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Memory for reward location is enhanced even though acetylcholine efflux within the amygdala is impaired in rats with damage to the diencephalon produced by thiamine deficiency

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Abstract

A rodent model of diencephalic amnesia produced by thiamine deficiency (pyrithiamine-induced thiamine deficiency [PTD]) was implemented to assess both changes in behavior and acetylcholine (ACh) efflux in the amygdala across four training sessions of a delayed alternation task. Two versions of the delayed alternation task were used. In one version, when a correct alternation was made a unique reward was paired with each spatial location ([left arm—chocolate milk] or [right arm—rat chow]). This paradigm is called the differential outcomes procedure (DOP). In the second version of the task, correct delayed alternation resulted in the same rewards but randomized across location (nondifferential outcomes procedure [NOP]). The PTD rats were impaired on the first session of delayed alternation testing. However, both control and PTD rats using the DOP performed significantly better on delayed alternation than rats trained with the NOP. This effect was driven primarily by the PTD rats in the DOP condition outperforming all other groups on sessions 2–4. Although ACh efflux in the amygdala increased during delayed alternation testing in all groups, the NOP-trained rats had a greater rise in training-related ACh release in the post-training period. This suggests that increased amygdalar cholinergic activation is more critical for processing spatial information than episodic reward information. These data correspond with the idea that cholinergic activation of the amygdala promotes processing in other neural systems.

Keywords

Acetylcholine; Amygdala; Memory; Delayed Alternation; Rat

The hippocampus and amygdala are independent, but interconnected components of the limbic system that are known to be vital for memory performance. These two structures form two memory systems that can be functionally distinguished in terms of the different kinds of information they process. Specifically, in rodents the hippocampus is critical for what has been termed allocentric learning (the associations between viewpoint-independent cues and locations); whereas the amygdala is important for connecting discrete environmental cues to biological relevant stimuli (e.g., food, danger). Damage to either component can sometimes improve learning of tasks handled by the other brain region (Ferbinteanu & McDonald,

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2001; Matthews & Best, 1995; McDonald & White, 1995). This suggests that these two regions have considerable independence and some of their interactions can be characterized as competitive (Gold, 2004). However, in the intact brain, there is evidence that these two regions can cooperate (McIntyre, Marriott & Gold, 2003). In particular, increased amygdala activation has been associated with enhanced hippocampal-dependent spatial memory.

The cognitive strategy employed by a subject on a particular task is accompanied by changes in neurochemical profile in different brain regions during learning (Chang & Gold 2004; Pych, Chang, Colon-Rivera & Gold, 2005; Vetreno, Anzalone & Savage, 2008). Manipulating the type of discrete cues available to an organism can determine the type of associations formed and potentially the brain region(s) needed for successful performance. For example, superimposing a Pavlovian differential outcomes procedure (DOP), which pairs to-be-remembered events with distinct rewards, onto a traditional hippocampal-dependent task can enhance short-term episodic memory functioning in amnesic patients (Hochhalter, Sweeney, Savage, Bakke & Overmier, 2000) and brain-damaged rats (Savage & Langlais, 1995; Savage, Buzzetti & Ramirez, 2004). The use of the DOP can switch a task from being dependent on the hippocampus to being dependent on an intact amygdala as reward information can be used in the cognitive strategy to solve the task (for a review see Savage & Ramos, 2009). The on-line assessment of neurochemical changes during and after performance of such a modified memory task that includes both episodic location and episodic reward information has not been done to date. In particular, we do not know the modulatory role of the cholinergic signal in the amygdala for either type of memory processing.

In the current experiment we assessed amygdalar function in normal and amnesic rats during training on the hippocampal-dependent maze task of delayed alternation (Rawlins & Olton, 1982; Aggleton, Neave, Nagle & Sahgal, 1995). We measured acetylcholine (ACh) release in the amygdala while rats were trained on delayed alternation with either the DOP or a control procedure in which reward-location pairing was random (Nondifferential Outcomes Procedure=NOP). In the NOP condition, a subject must retain the episodic memory of the previous location visited and use this information to guide the next behavioral response. The amnesic model was created using thiamine deficiency that causes diencephalic pathology and resultant amnesia in both humans (Wernicke-Korsakoff Syndrome [WKS]) and animals (pyrithiamine-induce thiamine deficiency [PTD-model], see Roland & Savage, 2007; Savage, Roland & Klintsova, 2007).

Thiamine plays a key role in the maintenance of normal cellular function and because of its high oxidative metabolism the brain is particularly vulnerable to thiamine deficiency (Butterworth, 1986). The rodent pyrithiamine-induced thiamine deficiency (PTD) model produces consistent bilateral lesions of the anterior, midline intralaminar, dorsolateral and posterior thalamic nuclei as well as the mammillary bodies, with more minor lesions in the pontine tegmentum and periaqueductal gray. (Langlais, Zhang & Savage, 1996; Mair, 1994). This damage in the animal model highly resembles the brain pathology seen in the human disorder of WKS (Witt, 1985). Although the extent of midline thalamic damage has been demonstrated to predict the severity of learning and memory impairment in the PTD model (Mair, Anderson, Langlais & McEntee, 1988; Mair, Knoth, Rabchenuk & Langlais, 1991), alterations in hippocampal and cortical functioning also contribute to the dysfunction observed in the model and in WKS patients (Oscar-Berman & Marinkovic, 2003; Reed et al, 2003). Our research group and others have found that after thiamine deficiency there are changes in hippocampal and cortical cholinergic function that correlate with behavioral impairment (Anzalone, Vetreno, Ramos & Savage, in press; Nakagawasai et al, 2000, Pires et al, 2001; 2005; Savage et al, 2003).

Although WKS patients and PTD-treated rats perform poorly on tasks of hippocampal function (Kopelman, Thomson, Guerrini & Marshall, 2009; Roland & Savage, 2007; Savage et al, 2003), both do have some spared cognitive functions (Kopelman, et al, 2009; Savage & Langlais, 1995; Vetreno et al, 2008). In particular, there is some evidence that they retain the ability to use Pavlovian-associated reward information to solve some tasks (Hochhalter et al., 2000; Savage & Langlais, 1995). Thus, the goal of this study was to determine the role and time course of ACh efflux in the amygdala in normal and amnesic rats when episodic reward information (DOP) or episodic spatial information (NOP) could be used to make delayed behavioral choices.

Method

Subjects

Forty-four male (3–4 months; 250–350g) Sprague-Dawley rats were used in this experiment. Initially, all subjects were pair-housed until the day of cannulae surgery, after which they were single housed. Subjects lived in a temperature controlled (68° F) vivarium that had a 12-hr light/12-hr dark cycle (light onset at 06:00h; offset at 18:00h).

PTD Treatment

Pyrithiamine treatment was similar to that previously described (Savage et al, 2007; Roland & Savage, 2009; Vetreno et al, 2008). Rats were first randomly assigned to one of the following treatments: (i) pair-fed control (PF, n = 22), or (ii) pyrithiamine-induced thiamine deficiency (PTD, n = 22) groups. Subjects in the PTD group were free-fed thiamine-deficient chow (Teklad Diets, Madison, WI) and given daily injections (0.25 mg/kg, i.p.) of pyrithiamine hydrobromide (Sigma, St. Louis, MO). On days 14–16 of treatment, signs of tonic-clonic movement of the front and hind limbs were displayed followed by generalized convulsions (seizures). Four hours after seizure onset PTD-treated rats received a large dose of thiamine (100 mg/kg, i.p.). The administration of thiamine and return to normal chow reverses the acute PTD symptoms within 8 hr. The pair-fed control (PF) subjects were fed daily thiamine deficient chow equal to the average amount consumed by the PTD treatment group (to mimic the anorexia effects) and given daily injections of thiamine hydrochloride (0.04 mg/kg, i.p.). Post-treatment all animals were provided an *ad libitum* balanced diet and were allowed to regain weight lost from treatment.

Recovery

All subjects were given 2.5 weeks to recover and return to a normal free-feeding weight. Weight was monitored daily and any animals that did not show signs of recovery, such as failure to gain weight, were euthanized with Sleep Away (1 mg/kg, i.p.; Fort Dodge Animal Health, Fort Dodge, IA)

Cannula Surgery

After the recovery period, all subjects were chronically implanted with bilateral cannulae (CMA/11, Carnegie Medicine Associates, Chelmsford, MA). All subjects were anesthetized with a Ketamine (8.25 ml)/Xylazine (1.75 ml) mixture (50 mg/kg i.p.) immediately before stereotaxic surgery. Stereotaxic coordinates were chosen from the atlas of Paxinos and Watson (1986) and are based on previous studies (see McIntyre et al, 2003). The coordinates for the amygdala were: Anterior-Posterior (AP): –3.0 mm (relative to bregma); Medial-Lateral (ML): 4.8 mm (+, – for left and right); Dorsal-Ventral (DV): –6.8 mm (from skull). Gentamicin (0.2 mg/kg, i.p., Abraxis, Schaumburg, IL) and buprenorphine (0.3 mg/kg, i.p., Reckitt Benckiser, Richmond, VA) were administered post-surgery to alleviate infection and pain. The animals were kept on a warm incubator until reflexes and muscle tone were recovered. Post-surgery,

all subjects were allowed to recover for 7 days with *ad libitum* access to food and water. The following week, 7 days prior to behavioral testing, all subjects were placed on food deprivation such that subjects were 85% of their *ad libitum* food weight by the first testing day. Five days before behavioral testing all animals were handled daily (5 min).

Behavioral testing and microdialysis

A plus-maze was used with one stem of the maze blocked off to create the T-maze used for delayed alternation. The maze was made of wood with clear Plexiglas sidewalls (12 cm high) and a painted black floor with the two goal arms of equal distance (55 cm) and the start arm a length of 66 cm. The maze was elevated 80 cm from the floor. Manually operated transparent Plexiglas guillotine doors separated the start box and the two goal boxes from the choice area. The reinforcers were Nestlé chocolate milk ® and Purina Rat chow ® mash (pellets mixed with tap water) that were delivered on a tray inserted through a slot in the correct goal box.

Subjects were habituated to the maze in a single exploratory session. After exposure to the testing room for 10 min, the rats were placed in the center of the plus-maze. All four arms were open and the rats were allowed to traverse the maze for 18 min. The day following maze habituation, testing with the delayed alternation procedure and *in vivo* microdialysis began. There were 4 sessions of delayed alternation with microdialysis. The amygdala in each hemisphere was sampled for 2 consecutive sessions. The order of which hemisphere was sampled first was counterbalanced across subjects.

On each delayed alternation testing session, subjects were transported to the testing room and placed in a microdialysis holding cage [acrylic cage (30 cm × 40 cm, depth 35 cm) with wood shavings at the bottom]. One probe (CMA/11-2 mm) was inserted into either the left or right (randomly determined) amygdala cannula. The microdialysis probe was connected to a microinfusion system and perfused continuously at a rate of 2 µl/min with artificial cerebrospinal fluid (127.6 mM NaCl, 4 mM KCl, 1.3 mM CaCl₂ dihydrate, 1.0 mM glucose, 0.9 mM MgCl₂, 0.9 mM NaH₂PO₄, and 2 mM Na₂HPO₄, brought to pH 7.0) and neostigmine bromide (500 nM). The neostigmine bromide was added to obtain consistent measurable levels of behaviorally relevant changes in ACh efflux (see McIntyre et al, 2003; Canal, Chang & Gold, 2008). There was a 60 min period of habituation in the maze room holding cage. Afterwards, dialysate samples were collected every 6 min (sample volume = 12 µl) for a period of 18 min prior to the maze task. This pre-maze phase was used to determine basal levels of ACh in awake rats.

After the 18 min baseline period, a rat was placed in the start box of the maze. On the first trial of each testing day, the rat was allowed a free choice to enter either the left or right arm of the maze and either response was rewarded. In the DOP condition, if a rat chose the left arm it was reinforced with mashed Purina chow upon approaching the left goal box. In contrast, if the rat chose the right arm it received chocolate milk upon entering the right goal box. This assignment of reward to each arm choice was consistent across trials when a correct choice was made (correct choice of the left arm=chow, correct choice of the right arm=chocolate milk). In contrast, rats in the NOP condition received a random reward presentation (either chocolate milk or mash) when a correct arm location was chosen and the rat approached the goal box. After consuming the reward (about 5 s), the rat was manually removed from the goal box and placed back into the start box. There was a 30 s delay interval between the end of the previous trial and the beginning of the next trial. The rat was expected to alternate into the opposite arm of that previously entered. A correct choice was reinforced with the corresponding reward condition (DOP, NOP). An incorrect response (repeat visit to the previous arm) was not rewarded and the rat was confined in the goal box for a 10 s time-out period before it was returned to the start box. The number of trials completed during a session was determined by each rat's pace of maze traversal, consumption time and number of time outs. As stated in the

results section, the number of trials completed during the 18 min testing period increased across sessions. During the delayed alternation, dialysate samples were collected every 6 min for a total of 18 min. At the completion of 18 min of maze training, the rat was removed from the maze and placed back into the microdialysis holding cage for an additional 18 min. Post-maze samples were collected for an additional 18 min. After the 18 min post-base period the probe was put into a standard solution (100 nM concentration of ACh and choline) to assess recovery rate. After collection, brain dialysis samples were frozen for later analysis using high performance liquid chromatography (HPLC).

Measurement of ACh output

Acetylcholine output was assayed by HPLC (Epison, BAS, West Lafayette, IN) that included an ion-exchange microbore analytical column (BAS, MR-8904), a microbore ACh/choline immobilized enzyme reactor containing acetylcholinesterase and choline oxidase (BAS MF-8903), an auxiliary electrode with a radical flow electrochemical thin-layer cell and 13 mm thin layer gasket, a wired enzyme electrode kit (a redox polymer film containing horseradish peroxidase coated in the surface of a 3 mm glassy carbon working electrode), and a low dispersion injected value with a 10 μ l polyetheretheketone loop. The mobile phase was a 50 mM dibasic potassium phosphate buffer (pH = 8.5) containing ProClin (BAS, CF-2150). The mobile phase was delivered at a rate of 140 μ l/min by a PM-91 pump (BAS). The detection level was in the 10 femtomole range. Acetylcholine standards (5 μ l of 20 and 100 nM of ACh + Ch) were injected before and after samples to verify detection stability.

Histology

At the conclusion of behavioral testing, animals were euthanized with 0.5 mg/kg, i.p. injection of Sleep-Away (26% sodium pentobarbital; 7.8% isopropyl alcohol, 20.7% propylene glycol; Fort Dodge Animal Health, Fort Dodge, IA). Their brains were removed, post-fixed in a 10% formalin solution for at least 72 h and then transferred to a 30% sucrose solution. Coronal sections were cut (60 μ m thick) on a sliding microtome (Sm2000r; Lecia Instruments, Germany) from the level of the anterior commissure to the level of the posterior pontine tegmentum. Every 4th section was stained with cresyl violet for assessment of thalamic damage and amygdalar cannulae location. Only animals with both bilateral cannulae located in the mid to lateral amygdala were included in the statistical analyses.

Statistics

The mean percent alternation and total number of arm entries during delayed alternation were analyzed with a mixed model ANOVA with two between-subjects factors (Group: PF, PTD; Reward Condition: DOP, NOP) and one within-subjects factor (Session: 1–4). The neurochemical microdialysis/HPLC data was analyzed with a mixed model ANOVA with two between-subjects factors (Group; Reward) and three within-subjects factors (Session: 1–4; Phase: Baseline, Maze, After; Sample time: 1–3).

Results

Histology

Figure 1 shows the standard PTD-induced thalamic pathology (A) and a PF normal thalamus (B) for comparison. The majority of PTD-treated rats had a midline thalamic lesion that encompassed the centromedian, paracentral, ventral lateral and posterior thalamic nuclei (see arrows). Panel C displays a prototypical amygdala cannula placement. The majority (over 90%) of the two millimeter probe was within the lateral amygdala. In some instances, the dorsal portion of the probe was in the striatum or the ventral tip extended slightly into the cortex (less than 10%). A number of subjects had misplaced cannulae that were located outside the

amygdala or had less than 90% of the probe within the amygdala. The final subject number per group was: PF-DOP=7; PF-NOP=7; PTD-DOP=6; PTD-NOP=7.

Behavior

The mean number of arm entries across sessions did not differ between Group (PF vs. PTD) or Reward (DOP, NOP) conditions (both $F's < 1$, $p's > .2$). Thus, PTD treatment did not significantly alter locomotor activity levels. However, the mean number of arm entries increased across sessions in both PTD and PF rats ($F[3, 69]=50.94$, $p<.01$) from a mean of $13.04(\pm 0.57 \text{ SEM})$ during session one to a mean of $20.01(\pm 0.62)$ on session four.

Figure 2 displays behavioral accuracy on the delayed alternation task. Regardless of reward condition (DOP, NOP), the PTD rats had lower alternation scores than the PF rats on the first session of delayed alternation training (Group X Session interaction: $F[3, 69]=2.73$, $p=.05$, *post hoc* t-test, $p<.01$). However, rats trained with the DOP had higher accuracy scores than rats trained in the NOP condition (main effect of Reward: $F[1, 24]=9.06$, $p<.01$). This effect was driven primarily by the PTD rats: PTD-treated rats trained with the DOP had significantly higher behavioral performance than those trained with the NOP on sessions 2 through 4 (Reward Condition X Session interaction: $F[3, 33]=4.73$, $p<.01$). Although the performance of PF rats trained with the DOP was slightly higher than PF rats trained with the NOP, the effect was not significant ($p>.10$).

Neurochemistry

As shown in Figure 3-A, relative to baseline conditions, all rats showed an increase in amygdalar ACh efflux during maze testing that persisted into the post- maze period (main effect of Phase: $F[2, 46]=58.87$, $p<.01$). There was no change in amygdalar ACh efflux as a function of session (all $F's < 1.3$, $p's > .20$). Thus, neither repeated sampling or repeated training significantly changed ACh levels in the amygdala.

Regardless of reward condition (DOP, NOP), PTD rats had a lower ACh efflux than the PF rats during the initial post-maze (A1) testing period (Group X Phase X Sample interaction: $F[4, 92]=3.02$, $p<.05$, *post hoc* t-test, $p<.05$ [see Figure 3-B]). However, rats trained with the NOP rule had a higher magnitude of ACh efflux in the amygdala during maze testing, but significantly so in the post-maze period compared to rats trained in the DOP condition (main effect of Reward Condition: $F[1, 23]=4.34$, $p<.05$; Reward Condition X Phase interaction: $F[2, 46]=3.27$, $p<.05$). No other main effects or interactions were significant.

Discussion

The present study provides further insight into the nature of the involvement of the amygdala in what has been traditionally labeled hippocampal-dependent behavior. There are three main findings from this study: (1) Rats with diencephalic lesions induced by thiamine deficiency have a greater tendency to use episodic reward information than control rats to solve a short-term spatial memory task; (2) Amygdalar ACh release significantly increases when rats perform a delayed alternation task. However, the data do not support the hypothesis that pairing unique rewards with specific locations would produce a greater amygdala ACh release. Actually, the greatest rise in amygdalar ACh release occurs in the NOP condition, which does not involve unique reward associations; (3) Diencephalic lesions do not result in greater activation of the amygdala, as measured by ACh efflux. In contrast, during the initial post-training period, control rats (PF) have a more persistent increase in amygdalar ACh level than PTD rats. The implications that these findings have in regards to the role of the amygdala in memory processing are addressed below.

Previous research has shown that PTD rats sustain damage to the anterior and midline thalamus as well as the mammillary bodies, and they are impaired at using spatial cues (Anzalone et al, in press; Langlais, Mandel & Mair, 1992; Langlais & Savage, 1995). In addition, there is a selective loss (25–30%) of cholinergic neurons in the medial septum/diagonal band that project to the hippocampus and on hippocampal-dependent tasks there is impaired hippocampal ACh efflux (>50% decrease relative to PF rats) that correlates with behavioral impairment in PTD-treated rats (Anzalone et al, in press; Roland & Savage, 2007; 2009). In contrast, the number of cholinergic neurons in the nucleus basalis magnocellularis that project the amygdala are not significantly decreased ($\approx 10\%$ reduction) and although spontaneous alternation performance was impaired, ACh efflux in the amygdala was normal in PTD rats (Savage et al, 2007). Furthermore, key structures in the reward pathway are relatively spared in the PTD model: There is no visible cytopathology in the nucleus accumbens (Irle & Markowitsch, 1982), amygdala (Mair et al, 1991) or the prefrontal infralimbic cortex (Langlais & Zhang, 1997). However, it is not known whether the structures in the reward pathway are functionally altered after thiamine deficiency. Similar to the hippocampal dysfunction observed in the PTD model, there could be a functional impairment of ACh efflux that is revealed during a cognitive challenge in those brain regions. However, functional alterations in the prefrontal cortex or nucleus accumbens have not been assessed after thiamine deficiency. In the current study, there was a significant--yet modest--reduction in amygdalar ACh efflux ($\approx 15\%$) in the PTD rats, relative to PF rats, during the immediate post-maze period. However, this effect occurred regardless of whether the rats were trained with the unique reward-location pairings or undifferentiated reward. Thus, PTD rats appear to have an intact reward system that permits them to appropriately respond to reward contingencies.

Similar to rats with lesions of the hippocampus (see McDonald & White, 1993; Matthews & Best, 1995), PTD rats also show a preference for non-hippocampal dependent stimulus features such as reward cues (Savage & Langlais, 1995) and response strategies (Vetreno et al, 2008). Such findings suggest that processing between different neural systems may compete for control over learning (type of associations formed). Although damage can decrease the contributions of a given memory system, it can result in enhanced processing -- and therefore improved learning-- in a different memory system (Gold, 2004). This was demonstrated in the later portion of the learning curve in the present study: PTD rats trained with the DOP have the best performance on sessions two through four. We believe this occurs because the dysregulation of the extended hippocampal system in PTD rats inhibits them from using spatial cues and thus reward cues become more distinct.

Although there was no direct test determining whether rats used amygdalar-based strategies when trained with the DOP, lesion studies (Ramirez & Savage, 2008; see also Blundell, Hill & Killcross, 2003) and temporary inactivation (Savage, Koch & Ramirez, 2007) of the amygdala selectively impairs performance on tasks that embed the DOP, while performance on tasks with the NOP is unaffected. Thus, inactivation and lesion studies suggest that the acquisition and later expression of the improvement in learning/memory by the DOP is dependent on an intact amygdala. In the current study we chose a delay interval (30 s) that created a taxing memory load for rats in order to reveal the benefits of the DOP enhancing acquisition. However, the effect of DOP was primarily seen in the PTD rats. This has been reported in a previous study that used a spatial maze version of the task (Savage & Langlais, 1995). These results suggest that on tasks that have a strong spatial component, such as maze location, intact rats do not display a significant tendency to use reward contingency information.

Of interest, the current study revealed that reward anticipation or expectancies produced by pairing specific arm locations with unique rewards does not selectively enhance amygdalar ACh release. In contrast, we see the greatest rise in amygdalar ACh efflux when locations have

no unique reward predictions (NOP condition). It appears that ACh efflux in the amygdala does not selectively correspond to reward contingencies, but rather has a greater role in processing of spatial information associated with general reward. Previous research has shown that ACh release in the amygdala positively correlates with performance on a hippocampal-dependent task (McIntyre et al, 2003). Furthermore, post-training increases in cholinergic tone within the amygdala can enhance later memory processing, whereas decreases in cholinergic tone can impair processing in other neural systems -- in particular the hippocampus (Gold, 2004; McGaugh, 2004).

A temporary reduction in cholinergic tone throughout the brain can be achieved by systemic administration of the muscarinic ACh receptor antagonist scopolamine hydro-bromide. Administration of this drug can reveal the importance of ACh functioning for certain behaviors. In an earlier paper, we demonstrated that the performance of normal rats trained on an operant matching-to-position task with the NOP was more disrupted by the systemic injection of scopolamine than was the performance of rats trained on the same task that implemented the DOP (Savage & Parson, 1997). It was also found that the DOP enhanced short-term memory performance of both PTD and PF rats in a non-drug state, as well as after the administration of scopolamine (Savage et al, 1999). These data support the notion that the cholinergic system is more critical when rats rely more on spatial cues than reward cues to guide behavioral choices. This could be directly tested by site-specific injections of cholinergic antagonist into the amygdala: such a manipulation should not affect DOP performance as much as NOP performance.

It has been demonstrated that reward anticipation or expectancies produced by Pavlovian procedures, that pair discriminative cues with reward, have affective or motivational and sensory properties that mediate explicit-type instrumental behavior (for reviews see Savage & Ramos, 2009; Urcuioli, 2005). Understanding the interactions between Pavlovian-instrumental behaviors was originally investigated under the two-process theory (Mowrer, 1947; 1956). Mowrer stated that cues paired with reward have the capacity to function as conditioned stimuli (CS) and can elicit conditioned responses—related to the outcome—that then can motivate instrumental or goal-directed behavior. Thus, the two-process theory evoked a Pavlovian conditioned anticipatory state (later termed expectancy) as a mediator between the discriminative cue and the instrumental response (Overmier & Linwick, 2001). The DOP does not make the task easier, a rat must still make the same behavioral decision (i.e., alternation). Differential reward information can improve choice accuracy by supplementing or by supplanting the memory of the discriminative cue-response association (see Savage, 2001). There is evidence that differential reward expectancies, like other dominant stimuli, can overshadow the discriminative cues when behavioral decisions are made (see Urcuioli, 2001). Thus, the expectation of unique reward in the DOP produces an implicit prime (classically conditioned S–S association [sample cue-reward]) which prompts behavioral choice. This type of associative process is different from the explicit remembrance of the previous spatial location alone.

Scopolamine has been shown, under some circumstances, to produce a dissociation between explicit memory and implicit memory: Explicit memory is impaired whereas implicit memory is left relatively intact (Knopman, 1991, Nissen et al, 1987; Rammsayer et al, 2000; Saar et al, 2001; von Linstow Roloff et al, 2007). The scopolamine data provide support for the idea that the cholinergic system is more critical for memory of spatial location than reward information. This data complements the ACh efflux data of the current study: The NOP-trained rats that must rely on spatial location cues had a greater activation of amygdalar ACh efflux than the DOP-trained rats that also have reward cues.

There are other lines of evidence that suggest the neurochemical activation of the amygdala enhances the remembrance of explicit information, such as distinct spatial locations. Specifically, Packard, Cahill and McGaugh (1994) found that amphetamine infusion directly into the amygdala after training on a Morris water maze task facilitated location retention. However, inactivation of the amygdala via lidocaine injections did not influence such memory. These findings suggest that hippocampal memory processing is not directly dependent on the amygdala, but neurochemical activation of the amygdala may assist in later memory formation that is initially supported by the hippocampus. Such data support McGaugh's view (2004) that the amygdala is a modulatory structure and one of its goals is to make memory for events (hippocampal-dependent) more distinct.

It was interesting that damage to the extended hippocampal system by thiamine deficiency did facilitate behavior when reward information could be used as an episodic cue, but this did not map onto significant increases in behaviorally-relevant ACh efflux in the amygdala. Although basal levels of ACh in the amygdala (62.76 femtomole) were not different as a function of Group (PF vs. PTD: $F < 1$) and the groups did not differ in ACh efflux during the time they were solving the task, PF rats had a greater increase, relative to PTD rats, in amygdalar ACh levels in the first post-maze retention period. Previous research using a less taxing memory paradigm did not find differences between PTD and PF rats during spontaneous alternation testing and there was a nonsignificant trend for ACh levels to be higher in the amygdala in PTD rats (Savage et al., 2007). These results suggest that amygdalar ACh efflux is sensitive to hippocampal memory demand. The post-training increase in amygdalar ACh levels suggests a modulatory affect on consolidation. This data is in agreement with pharmacological studies demonstrating that cholinergic agonists infused into the amygdala immediately after training enhance memory retention (Dalmaz, Introini-Collison & McGaugh, 1993), whereas intra amygdalar infusions of cholinergic antagonists impair memory retention (Izquierdo, da Cunha, Rosat, Jerusalinsky, Ferreira & Medina, 1992).

The current data suggest that when memory demands are high, ACh efflux in the amygdala participates in the processing of spatial information. The fact that amygdalar ACh release is greater in the NOP version of the delayed alternation task supports the view that the amygdala does modulate memory formation processed by different brain regions. This is true even when inactivation and lesion studies suggest that the amygdala is not required for the formation of such memories (McGaugh, 2004; McGaugh, Cahill & Roozendaal, 1996). Thus, the cholinergic signal in the amygdala has a unique profile: It does not reach asymptotic levels under conditions of reward expectation; rather, the greatest ACh efflux occurs in the immediate post-training phase of hippocampal-dependent performance. Thus, there is not a one-to-one correspondence between improvements in amygdala-dependent behavior and increases in amygdalar ACh release.

In summary, it appears that the primary role of post-training cholinergic activation in the amygdala is to modulate memory processing in many brain systems—including the hippocampus. This has been directly shown through studies in which post-training pharmacological cholinergic receptor activation or blockade within the amygdala produced enhancement or impairment, respectively, of memory for many kinds of training (see McGaugh, 2004). The present study supports these findings by showing differential amygdalar ACh levels during and after delayed alternation as a function of brain integrity and hippocampal demand.

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Figure 1.

Panel A. Photomicrograph (4X) of the midline thalamus of a PTD rat displaying the prototypic PTD-induced lesion (arrows). Panel B. Photomicrograph (4X) of the midline thalamus of a PF rat. Panel C. An example of an acceptable probe placement in the amygdala. The placement of the 2-mm probe membrane is represented by the arrows. Although the probes were directed at the lateral amygdala, dialysis most likely included acetylcholine drawn from much of the amygdala.

Abbreviations: BLA= basolateral amygdaloid nucleus, anterior; BLP= basolateral amygdaloid nucleus, posterior; CeL=central amygdaloid nucleus, lateral; CM= central medial thalamic nucleus; Lhb= lateral habenular nucleus; MDL=mediodorsal thalamic nucleus, lateral; PC= paracentral thalamic nucleus; Po= posterior thalamic nucleus;

Figure 2.

Panel A. Differences in accuracy performance on two variations of a delayed alternation task as a function of training across four sessions. PTD rats are represented by the red circles, whereas the PF rats are represented by the black squares. Rats trained with the DOP have solid lines and those trained with the NOP have dashed lines. Panel B. The PTD rats, regardless of reward parameters, were impaired on the first session of delayed alternation, relative to PF control rats ($p < .01$). Panel C. There was a main effect of training procedure ($p < .01$). Specifically, PTD rats (red circles) trained with the DOP had higher choice accuracy than those trained in the NOP condition ($p < .05$). Although there was a trend for the same phenomenon to occur in the PF rats (black squares), the effect did not reach significance.

Figure 3.

Panel A. Increases in release of acetylcholine (ACh) in the amygdala during (M1, M2, M3) and after (A1, A2, A3) training on T-maze version of delayed alternation. Note that training resulted in increased release of ACh efflux in both PF and PTD rats ($p < .01$). However, the duration of release was reduced in PTD rats in the acute post maze period ($p < .05$). Panel B. Regardless of brain damage (PF [black square] vs. PTD [red circle]), when spatial location was not correlated with unique reward (NOP condition) there was higher ACh release in the amygdala during training (trend $p = .09$) and into the post training period ($p < .05$) than when spatial location was paired with unique rewards (DOP condition).