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Brief Communication

Inactivation of the anterior cingulate cortex blocks expression of remote, but not recent, conditioned taste aversion memory

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Previous studies have shown that medial prefrontal cortical regions, such as the anterior cingulate cortex (ACC), play a key role in the expression of remote spatial and contextual memory. To evaluate whether this role is conserved in hippocampal-independent tasks we trained mice in the conditioned taste aversion (CTA) paradigm. Lidocaine-induced inactivation of the ACC blocked the expression of CTA tested one month (remote), but not one day (recent), after conditioning with either a weak or strong unconditioned stimulus (US). These data suggest that the ACC may play a conserved role in remote memory, regardless of memory strength or content.

The hippocampus plays an essential role in the formation of spatial, contextual, and trace conditioning memories (Morris et al. 2003; Eichenbaum 2004; Kesner and Hopkins 2006). However, as these memories mature they may become additionally (or exclusively) dependent on extra-hippocampal structures, including the medial prefrontal cortex (mPFC) (Frankland and Bontempi 2005; Squire and Bayley 2007). For example, lesions of the mPFC (including the ACC and prelimbic cortex) disrupt expression of month-old (remote), but not day-old (recent), trace eye-blink conditioning memories (Takehara et al. 2003). Similarly, pharmacological inactivation of the ACC specifically disrupts expression of remote, but not recent, contextual fear (Frankland et al. 2004), five-arm discrimination (Maviel et al. 2004), and water maze (Teixeira et al. 2006) memories. These studies are consistent with the idea that while the hippocampus may play an essential role in the expression of recent memory (e.g., by integrating information stored in distributed cortical modules representing various features of an experience), regions such as the ACC might assume a similar integrative function at more remote time points (Frankland and Bontempi 2005). However, whether this role extends to memories that do not initially depend on the hippocampus is not known.

To address this issue we used a conditioned taste aversion (CTA) paradigm (Garcia et al. 1955). In this task mice learn to avoid a novel taste (e.g., saccharin-flavored water [SACC]) that is paired with a malaise-inducing agent (such as lithium chloride [LiCl]). When later given a choice between SACC and water, mice that were previously treated with LiCl will avoid the SACC and drink the water. In these and subsequent experiments, C57B6/129 mice (F1 offspring from a cross between C57B6Tac and 129Svev, Taconic Farms) were water-restricted for the duration of the experiment. During the initial habituation phase, mice were placed in a cage and given access to two water bottles. Over the course of 5 d, mice learned to concentrate their daily water consumption in these 30 min sessions. On the training day, the water bottles were replaced by a single bottle containing SACC

(0.1%) and the mice were allowed to drink for 30 min. Forty minutes later mice were treated with LiCl (0.15 M, 2% body weight, i.p.; $N = 10$) or PBS ($N = 10$). In the choice test 1 d later, mice were placed back into the same cage and had access to bottles containing either water or SACC. LiCl-treated mice exhibited a preference for water over SACC ($t_{(9)} = 5.09$, $P < 0.01$) (Fig. 1A). In contrast, PBS-treated mice drank roughly equal amounts of SACC and water in the choice test ($t_{(9)} = -0.36$, $P > 0.05$) (Fig. 1B), similar to naïve mice ($N = 20$) given a free choice between SACC and water ($t_{(19)} = -1.53$, $P > 0.05$) (Fig. 1C). These data indicate that a single SACC-LiCl pairing is sufficient to establish CTA in C57B6/129 mice.

Hippocampal manipulations have no effect or only a mild effect on CTA memory formation, suggesting that the hippocampus does not play an essential role in CTA (Yamamoto and Fujimoto 1991; Josselyn et al. 2004). To verify that CTA memory expression does not depend on the dorsal hippocampus (dHPC) using our protocol, cannulae were implanted bilaterally above the dHPC using standard stereotaxic procedures (Teixeira et al. 2006). At least 1 wk following surgery, mice were habituated (as above) and conditioned (0.1% SACC paired with 0.15 M LiCl). CTA memory was assessed one day later in a choice test. Ten minutes before this test, mice received an infusion of PBS ($N = 8$) or lidocaine ($N = 9$; 0.5 μ L, 4% w/v) into the dHPC. Lidocaine is a sodium channel blocker and therefore transiently suppresses both excitatory and inhibitory neural activity (Sandkuhler et al. 1987). In the choice test, both PBS-treated and lidocaine-treated mice consumed significantly more water compared with SACC (PBS: $t_{(7)} = 7.13$, $P < 0.01$; lidocaine: $t_{(8)} = 5.98$, $P < 0.01$), suggesting that inactivation of the dHPC does not affect the expression of CTA (Fig. 1D,E). In contrast, using identical procedures we previously showed that intra-dHPC lidocaine infusions block the expression of water maze memories in C57B6/129 mice (Teixeira et al. 2006).

Lidocaine-induced inactivation of the ACC blocks the expression of remote, but not recent, spatial (water maze [Teixeira et al. 2006] or five-arm discrimination [Maviel et al. 2004]), and contextual (Frankland et al. 2004) memory. Therefore, we next tested whether the ACC plays a similar role in the expression of remote CTA memory. Cannulae were implanted in the ACC

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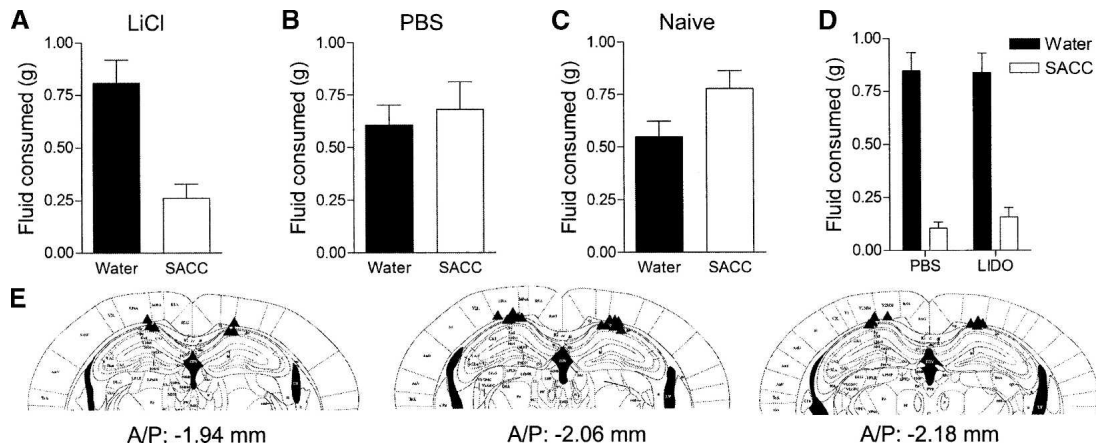


Figure 1. (A–C) CTA in normal mice. Mean consumption (\pm SEM) of water (black) and SACC (white) in the choice test is shown for three groups of mice: (A) mice where SACC was paired with 0.15 M LiCl during training; (B) mice where SACC was paired with PBS during training; and (C) naïve mice (habituated as above and then given a choice test). (D) Inactivation of the dHPC does not block expression of CTA. During training SACC was paired with 0.15 M LiCl. Mean consumption (\pm SEM) of water (black) and SACC (white) in the choice test 1 d following training is shown for mice receiving pre-test intra-dHPC infusions of either PBS or lidocaine. (E) Representative cannula placements (triangles) in the dHPC.

(Teixeira et al. 2006), and 1 wk later mice were habituated and then conditioned (0.1% SACC paired with 0.15 M LiCl). CTA was then assessed in separate groups of mice either 1 d (recent test) or 30 d (remote test) later. Ten minutes prior to this test, mice received an infusion of PBS or lidocaine (0.5 μ L; 4% w/v) into the ACC. In the choice test, PBS-treated mice consumed significantly more water than SACC at both the recent ($t_{(15)} = 2.61$, $P < 0.05$, $N = 16$) and remote ($t_{(13)} = 2.42$, $P < 0.05$, $N = 14$) time points, indicating that a single SACC-LiCl pairing is sufficient to produce a CTA that lasts as long as 1 mo. In contrast, intra-ACC infusion of lidocaine blocked CTA expression in the remote test ($t_{(11)} = 0.16$, $P > 0.05$, $N = 12$), but spared CTA in the recent test ($t_{(13)} = 2.94$, $P < 0.05$, $N = 14$) (Fig. 2A,B). Importantly, lidocaine infusions had no effect on overall liquid consumption at either

retention delay (no main effect of infusion [$F_{(1,52)} = 2.59$, $P > 0.05$] nor infusion \times delay interaction: [$F_{(1,52)} = 0.75$, $P > 0.05$] (Fig. 2C), indicating that ACC inactivation did not have any nonspecific effects on drinking behavior. The effects of ACC inactivation on remote CTA memory parallel those observed in contextual fear conditioning (Frankland et al. 2004) and water maze (Teixeira et al. 2006).

Increasing the intensity of the US should increase the strength of the CTA memory (Garcia et al. 1955). Therefore, in the next series of experiments we evaluated whether lidocaine-induced inactivation of the ACC would also block the expression of a remote CTA following training using a stronger protocol. As before, mice were implanted with cannulae in the ACC, and, 1 wk later, habituated and then conditioned. On the conditioning

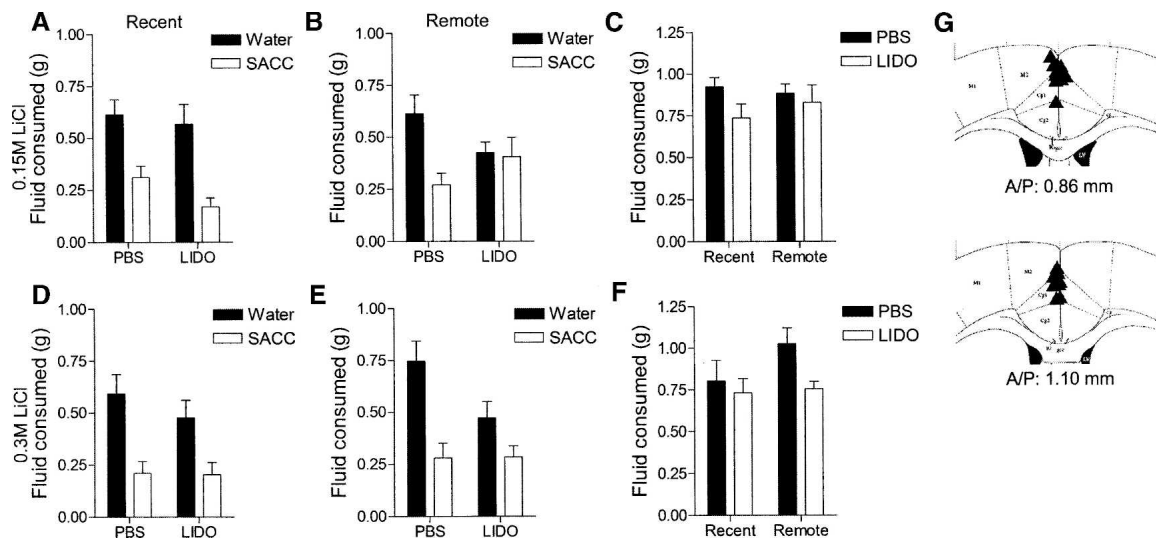


Figure 2. Inactivation of the ACC blocks expression of remote CTA memory following training with a weak or strong US. (A–C) Conditioning with weak US (0.15 M LiCl). During training SACC was paired with 0.15 M LiCl, and mice were given a choice test either 1 d (recent) (A) or 30 d (remote) (B) later. For each choice test, mean consumption (\pm SEM) of water (black) and SACC (white) is shown. (C) The total fluid consumed (water + SACC) is shown for PBS-treated (black) and lidocaine-treated (white) mice in the recent and remote choice tests. (D–F) Conditioning with strong US (0.3 M LiCl). During training SACC was paired with 0.3 M LiCl, and mice were given a choice test either 1 d (recent) (D) or 30 d (remote) (E) later. For each choice test, mean consumption (\pm SEM) of water (black) and SACC (white) is shown. (F) The total fluid consumed (water + SACC) for PBS-treated (black) and lidocaine-treated (white) mice in the recent and remote choice tests. (G) Representative cannula placements (triangles) in the ACC.

day, SACC was paired with 0.3 M LiCl (2% body weight, i.p.). Either 1 d (recent test) or 30 d (remote test) later, mice were given a choice test. Ten minutes prior to this test, mice received an infusion of PBS or lidocaine (0.5 μ l; 4% w/v) into the ACC. In the choice test, PBS-treated mice consumed more water than SACC at both the recent ($t_{(12)} = 3.85$, $P < 0.01$, $N = 13$) and remote ($t_{(15)} = 3.33$, $P < 0.01$, $N = 16$) time points. In contrast, intra-ACC infusion of lidocaine blocked CTA expression in the remote ($t_{(11)} = 1.46$, $P > 0.05$, $N = 12$), but not the recent ($t_{(12)} = 2.56$, $P < 0.05$, $N = 13$), test (Fig. 2D,E). While there was a trend for reduced liquid consumption in the lidocaine-treated mice in the remote group, this effect was not statistically reliable: an ANOVA revealed no main effect of infusion ($F_{(1,50)} = 3.20$, $P > 0.05$) nor infusion \times delay interaction ($F_{(1,50)} = 1.08$, $P > 0.05$) (Fig. 2F). Representative cannula placements for these experiments are shown in Figure 2G. Similar to our first study, these results indicate that lidocaine-induced inactivation of the ACC blocks the expression of remote CTA memory, even following training with a higher intensity US.

Comparison of the two experiments reveals that water preference was generally (but not significantly) stronger in control mice trained with 0.3 M LiCl ($F_{(1,55)} = 1.80$, $P = 0.18$) (Fig. 3A). This lack of statistical reliability raises the possibility that increasing the intensity of the US did not necessarily induce a stronger CTA memory. However, when the PBS-treated mice from the two remote groups were retested 1 d later, extinction was only evident in the weakly-conditioned group. Whereas the mice conditioned with 0.3 M LiCl continued to express a preference for water over SACC ($t_{(15)} = 2.83$, $P < 0.05$) (Fig. 3B), mice conditioned with 0.15 M LiCl consumed similar quantities of water and SACC ($t_{(13)} = 0.72$, $P > 0.05$) (Fig. 3C). Resistance to extinction in the group of mice conditioned with 0.3 M LiCl is consistent with the interpretation that CTA memory was stronger in this group. We further evaluated the efficacy of the LiCl treatment in an additional experiment. Mice were treated with either

PBS, 0.15 M or 0.3 M LiCl (all $N = 7$) and then immediately placed in an open field ($44 \times 44 \times 19$ cm), and their activity was monitored for 24 min. Exploration declined as a function of time ($F_{(3,54)} = 86.55$, $P < 0.01$), indicating that all groups of mice habituated to the open field (Fig. 3D). Most importantly, LiCl reduced overall levels of exploration in a dose-dependent manner (Fig. 3E) ($F_{(1,18)} = 10.17$, $P < 0.01$; Newman-Keuls post-hoc analyses indicate that $PBS_{\text{total activity}} > 0.15 M_{\text{total activity}} > 0.3 M_{\text{total activity}}$, $P_s < 0.05$). The more pronounced reduction in exploration in the group of mice treated with 0.3 M LiCl, suggests this higher dose of LiCl is more efficacious in inducing malaise in C57B6/129 mice.

Lesion, electrophysiological, and immediate early gene mapping studies have helped to establish that CTA engages a network of regions including the nucleus of the solitary tract, the parabrachial nucleus of the pons, the medial thalamus, the amygdala, and the insular cortex (Houpt et al. 1994; Yamamoto et al. 1994; Lamprecht and Dudai 1995; Welzl et al. 2001; Bermudez-Rattoni 2004; Bernstein and Koh 2007). Whereas previous studies have primarily been concerned with identifying circuits involved in the encoding and initial recall of CTA memories, we have instead focused on CTA expression at remote time points following training. Our primary observation is that inactivation of the ACC blocks the expression of remote (month-old), but not recent (day-old), CTA memory.

Similar to contextual and spatial memory, these data indicate that expression of a CTA memory at remote retention delays necessarily involves the ACC. The time-dependent recruitment of the ACC into circuits supporting this and other forms of memory might reflect one of two processes. First, it might reflect a role for the ACC in effortful recall in that older memories might be harder to access and therefore likelier to engage the ACC (Rudy et al. 2005). Our experimental design addresses this issue since we manipulated both memory age and strength. Whereas an effortful recall account would predict that ACC inactivation

would affect weak (rather than strong) memory, regardless of age, we found the opposite pattern: ACC inactivation disrupted remote (rather than recent) memory, regardless of strength. While we did not exhaustively vary memory strength in these studies, the data nonetheless do not support an effortful recall account.

A second (but not necessarily mutually exclusive) possibility is that the recruitment of the ACC reflects a time-dependent process of memory reorganization (Frankland and Bontempi 2005). That the ACC seems to play a similar role in the expression of remote memory in contextual and spatial tasks suggests that its role in remote memory does not depend on (1) the content of learning and (2) whether or not the task is initially hippocampal-dependent. This last observation is perhaps the most surprising since it implies that time-dependent systems-wide reorganization might be a general process that is conserved across memory systems.

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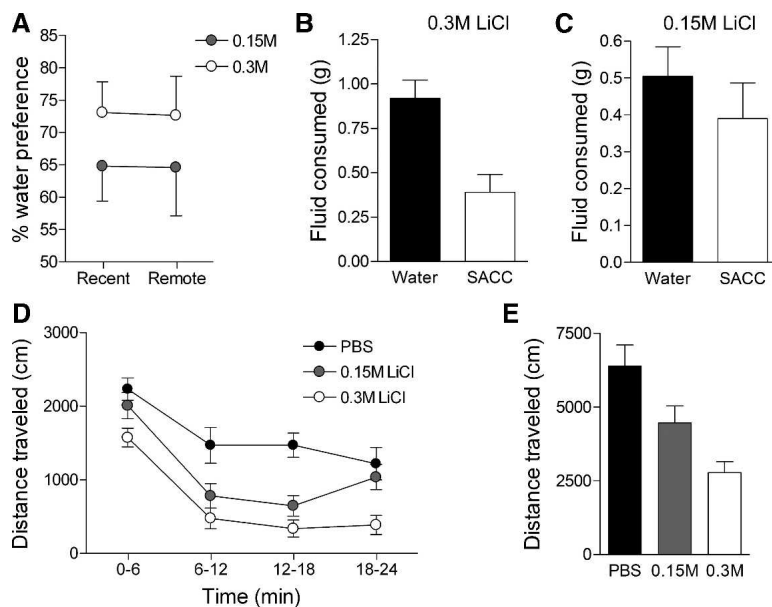


Figure 3. (A) Water preference in recent and remote choice tests in PBS-treated mice trained with either 0.15 M (gray circles) or 0.3 M (black circles) LiCl. (B–C) When retested 1 d later the mice trained with 0.3 M LiCl (B) continued to prefer water (black) over SACC (white), whereas the mice trained with 0.15 M LiCl (C) consumed roughly equal amounts of both. (D–E) Effects of LiCl treatment of activity in open field. (D) Distance traveled in open field is plotted in 6 min blocks for mice treated with PBS (black), 0.15 M (gray), or 0.3 M (white) LiCl. (E) Total distance traveled in 24 min test is shown for mice treated with PBS (black), 0.15 M (gray), or 0.3 M (white) LiCl.

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