Hippocampal inactivation enhances taste learning

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Learning tasks are typically thought to be either hippocampal-dependent (impaired by hippocampal lesions) or hippocampal-independent (indifferent to hippocampal lesions). Here, we show that conditioned taste aversion (CTA) learning fits into neither of these categories. Rats were trained to avoid two taste stimuli, one novel and one familiar. Muscimol infused through surgically implanted intracranial cannulae temporarily inactivated the dorsal hippocampus during familiarization, subsequent CTA training, or both. As shown previously, hippocampal inactivation during familiarization enhanced the effect of that familiarization on learning (i.e., hippocampal inactivation enhanced latent inhibition of CTA); more novel and surprising, however, was the finding that hippocampal inactivation during training sessions strongly enhanced CTA learning itself. These phenomena were not caused by specific aspects of our infusion technique—muscimol infusions into the hippocampus during familiarization sessions did not cause CTAs, muscimol infusions into gustatory cortex caused the expected attenuation of CTA, and hippocampal inactivation caused the expected attenuation of spatial learning. Thus, we suggest that hippocampal memory processes interfere with the specific learning mechanisms underlying CTA, and more generally that multiple memory systems do not operate independently.

With few exceptions, learning tasks are thought to be either "hippocampal-dependent" (i.e., impaired by hippocampal lesion) or "hippocampal-independent" (i.e., unaffected by hippocampal lesion) (for review, see Eichenbaum 2004). Conditioned taste aversion (CTA), a form of learning in which a normally appetizing taste becomes aversive following its association with gastric distress, requires a neural circuit that includes the pons, taste cortex, and amygdala (Gallo et al. 1992; Spector 1995; Lamprecht et al. 1997; Yasoshima and Yamamoto 1997; Schafe et al. 1998) and is generally thought to be hippocampal-independent (Yamamoto et al. 1995; Bures et al. 1998).

Interpretation of the data suggesting that CTA is a hippocampal-independent form of learning is complicated, however, by the fact that all of the above-mentioned studies used permanent lesions. Such lesions have complex effects, downregulating some systems and upregulating others; during the 1–2 wk of recovery that must pass before rats with permanent lesions are healthy enough to participate in experiments, these changes evolve in a way that may extend, mask, or compensate for acute loss of hippocampal activity (e.g., Hanniğan Jr. et al. 1984; Simon et al. 1988; Lipska et al. 1992). Because particular parts of the systems that interact with the hippocampus are deeply involved in sensory aspects of CTA (Reilly and Bornovalova 2005), it is possible that temporary hippocampal inactivations might have an effect, even a facilitatory one, on CTA, despite the lack of such effects observed on the majority of tasks.

Another reason to hypothesize that taste learning in particular might actually be facilitated by loss of the hippocampus has to do with latent inhibition—the process whereby familiarization with a taste reduces later learning of a CTA for that taste. Latent inhibition of CTA itself appears to be enhanced by hippocampal lesions (Reilly et al. 1993; Gallo and Candido 1995; Purves et al. 1995), despite the fact that in most paradigms latent inhibition is impaired by damage to the hippocampus or related structures (Kaye and Pearce 1987; Schmajuk et al. 1994; Yee et al. 1995; Coutureau et al. 1999; Grecksch et al. 1999; Shohamy et al. 2000). Such data suggest that the role of hippocampus in learning may depend upon the specific paradigm and system being studied. Related work suggests that hippocampal spatial learning processes interfere with striatal-dependent "habit learning" processes, such that habit learning is enhanced following hippocampal lesions (Schroeder et al. 2002; Poldrack and Packard 2003). It is possible that a similar enhancement of amygdala-dependent CTA learning may follow removal of the hippocampus from the circuit, although such a phenomenon has never before been reported.

Here we replicate the finding that removal of the hippocampus enhances latent inhibition of CTA, and further show that temporary, muscimol-induced inactivation of the dorsal hippocampus does in fact enhance CTA: Rats with inactivated hippocampi learn stronger aversions than normal rats. Identical inactivations caused the expected impairment of spatial learning, and further control experiments/analyses demonstrated that this result reflects neither specific aspects of our training regimen nor of muscimol infusion. Based on these data, we conclude that hippocampal learning processes interfere with those more centrally involved in CTA, and further suggest that latent inhibition circuits are to some degree separate from basic CTA circuits. At a more general level, our data serve as strong evidence that hippocampal and amygdalar learning circuits are not independent.

Results

Figure 1 presents a highly representative coronal slice through the dorsal hippocampus (dHIPP) in a randomly selected rat that received muscimol (MUS) infusions during CTA conditioning sessions. The guide and infusion cannulae descend through cortex and end in the CA1 cell fields of dHIPP. Figure 1B shows a schematic, taken from Paxinos and Watson (1997), in which arrows point to the boxed regions found to contain all guide cannula tips. While it is difficult to estimate the precise spread of MUS or any non-lesioning agent, our slowly delivered infusion into dHIPP, just below and around the cannula tips, is almost certainly responsible for the effects described below, and also makes it clear that the damage caused by the 23-gauge guide...
cannulae (accentuated by slight tissue shrinkage during processing) had little impact on our results.

We set out to examine whether CTA is enhanced by temporary dHIPP inactivation. Our “within-subject” protocol also enabled us to replicate previously demonstrated lesion-induced enhancements of latent inhibition (Purves et al. 1995); each rat was trained to avoid two different tastes, one familiar (taste-fam) and one novel (taste-nov). Because the results of Purves et al. were essentially identical to those demonstrated in a between-group procedure (Reilly et al. 1993), it can be assumed that the within-group procedure did not cause undue changes to the learning processes involved.

The tastes used were 0.1 M sucrose and 0.1 M NaCl (counterbalanced for familiarity). For one of five groups, MUS was infused into dHIPP just before the four familiarization sessions with taste-fam, and vehicle (VEH) was infused just before the four conditioning sessions with taste-fam/taste-nov followed by intra-peritoneal LiCl injections. The second group received the opposite treatment, the third group received MUS in all familiarization and training sessions, the fourth (control) group received VEH in all sessions, and the fifth group was an unoperated control that received no infusions. In all cases, the behavior of unoperated controls was not statistically different from that of any conditioning involving only VEH infusion.

The amount (in mL) of fluid consumed in the sole testing sessions by rats that received MUS infusions during familiarization or training sessions (but not both, see below) is shown in Figure 2, along with control data. The success of the familiarization sessions in producing latent inhibition is clear because rats consistently drank less taste-nov than taste-fam.

Hippocampal inactivation had two main impacts on learning, both driving down consumption of taste-nov and driving up consumption of taste-fam. Rats that received MUS infusions just before training sessions developed stronger than normal CTAs; that is, they learned to drink less taste-nov than rats that received infusions of VEH (or nothing). Rats that received MUS infusions during familiarization sessions, meanwhile, drank more taste-fam than VEH/control rats; MUS enhanced latent inhibition of CTA.

These effects were borne out in a two-way ANOVA for infusant (MUS, VEH/nothing) and taste (taste-fam, taste-nov), which showed a significant effect of taste ($F_{(1,60)} = 25.54$, $P < 0.00001$) and a significant interaction between taste and infusant ($F_{(1,60)} = 5.50$, $P = 0.02$). Subsequent analysis revealed that MUS reduced drinking of taste-nov (Scheffe $F = 4.80$, $P < 0.001$) but increased drinking of taste-fam (Scheffe $F = 2.79$, $P = 0.03$). To further probe this latter effect, which we suspect was limited by a ceiling on within-session drinking, we compared both MUS- and VEH-taste-fam groups to the unconditioned, NaCl-injected controls: The MUS-taste-fam rats proved indistinguishable from the ceiling provided by controls ($t_{(13)} = 1.1$, $P = 0.28$), while VEH-taste-fam rats drank significantly less than controls ($t_{(22)} = 3.74$, $P < 0.002$). Hippocampal inactivation during familiarization sessions enhanced latent inhibition (i.e., increased drinking in testing sessions) to the largest degree possible in our paradigm.

Differences in taste learning may sometimes be the result of differences in initial drinking; a rat that drinks less of a taste during a conditioning session (for instance, perhaps because a hippocampal infusion reduced desire for fluids) will later prove to have learned less about that taste. In anticipation of this possibility, we ensured that our rats had less to drink than they desired, such that all drank the same amount. Each and every rat drank the full 10 mL of fluid in <30 min, regardless of condition. Clearly, any small effect that hippocampal MUS infusions had on normal drinking behavior did not interfere with rats drinking the full amount available. Therefore, learning differences between the groups do not reflect differential exposure to either taste. These data also suggest that intracranial infusions of MUS do not themselves cause CTA; a single pairing of taste and LiCl-induced illness was sufficient to reduce consumption of NaCl or sucrose to ~6 mL (data not shown), but rats never drank less than a full 10 mL in the familiarization sessions, even following a previous pairing of taste-fam and muscimol infusion.

It might also be argued that some aspect of the two-taste paradigm caused the observed effects. Perhaps the two tastes “competed” for associability with illness, such that a MUS effect on taste-fam affected learning to taste-nov (and vice versa). The data, however, do not suggest that such between-taste interaction occurred. For instance, two groups—unoperated controls, and rats that received MUS during familiarization but VEH during conditioning—had similar experiences with taste-nov but different experiences with taste-fam. Any between-taste interaction would have been apparent in a difference between the two groups in taste-nov consumption. No such difference was found ($P > 0.1$). More generally, there is no significant correlation between consumption of taste-nov and taste-fam by single rats.
(r² = 0.05 once the lack of normality in consumption of taste-nov was corrected; r² = 0.19 before; Fig. 3); a strong correlation would be expected if tastes had competed for associability.

Previous studies examining the issue using permanent lesions have found that the hippocampus plays no role in CTA (Yamamoto et al. 1995; Bures et al. 1998); even the studies that previously reported enhancements of latent inhibition following permanent hippocampal lesions, including the one on which we based our protocol (Purves et al. 1995), did not report enhancements of CTA (Reilly et al. 1993). Therefore, we ran an additional group of rats in which we more closely mimicked the permanent lesions used by Purves et al. and Reilly et al.: These rats received MUS infusions before all four familiarization sessions and all four conditioning sessions. This group could be thought of as a “virtual lesion,” in that rats received dHIPP inactivation before every training session. (To make the analogy complete, of course, rats would have to receive MUS infusions before the testing sessions, as well, which we did not do.)

Figure 4 presents the amount of taste-nov drunk in testing sessions by rats that had received either MUS in all previous sessions or VEH in all previous sessions. These data demonstrate that MUS infusions significantly enhanced learning of an aversion to taste-nov (directional t(24) = 1.71, P = 0.05). MUS infusion during all familiarization and training sessions did not increase latent inhibition for taste-fam (data not shown), but this was as expected: dHIPP inactivation during familiarization sessions should increase consumption (i.e., enhance latent inhibition), and dHIPP inactivation during training sessions should reduce consumption (i.e., enhance learning); the two effects cancel each other out.

While our data suggest that the inactivation-induced enhancement of CTA was not due to any LiCl-like effect of the MUS itself, it remains possible that some tendency for taste-MUS pairings lead to CTA was masked by the 10-mL cap on drinking in familiarization sessions. To provide further evidence that this was not the case, and to test the reliability of our training and infusion procedures, we prepared a subset of rats with cannulae in the primary gustatory cortex, a brain region known to be required for normal CTA learning (Gallo et al. 1992; Naor and Dudai 1996; Gutierrez et al. 2003). We trained these rats on a simplified version of the regimen (see Materials and Methods), infusing either MUS or VEH into cortex during conditioning sessions. We used 0.02 M citric acid, a solution that the rats were willing to drink but do not normally prefer over water, as the conditioned taste, because a subset of these rats had already been exposed to both sweet and salty tastes.

Figure 5 presents fluid consumption for rats with and without cortical inactivation during the sole testing session, as well as consumption by untrained rats that received unpaired presentations of the taste and LiCl. Far from enhancing learning, single-site cortical inactivation during the conditioning session was enough to totally disrupt learning of the aversion to mild citric acid (t(11) = 4.30, P < 0.001), consistent with the results of many previous studies (Lasiter et al. 1985; Gallo et al. 1992; Yamamoto et al. 1995; Bielavskas and Roldan 1996; Naor and Dudai 1996; Schafe and Bernstein 1998). We conclude that our infusion technique was reliable and robust: Rats learned a normal, cortically dependent aversion in our paradigm, and MUS infusions did not, in and of themselves, lead to enhanced taste learning. Only MUS infusions into dHIPP enhanced CTA.

Finally, we addressed the possibility that our infusions were not specific to dHIPP. In a control experiment, we tested the efficacy of our hippocampal infusions by examining performance of dHIPP rats on a reliably hippocampal-dependent spatial learning task, the Morris Water Maze task, in which rats must learn the relationships between distal spatial cues to find a submerged platform in a pool of milky water. Water maze learning has been shown to be impaired by dHIPP lesion (Bannerman et al. 1999), and performance deficits in this task are remarkably similar regardless of whether dHIPP is removed via excitotoxic lesions or single-site infusions of muscimol (Moser and Moser 1998). We replicated the latter study with rats prepared as for the CTA experiments, testing retention and new learning with intact and inactivated hippocampi.

Figure 6A shows the basic learning curves before surgery. Rats learned to find the submerged platform across 16 trials, after which performance asymptoted. This learning is also reflected in probe trials without a platform (Fig. 6B), which caused rats to preferentially search the quadrant of the pool in which the platform should have been. Here, we also show that the performance of rats that later received MUS infusions (dark bars) was indistinguishable from that of other rats (light bars).

Figure 6C reveals that dHIPP inactivation had the expected impact on retention 1 wk following training and surgery. Control rats retained the learning, in that they continued to spend much more time in the “appropriate” quadrant than would be predicted by chance (25%). The performance of rats that had received MUS infusions into dHIPP, meanwhile, did not differ from chance. MUS had an even stronger impact on relearning, as shown in the results from the third probe trial in Figure 6D: Control rats learned to spend their time where they expected the

Figure 3. Lack of competition between taste-nov and taste-fam. Within-rat analysis of amount consumed during testing sessions, with taste-fam on the x-axis and taste-nov on the y-axis. The fact that most points fall in the lower right half of the space reflects latent inhibition—greater consumption of taste-fam. The lack of an obvious relationship between the two variables (which is insignificant when the non-normality of taste-nov consumption is controlled for) makes it clear that the two probably did not compete for associability. See text for details.

Figure 4. Enhanced CTA could be seen even when MUS was infused in all familiarization and training sessions. The y-axis shows mL of taste consumed during the testing sessions (x-axis) for rats receiving MUS (“virtual lesion”) or VEH (“unlesioned control”) infusions across the entire training regimen—familiarization sessions with one taste, and training sessions with both tastes. MUS infusions before conditioning sessions decreased consumption of taste-nov.
of turning in a learning that is striatal-MUS in gustatory cortex impairs CTA. The (i.e., dHIPP inactivation) during testing, which implies (Berridge and Robinson 1998).

— Several possible confounds can be ruled out as causes of this learning enhancement: (1) all rats drank the same amount during familiarization, and thus differences in the volume of pre-exposure cannot explain our results; (2) inactivation of dHIPP during familiarization sessions 1–4 did not lead to reduced drinking, suggesting that intra-hippocampal MUS infusions did not, in and of themselves, cause CTA; (3) identical inactivation of gustatory cortex had the expected, previously observed effect on CTA, demonstrating that our training and infusion techniques worked appropriately and proving conclusively that intracranial MUS infusions do not ubiquitously cause or enhance CTA; (4) MUS infusion into dHIPP had the expected, previously observed effect on spatial learning and performance, demonstrating that we were in fact inactivating hippocampal neurons; and (5) learning was specifically enhanced for rats that underwent a change from an altered “state” (i.e., dHIPP inactivation) during conditioning to a normal “state” during testing, which implies that “state-dependent learning”, according to which changes in state should impair learning (Santini et al. 2001; Bast et al. 2003), cannot explain our findings.

The learning enhancement that accompanied hippocampal inactivation was unexpected—it has repeatedly been reported that permanent hippocampal lesions have no impact on CTA (Reilly et al. 1993; Purves et al. 1995; Yamamoto et al. 1995; Bures et al. 1998). Two factors probably contribute to the difference between our results and those reported previously. First, aversions expressed in our experiment were smaller than those typically induced. Often, taste aversion conditioning causes rats to nearly cease consumption of the taste, a ceiling effect for learning that makes further enhancements difficult to detect. Our rats, meanwhile, were supplied with only one lick spout (which strongly encouraged at least some consumption in our thirsty rats, even of a taste which to them was aversive) and were trained in a relatively complex, two-tastant training protocol; this combination of factors likely led them to express a relatively small reduction of consumption to even the novel taste, a fact that made our paradigm more sensitive to enhancements of learning.

Second, our temporary inactivations probably had fewer secondary effects on learning-related systems than do the more commonly used permanent lesion techniques. During the 1–2 weeks of recovery that must necessarily pass before rats with permanent lesions recover enough to participate in experiments, secondary extra-hippocampal changes emerge, changes that may mask or compensate for the acute effects of hippocampal removal (see below). It is entirely possible that a learning enhancement induced by hippocampal inactivation, such as is apparent in our data set, could vanish across such a time period.

While it is possible to argue that our results could be specific to a two-stimulus paradigm, several facts make such an explanation unlikely. First, the use of multiple-stimuli paradigms has a long history in behavioral neuroscience (e.g., Hall and Minor 1984; Bellingham et al. 1985; Kehoe et al. 1993) and is generally thought to involve the same processes as single-stimulus training. Second, the results of the within-subject, permanent lesion experiment upon which we based our paradigm (Purves et al. 1995) were essentially identical to those of a between-subject experiment that also used permanent lesions (Reilly et al. 1993). Finally, there is no evidence of inter-stimulus “competition” in our data set, nor of MUS effects transferring from one taste to the other. Thus, the most important differences between our study and earlier studies probably have to do with our relatively small aversion effects and our use of muscimol.

If conditioned taste aversions are learned supra-optimally when the hippocampus is incapacitated, the implication is that a normally functioning hippocampus may interfere with the learning of a CTA. While this corollary is difficult to test, it is consistent with the findings of Packard and colleagues, who have suggested that normal hippocampal function interferes with striatal-dependent learning processes (Poldrack and Packard 2003). These researchers trained normal and lesioned rats to perform radial arm maze tasks in which spatial cues either indicated or were irrelevant to the arm that had been baited with food (this latter task required that the rats learn the “habit” of turning in a particular direction at the maze center—learning that is striatal-dependent), and reported that hippocampal lesions impaired spatial learning while enhancing habit learning (Schroeder et al. 2002).

Could a similar process be occurring here? The hippocampus is known to be involved in spatial foraging (Baird Day et al. 1999; Pravosudov and Clayton 2002), but it seems doubtful that spatial learning processes actually compete and interfere with chemosensory learning processes in our one-bottle instantiation of the CTA paradigm, at least not in the same way that spatial and habit learning processes interfere with each other when a rat must choose a lane in a radial arm maze (Poldrack and Packard 2003). It does not appear that rats use spatial information to ensure avoidance of a toxin even when that spatial information is the only viable cue (Mediavilla et al. 2001). Still, perhaps the hippocampus’ “attempt” to map spatial locations onto food sources interferes with the explicitly non-spatial task, in this case amygdalar learning of stimulus palatability. It is known that lesions of the amygdala (which impair CTA; see Rolls et al. 1989; Lamprecht et al. 1997; Schaie et al. 1998; Morris et al. 1999; Yasoshima et al. 2000; Reilly and Bornovaletova 2005) affect the strategies that rats use in maze learning tasks (Packard and Wiggard 2004). This fact is consistent with the possibility that the CTA machinery and hippocampus may interact in some way.

At another level, the observed learning enhancement may be a function of the complexities of the hippocampus’ relationship with the distributed dopamine system, which is involved in learning about stimulus “value” (Berridge and Robinson 1998). In many circumstances, the hippocampal projections to the dopamine system work in opposition to striatal, prefrontal, and

**Figure 5.** MUS in gustatory cortex impairs CTA. The y-axis shows mL of citric acid consumed by rats receiving MUS infusion or VEH/no infusion. As expected, MUS infusion doubled the amount of tastant consumed, eliminating CTA to the citric acid (MUS rats consumed as much as unpaired controls, consumption of which is shown by the horizontal dashed line).
Figure 6. Inactivation of dorsal hippocampus impairs spatial learning. (A) In a replication of Moser and Moser (1998), rats were first trained in the hidden-platform version of the Morris Water Maze task. (x-axis) Session, (y-axis) latency to find the platform. Rats learned the task in four sessions, as evidenced by the gradual asymptote in speed of finding the platform. (B) The standard transfer task, in which the platform is removed before the rats enter the water, showed that rats had learned to spend the bulk of their time searching for the platform in the appropriate quadrant (where it had been before removal), relative to the average time spent in other quadrants. For this and all subsequent panels, the y-axis is percentage of total swimming time. All rats were identical at this point, but we have divided them into two groups to demonstrate the lack of initial differences between the rats that would later receive MUS inactivation of dHIPP (dark bars) and those that would not (light bars). Following performance on the transfer task, rats received surgery, 1 wk of recovery separated panel B from panel C. (C) Retention, tested with the transfer task, was relatively good for control rats (light bar), but rats with inactivated hippocampi (dark bar) spent relatively little time in the appropriate quadrant. (D) Subsequent retraining with the platform in a new spatial location, again tested with a subsequent transfer task, went quickly for control rats (light bar). Following hippocampal inactivation (dark bar), however, rats did not learn to spend time in the appropriate quadrant.

Materials and Methods

Subjects

All methods conform to Brandeis University’s animal use and care guidelines. Studies were performed using female Long-Evans rats (Charles River Laboratories) with body weights of 250–320 g at the time of surgery; while female rats condition somewhat more consistently than male rats (Chambers et al. 1981), this fact in no way interferes with our results. Subjects were housed individually in an animal facility with a 12 h light/12 h dark cycle (lights out at 7 p.m.) with ad libitum access to standard chow. Experiments were run in the afternoon in squads of four.

Surgery

Rats were anesthetized via intraperitoneal (ip) injection (ketamine, 100 mg/kg; xylazine, 5 mg/kg; acepromazine, 1 mg/kg) and given update doses of ketamine ip as needed to maintain deep anesthesia, determined by breathing rate and response to foot pinch. Subjects were placed in a stereotaxic frame, the scalp was incised and retracted, and the skull was leveled. Small holes were drilled into the skull for bilateral placement of guide cannulae (23-gauge, 15 mm in length) in the dorsal hippocampus (dHIPP, coordinates relative to bregma: AP −3.8 mm, ML ±2.5 mm, DV −1.8 mm from dura; n = 28); muscimol infused at these single sites is sufficient to impair performance of a spatial learning task (Moser and Moser 1998). A subset of animals also re-
ceived guide cannulae placed in the taste-responsive region (Ko-sar et al. 1986; Katz et al. 2001) of insular cortex (GC, AP +1.4 mm, ML ± 5 mm, DV − 2.5 mm from dura; n = 9). Cannulae were anchored to the skull with four 0-80 screws and dental acrylic. Stainless steel stylets (30-gauge, 15 mm in length) were inserted into the guide cannulae to ensure patency.

Training protocol

Following 1 wk of recovery from surgery, rats began a training regimen wherein each was familiarized to one tantast (taste-fam) and conditioned to both that tantast and a novel tantast (taste-nov). First, rats were acclimated to taking fluids in a testing chamber with a single lick spout in one end. After adaptation sessions, they were familiarized with taste-fam; such repeated taste pre-exposures lead to latent inhibition (LI)—later difficulty associating the taste with sickness. Next they were trained, using two pairings of each taste with LiCl-induced gastric distress. The learning was evaluated in taste-alone tests: One taste was presented at the single lick spout, the rats’ desire to drink was recorded, and no LiCl was delivered.

In more detail, the protocol was as follows (as schematized in Table 1):

Days 1–4 (acclimation)

Rats were trained to obtain a daily fluid ration in two drinking periods per day. Afternoon 30-min sessions took place shortly after noon, in experimental chambers located in a sound-attenuating enclosure. For these sessions, rats were given 30 min to consume 10 mL of water from a single lick spout, after which they were returned to their home cages in the animal facility. At midnight, animals received 15 min of ad lib access to water in their home cages (this midnight session continued through all subsequent days). The midnight feeding, delivered during the rats’ active period, ensured that rats never went more than ~12 h without water, but also ensured that they were very thirsty for the actual sessions.

Days 5–8 (familiarization)

Rats received either VEH or MUS into bilateral dHIPP 30 min prior to being moved to the experimental chamber for their afternoon drinking sessions; for these sessions, rats were given 30 min to consume 10 mL of taste-fam.

Days 9–12 (conditioning)

Rats received bilateral infusions of either VEH or MUS into dHIPP 30 min prior to being moved to the experimental chambers for their afternoon drinking sessions. For these sessions, they were given 30 min access to 10 mL of either taste-fam or taste-nov at the single lick spout. Immediately following this session, animals received 10 mL/kg of 0.15 M LiCl via ip injection in their home cages; this injection was sufficient to induce visual evidence of gastric distress. In total there were two conditioning sessions for each tantast, delivered in alternation (i.e., taste-nov on Day 9, taste-fam on Day 10, taste-nov on Day 11, and taste-fam on Day 12).

Days 13–14 (testing)

Rats were returned to the experimental chambers for their afternoon drinking sessions, where they were given 30 min ad lib access (i.e., in these sessions the rats could conceivably drink >10 mL) to either taste-fam or taste-nov at the single lick spout. One taste was tested in each session.

Intra-cranial infusions

The infusion procedure was adapted from Holt and Maren (1999). Stylets were removed and 30-gauge infusion cannulae (15 mm long for dHIPP infusion; 17 mm long for GC infusion, for final DV coordinate −4.5 mm) connected via PE-10 tubing to 10-µL Hamilton syringes mounted in a dual syringe pump (Harvard Apparatus) were inserted into the guide cannulae. 0.5 µL of either 1 µg/µL muscimol (MUS: 5-(Aminomethyl)-3-isoxazolol; Sigma) in 0.9% saline or the 0.9% saline vehicle (VEH) alone was infused bilaterally over 60 sec; these infusions hyperpolarize, and thus effectively cease the firing of, virtually any neurons in the vicinity with GABA synapses (Krupa et al. 1999). During infusions, rats sat in the experimenter’s lap and were discouraged from grooming or excessive motion. Infusion cannulae were left in place for 2 min post-infusion to ensure that the liquid had diffused from the injection site, and the stylets were replaced.

Rats were randomly assigned to groups that differed with regard to hippocampal infusant, muscimol (MUS) or vehicle (VEH). Groups, as summarized in Table 2, included the following: Some received MUS infusions during the familiarization phase only, while others received MUS infusions during conditioning sessions only (Fig. 2); in addition, some received MUS during both familiarization and conditioning sessions (Fig. 4). Two infusion control groups were run: One received VEH infusions before familiarization and conditioning sessions, and one received no infusions at all.

Finally, sham-conditioned (taste paired with ip saline injections) controls allowed us to examine drinking in rats that had been subjected to otherwise identical training.

Stimuli

The tantasts used were 0.1 M NaCl and 0.1 M sucrose, chosen to facilitate comparison with electrophysiological studies (e.g., Katz et al. 2001). Pilot studies revealed that rats preferred these concentrations of sucrose and NaCl over water (although not precisely the same degree) in a two-bottle test. Each tantast served as taste-fam for half of the subjects in each group; while taste was counterbalanced, this variable had no impact on the results, and the data from rats for which NaCl served as taste-fam were combined with the data from rats in the same group for which sucrose served as taste-fam.

CTA training with cortical inactivation

One group of rats, including a subset of rats from the dHIPP study, were subsequently run in a simpler CTA paradigm in the same apparatus, to test that our infusion and training procedures produced expected effects. The tantast used was 0.02 M citric acid (which was not preferred over water, but which was not highly unpalatable). The procedures used were a subset of those used for the two-taste experiment; no familiarization sessions were run, and rats received only one conditioning and one testing session. Subjects were randomly assigned to one of two groups, differing with regard to cortical infusant, VEH or MUS. Consumption of these rats was further compared with drinking in a “pseudo-conditioned” (unpaired presentation of taste and LiCl) group.

Table 1. Day-by-day description of training

<table>
<thead>
<tr>
<th>Session number (time →)</th>
<th>Acclimation</th>
<th>Familiarization</th>
<th>Conditioning</th>
<th>Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste-fam</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Taste-nov</td>
<td>9</td>
<td>10</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Water</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

The order of conditioning and testing trials was counterbalanced, such that for half of the rats session 9 was the first taste-fam conditioning session, etc.

Quantification and analysis of CTA learning

Amount of fluid consumed in acclimation, familiarization, conditioning, and testing sessions was quantified to the nearest 0.5 mL. CTA learning could be recognized as a reduction (from baseline) in the amount of tantast consumed, and latent inhibition could be recognized in the difference between the amount of taste-fam and taste-nov consumed. The significance of any differences in consumption was evaluated using t-tests and mixed effects anovas during the single testing sessions, as described in the Results section, using the statistics package S-plus (In-...
Table 2. Infusion schedule for the five dHIPP and CTA groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Surgery</th>
<th>Familiarization (4 d)</th>
<th>Conditioning (4 d)</th>
<th>Testing (2 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (dHIPP &amp; familiarization)</td>
<td>dHIPP cannulae</td>
<td>Muscimol (MUS)</td>
<td>Vehicle (VEH)</td>
<td></td>
</tr>
<tr>
<td>Group 2 (dHIPP &amp; taste learning)</td>
<td>dHIPP cannulae</td>
<td>VEH</td>
<td>MUS</td>
<td></td>
</tr>
<tr>
<td>Group 3 (virtual lesion)</td>
<td>dHIPP cannulae</td>
<td>MUS</td>
<td>MUS</td>
<td>VEH</td>
</tr>
<tr>
<td>Group 4 (vehicle control)</td>
<td>dHIPP cannulae</td>
<td>VEH</td>
<td>MUS</td>
<td>VEH</td>
</tr>
<tr>
<td>Group 5 (unoperated control)</td>
<td>none</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Groups 1 and 2 provided data for Figure 2. Group 3 provided data for Figure 4. Groups 4 and 5 provided data for both figures. (dHIPP) Dorsal hippocampus, (CTA) conditioned taste aversion, (MUS) muscimol, (VEH) vehicle.

Morris Water Maze training with dHIPP muscimol infusion

Our water maze test was similar to that described by the Moser group (Moser and Moser 1998). Rats were trained to swim through water made opaque by the addition of white, nontoxic paint to a submerged platform in a 150-cm diameter pool surrounded by walls with large, high-contrast black and white pictures. The submerged platform was consistently placed from trial to trial for each rat (but differently placed for different rats), such that the location of the platform was learnable in relation to the distal spatial cues.

In each trial the rat was dropped into the pool, facing the wall. The starting point was selected pseudo-randomly, such that each rat eventually started at each of eight points around the perimeter in eight trials. Once in the water, the rat was given 120 sec to find the platform on its own; failing that, it was guided to the platform by hand, and allowed to rest there for 5 sec until the next trial. Latency to find the platform was noted with a timer and verified via digital recordings. Each rat received eight trials per day (two sessions of four trials, with 30 min between session) across 8 days, for a total of 32 trials.

On the day following the completion of training, rats received a single probe trial without a platform. The platform was removed from the pool, and the rat was released in a spot opposite to the previous location of the platform. The rat’s search pattern across the subsequent 20 sec was digitally recorded; the percentage of time spent in each quadrant was later coded by an undergraduate blind to condition, and the percentage of time spent in the quadrant with the platform was used as an index of learning. Following this trial, the animal was removed from the pool, the platform was replaced, and the animal was placed once more into the pool and allowed to locate the platform.

Next, the rats were surgically prepared as for the CTA experiment, and after recovery from surgery the probe trial was repeated (this trial was called the “relearning test”), 15 min following MUS (or control) infusions into dHIPP. Such infusions have been previously shown to disrupt probe trial performance (Moser and Moser 1998). Immediately following this second probe trial, the distal cues were rearranged (disrupting any of the originally learned spatial relationships), the platform was placed in the new location, and the infused rats were retrained across four more blocks of two trials. Blocks were separated by 5 min. Finally, a third probe trial (the “relearning test”) assessed whether inactivation of dHIPP interfered with new spatial learning.

Histology

Cannula placement was confirmed histologically after the experiment. Rats were deeply anaesthetized and perfused transcardially with 10% saline followed by 10% formol-saline. Brains were removed and refrigerated in 30% sucrose/10% formalin solution for several days. Coronal sections (40 µm) were cut on a cryostat and mounted to slides. Sections were stained with cresyl violet to visualize cannula tracks.

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