (10 mg/ml) injected for lesions of the DH, VH, and EC.

each case.

Following testing, rats were anesthetized using a lethal dose of salt pentobarbital (100 mg/kg; i.p.) and were perfused intracardially with 0.9% saline followed by 10% formol-saline. The brains were removed from the skull and were stored in 10% formol-saline. Frozen coronal sections 30 μ m thick were cut through the lesion area and every fifth section was mounted on glass microscope slides. The sections were stained using thionin and were examined with a microscope to determine the extent of the lesion.

Results and Discussion

Histology

The minimum and maximum lesion sizes for each of the lesion types are illustrated in Figure 17. In the FF lesion group (23 rats retained after histological examination), damage was generally restricted to the fimbria-fornix, triangular septal and lateral septal regions, but the lesion spread to the anterior subfornical organ in some rats. This did not affect their salt solution consumption. In the LA lesion group (23 rats retained), damaged areas extended beyond the lateral nucleus itself to include the lateral dorsolateral and basolateral amygdala nuclei. In addition, the ventral endopiriform nucleus and capsular part of the central amygdala nucleus were damaged in some rats. In the DH lesion group (30 rats retained), damage was generally restricted to the dorsal CA1, CA2, CA3 and dentate gyrus regions, although some cortical damage was created directly dorsal of the hippocampus in two rats. In the VH lesion group (24 rats retained), damage was restricted to the CA2 and CA3 regions in ventral hippocampus and to the molecular and polymorph layers of the dentate gyrus, the oriens and radiatum layers of hippocampus, and the pyramidal cell layer of hippocampus. In the EC lesion group (25 rats retained), damage was generally restricted to the caudomedial, medial, dorsal intermediate and dorsolateral entorhinal cortex, but the ventral subiculum was also damaged in some rats.

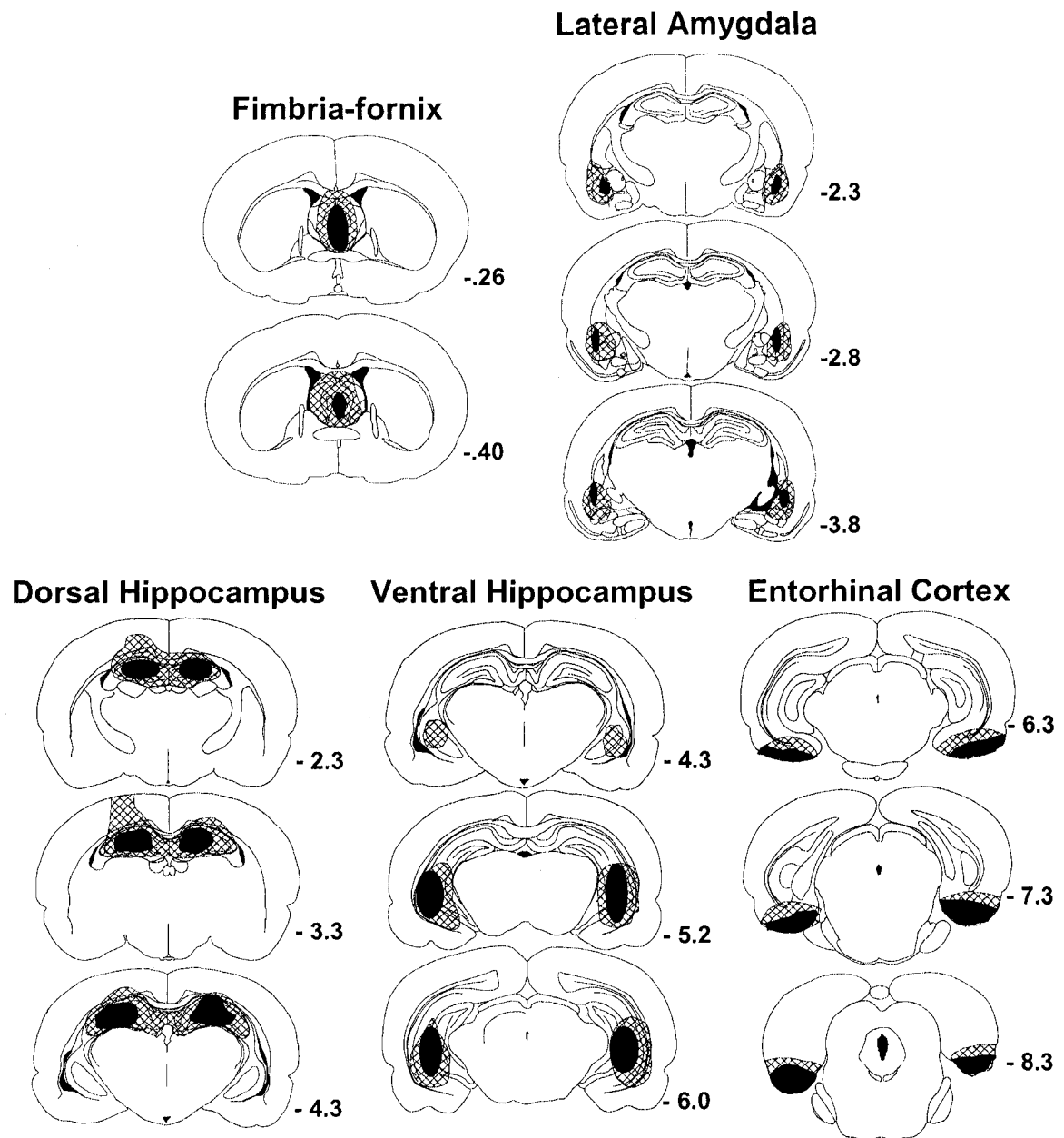


Figure 17. The maximum (crosshatching) and minimum (black) extent of lesion damage for each of the lesion groups used in the LCP and CCP tasks.

Behavioral Results: LCP Task

Analyses of training trial solution consumption indicated there were no differences in the mean amount of salt solution and water consumed by each lesion group [SH: 18.5 ml salt, 19.1 ml water; FF: 20.3 ml salt, 19.7 ml water; LA: 22.6 ml salt, 21.3 ml water; DH: 20.8 ml salt, 19.8 ml water; VH: 19.2 ml salt, 19.4 ml water; EC: 18.6 ml salt, 19.4 ml water]. The post-test salt solution consumption for each lesion group is illustrated in Figure 18. To verify the rats' salt deprivation state during the test trial, a two-way (Lesion x Deprivation State) ANOVA on the post-LCP consumption of salt solution was computed. There was a significant main effect for Lesion type [$F(5, 93) = 2.551, p = 0.03$] and a significant main effect for Deprivation State [$F(1, 93) = 73.067, p < 0.001$]. The Lesion x Deprivation State interaction effect was not significant [$F(5, 93) = 0.930, p = 0.47$]. The main effect for Deprivation State demonstrates that rats given furosemide consumed significantly more salt solution than rats given saline.

Planned comparisons revealed that the main effect of Lesion type was due to the fact that rats with LA lesions consumed more of the salt solution than did rats with all other lesion types [$F(1, 93) = 8.294, p < 0.01$]. A total of 9 rats were eliminated for not meeting the salt-deprivation criterion described in the General Methods chapter. After eliminations using this criterion and following histological examination, the analyses of the compartment preference times were based on the following Ns: FF (15), LA (14), DH (20), VH (15), EC (16), and SH (24).

Figure 19 shows the compartment preference times for the rats in the salt-LCP condition (salt+water deprived during testing) and the water-CCP condition (water-only deprived during testing) of the LCP task. As previously demonstrated with normal rats, SH rats in the salt-LCP condition spent more time in their salt-paired compartments and the SH rats in the water-CCP condition preferred their water-paired compartments. The lesions had different patterns of effects on the two behaviors. A three-way (Lesion x Deprivation State x Compartment) ANOVA revealed significant interactions for Compartment x Lesion [$F(5, 93) = 3.363, p < 0.01$], Compartment x Deprivation State [$F(1, 93)$

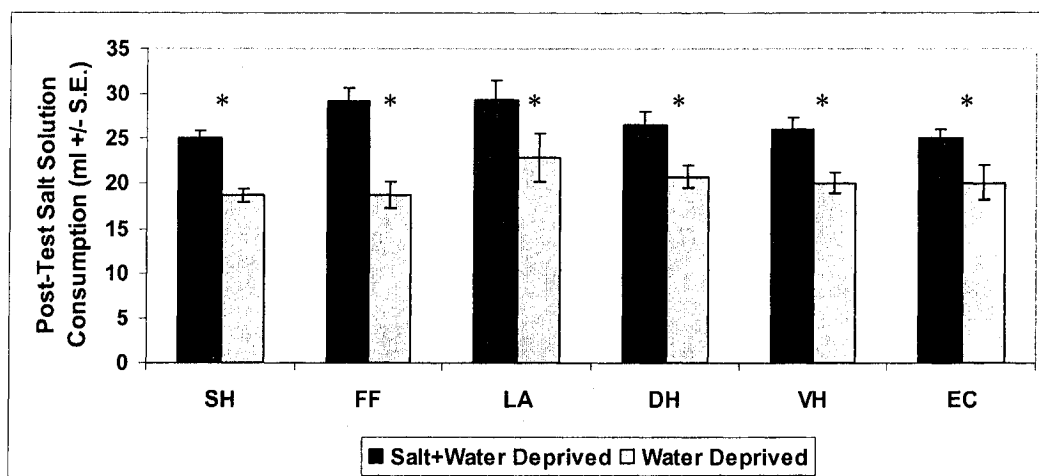


Figure 18. Experiment Six post-test salt solution consumption for rats in the LCP task. Rats classified as salt deprived using the criteria outlined in the General Methods chapter consumed significantly more salt solution than rats classified as water-only deprived, verifying salt deprivation. * = $p < 0.05$.

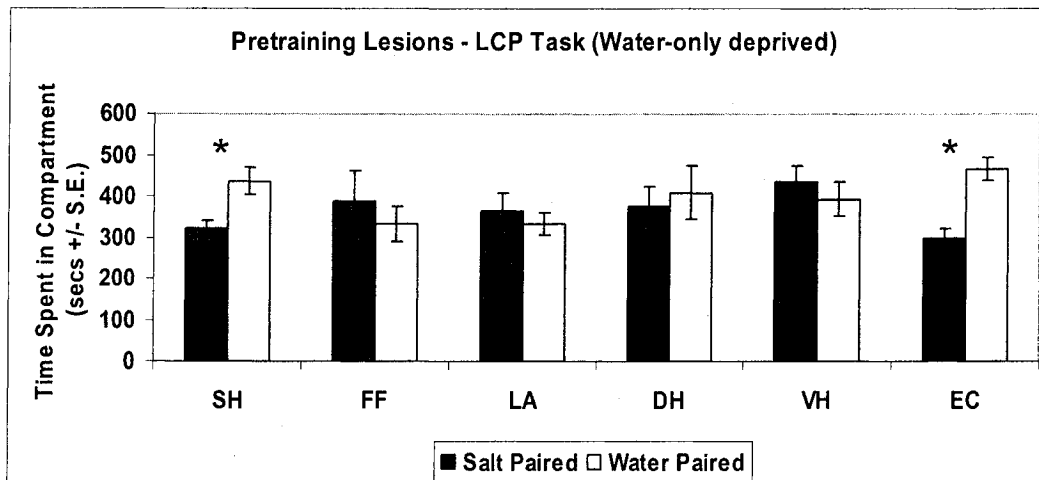
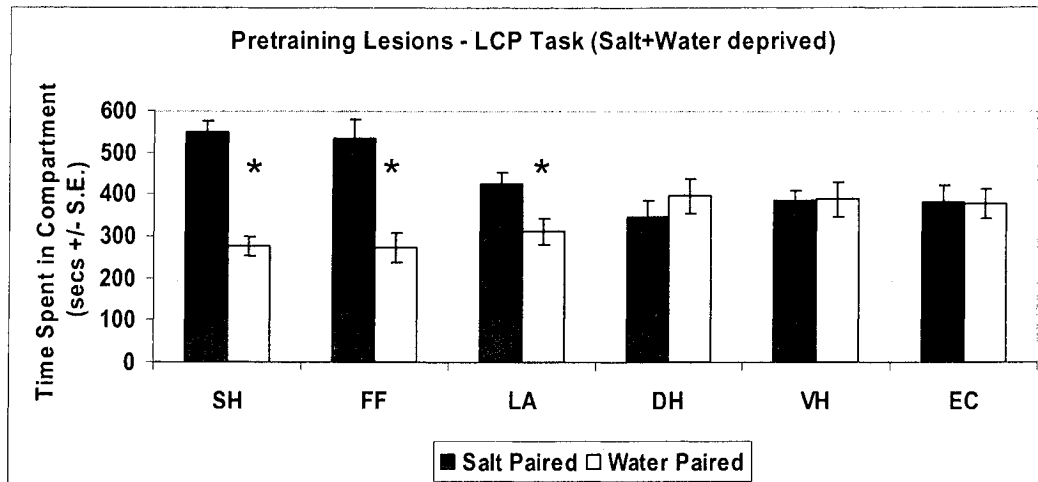


Figure 19. Experiment Six compartment preference times for rats that were salt+water deprived during the preference test (top) and for rats that were water-only deprived during the preference test (bottom). Lesions of the dorsal hippocampus (DH), ventral hippocampus (VH), and entorhinal cortex (EC) disrupted the preference for the salt-paired chamber, while lesions of the fimbria-fornix (FF), lateral amygdala (LA), DH, and VH disrupted the preference for the water-paired chamber. * = $p < 0.05$.

= 11.436, $p < 0.001$], and Compartment x Lesion x Deprivation State [$F(5, 93) = 3.150$, $p = 0.01$]. No other effects were significant [Lesion, $F(5, 93) = 1.257$, $p = 0.29$; Dep State, $F(1, 93) = 0.58$, $p = 0.450$; Lesion x Dep State, $F(5, 93) = 1.730$, $p = 0.14$; Compartment, $F(1, 93) = 3.259$, $p = 0.07$].

Planned comparisons revealed that in the salt-LCP condition, rats with SH, FF, and LA lesions spent significantly more time in their salt-paired compartments than in their water-paired compartments [SH: $F(1, 93) = 30.74$, $p < 0.001$; FF: $F(1, 93) = 11.68$, $p < 0.001$; LA: $F(1, 93) = 6.60$, $p = 0.01$]. Rats with DH, VH and EC lesions did not show this preference [DH: $F(1, 93) = 0.61$, $p = 0.44$; VH: $F(1, 93) = 0.003$, $p = 0.96$; EC: $F(1, 93) = 0.02$, $p = 0.89$]. Rats in the water-CCP condition with SH and EC lesions spent significantly more time in their water-paired than in their salt-paired compartments [SH: $F(1, 93) = 4.50$, $p < 0.05$; EC: $F(1, 93) = 5.09$, $p < 0.05$]. Rats with FF, LA, DH and VH lesions did not show this preference [FF: $F(1, 93) = 0.62$, $p = 0.43$; LA: $F(1, 93) = 0.17$, $p = 0.68$; DH: $F(1, 93) = 0.28$, $p = 0.60$; VH: $F(1, 93) = 0.32$, $p = 0.57$]. This pattern of results demonstrates that salt-LCP learning is dependent on the EC, DH, and VH, but not the FF or LA, while water-CCP learning is dependent on the FF, LA, DH, and VH, but not the EC. Therefore, within the LCP task, two types of learning take place, and each of these types of learning are mediated by dissociable neural circuits.

Behavioral Results: CCP Task

The consumption of the salt solution and water during training trials is illustrated in Figure 20. In order to verify that rats in the CCP task were salt deprived during training, their consumption of the salt solution and water during the training trials was examined with a three-way (Lesion x Trial x Solution) ANOVA. There was a significant main effect for Solution [$F(1, 61) = 274.238$, $p < 0.001$], revealing that the rats consumed more of the salt solution than water during training, indicating that they were salt deprived during the training trials. Two rats in the VH group had to be eliminated from the experiment because of a lack of salt deprivation during the training trials. After elimination following histological examination, analyses of the compartment preference times were

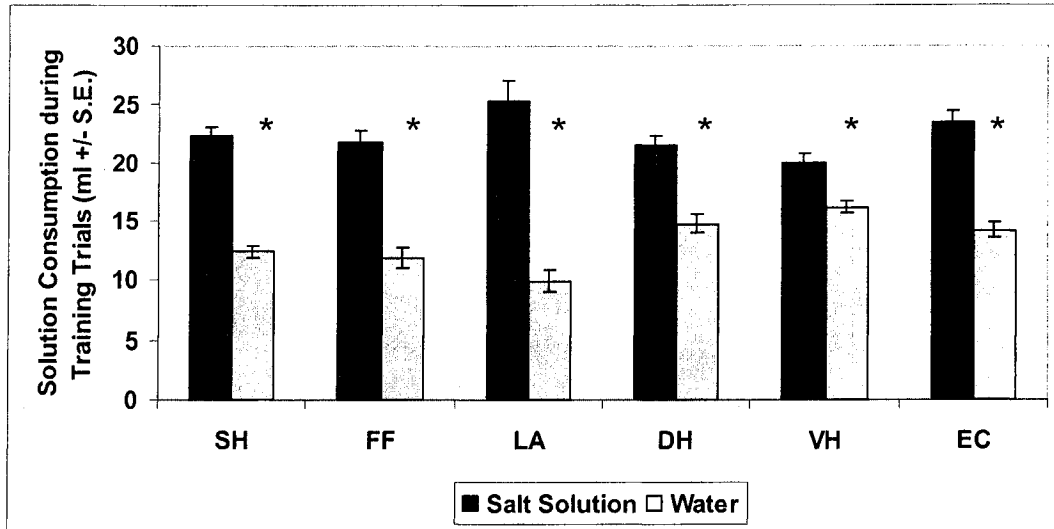


Figure 20. Experiment Six solution consumption during training trials by rats in the CCP task. Rats in each lesion group consumed more salt solution than water, verifying salt deprivation during the training trials. * = $p < 0.05$.

based on the following Ns: FF (7), LA (9), DH (10), VH (8), EC (9), and SH (22).

Figure 21 shows the compartment preferences times for the CCP task (salt+water deprived during training and testing). As previously demonstrating using normal rats, the SH rats spent more time in their salt-paired compartments than in their water-paired compartments. A two-way (Lesion x Compartment) ANOVA revealed a significant Lesion x Compartment interaction [$F(5, 61) = 2.465, p = 0.04$] and a significant main effect for Compartment [$F(1, 61) = 7.075, p = 0.01$] but not for Lesion type [$F(5, 61) = 1.618, p = 0.169$]. Planned comparisons revealed that rats in the SH and EC lesion groups spent significantly more time in their salt-paired compartments than their water-paired compartments [SH: $F(1, 61) = 12.17, p < 0.001$; EC: $F(1, 61) = 11.87, p = 0.001$]. Rats with FF, LA, DH, and VH lesions did not show this preference [FF: $F(1, 61) = 0.01, p = 0.92$; LA: $F(1, 61) = 0.07, p = 0.79$; DH: $F(1, 61) = 0.15, p = 0.70$; VH: $F(1, 61) = 0.05, p = 0.82$].

These results are identical to the pattern of lesion effects seen in the water-CCP groups in the LCP task. This provides further evidence that the preference for the salt-paired compartment in the salt-CCP condition and the preference for the water-paired compartment in the water-CCP condition of the LCP task are both conditioned preferences resulting from the consumption of rewarding substances coinciding with the rats' deprivation state during the training trials. This pattern of results is dissociable from the pattern for salt-LCP learning, demonstrating that LCP and CCP learning involve different neural circuits.

Summary of Pre-training Lesion Findings

The results of pre-training lesions on LCP and CCP learning are summarized in Table 3. Pre-training lesions of the DH, VH, or EC disrupted LCP learning, while lesions of the FF or LA did not. Conversely, pre-training lesions of the FF, LA, DH, or VH disrupted both water- and salt-CCP learning, while lesions of the EC did not. These findings indicate dissociable neural pathways responsible for LCP and CCP learning. Involvement of the EC, DH, and VH in LCP learning indicates cortical-to-hippocampal processing, while the involvement of the FF, LA, DH, and VH in CCP learning indicates subcortical-to-hippocampal

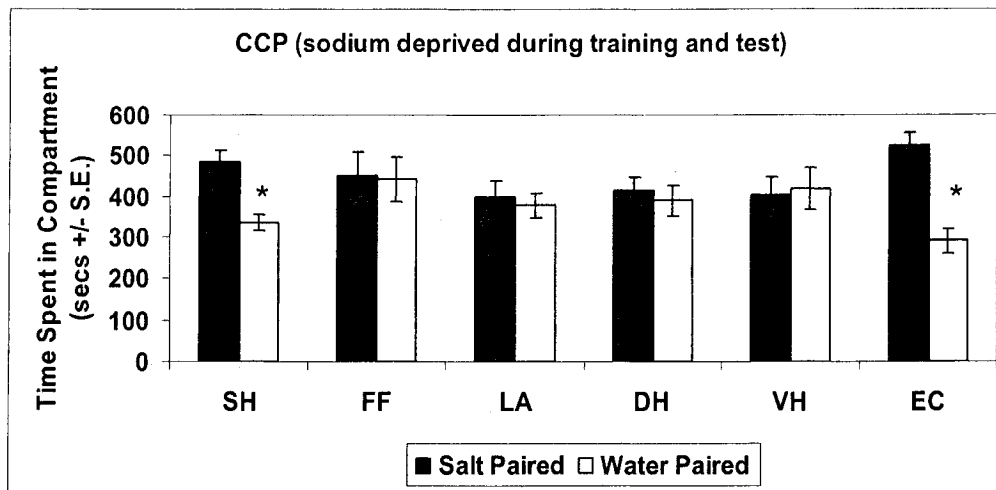


Figure 21. Experiment Six compartment preference times for rats in the CCP task (salt+water deprived during both training and testing). Lesions of the FF, LA, DH, and VH disrupted the preference for the salt-paired compartment, a pattern of effects identical to the pattern of lesions that disrupted the water-CCP in the LCP task. * = $p < 0.05$.

Region	Latent Learning of Salt-Cues Relationship	Conditioning to Water- or Salt-Paired Cues
Fimbria-Fornix	Normal	Impaired
Lateral Amygdala	Normal	Impaired
Dorsal Hippocampus	Impaired	Impaired
Ventral Hippocampus	Impaired	Impaired
Entorhinal Cortex	Impaired	Normal

Table 3. Summary of pre-training lesion effects on latent learning of the salt-compartment cues relationship (salt-LCP) and conditioning to either water- or salt-paired compartment cues (water- and salt-CCP). Latent learning involved the cortical-to-hippocampus processing via the entorhinal cortex, while conditioning involved subcortical-to-hippocampus processing via the fimbria-fornix and the amygdala.

processing (Amaral and Witter, 1989).

Experiment Seven: Temporary Inactivation of EC, DH, and VH in LCP Task

Methods

One hundred sixty rats were divided into three cannula placement: dorsal hippocampus (DH; $n = 51$), ventral hippocampus (VH; $n = 57$), and entorhinal cortex (EC; $n = 52$). Control rats (CNTR) were taken from each of the three cannula placement groups (11 DH rats, 13 VH rats, and 11 EC rats).

Prior to training rats were placed into one of the three cannula placement surgery groups (DH, VH, or EC). All rats were anesthetized with isoflurane (5% induction, 2.5% maintenance), and stainless steel guide cannula were implanted using stereotaxic coordinates derived from the brain atlas of Paxinos and Watson (2005). DH cannula placements were at the following coordinates: -3.8 mm A/P, ± 2.5 mm M/L, and -2.7 mm D/V. VH cannula placements were at the following coordinates: -5.3, ± 5.0 , and -5.5. EC cannula placements were at the following coordinates: -7.3, ± 5.0 , and -7.0. All rats were given an intramuscular injection of Dipyrone (analgesic, 0.5 mg) approximately 30 minutes prior to recovery from anesthesia.

All rats were allowed to recover from surgery for 7 days and were then placed on the water deprivation schedule. All rats were trained using the LCP procedure. The rats in each cannula placement group were run separately, together with a group of CNTR rats in each case.

Inactivation during Training Trials

Groups of rats with each cannula placement were given infusions of muscimol hydrobromide (0.5 μ L of a 1 mg/ml solution in physiological saline, bilaterally infused over 60 sec) 30 minutes prior to being placed into their salt-paired compartment. These rats were given infusions of physiological saline (identical injection volume and parameters) 30 minutes prior to being placed into their water-paired compartments. This procedure was intended to impair acquisition of the association between the salt and the cues in the salt-paired compartment but have no effect on the acquisition of the association between the water and the cues in the other compartment. Control rats were infused with

physiological saline prior to being placed in both compartments during training.

Inactivation during Preference Test

Other groups with each cannula placement underwent the LCP training procedure as described above, with no infusions. They were given infusions of muscimol identical to the pre-training infusions 30 minutes prior to the preference test. Control rats were infused with physiological saline 30 minutes prior to the preference test.

Histology

Following testing, the rats were given a lethal dose of salt pentobarbital (100 mg/kg; i.p.) and perfused intracardially with 0.9% saline followed by 10% formol-saline. Their brains were removed from the skull and were stored in 10% formol-saline. Frozen coronal sections 30 μ m thick were cut through the cannula placement areas and every fifth section was mounted on glass microscope slides. The sections were then stained with thionin and examined with a microscope to determine the placements of the cannulae.

Results and Discussion

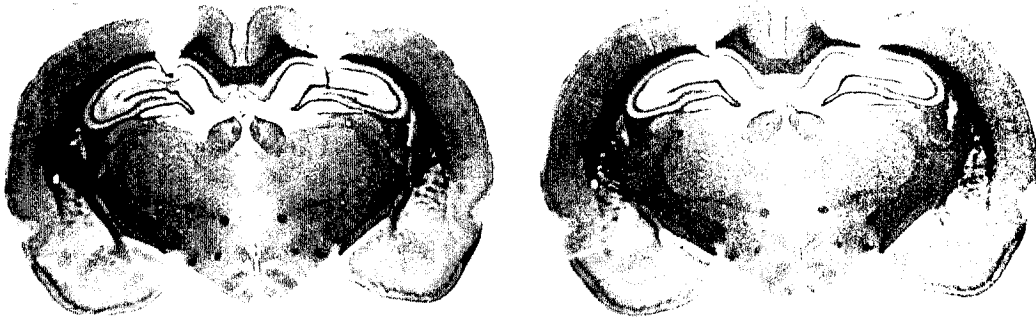
Histology

Following histological examination several rats were eliminated from each group due to inaccurate placement of one or both of the cannulae. Three rats were eliminated from the DH group, 2 from the VH group, and 2 from the EC group. In addition, 2 rats died following surgery in the DH group. Images of typical cannula placements for each group are shown in Figure 22.

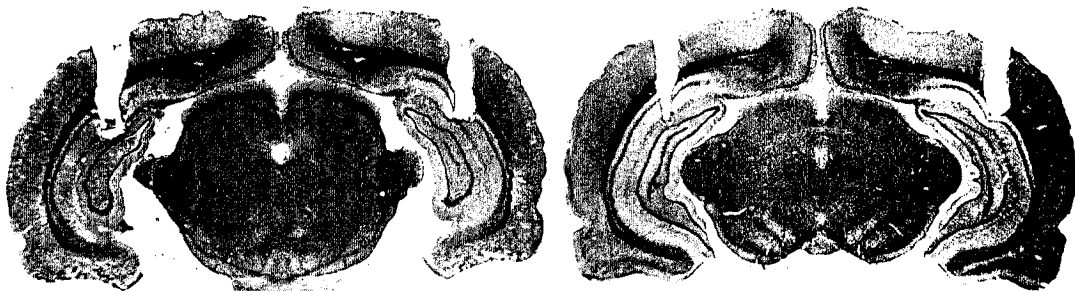
Salt Consumption

Consumption of the salt solution during the training trials and the post-test presentation of the salt solution in the rats' home cages were examined to determine the influence of salt-deprivation on the rats' preference test behavior. The amounts consumed during the training trials and the post-test presentations are shown in Figure 23.

Dorsal Hippocampus



Ventral Hippocampus



Entorhinal Cortex



Figure 22. Representative photographic images of the placements of the outer guide cannula for the dorsal hippocampus, ventral hippocampus, and entorhinal cortex groups. Injection cannulas extended below the tip of the guide cannulas by 0.5 mm.

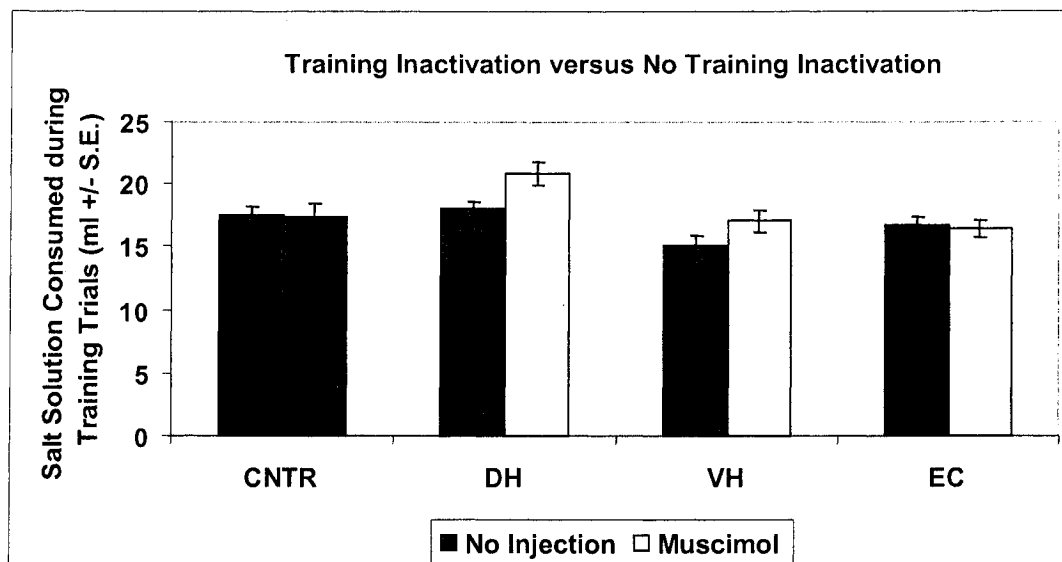
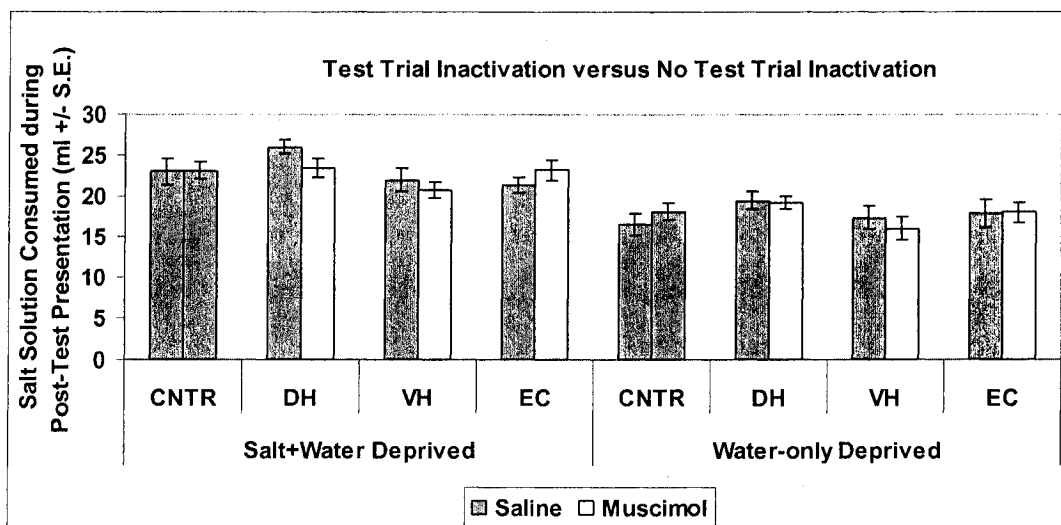
A**B**

Figure 23. Experiment 7 salt solution consumption. **A.** Salt solution consumption during training trials, by rats that received muscimol inactivation compared to rats that received no injections. **B.** Salt solution consumption during the post-test period, by rats that received muscimol inactivation compared to rats that received a saline injection.

Figure 23A compares the amounts of salt solution consumed during the training trials by rats in the training trial inactivation groups, and by rats in the test trial inactivation groups that received no muscimol injections prior to the training trials. A two-way (Group x Training Treatment) independent groups ANOVA revealed significant main effects for Group [$F(3, 136) = 9.067, p < 0.001$] and Training Treatment [$F(1, 136) = 5.468, p = 0.02$], and a significant Group x Training Treatment interaction [$F(1, 136) = 2.667, p = 0.05$].

Tukey's HSD post-hoc comparisons on the Lesion Group x Training Treatment interaction revealed that DH rats that received muscimol inactivation during training trials consumed more salt solution during training than all other groups of rats, p 's < 0.05 . There were no other significant differences in salt solution consumption during training as the result of muscimol infusion during training. This result was unexpected because pre-training lesions of the DH did not produce any change in salt solution consumption during training (Experiment 6). The DH rats that showed this increase were not salt deprived during the training trials, therefore any changes in salt consumption would have been due to changes in salt preference (need-free salt intake based on the taste properties of salt solutions) rather than changes in salt appetite (need-induced intake due to an increase in circulating aldosterone and angiotensin). There is little evidence linking salt preference and hippocampal function. Although Krecek et al. (1972) showed a positive relationship in male rats between salt preference and hippocampal RNA expression, Kim (1960) corroborated the earlier finding that DH lesions had no effect on salt preference (Experiment Six).

Figure 23B compares the amount of salt solution consumed during the post-test presentation by rats in the test trial inactivation groups that received either muscimol inactivation or a saline injection prior to the preference test trial. A three-way (Deprivation State x Group x Testing Treatment) revealed significant main effects for Deprivation State [$F(1, 128) = 69.600, p < 0.001$] and for Group [$F(3, 128) = 4.249, p < 0.01$]. There were no other significant effects [Testing Treatment, $F(1, 128) = 0.152, p = 0.697$; Deprivation State x Group, $F(3, 128) = 0.259, p = 0.855$; Deprivation State x Testing Treatment, $F(1, 128) = 0.136, p =$

0.713; Group x Testing Treatment, $F(3, 128) = 1.183, p = 0.319$; Deprivation State x Group x Testing Treatment, $F(3, 128) = 0.553, p = 0.647$].

The significant effect for Deprivation State demonstrates that rats that received furosemide prior to the preference test consumed significantly more salt solution in the post-test presentation than rats that received the PBS injection. Tukey's HSD post-hoc comparisons conducted on the significant effect for Group revealed that DH rats (mean = 22.17 ml) consumed more salt solution during the post-test presentation than VH rats (19.13 ml), regardless of Deprivation State or Testing Treatment, $p < 0.05$. However, neither DH nor VH rats differed from CNTR rats (20.31 ml), p 's > 0.05 , indicating that these differences in salt solution consumption are negligible. There were no other significant differences in salt solution consumption during the post-test presentation.

Preference Test – Inactivation during Training

Figure 24 shows the mean preference times for the rats that received pre-training injections of muscimol in the salt-LCP condition (water+salt deprived during testing) and the water-CCP condition (water-only deprived during testing). Four rats were eliminated for failing to meeting the salt deprivation criterion described in the procedure section. After eliminations using this criterion and due to misplaced cannulas already mentioned, analyses of the compartment preference times for the DH (salt-LCP $n = 8$; water-CCP $n = 8$), VH (salt-LCP $n = 10$; water-CCP $n = 10$), EC (salt-LCP $n = 9$; water-CCP $n = 9$), and CNTR (salt-LCP $n = 9$; water-CCP $n = 8$) groups were conducted. The CNTR group consisted of 5 rats with DH cannulas (salt-LCP $n = 3$; water-CCP $n = 2$), 6 rats with VH cannulas (salt-LCP $n = 3$; water-CCP $n = 3$), and 6 rats with EC cannulas (salt-LCP $n = 3$; water-CCP $n = 3$).

As previously demonstrated for normal rats, the CNTR rats trained in the salt-LCP condition preferred their salt-paired compartments. Inactivation of the DH or EC disrupted this preference. In the water-CCP condition, all groups showed a preference for the water-paired compartment. A three-way (Group x Deprivation State x Compartment) ANOVA revealed a significant three-way interaction among these factors [$F(3, 63) = 2.89, p < 0.05$] and for the

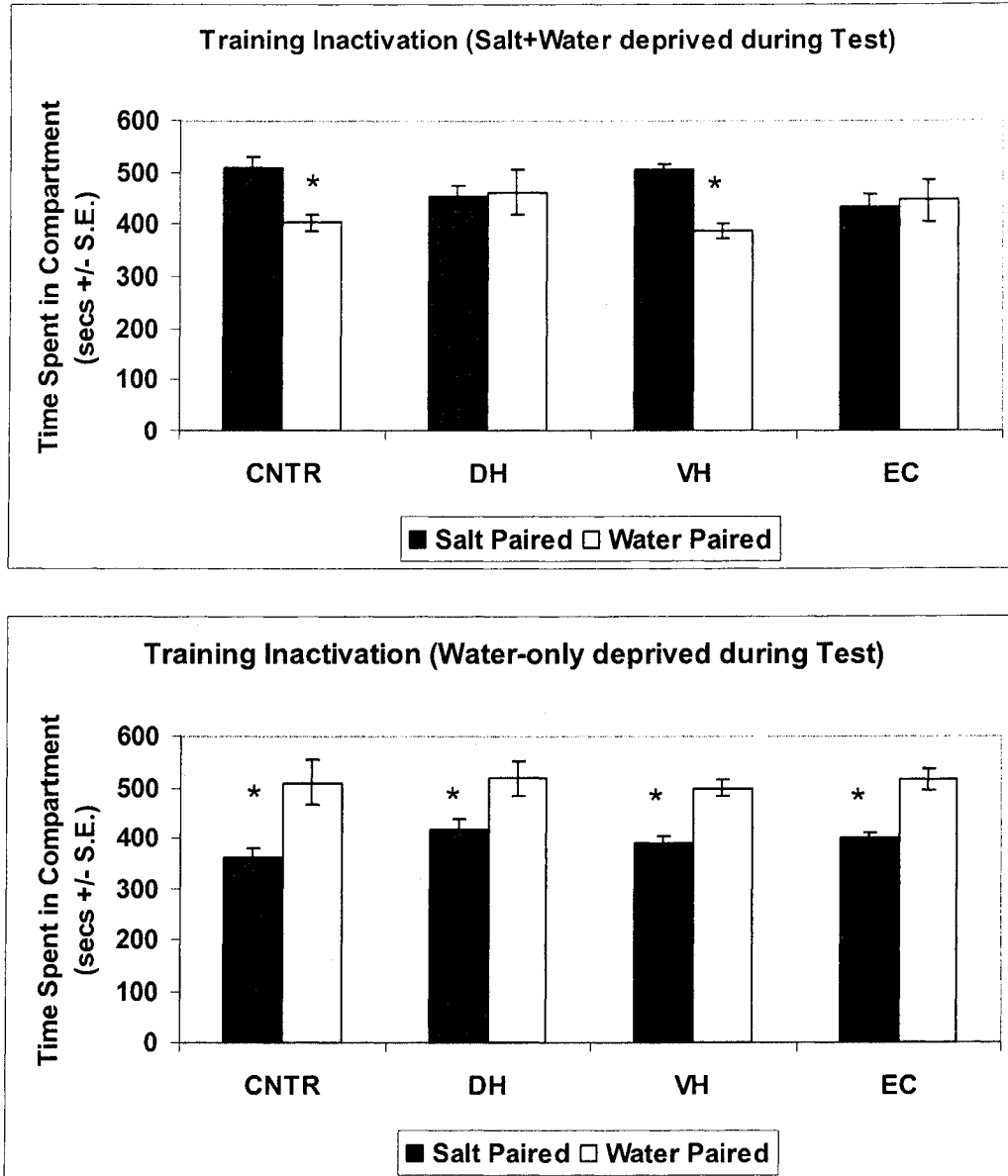


Figure 24. Experiment 7 compartment preference times for rats in the salt-LCP condition (top) and for rats in the water-CCP condition (bottom) that received the training trial inactivation procedure. Inactivation of the dorsal hippocampus (DH) or entorhinal cortex (EC) impaired acquisition of latent learning, while inactivation of the ventral hippocampus (VH) did not. Water conditioning was unimpaired in all groups. * = $p < 0.05$.

Compartment x Deprivation State interaction [$F(1, 63) = 36.72, p < 0.001$]. No other effects were significant [Group, $F(3, 63) = 1.13, p = 0.35$; Deprivation, $F(1, 63) = 0.01, p = 0.91$; Group x Deprivation, $F(3, 63) = 2.42, p = 0.07$; Compartment, $F(1, 63) = 3.47, p = 0.07$; Compartment x Group, $F(3, 63) = 1.54, p = 0.21$].

For the salt-LCP condition, planned comparisons showed that rats in the CNTR and VH groups spent significantly more time in their salt-paired than in their water-paired compartments [CNTR: $F(1, 63) = 13.76, p < 0.001$; VH: $F(1, 63) = 9.03, p < 0.01$]. Rats in the DH and EC groups showed no preferences [DH: $F(1, 63) = 0.05, p = 0.83$; EC: $F(1, 63) = 0.11, p = 0.74$]. Planned comparisons also revealed that all rats in the water-CCP condition spent significantly more time in their water-paired than in their salt-paired compartments [CNTR: $F(1, 63) = 10.99, p < 0.01$; DH: $F(1, 63) = 5.08, p < 0.05$; VH: $F(1, 63) = 7.94, p < 0.01$; EC: $F(1, 63) = 7.54, p < 0.01$].

Preference Test – Inactivation during Testing

Figure 25 shows the mean preference times for the rats that received pre-testing injections of muscimol. Three rats were eliminated for failing to meet the salt deprivation criterion described in the procedure section. After eliminations using this criterion and due to misplaced cannulas, analyses of the compartment preference times for the DH (salt-LCP $n = 9$; water-CCP $n = 9$), VH (salt-LCP $n = 10$; water-CCP $n = 9$), EC (salt-LCP $n = 9$; water-CCP $n = 9$), and CNTR (salt-LCP $n = 9$; water-CCP $n = 9$) groups were conducted. The CNTR group consisted of 6 rats with DH cannulas (salt-LCP $n = 3$; water-CCP $n = 3$), 6 rats with VH cannulas (salt-LCP $n = 3$; water-CCP $n = 3$), and 6 rats with EC cannulas (salt-LCP $n = 3$; water-CCP $n = 3$).

The CNTR rats in the salt-LCP condition preferred their salt-paired compartments; again, inactivation of the DH or EC disrupted this preference, while inactivation of the VH reversed the preference. In the water-CCP condition, all groups except for the DH group preferred their water-paired compartments. The ANOVA revealed a significant three-way interaction (Group x Deprivation State x Compartment: $F(3, 65) = 3.72, p < 0.05$), significant main

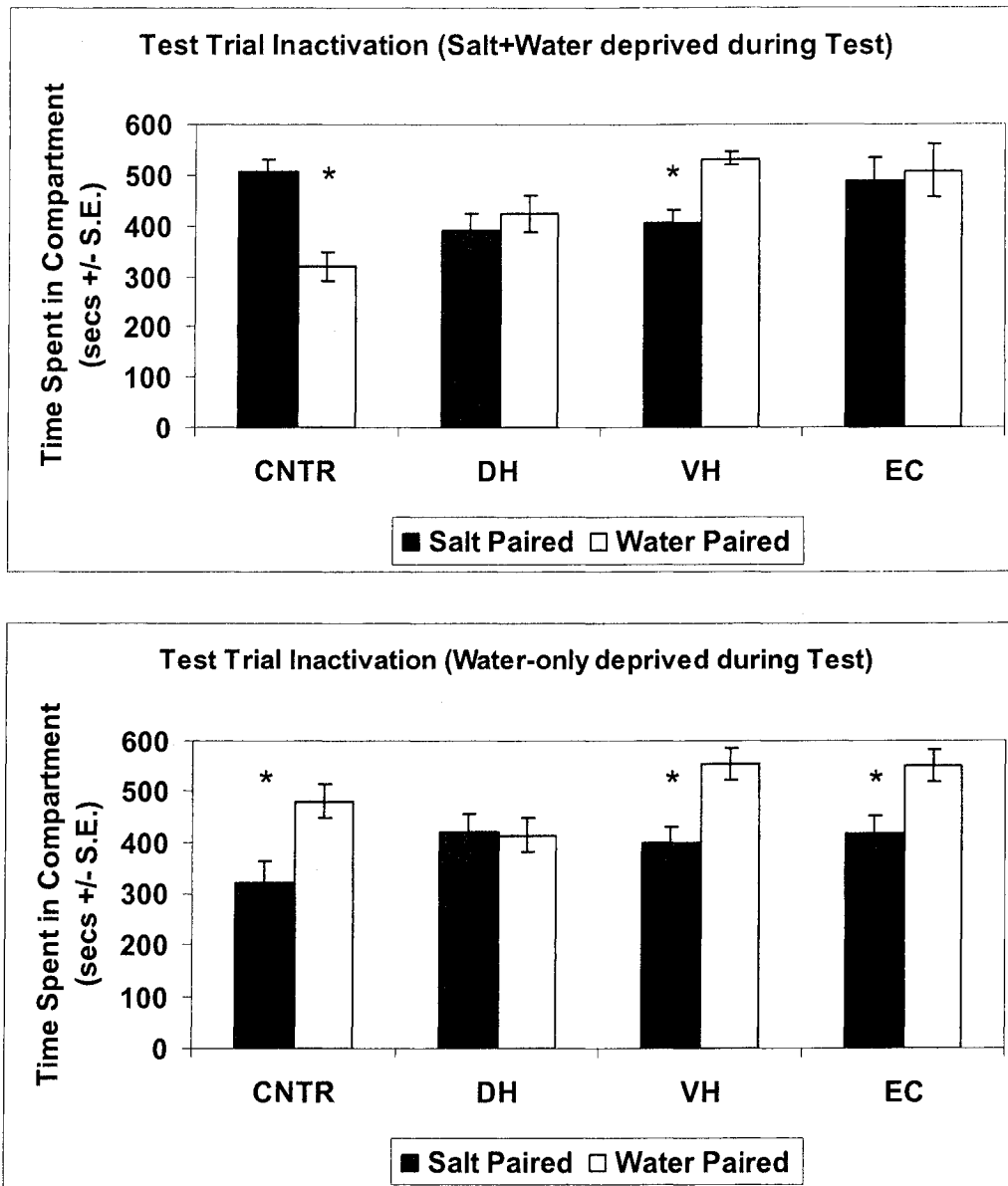


Figure 25. Experiment 7 compartment preference times for rats in the salt-LCP condition (top) and for rats in the water-CCP condition (bottom) in the test trial inactivation procedure. Inactivation of the dorsal hippocampus (DH) impaired the expression of latent learning and conditioning, while inactivation of the entorhinal cortex (EC) impaired the expression of only latent learning. Inactivation of the VH impaired the expression of latent learning and allowed conditioning to be expressed in both conditions. * = $p < 0.05$.

effects of Group [$F(3, 65) = 23.09, p < 0.001$] and Compartment [$F(1, 65) = 6.37, p < 0.05$], and significant interactions for Compartment x Deprivation State [$F(1, 65) = 6.77, p < 0.05$]. No other effects were significant [Deprivation, $F(1, 65) = 0.11, p = 0.74$; Group x Deprivation, $F(3, 65) = 0.48, p = 0.70$; Compartment x Group, $F(3, 65) = 2.51, p = 0.07$].

For the salt-LCP groups, planned comparisons showed the rats in the CNTR group spent significantly more time in their salt-paired than in their water-paired compartments [$F(1, 65) = 9.06, p < 0.01$] while rats in the VH group spent significantly more time in their water-paired compartments [$F(1, 65) = 4.61, p < 0.05$]. Rats in the DH and EC groups had no significant preferences [DH: $F(1, 65) = 0.30, p = 0.58$; EC: $F(1, 65) = 0.13, p = 0.72$]. For the water-CCP condition, rats in the CNTR, VH and EC groups spent significantly more time in their water-paired than in their salt-paired compartments [CNTR: $F(1, 65) = 6.88, p < 0.01$; VH: $F(1, 65) = 6.28, p < 0.05$; EC: $F(1, 65) = 4.26, p < 0.05$]. Rats in the DH group had no significant preference [$F(1, 65) = 0.01, p = 0.93$].

The results of the temporary inactivation experiments are summarized in Table 4. It needs to be reiterated at this time that during training on the LCP task two types of learning are acquired in parallel: (1) a latent association between the neutral salt solution and salt-paired compartment cues, and (2) conditioned responses to the water-paired compartment cues. The type of learning that is expressed during testing depends on the motivational state of the rat: (1) the latent association is expressed if the rat is salt+water deprived during testing, and (2) the conditioned responses are expressed if the rat is water-only deprived during testing.

The acquisition of the latent association was impaired by inactivation of DH and EC during training trials. Although none of the pre-training injections of muscimol impaired acquisition of the conditioned responses to water-paired cues, the CCP learning was not expressed in any of the LCP condition groups. In other words, despite the fact that the latent association was disrupted in these rats, the intact conditioned responses were not expressed when rats were salt+water deprived during testing, suggesting that the state of salt+water deprivation had an

	Salt-LCP		Water-CCP
Brain Area	Acquisition	Expression	Expression
Entorhinal Cortex	Impaired	Impaired	Normal
Dorsal Hippocampus	Impaired	Impaired	Impaired
Ventral Hippocampus	Normal	Impaired (expresses water-CCP)	Normal

Table 4. Temporary inactivation effects demonstrated in Experiment Seven. The entorhinal cortex and dorsal hippocampus were necessary for the acquisition and expression of the latent association between salt and salt-paired compartment cues, while the ventral hippocampus was necessary only for the expression of this latent association.

effect on the expression of the conditioned responses.

Expression of the latent association was impaired by inactivation of VH, DH, and EC during the preference test. Importantly, the VH group exhibited a preference for the water-paired compartment, behaving as though they were water-only deprived despite being salt+water deprived. In contrast, although EC inactivation impaired expression of the salt-LCP but not the water-CCP there was again no expression of the water-CCP when these rats were salt+water deprived, again suggesting that the state of salt+water deprivation had an effect on the expression of the conditioned responses.

Motivational Competition Hypothesis

The behavioral pattern of the salt-LCP and water-CCP learning can be viewed in terms of motivational competition. In the LCP task, two types of information are acquired during training. One, the water-paired compartment cues gain the ability to elicit a conditioned approach response because of their association with the rewarding water. Two, a latent association is formed between the salt-paired compartment cues and the neutral salt solution. During the preference test, a water-only deprived rat expresses the conditioned responses to the water-paired cues. However, if a rat is salt+water deprived a tendency to seek out the salt-paired cues may compete with the conditioned responses to the water-paired cues. Within this framework, it can be suggested that inactivation of the VH during the preference test eliminated this motivational competition, which resulted in the expression of the conditioned approach responses to the water-paired cues and a water-CCP.

Experiment Eight: Testing the Motivational Competition Hypothesis

The expression of salt-LCP learning involves two components: (1) the retrieval of the latent association between salt and salt-paired compartment cues, and (2) the motivational signals produced by salt deprivation. The motivational competition hypothesis described above states that the motivational signals produced by salt deprivation eliminate the tendency to perform the conditioned responses to the water-paired cues. There are two possibilities for this motivational competition: (1) the VH could act directly on the amygdala to inhibit

the expression of the conditioned responses, or (2) the VH could initiate salt-seeking behaviors that behaviorally compete with the conditioned responses mediated by the amygdala.

The purpose of the following experiment was to test the motivational competition hypothesis by isolating the motivational competition of the water-CCP response, in the absence of the stimulus-stimulus association involving salt and compartment cues. If this hypothesis is correct, then a rat that acquires a water-CCP during training but then is salt deprived during the preference test should show no preference for the water-paired compartment, even in the absence of information about salt availability in a salt-paired compartment. This would be analogous to rats with lesions or inactivation of the DH or EC that cannot recall the neutral S-S association between salt and compartment cues but still do not demonstrate a water-CCP when they are salt+water deprived.

Methods

Seventeen rats were divided into two behavioral groups: SDep/CCP ($n = 9$) rats were water-only deprived during training and then salt+water deprived during testing; W-CCP ($n = 8$) rats were water-only deprived during both training and testing.

A water-CCP training procedure was used. Following the acclimation to the water deprivation schedule, all rats were given two training trials over four days, during which they were given access to water in one compartment for 30 minutes on one day, and then no solution in the other compartment for 30 minutes the next day. On the training days in which water was not available in the apparatus, rats were given 30 minutes supplemental access to water in their home cages after a period of at least one hour following their training session.

Results and Discussion

Salt Consumption

Consumption of the salt solution during the post-test presentation in the rats' home cages were examined to verify that rats in the SDep/CCP group were salt deprived during the preference test. An independent groups t-test revealed that rats in the SDep/CCP group (mean = 21.6 ml) consumed significantly more

salt solution during the post-test presentation than rats in the W-CCP group (mean = 13.1 ml), $t(15) = 5.396, p < 0.001$. This verifies that the SDep/CCP rats were salt deprived during the preference test.

Preference Test

Figure 26 shows the mean preference times for the rats in the W-CCP and SDep/CCP groups. The W-CCP rats preferred their water-paired compartments, while the SDep/CCP groups showed no compartment preferences. The ANOVA revealed a significant main effect for Compartment [$F(1, 15) = 16.098, p < 0.001$] and a significant Compartment x Group interaction [$F(1, 15) = 10.944, p = 0.005$]. Planned comparisons showed the rats in the W-CCP group spent significantly more time in their water-paired than in their unpaired compartments [$F(1, 15) = 25.305, p < 0.001$] while rats in the SDep/CCP group showed no significant preferences for either compartment [$F(1, 15) = 0.263, p = 0.62$].

These results confirmed the motivational competition hypothesis. Despite having the same training as the W-CCP group that demonstrated a significant water-CCP during the preference test, the SDep/CCP group did not demonstrate this water-CCP because they were salt deprived during the preference test. This state of salt deprivation competed with and eliminated the conditioned responses to the water-paired compartment cues, resulting in no compartment preference. The fact that the conditioned response of water-CCP learning is mediated by a different neural circuit (FF and LA, in addition to the DH and VH) than is salt-LCP learning (EC, DH, and VH) suggests that the elimination of the water-CCP by motivational competition is due to competition between the two different neural systems at the level of behavioral output.

The results from Experiment Seven would imply that the VH is necessary for the use of stimulus information about internal cues of salt deprivation to alter the salience of the salt-paired cues. This information about internal cues of salt deprivation was not present during the training trials, and therefore inactivation of the VH during training did not disrupt salt-LCP learning. Inactivation of the EC or DH during testing also disrupted expression of the salt-LCP, but did not affect motivational competition with the water-CCP. This may be due to the fact that

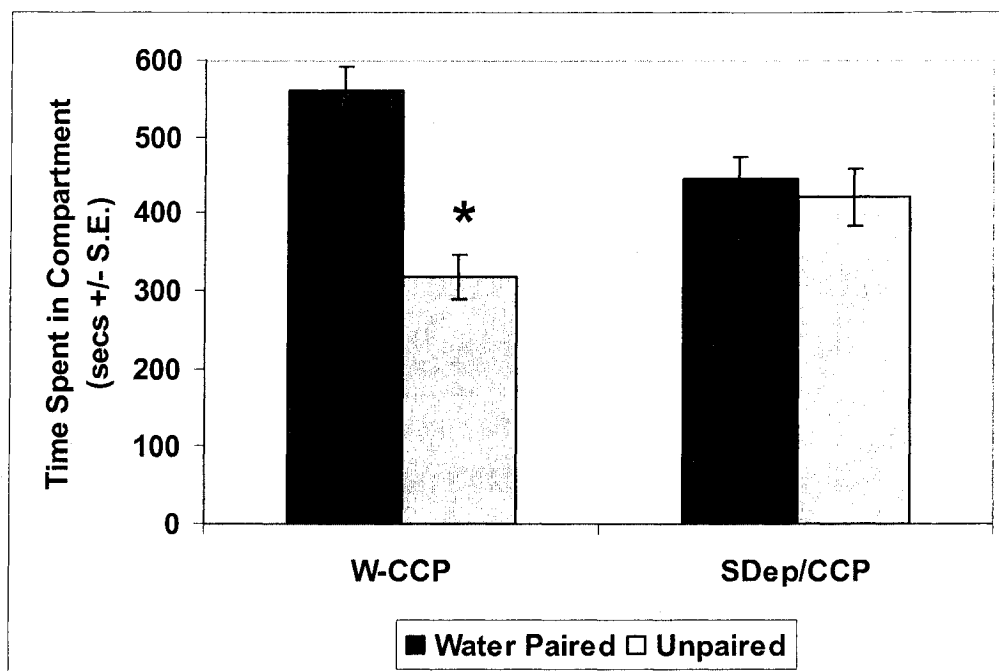


Figure 26. Experiment eight compartment preference times for rats that received water-CCP training. The motivational information about salt deprivation competed with the conditioned responses to the water-paired cues, eliminating the water-CCP.

these rats had an intact VH during testing, allowing them to utilize the stimulus information about internal cues of salt deprivation in order to elicit salt seeking behaviors, which then competed with the conditioned approach responses to the water-paired cues. However, because of inactivation of the EC or DH, these rats could not recall the latent association between salt-paired compartment cues and the salt solution. Therefore, they could not differentiate between the salt-paired and water-paired cues, resulting in no preferences for either compartment.

CHAPTER SIX: GENERAL DISCUSSION

The eight experiments discussed in this thesis produced several important findings. One, the Latent Cue Preference task was shown to be a reliable task for the demonstration of irrelevant-incentive latent learning in rats. Rats acquire an association between the neutral salt solution and the neutral salt-paired compartment cues in the box apparatus that can be used flexibly to guide behaviors when the latent association becomes relevant to the rat. Two, irrelevant-incentive latent learning and conditioning were shown to be behaviorally dissociable. Although both types of learning result in a rat spending more time in the salt-paired compartment than the water-paired compartment, the two types of learning are different in their requirements of motivational information for expression. Three, irrelevant-incentive latent learning and conditioning were also shown to be dependent on separate and dissociable neural circuits. Irrelevant-incentive latent learning was dependent on cortical-to-hippocampus processing, while conditioning was dependent on subcortical-to-hippocampus and amygdala processing. Finally, different neural circuits were shown to be involved in the acquisition versus the expression of irrelevant-incentive latent learning, leading to an important dissociation between dorsal hippocampus and ventral hippocampus function.

Behavioral Analysis of LCP and CCP Learning

Past researchers have used a variety of tasks to demonstrate that rats can latently acquire an association between the presence of salt and specific environmental cues, and then use that latent association at a time when the rat becomes deprived of salt (e.g. Kriekhaus and Wolf, 1968; Khavari and Eisman, 1971; Paulus et al., 1984; Berridge and Schulkin, 1989; Coldwell and Tordoff, 1993a; 1993b). However, these earlier tasks that demonstrated irrelevant-incentive latent learning about salt all had features that prevented their use in determining the brain structures and circuits necessary for this type of learning. The irrelevant-incentive latent learning task developed by Kriekhaus and Wolf (1968) used an increase in bar-pressing as the measure of latent learning. However, lesions of the hippocampal circuitry produce behavioral disinhibition

(Douglas, 1967; Coutureau et al., 2000) that results in an increase in bar-pressing that is independent of learning (Owen and Butler, 1980; Shull and Holloway, 1985). The spatial latent learning task originally developed by Blodgett (1929) depends on increased performance on reinforced maze trials to demonstrate latent learning that occurred during unrewarded pre-exposure trials. However, lesions of the hippocampal circuitry produce deficits on these reinforced maze trials compared to sham-lesioned controls even when latent learning has occurred (Kimble and BreMiller, 1981; Kimble et al. 1982), making interpretation of the lesion effect difficult. One goal of this thesis was to develop a behavioral task that was immune to these effects. This was the Latent Cue Preference (LCP) task.

LCP Training

In the LCP task, a water deprived rat experiences two things during training. One, the rat drinks a salt solution in one distinct compartment of the CCP box apparatus. This salt solution is not rewarding to the water deprived rat (Experiment 5), making the taste of the salt solution a neutral item of information. The specific visual, tactile, and olfactory cues in the compartment are also neutral items of information. However, during this time the rat is still able to form a latent association between the taste of salt and the specific cues that are present in the compartment. This latent association by itself does not influence behavior in any way by (Experiment 5). This is latent learning. Two, the water deprived rat drinks water in the other distinct compartment of the CCP box apparatus. Consuming the water satisfies its deprivation state, making the water a rewarding or unconditioned stimulus (US). The US produces unconditioned responses (UR) that result in the rat approaching and spending time with the water. The previously neutral cues in the compartment that are associated with the US (water) become conditioned stimuli (CS). This association between the US and CS continues to gain strength during the training trials, until eventually the CS by itself is able to elicit conditioned responses (CR) that are similar to the UR produced by the US. This is conditioning.

LCP Testing

During the compartment preference test, the rat is allowed to freely explore both compartments of the box with no solutions present. If the rat is salt deprived, an internal motivational state that sensitizes rats to the taste of salt is produced (Richter, 1939; Nachman, 1962; Handal, 1965). The results described in this thesis show that this sensitization extends to the latent association between the taste of salt and salt-paired compartment cues, as the motivational state of salt deprivation initiates the retrieval of this latent association. In addition, the motivational state of salt deprivation competes with and eliminates the CR to the water-paired compartment cues (CS) that were also acquired during training (Experiment 8). This elimination of the CR could occur through two mechanisms. One, VH could directly inhibit the amygdala, which mediates the CR to the water-paired cues. The CA1 subfield in the ventral hippocampus has substantial reciprocal connections with the lateral and basal nuclei of the amygdala, and lighter reciprocal connections with the central and medial nuclei (Pitkanen et al., 2000) that would allow for this direct inhibition. Two, the state of salt deprivation could initiate salt-seeking behaviors that compete with and eliminate the conditioned responses to the water-paired compartment cues.

The co-occurrence of the latent association between salt and salt-paired compartment cues and a state of salt deprivation during testing results in a rat showing a preference for the salt-paired compartment, a salt-LCP. However, the influence of each of these two factors individually is not enough to produce a preference for the salt-paired compartment. The results of Experiment 5 demonstrated that the latent association by itself produces no compartment preferences during testing, and the results of Experiment 8 showed that a state of salt deprivation by itself produces no compartment preferences.

One view of this process by which latent associations are retrieved by motivational states is provided by contextual retrieval theory (Hirsh, 1974). This theory postulates that internal motivational states such as hunger and thirst, and in this case salt appetite, are contextual cues that result in the recall of stored situational information relevant to the contextual cue. According to this idea, in

the LCP task salt deprivation results in the recall of the latent association between the taste of salt and the salt-paired compartment cues made during training because the latent association is relevant to the contextual cue of salt deprivation. Evidence for this idea is provided by studies in which rats running in a T- or Y-maze learned to turn in one direction for food when hungry and in the other direction for water when thirsty (Hsiao and Isaacson, 1971; Hirsh, et al., 1978; Hirsh, et al., 1979). In these experiments the maze cues remained constant, but the rats made responses appropriate to their internal state. However, in these T- and Y-maze tasks rats were food- and water-deprived during training so their behavior was based on reinforced responses. In the LCP task the rats were not salt deprived during the training trials, so the association made between the taste of the salt and the salt-paired compartment cues was neutral rather than rewarded or reinforced. Therefore, the information retrieved by the contextual cue of salt deprivation consisted of this latently learned association. This ability to form a latent association and retrieve it during a state of salt deprivation was demonstrated and replicated throughout the eight experiments described in this thesis.

If that rat is not salt deprived during the compartment preference test (water-only deprived or not deprived of either substance) then no motivational information about salt deprivation is present. Therefore, contextual retrieval of the latent association between the taste of salt and salt-paired compartment cues does not take place, and no salt-seeking behaviors exist to compete with the CR to the water-paired compartment cues (CS) acquired during training. When the rat explores both compartments the CS present in the water-paired compartment elicit the CR, which results in the rat approaching and spending time with the water-paired compartment cues. This produces in a preference for the water-paired compartment, or a water-CCP.

CCP Training and Testing

The preference for the salt-paired compartment in the CCP task is based on the same learning mechanism that underlies the preference for the water-paired compartment in the LCP task. In the CCP task, a salt+water deprived rat drinks a

salt solution in the one distinct compartment of the CCP box apparatus, and consumes water in the other compartment. Consuming the salt solution satisfies its deprivation state, making the salt solution a rewarding or unconditioned stimulus (US). The US produces unconditioned responses (UR) that result in the rat approaching and spending time with the salt solution. The previously neutral cues in the salt-paired compartment become conditioned stimuli (CS) because they have been associated with the US (salt). This association between the US and CS gains strength during the training trials, and eventually the CS by itself is able to elicit conditioned responses (CR) that are similar to the UR produced by the US. During the preference test, the rat freely explores both compartments of the CCP box. Encountering the salt-paired compartment cues (CS) elicits CR that result in the rat demonstrating a preference for the salt-paired compartment, or a salt-CCP.

Latent Learning and Conditioning are Behaviorally Dissociable

Irrelevant-incentive latent learning and conditioning can be dissociated behaviorally. Irrelevant-incentive latent learning requires that a deprivation state related to the previously irrelevant-incentive be present during testing in order for the latent association between the irrelevant-incentive and other stimuli to be expressed. In the LCP task, if a rat was not salt deprived during the preference test it either showed a preference for the water-paired compartment (Experiments 1 and 2) due to the existence of CR to the water-paired compartment cues (CS) or it showed no preference for either compartment (Experiment 5) if there was no water (US) present during training. The expression of the salt-LCP required both the latent association between salt and salt-paired compartment cues acquired during training and the motivational information about salt deprivation.

However, conditioning does not require a deprivation state to be present during testing in order for the CS to elicit CR. In both a water-CCP and salt-CCP task, if a rat formed an association between a US (either water or salt) and the CS in the compartments, then encountering the CS during the preference test was enough to elicit the CR, resulting in a preference for the water- or salt-paired compartment. No matching deprivation state was required in order for the CS to

elicit the CR (Experiments 4 and 5). In other words, a rat did not need to be salt deprived to express a salt-CCP, nor did it need to be water deprived to express a water-CCP.

Neural Circuitry involved in LCP learning and its Dissociation from CCP Learning

Irrelevant incentive latent learning of the association between compartment cues and the taste of salt was impaired by pre-training lesions of entorhinal cortex, dorsal hippocampus, or ventral hippocampus, but not by pre-training lesions of the fimbria-fornix or lateral amygdala (Experiment 6). The acquisition of this latent association was impaired by temporary inactivation of the entorhinal cortex or dorsal hippocampus, while the expression of the latent association was impaired by temporary inactivation of the ventral hippocampus, in addition to the entorhinal cortex and dorsal hippocampus (Experiment 7). Conversely, conditioned responses to compartment cues associated with either salt or water reinforcers was impaired by pre-training lesions of fimbria-fornix, lateral amygdala, dorsal hippocampus, or ventral hippocampus, but not by pre-training lesions of the entorhinal cortex (Experiment 6). This pattern of lesion and inactivation effects suggests that latent learning about salt and conditioned responses to both salt- and water-paired compartment cues involve different neural circuits. Irrelevant-incentive latent learning required cortical-to-hippocampus processing, while conditioning required subcortical-to-hippocampus and amygdala processing.

Neural Circuit Involved in Acquisition of LCP Learning

Figure 27 illustrates the neural circuits involved in the acquisition and expression of the salt-LCP. The interconnections between the entorhinal cortex and the hippocampus constitute a main input-output pathway between sensory cortical areas and the hippocampus (Amaral and Witter, 1989). The salt-LCP, a form of stimulus-stimulus learning, requires this pathway to process the neutral association between compartmental cues and the taste of salt, and to use that association when it becomes relevant during a subsequent state of salt deprivation. The acquisition of the neutral association between the taste of salt and salt-paired

Latent Cue Preference

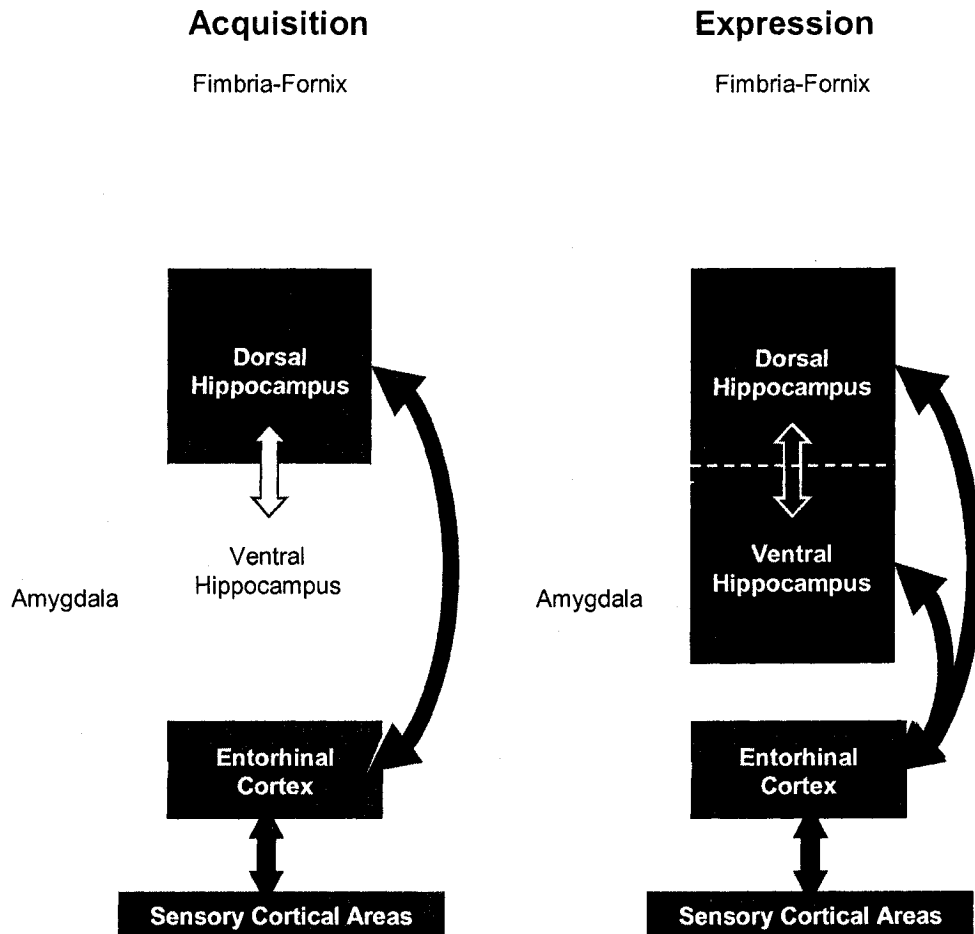


Figure 27. Illustration of the neural circuits involved in the acquisition and expression of the salt latent cue preference (salt-LCP). Dark areas indicate involvement, while light areas indicate the area is not involved. Acquisition of the latent association between salt and salt-paired compartment cues requires information from the sensory cortical areas to be sent to the entorhinal cortex and dorsal hippocampus. Expression of this latent association recruits the ventral hippocampus as well, which is necessary for utilizing internal cues about salt deprivation in the retrieval of the latent association processed in the entorhinal cortex/dorsal hippocampus circuit.

compartment cues is dependent on an intact entorhinal cortex/dorsal hippocampus circuit. Anatomically, the entorhinal cortex has diverse connections between sensory cortical regions and the hippocampus (Insausti et al., 1997; Burwell and Amaral, 1998a; 1998b; Witter et al., 2000; Sewards and Sewards, 2003). Various unimodal and multimodal sensory cortical areas have reciprocal connections with the perirhinal cortex, which in turn has numerous reciprocal connections with the entorhinal cortex. Layers II and III of the entorhinal cortex have projections that form the perforant pathway to the dentate gyrus and all CA subfields of the dorsal hippocampus, as well as the subiculum.

Neurons in the dentate gyrus send a major projection called the mossy fibers to the CA3 region of the dorsal hippocampus. The CA3 neurons send projections called the Schaffer collaterals to the CA1 subfield of the dorsal hippocampus, but these CA3 neurons also have many recurrent projections that synapse back onto neurons in the CA3 region. Neurons in the CA1 subfield of the dorsal hippocampus and the subiculum have direct projections to the deep layers (IV and V) of the entorhinal cortex, which in turn have direct projections to numerous cortical areas including association cortex. This entorhinal cortex/dorsal hippocampus circuitry is involved in a variety of learning and memory tasks, including spatial learning (Aggleton et al., 2000; Fyhn, et al., 2004; Steffenach et al., 2005), nonspatial learning (Jarrard et al., 2004; Hargreaves et al., 2005), inhibitory avoidance learning (Izquierdo and Medina, 1993; Izquierdo et al., 1993; Pereira et al., 2001), and attention (Burwell, 2000).

Although both components of the entorhinal cortex/dorsal hippocampus circuit are required for the acquisition of the latent association between the salt solution and salt-paired compartment cues, each may mediate a different aspect of this association. The dorsal hippocampus appears to mediate learning about the relationships among external and internal stimuli (Rudy and Sutherland, 1989; Good and Honey, 1991; Richmond et al., 1999; Kennedy and Shapiro, 2004; Rudy and Matus-Amat, 2005). The two compartments of the apparatus had distinctive visual, tactile, and olfactory cues, and salt-LCP learning required the additional use of gustatory cues produced by the taste of salt. The compartmental

cues had to be incorporated into one representation or context, which was then associated with the gustatory information about the taste of the salt solution, recruiting the dorsal hippocampus. The formation of associations between various stimuli may take place in the CA3-to-CA3 recurrent synapses and the CA3-to-CA1 synapse in the dorsal hippocampus (e.g. Ozaki et al., 1983; Kesner et al., 2004; Gilbert and Kesner, 2006). Additional evidence for the general role of the dorsal hippocampus in processing representational or contextual learning comes from the finding that inactivation of the dorsal hippocampus during the compartment preference test also impaired the expression of the water-CCP (Experiment 7), which is based on a representation of the water-paired compartment cues (CS) that elicit CR to the those cues, resulting in a preference for the water-paired compartment.

Inactivation of the dorsal hippocampus also impairs another form of latent learning involving contextual learning of cues within a small apparatus, the context pre-exposure facilitation effect in fear conditioning (Barrientos et al., 2002; Matus-Amat et al., 2004; Rudy and Matus-Amat, 2005). In this type of learning, animals are pre-exposed to a test cage, which allows them to form associations between the cues present in the cage. This latent contextual learning facilitates the subsequent acquisition of a conditioned association between the contextual cues and the effects of foot-shock. In contrast, a functional dorsal hippocampus is not required for latent learning of spatial information (Kimble and BreMiller, 1981; Kimble et al. 1982; White and Waller, 2000; White et al., 2003; Gaskin et al., 2005). In these experiments, rats with lesions of the dorsal hippocampus (either permanent or temporary) still retained the ability to acquire neutral spatial information during an unrewarded pre-exposure period. Interestingly, pre-training lesions of the fimbria-fornix impair this spatial latent learning (Chai and White, 2004) while damage to the fimbria-fornix did not disrupt the irrelevant-incentive learning described in this thesis (Experiment 6).

Although contextual learning and spatial learning would seem to require the same integration of multiple cues, these two types of learning appear to be mediated by different neural processes (Good and Honey, 1997; Richmond et al.,

1999; Burwell et al., 2004). A possible reason for this difference could be that spatial learning requires the integration of visual cues in an environment over time, while an animal moves around in the environment, while contextual learning requires integration of multimodal cues in a small environment that can be perceived simultaneously. The findings suggest the possibility that spatial learning may be mediated by a fimbria-fornix/entorhinal cortex circuit. Learning in the LCP task involved the integration of visual, tactile, olfactory, and gustatory cues in a small environment that were perceived simultaneously, which appears to require a functional dorsal hippocampus.

The entorhinal cortex may be the part of the entorhinal cortex/dorsal hippocampus circuit that processes neutral sensory information about the salt solution and the compartment cues. Witter et al. (2000) have proposed that the entorhinal cortex is part of a network that detects novel stimulus information (i.e. the taste of salt, distinctive compartment cues), and consists of a lateral input carrying external sensory information from the perirhinal or postrhinal cortices to the dorsal hippocampus, and a medial input carrying motivational information that reaches the ventral hippocampus. According to this theory, the entorhinal cortex and dorsal hippocampus would be necessary for mediating the acquisition of the sensory association between the visual and tactile compartment cues and the taste of salt. During testing, the entorhinal cortex and ventral hippocampus would be necessary for the expression of this association by mediating the transmission of stimulus information about the internal state of salt deprivation to the ventral hippocampus. The present temporary inactivation results (Experiment 7) are consistent with this model of entorhinal cortex function.

In addition, there is evidence from other lesion studies that the entorhinal cortex may be necessary for processing neutral or unrewarded information, as damage to the entorhinal cortex has been shown to disrupt latent inhibition (Coutureau et al., 1999; Coutureau et al., 2002). These latent inhibition tasks are essentially the inverse of the context pre-exposure facilitation effect, as pre-exposure to the neutral cues in the conditioning apparatus results in a retardation of subsequent conditioning. In both latent inhibition learning and salt-LCP

learning, associations are formed among neutral stimuli that do not affect immediate behavior, but can affect subsequent learning and behavior. In both types of learning, it appears that the entorhinal cortex is necessary for processing these neutral stimuli and possibly forming associations among them.

Although several researchers have made functional dissociations between the medial and lateral entorhinal cortex (Ferbinteanu et al., 1999; Hargreaves et al., 2005; Witter et al., 2000) the present pre-training lesions and temporary inactivation of the entorhinal cortex included disruption to both the medial and lateral regions, so a dissociation of function between these regions in LCP learning cannot be made. However, it can be noted that the coordinates of the entorhinal cortex lesions used in Experiment 6 were similar to those used in several latent inhibition experiments (Yee et al., 1995; 1997). In contrast, inactivation of the entorhinal cortex more posterior and dorsal to the present lesions (see Table 1) impairs both spatial learning (Steffenach et al, 2005) and spatial latent learning (Gaskin and White, in preparation). In the LCP task the rats are confined in a small box apparatus, while in spatial latent learning tasks rats freely explore mazes in large open rooms. Although additional experiments are needed, these findings provide initial evidence of a functional dissociation within the entorhinal cortex with respect to spatial versus contextual learning.

Neural Circuit Involved in Expression of LCP Learning

During the expression/test phase of the LCP task, rats are allowed to freely explore both the water-paired and salt-paired compartments. If a rat is water-only deprived, it spends significantly more time in the water-paired compartment in a demonstration of a water-CCP. However, if a rat is salt+water deprived during this expression phase, it must overcome the conditioned responses (CR) to the water-paired cues (CS) in order to express the preference for the salt-paired compartment cues to demonstrate the salt-LCP. The motivational information about its internal state of salt deprivation initiates the recall of the latent association between the salt solution and the salt-paired compartment cues, as well as competes with eliminates the CR to the water-paired compartment cues. This requires the involvement of the ventral hippocampus along with the

entorhinal cortex/dorsal hippocampus circuit (see Figure 27).

It has been demonstrated that an intact ventral hippocampus is necessary for the expression of several types of behaviors that require the utilization of internal motivational signals. For example, damage to the ventral hippocampus results in the inability of anxiety or fear signals to reduce exploratory behavior (Kjelstrup et al., 2002; Bannerman et al., 2003; McHugh et al., 2004; Trivedi and Coover, 2004) or willingness to eat in a potentially harmful environment (Bannerman et al., 2002; McHugh et al., 2004). In addition, animals with ventral hippocampus lesions have a reduced ability to use hunger cues to generate feeding behaviors (Davidson and Jarrard, 1993; Tracy et al., 2001). These findings point to a role for the ventral hippocampus in the use of internal motivational cues in the production of appropriate behaviors. This is similar to the ideas postulated in contextual retrieval theory (Hirsh, 1974) described earlier. Again, this theory states that internal motivational states are contextual cues that recall specific situational information relevant to the contextual cue. In the case of salt-LCP learning, the motivational information about salt deprivation was processed by the ventral hippocampus, an initiated the retrieval of the latent association between salt and salt-paired compartment cues that was processed by the entorhinal cortex/dorsal hippocampus circuit (see preceding section).

The ventral hippocampus was only necessary for the expression of the latent association between the salt solution and salt-paired compartment cues, which required the use of information about the internal state of salt deprivation. When this information was not available during ventral hippocampus inactivation, the rats behaved as though they were not salt deprived and spent more time in the water-paired compartment, expressing the water-CCP (Experiment 7). It is interesting to note that inactivation of the ventral hippocampus during the compartment preference test did not disrupt the water-CCP shown by rats that were water-only deprived (Experiment 7). This would imply that motivational information about the internal state of water deprivation was not necessary for the expression of the water-CCP. The behavioral results of Experiment 5 confirm that this is indeed the case. These findings again highlight an important

difference between LCP and CCP learning: motivational information is required for the expression of LCP learning while it is not required for the expression of CCP learning.

Neural Circuit of CCP Learning

Figure 28 illustrates the neural circuit involved in water-CCP and salt-CCP learning. Behaviors that depend on conditioning to compartmental cues based on a deprivation state during training (the salt-CCP and water-CCP) involve a neural circuit that includes the fimbria-fornix, lateral amygdala, and the hippocampus. The salt- and water-CCPs, forms of stimulus-affect learning, require the integration of compartmental cues and signals about the positive affective state that is produced when the rat's deprivation state is relieved during training by the consumption of either the salt solution (salt-CCP) or water (water-CCP). This processing involves the main input-output pathway between subcortical areas, amygdala, and the hippocampus (Amaral and Witter, 1989).

Role of the hippocampus circuitry in CCP learning

There is considerable evidence that the hippocampus mediates learning about the relationships among external and internal cues (Rudy and Sutherland, 1989; Good and Honey, 1991; Richmond et al., 1999; Kennedy and Shapiro, 2004; Rudy and Matus-Amat, 2005). In this capacity the dorsal hippocampus may form relationships among the visual, tactile, and olfactory cues present in the compartments of the CCP box apparatus, and create distinct representations of each compartment. This representation or context would then serve as the CS that is associated with the US in the US-CS conditioning relationship that is formed by the amygdala. This type of learning in which contextual representations serve as the CS is called contextual conditioning, and has been shown previously to require an intact dorsal hippocampus (e.g. Winocur et al., 1987; Selden et al., 1991; Good and Honey, 1991; Phillips and LeDoux, 1992; Honey and Good, 1993; Hall et al., 1996; Maren et al., 1997), as well as an intact fimbria-fornix (Phillips and LeDoux, 1995; Maren and Faneslow, 1997; Bannerman et al., 2001) indicating that the transfer of information from subcortical areas to the hippocampus is necessary for contextual conditioning (Experiment 6). However,

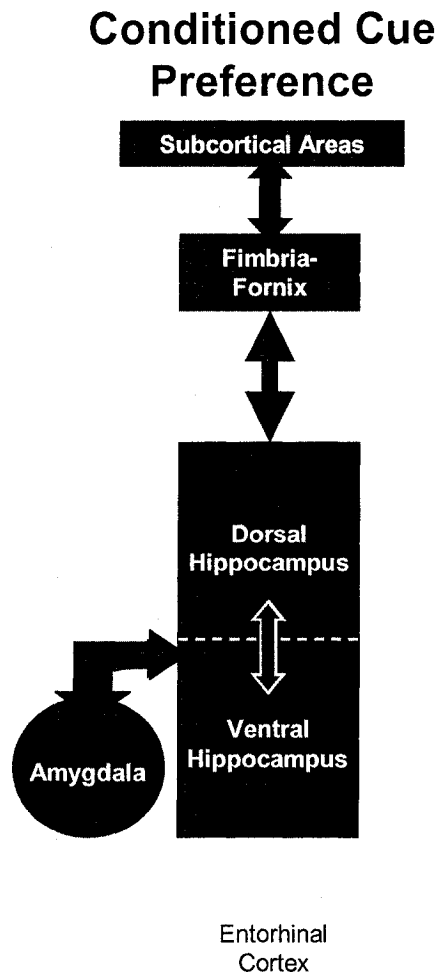


Figure 28. Illustration of the neural circuit involved in both water-CCP and salt-CCP learning. Dark areas indicate involvement, while light areas indicate the area is not involved. The formation of the association between the US (water or salt) and the CS (compartment cues) requires information to be sent from subcortical areas through the fimbria-fornix to the dorsal hippocampus. The dorsal hippocampus then integrates the various compartment cues into one representation (CS) that is sent to the amygdala. The amygdala then forms the association between the US and CS. The ventral hippocampus is recruited because motivational information about a deprivation state is present during training. This information may be required to facilitate the rewarding effect of the US.

the present results indicate that the transfer of information from the sensory cortical areas to the hippocampus via the entorhinal cortex is not necessary for contextual conditioning (Experiments 6 and 7). This is corroborated by previous findings that demonstrated that the entorhinal cortex input into the hippocampus is not necessary for contextual fear conditioning (Phillips and LeDoux, 1995; Good and Honey, 1997; Bannerman et al., 2001).

The ventral hippocampus may be a necessary component of the hippocampal circuitry because it processes the motivational information about deprivation states that occur during training. Pretraining lesions of the ventral hippocampus disrupted both salt- and water-CCPs (Experiment 6). As shown in Experiment 5, the salt solution used in the present experiments has no inherent reward value. It is only when the consumption of the salt solution interacts with a state of salt deprivation does the salt solution gain reward value and become an unconditioned stimulus and is able to elicit unconditioned responses. The ventral hippocampus may be involved in the utilization of the motivational information about salt deprivation that is required to give the salt solution reward value. If the ventral hippocampus is damaged, the salt solution does not gain reward value and does not become an unconditioned stimulus that is necessary for conditioning.

Role of the amygdala in CCP learning

Lesions of the lateral amygdala impaired CCP learning for both salt and water. This finding is consistent with many previous reports that damage to or disruption of the amygdala impairs many types of conditioning, including appetitive conditioning in the radial arm maze (White and McDonald, 1993; McDonald and White, 1993; McIntyre et al., 1998; Holahan, 2005) and CCP box apparatus (Hiroi and White, 1991; Brown and Fibiger, 1993; Hsu et al., 2002; Schroeder and Packard, 2002), as well as aversive conditioning (Goldstein, 1965; Hitchcock and Davis, 1986; Hitchcock and Davis, 1987; Cador et al., 1989; Cahill and McGaugh, 1990; LeDoux et al., 1990; Selden et al., 1991; Helmstetter, 1992; Sananes and Davis, 1992; Holahan and White, 2002).

The amygdala is necessary for the formation of an association between a discrete conditioned stimulus (CS) and an unconditioned stimulus (US). The

lateral and central nuclei of the amygdala receive numerous projections from sensory cortical areas and subcortical input from the thalamus (Price, 2003). In addition, the amygdala has many reciprocal connections with the hippocampus (Pitkanen et al., 2000) that allow for the transfer of the CS (compartment context) information processed in the hippocampus to the amygdala. Once the CS information has reached the amygdala it can be associated with the US information about the salt solution or water. After the CS-US association is made, if the CS (compartment context) is encountered again it will elicit conditioned responses mediated by the central nucleus of the amygdala, the main output nucleus of the amygdala that has direct connections to various motor and autonomic function brain regions (Price, 2003).

Updating the MMS Model

The results of the pre-training lesion experiment (Experiment 6) and the temporary inactivation experiment (Experiment 7) provide some knowledge of the neural circuits involved in latent learning in the LCP task. This information can now be added to the current MMS model, as illustrated in Figure 29.

The cognitive memory system is responsible for forming stimulus-stimulus (S-S) associations in which one of the stimuli in the association has some reward value (e.g. food that is present in a maze). Encountering that stimulus produces an immediate change in behavior. These S-S associations can then be used to guide novel, controlled behaviors at later times. For example, an S-S association involving the location of food in a maze can be later used to produce a novel route in the maze to get to the food location. This type of learning involves the hippocampus as its central structure.

The latent learning system is responsible for forming S-S associations in which all of the stimuli are neutral and do not immediately influence behavior. However, these S-S associations can be recalled and utilized to guide novel, controlled behaviors when they become relevant to the current situation. For example, in the LCP task a rat learns to associate the neutral taste of salt with neutral compartment cues. None of these stimuli immediately influence compartment preference behavior. It is only when the rat becomes salt deprived

<i>Type of Learning</i>	<i>What is Learned?</i>	<i>What is Expressed?</i>	<i>Central Structure</i>
Cognitive (S-S)	Stimulus-stimulus (S-S) associations, in which one of the stimuli has reward value, which immediately influences behavior	The S-S associations are used to guide novel controlled behaviors	Hippocampal formation
Latent (S-S)	Stimulus-stimulus (S-S) associations that involve neutral stimuli that do not influence immediate behavior	The S-S associations are expressed when they become relevant to the current situation and guide novel behaviors	Entorhinal Cortex
Habit (S-R)	Stimulus-response (S-R) associations that are strengthened through repeated pairing with a reinforcer	Responses are elicited by presentation of the stimulus, even in the absence of the reinforcer	Dorsal Striatum (Cerebellum)
Conditioning (S-Af)	A neutral stimulus (CS) is associated with a stimulus (US) that produces an affective state and an unlearned behavioral response (UR)	When the CS is encountered it elicits a response (CR) similar to the one originally elicited by the US	Amygdala

Figure 29. The proposed update of the current multiple memory systems model that includes latent learning. Evidence presented in this thesis show the entorhinal cortex as a potential central structure involved in the formations of latent associations among neutral stimuli that do not effect immediate behavior but can be recalled and utilized when they become relevant to the animal.

does that latent association influence preference behavior. This type of learning appears to involve the entorhinal cortex as its central structure.

The entorhinal cortex is the one brain region that is unique to salt-LCP learning, as the dorsal hippocampus and ventral hippocampus are also involved in CCP learning. As mentioned previously, damage to the entorhinal cortex also disrupts latent inhibition (Coutureau et al., 1999; Coutureau et al., 2002), which involves the latent acquisition of neutral associations as well. In addition, the entorhinal cortex may also be necessary for spatial latent learning (Gaskin and White, in preparation). Therefore, the entorhinal cortex appears to be a central structure for the latent acquisition of associations among neutral stimuli that do not influence immediate behavior, but can be recalled and utilized when they become relevant to the animal.

However, in the LCP task the dorsal hippocampus was also necessary for the formation of the latent association between salt and salt-paired compartment cues. This may have been due to the fact that the compartment cues used in the task involved several multimodal cues that had to be formed into one representation or context. This process may have made the dorsal hippocampus a necessary component in salt-LCP learning. However, if the latent association involved only one discrete cue (rather than several multimodal cues) that was associated with the salt stimulus, then the dorsal hippocampus may not have been necessary for the formation of the latent association.

The ventral hippocampus was also recruited during the expression of the latent association between salt and salt-paired compartment cues because the recall of the latent association was dependent on the use of internal motivational information about salt deprivation. Again, this quality is unique to the LCP task. If a task were created that did not utilize internal motivational states to initiate the retrieval of the latent association, then the ventral hippocampus would not be predicted to be a necessary component. However, the entorhinal cortex does appear to be a necessary component for many latent learning tasks, making it a prime candidate as the central structure in latent learning.

Habit learning and conditioning are unchanged from the current MMS model. The habit learning system is responsible for forming stimulus-response (S-R) associations. These S-R associations are strengthened by their repeated pairing with a reinforcer. Once the S-R association has been sufficiently strengthened, the response element of the association will be elicited automatically when the stimulus element of the association is encountered. For example, a food-deprived rat learns to enter and run down to the end of an arm (response) on the radial maze that is paired with a light (stimulus) in order to obtain food (reinforcer). After repeated training, the rat will enter and run down to the end of any lit arm on the maze. This type of learning involves the dorsal striatum as its central structure.

Finally, the conditioning system is responsible for forming associations between an unconditioned stimulus (US) and a conditioned stimulus (CS). The US normally elicits an unconditioned response (UR), and when the CS is repeatedly paired with the US it gains the ability to elicit conditioned responses (CR) that are similar to the original UR produced by the US. For example, if a rat is given an electrical shock (US) it will freeze (UR). If a light (CS) is repeatedly presented with the shock, the light by itself will be able to elicit freezing behavior (CR). This type of learning involves the amygdala as its central structure.

Although these four types of learning occur in parallel, there are instances in which they can interact. For example, latent learning and conditioning can either compete with each other for expression or can cooperate in the production of behaviors. In the LCP task rats acquire both latent learning and conditioning during training. If a rat is water-only deprived during testing then conditioning is expressed. However, if a rat is salt deprived during testing then the latent learning competes with the conditioning for expression. In latent inhibition tasks (Coutureau et al., 1999; Coutureau et al., 2002) rats are pre-exposed to contexts during which they acquire latent learning. This latent learning later competes with and retards the acquisition of conditioning in the same context. Conversely, the context pre-exposure facilitation effect for fear conditioning (Barrientos et al., 2002; Matus-Amat et al., 2004; Rudy and Matus-Amat, 2005) involves pre-

exposure to contexts during which latent learning occurs. This latent learning later cooperates with and facilitates the acquisition of conditioning in the same context.

Concluding Statement

The experiments described in this thesis provide several important contributions to the field of learning and memory research, and specifically to the current research on multiple memory systems. I created a new behavioral task to evaluate latent learning in rats, and successfully used that task to determine several brain structures and circuits that are necessary for this latent learning. These are contributions to an area of learning and memory research that has been left behind over the last 20 years, but is an area that may underlie many of our biographical memories. My hope is that these initial findings will initiate a resurgence in latent learning research that will result in the discovery of more information about its neural mechanisms. Uncovering the details of these neural mechanisms may then lead to a greater understanding of learning and memory systems that are present in the mammalian brain, and may result in a greater understanding of biographical memory and its disorders. Then, maybe ten years from now, a Google search of Hugh Blodgett's name will actually produce information on latent learning as its first match.

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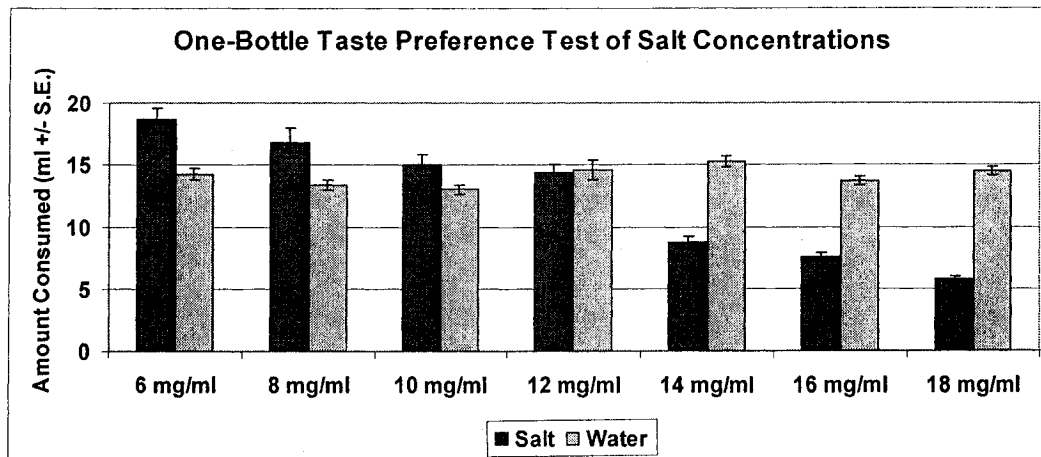
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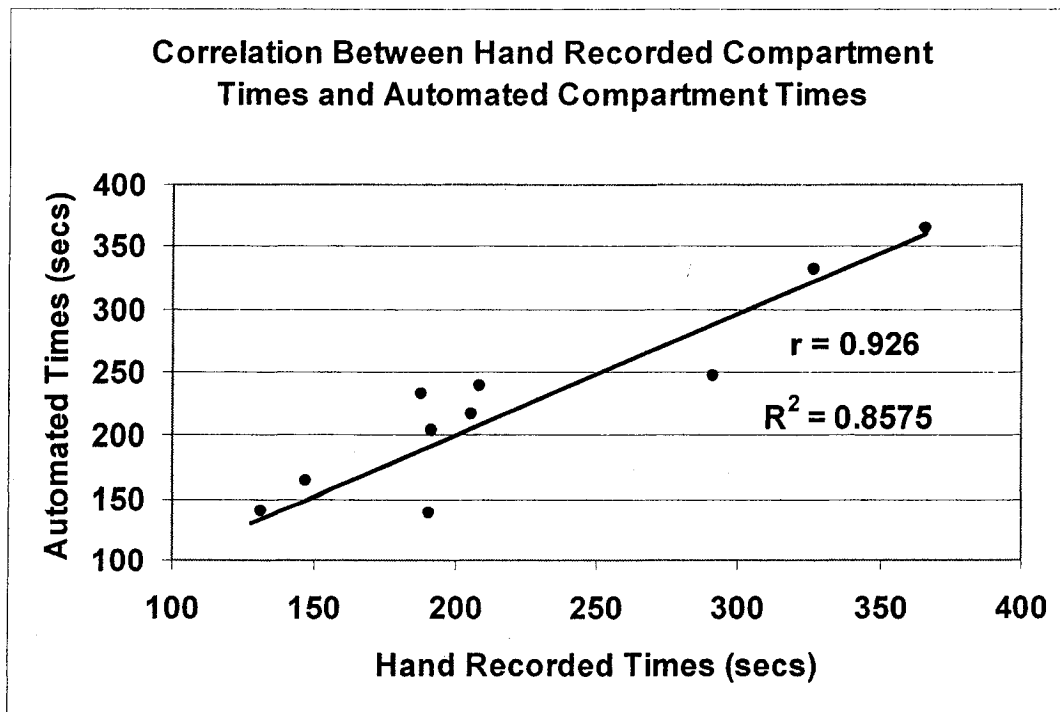
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Appendix A



Using a one-bottle taste preference test similar to the situation that would occur during training trials of the LCP task, I was able to determine that a salt solution concentration of 12 mg/ml was preferred equally to tap water by water deprived rats. This concentration was later revised to 12.5 mg/ml with repeated testing.

Appendix B



There was a strong correlation between the hand-recorded compartment times and the compartment times recorded by the automated system ($r = 0.926$).

This finding validates the use of the automated system for recording the compartment times.

Appendix C

Animal Research Protocol Certificate