Brief Communication

A latent cue preference based on sodium depletion in rats

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Three experiments show latent (or incidental) learning of salt-cue relationships using a conditioned cue-preference paradigm. Rats drank a salt solution while confined in one compartment and water in an adjacent, distinct compartment on alternate days. When given access to the two compartments with no solutions present, sodium-deprived rats preferred their salt-paired compartments; normal rats preferred their water-paired compartments. Reversing the deprivation states of the two groups reversed their preferences. These results show that rats can latently acquire associations between environmental cues and the taste of salt, and can use this information flexibly to guide behavior based upon internal cues produced by sodium deprivation.

Latent learning is the incidental, unreinforced acquisition of 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. Its original demonstration (Blodgett 1929; Tolman and Honzik 1930) refuted two basic assertions of associative learning theory (Thorndike 1932; Hull 1943) by showing (1) reinforcement is not necessary for learning to occur, and (2) learning can occur without performing the behaviors that ultimately reveal its existence (Thistlethwaite 1951). Hirsh's (1974) hypothesis that learning and performance are mediated in different neural systems, with the hippocampus involved in explicitly mnemonic processes separate from a "performance line" that functioned according to the predictions of associative theory, provided a different view of the relationship between learning, performance, and reinforcement. The idea that the acquisition of information and learning reinforced responses are mediated in different neural systems is consistent with the results of numerous experiments (for review, see White and McDonald 2002). There is also evidence that learning unreinforced information and the use of that information to generate behavior are also mediated by different parts of the brain (Kimble et al. 1982; Gaskin and White 2005).

The main goal of the present study was to investigate the interaction between the incidental acquisition of unreinforced information and an internal state in determining how this information is used to guide behavior. A further goal was to study this interaction using a task that does not rely primarily on spatial learning. We used irrelevant-incentive learning, a type of latent learning that occurs when a rat is pre-exposed to a reinforcer (usually food or water) in a satiated state. When later deprived of the reinforcer the rats acquire a new behavior that allows them to obtain it (Thistlethwaite 1951). We adapted irrelevant-incentive learning to the conditioned cue-preference (CCP) paradigm (also called conditioned place preference), which has been used to determine the neural bases of conditioning for food and drug reinforcers (Carr et al. 1989; Schechter and Calgagnetti 1993, 1998; Tzschentke 1998).

The irrelevant incentive used in these experiments was the taste of a salt solution, which has been used in previous demonstrations of incidental learning. For example, rats were trained to press one bar for water and another bar for a salt solution (Kriek-

haus and Wolf 1968). When later sodium deprived, the rats showed greater resistance to extinction on the salt-paired bar than on the water-paired bar. Fimbria-fornix (Owen and Butler 1980), but not cortical (Wirsig and Grill 1982) lesions, eliminated the difference in resistance to extinction of the normal and salt-deprived rats, but the conclusion that this was due to elimination of latent learning was questioned (Owen and Butler 1980; Shull and Holloway 1985) due to the disinhibitory effects of fimbria-fornix and hippocampal lesions on rates of responding (Douglas 1967; Coutureau et al. 2000). The CCP paradigm, however, is insensitive to rates of responding (Hiroi and White 1991; White and McDonald 1993).

When a salt solution was paired with different flavors during training, rats preferred the salt-paired flavors during later sodium-deprivation (Fudim 1978; Westbrook et al. 1995). A similar experiment showed that rats exposed to flavors that were paired with different salted and unsalted foods preferred the salt-paired flavor when later sodium deprived (Coldwell and Tordoff 1993). These findings demonstrate that rats can incidentally acquire associations between salt and environmental cues and can express these associations with appropriate behaviors when in a state of sodium deprivation.

The apparatus used in the present study is illustrated in Figure 1. To limit spatial learning, several measures were taken to reduce the possibility that the rats could identify the locations of the salt using spatial cues. First, visibility of cues outside the apparatus was reduced by the height of its opaque walls (45 cm). Second, the apparatus was rotated 180 degrees between training trials so that no external cues that remained visible above the apparatus were consistently associated with the stimuli in either compartment. Third, to make relational learning among the cues in the compartments difficult, the apparatus was designed so that rats could see the cues in only one of the compartments from any location within either paired compartment or from the connecting compartment. Fourth, given evidence that movement restriction prevents or greatly retards spatial learning (McDonald and White 1995; White and Ouellet 1997), the size of the compartments was reduced to restrict rats' movement within the apparatus during training.

In all experiments, naive male Long-Evans rats were acclimated to a water-deprivation schedule in which they received access to water for 30 min each day for 6 d. In Experiment 1, rats were given four training trials. On each 2-d training trial, rats in the experimental group were placed into a compartment with either the salt solution (12.5 mg/mL NaCl) or water in the drink-

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Figure 1. Top view of the apparatus. Compartments 1 and 3, used as the salt- and water-paired compartments (counterbalanced) measured 28×8 cm. Compartment 1 was black and the floor was covered with wood-chip bedding. Compartment 3 had black and white vertical stripes and the floor was covered with a 1-cm wire mesh. Compartment 2 (17×8 cm) was the neutral compartment from which rats had free access to 1 and 3 during the test trials; the walls and floor of compartment 2 were gray and the floor was wood. The partitions separating the compartments were made of black Plexiglas (with white stripes on the side facing compartment 3). Dashed lines indicate doors (6×6 cm) from compartment 2 to compartments 1 and 3, which were closed during training trials and open during the test trial. The walls and partitions were 45-cm high. The top of the apparatus was covered with clear Plexiglas doors. Water tubes were mounted at the ends of compartments 1 and 3 with their spouts protruding into the compartments. Infrared motion detectors (RadioShack model 49-208A; using wide-angle lens, modified to be optimally sensitive to the infrared wavelength emitted by rodents—R.E. Brown, pers. comm.) were mounted in each compartment, facing down, ~30 cm above the floor.

ing tube for 30 min on 1 d, then in the other compartment with the other substance the next day. The compartments assigned as salt paired and water paired and the orders of salt and water presentation were counterbalanced. A control group was given water in both compartments. No supplemental water was given during training. Mean amounts of salt solution consumed on each training trial ranged between 12.5 and 15.3 mL, while the mean water consumption ranged between 13.7 and 15.8 mL. ANOVA revealed no significant differences in solution consumption during training.

On the day following the training trials, half of the rats in each group were given a low-sodium diet (Harlan Teklad, <0.01% sodium) for 14 d (demonstrated to induce sodium deprivation) (Stricker et al. 1991; Curtis et al. 2001). They were also given distilled water on the water-deprivation schedule. The other half of each group was kept on a normal diet for the same period and given tap water. A preference test was given on day 15 following the training trials. Rats were placed into compartment 2 (see Fig. 1) and given free access to all compartments for 20 min with no solutions present. Information about the location of the rats in the apparatus provided by the motion detectors was used to calculate the total time spent in the salt- and water-paired compartments. Immediately following the preference test, all rats were given a final 30-min access to the salt solution or water in their home cage. A rat was defined as sodium deprived if it consumed an amount more than two standard deviations above the average amount of salt solution it had consumed during the training trials. In Experiment 1, three rats failed to meet this criterion and were eliminated from the analysis.

The compartment preference data are shown in Figure 2. Planned comparisons on the significant interaction effect [$F_{(3,39)} = 4.352$, $P < 0.01$] showed that rats in the SWDep group preferred their salt-paired compartments [$F_{(1,39)} = 6.24$, $P < 0.02$]. This preference constitutes irrelevant-incentive learning, or a latent cue preference (LCP) for salt-paired cues. The rats acquired an association between the taste of salt and the cues in the com-

partment containing salt during training. During testing, this association and the salt appetite produced by sodium deprivation interacted to produce behaviors that led to a preference for the salt-paired compartment. Planned comparisons also showed that rats in the SWND group preferred their water-paired compartments [$F_{(1,39)} = 6.73$, $P < 0.02$]. This preference may have been based on an LCP for the water-paired cues because these rats were water deprived during testing, much like the SWDep rats were sodium deprived during testing. Another possibility is that these rats developed a CCP for the water-paired cues because they were water deprived during training, thereby pairing those cues with a reinforcer relevant to their deprivation state. The rats in the WWDep and WWND groups showed no preferences, regardless of their deprivation state during testing.

Analysis of the final consumption test data revealed a significant interaction effect [$F_{(1,41)} = 29.222$, $P < 0.0001$]. Tukey's post-hoc tests showed that sodium-deprived rats drank more salt solution than normal rats and more salt solution than normal rats drank water, $P < 0.05$. These findings confirm that the low-sodium diet increased salt appetite.

In Experiment 2, naive rats underwent the same training procedure as in Experiment 1, but were given injections of furosemide (10 mg/mL, i.p., which produces sodium deprivation within 48 h) (Starbuck et al. 1997; Thunhorst et al. 1999) on the day following the last training trial. These rats were then given the low-sodium diet and distilled water on the water-deprivation schedule. Control rats were injected with an equal volume of phosphate-buffered saline and given the normal diet and tap water. The preference test was given 48 h after the injections, followed by the final consumption test.

Mean consumption of the solutions during training was again similar (salt: 17.0–17.3 mL; water: 16.3–19.0 mL; no group differences). Figure 3 shows the compartment preference data. Planned comparisons on the significant interaction [$F_{(2,27)} = 4.823$, $P < 0.02$] showed that rats in the SWDep group preferred their salt-paired compartments [$F_{(1,27)} = 5.23$, $P < 0.05$]. Rats in the SWND group preferred their water-paired compartments [$F_{(1,27)} = 4.46$, $P < 0.05$]. These findings replicate those of Experiment 1. Rats in the WWDep group showed no preference for either compartment.

Analysis of the consumption data on the final test revealed a significant effect for solution [$F_{(2,27)} = 27.934$, $P < 0.0001$]. Tukey's post-hoc tests showed that both furosemide-treated groups (SWDep and WWDep) consumed more salt solution than the SWND group, $P < 0.05$. These results confirm increased sodium appetite in the rats that received furosemide. However, two

Figure 2. Experiment 1 compartment preference results. Analyses revealed that the SWDep group showed a significant preference for the salt-paired compartment, while the SWND group showed a significant preference for the water-paired compartment. * $P < 0.05$.

Figure 3. Experiment 2 compartment preference results (first preference test). Analyses revealed that the SWDep group showed a significant preference for the salt-paired compartment, while the SWND group showed a significant preference for the water-paired compartment. * $P < 0.05$.

rats failed to meet the sodium-deprivation criterion and were excluded from the analysis.

Following the test trial, rats in the SWDep and SWND groups were rested for 5 d, during which those in the SWDep group were given access to the salt solution in their home cages on three occasions to eliminate any residual salt deprivation. The rats in both groups were then given one additional 2-day training trial. After this trial, rats in the SWND group were injected with furosemide (SWND/Dep) and given the low-sodium diet and distilled water for 48 h. Rats in the SWDep group were given control injections (SWDep/ND) and the normal diet and water for 48 h. Both groups were then given a second 20-min preference test. Only weak preferences were shown during this test, but these were much larger during the early part of the test than during the later part. Therefore, the 20-min trial was split into two 10-min parts, and the data for these parts are shown in Figure 4. Tukey's post-hoc tests on the significant interaction [$F_{(1,17)} = 12.973$, $P < 0.01$] showed that rats in the SWND/Dep group preferred their salt-paired compartments during the first 10-min period, and rats in the SWDep/ND group preferred their water-paired compartments during the first 10-min period (P s < 0.05). Importantly, this shows the latently acquired association between the salt and compartment cues could be used flexibly, depending upon whether the rat was in a state of sodium deprivation or was sodium replete. During the second 10-min period, neither group showed significant preferences.

On the final consumption test, the SWND/Dep group consumed more salt solution than the SWDep/ND group [$t_{(18)} = 5.631$, $P < 0.0001$]. Furthermore, there was no significant difference in the amount of salt solution consumed by the rats that received furosemide in the two parts of the experiments (27.5 mL in part one, 28.4 mL in part two). This indicates that the levels of sodium deprivation during the preference tests in the two experiments were approximately equal, suggesting that differences in salt appetite cannot explain the relatively weak compartment preference on the second test. Another explanation for the weak preference could be extinction of the latently acquired association between the taste of salt and the compartment cues. Even though rats were given an additional training trial between preference tests, learning that salt and compartment cues were not always associated may have occurred after a total of 30 min (20 on the first test and 10 on the second test) of exposure to the cues in the absence of the salt solution.

In Experiment 3, the procedure was identical to that of Experiments 1 and 2, but the rats were sodium deprived during

training. Following training, half of the rats were sodium deprived prior to the preference test and half were not sodium deprived. As shown in Figure 5, both groups preferred their salt-paired compartments, an impression confirmed by a significant main effect of compartment [$F_{(1,15)} = 15.606$, $P < 0.002$].

Two events occur when rats consume a salt solution, i.e., they taste the salt and they ingest some salt. In Experiments 1 and 2, the rats tasted the salt during training and learned about its relationship to the external cues, but since they were not deprived, the ingestion of salt was without consequence. The learned information about the availability of salt interacted with the deprivation state during the test to produce behaviors that resulted in a preference for the salt-paired cues. This kind of learning is a latent cue preference (LCP). In contrast, in Experiment 3 the rats learned about the availability of salt, and because they were salt deprived, the ingested salt was reinforcing. In this case, the behaviors that produced the preference did not depend on the deprivation state during the test. This kind of learning is a conditioned cue preference (CCP).

These findings suggest that the LCP procedure can reliably demonstrate latent learning for an association between the taste of salt and external cues present when the salt is tasted. Sodium deprivation produces an internal state that sensitizes rats to the taste of salt (Richter 1939; Nachman 1962; Handal 1965). The present findings show that this sensitization extends to learned associations between the taste of salt and associated cues. One view of this process is provided by contextual retrieval theory (Hirsh 1974). This theory postulates that internal motivational states such as hunger and thirst, and by extension sodium appetite, are contextual cues that result in the recall of stored information about specific information relevant to the contextual cue. Evidence for this idea is provided by studies in which rats running in a T-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, 1979). In these experiments the maze cues remained constant, but the rats made responses appropriate to their internal states. In these tasks the rats were food and water deprived during training, so their behavior was based on reinforced responses. However, in the LCP task the rats were not sodium deprived during the training trials, so any associations made between the taste of the salt and the cues in the compartment were unreinforced. Therefore, the information retrieved by the contextual cue (sodium deprivation) consisted of a latently learned association.

Figure 4. Experiment 2 compartment preference results (second preference test). Analyses revealed that the SWND/Dep group showed a significant preference for the salt-paired compartment during the first 10 min of the test trial, while the SWDep/ND group showed a significant preference for the water-paired compartment during the first 10 min of the test trial. * $P < 0.05$.

Figure 5. Experiment 3 compartment preference results. Analyses revealed that both groups showed a significant preference for the salt-paired compartment, indicating that this preference was not dependent on deprivation state during the test period. * $P < 0.05$.

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