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Consolidation of altered associability information by amygdala central nucleus

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Abstract

The surprising omission of a reinforcer can enhance the associability of the stimuli that were present when the reward prediction error was induced, so that they more readily enter into new associations in the future. Previous research from this laboratory identified brain circuit elements critical to the enhancement of stimulus associability by the omission of an expected event and to the subsequent expression of that altered associability in more rapid learning. These elements include the amygdala, the midbrain substantia nigra, the basal forebrain substantia innominata, the dorsolateral striatum, the secondary visual cortex, and the posterior parietal cortex. Here, we found that consolidation of a surprise-enhanced associability memory in a serial prediction task depends on processing in the amygdala central nucleus (CeA) after completion of sessions that included the surprising omission of an expected event. Post-surprise infusions of anisomycin, lidocaine, or muscimol prevented subsequent display of surprise-enhanced associability. Because previous studies indicated that CeA function is unnecessary for the expression of associability enhancements that were induced previously when CeA function was intact (Holland & Gallagher, 2006), we interpreted these results as indicating that post-surprise activity of CeA (“surprise replay”) is necessary for the consolidation of altered associability memories elsewhere in the brain, such as the posterior parietal cortex (Schiffino, et al., 2014a).

Graphical Abstract

Post-surprise perturbation of central amygdala activity prevents surprise-induced enhancements in learning

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Keywords

consolidation; attention; cue associability; Pearce-Hall theory; amygdala

1. Introduction

According to many theories of associative learning (e.g., LePelley, 2004; Pearce & Hall, 1980; Pearce & Mackintosh, 2010), the surprising omission of an expected reinforcer within a learning trial can enhance subsequent attention to cues present on that trial, as reflected in the rate at which those cues enter into new associations later. This assertion has been supported in a variety of experimental procedures (Dickinson, Hall, & Mackintosh, 1976; Hall & Pearce, 1979; Haney et al., 2010; Holland & Gallagher, 1993a, b; Rescorla, 1991; Swan & Pearce, 1988; Wilson et al., 1992). Notably, these surprise-induced enhancements in cue associability do not occur in rats with compromised function of the amygdala central nucleus (CeA; Haney et al., 2010; Holland, 2016; Holland & Gallagher, 1993a, b; Holland, Thornton, & Ciall, 2000; Wheeler & Holland, 2011).

Holland and Schiffino (2016) noted that the search for neural bases of surprise-induced enhancements of cue associability profitably distinguishes among *surprise* systems responsible for altering associability at the time of surprise, *storage* systems that represent altered associability values, and *expression* systems that access those altered values to implement more rapid learning later. A serial prediction task (Wilson et al., 1992) has proved particularly useful for this search, because the induction of surprise (and presumably the recalculation of cue associability) occurs in one phase of the experiment, but the assessment of the expression of altered cue associability as more rapid learning occurs in a subsequent phase. By separating these key functions widely in time (for example, across days), this procedure allows us to separately examine the effects of brain manipulations on the induction, storage, and expression of associability changes. In this procedure (Table 1), rats first receive consistent serial light→tone pairings in an *expectancy* phase to establish the light as a valid predictor of the tone. Half of those serial compounds are reinforced with food

delivery and half are not. Next, in a *surprise* phase, for *shift* rats the tone is omitted on the nonreinforced trials, whereas *consistent* rats continue receiving the same trials as in the expectancy phase. Finally, the associability of the light is assessed in a test phase in which the light is directly paired with food. Within models such as that of Pearce and Hall (1980), as the light comes to predict the tone in the expectancy phase, its associability decreases. However, omission of the tone in the shift condition in the surprise phase restores or enhances the light's associability, allowing it to acquire associations with the food in the final test phase more rapidly than rats in the consistent condition.

By transiently perturbing neural activity during either the surprise or test phases, we have identified brain regions that uniquely contribute to surprise or expression systems. Both the CeA (Holland & Gallagher, 2006) and its interactions with the substantia nigra pars compacta (SNc; Lee et al., 2006, 2008) are critical at the time of surprise but not at the time of expression of enhanced cue associability in test, whereas the cholinergic substantia innominata/nucleus basalis magnocellularis (SI/nBm; Holland & Gallagher, 2006), dorsolateral striatum (Asem et al., 2015), and secondary visual cortex (Schiffino & Holland, 2016) are critical at the time of expression, but not at the time of surprise.

Interestingly, Schiffino et al. (2014a) found that function of the posterior parietal cortex (PPC) was critical at both the time of surprise and the time of expression. Because PPC function was required both when surprise-induced changes in associability were encoded and when they were retrieved for new learning, Schiffino et al. (2014a) suggested that PPC might be the site of a relatively permanent memory of altered cue associability. In support of that assertion, Schiffino et al. (2014a) also found that infusion of the protein-synthesis inhibitor anisomycin into PPC immediately after surprise sessions prevented (in a time-dependent manner) cue associability enhancements, as if it prevented the consolidation of an altered associability memory. This consolidation process likely relies upon interactions among multiple brain regions, and given the historical importance of amygdala function to memory consolidation (McGaugh, 2004) and the observation that CeA activity during encoding is critical to associability enhancements (Holland & Gallagher, 2006) it seems reasonable to consider a role for this nucleus in the consolidation of altered associability memories.

Most explorations of amygdala function in memory consolidation have focused on the basolateral amygdala (BLA) and its role in the stabilization of memories about motivationally significant experiences (Hermans et al., 2014; Huff et al., 2013; McGaugh, 2004; Pare, 2003; Schafe et al., 2001). Considering other attributes of BLA function, it seems likely that those memories represent specific sensory properties of the motivationally significant event (Balleine & Killcross, 2006; Clark et al., 2012; Everitt et al., 2000; 2003; Gallagher & Schoenbaum, 1999; Holland & Gallagher, 1999; Seymour & Dolan, 2008). However, cue associability is a parameter of attentional control, not an associative memory imbued with motivational significance. Furthermore, although evidence suggests that information necessary for associability changes (that is, prediction error) is coded in BLA (e.g., Esber et al., 2012; Esber & Holland, 2014; Roesch et al., 2010), performance in the serial prediction task is normal in rats with neurotoxic lesions of BLA (Holland et al., 2001).

Thus, it is unclear how well much of the existing literature on BLA's role in memory consolidation applies to consolidation of associability memories.

The role of CeA in memory consolidation has been less-explored, and most if not all of those studies used aversive conditioning procedures (e.g., Campolongo et al., 2009; DaCunha et al., 1999; LaLumiere et al., 2004; Parent & McGaugh, 1994; Pitts & Takahashi, 2011; Quiarte et al., 1997; Roozendaal & McGaugh, 2011; Roozendaal et al., 2009; Wilensky et al., 2006). Nevertheless, CeA involvement in memory consolidation warrants examination, given its direct and widespread access to numerous autonomic, endocrine, and diffuse projecting neuromodulatory systems that regulate consolidative processes (Izquierdo & McGaugh, 2000; Izquierdo & Medina, 1997; Jolkkonen et al., 2002; Pitkanen, 2000; Power et al., 2003; Roesler & Schroder, 2011; Roozendaal, 2000, 2007; Swanson & Petrovich, 1998; Wallace et al., 1992). In particular, consolidation of associability memories might involve protracted interactions of CeA with systems involving the dopaminergic SNc (Lee et al., 2006, 2008), the cholinergic SI/nBm (Chiba et al., 1995; Han et al., 1999), and the orexigenic lateral hypothalamus (Wheeler et al., 2014), all of which have been found to influence performance in the serial prediction task.

Here we examined the importance of CeA function for the consolidation of associability memories in the serial prediction task by infusing drugs into that nucleus after surprise sessions. In Experiment 1, the translation blocker anisomycin was infused bilaterally to inhibit protein synthesis. In Experiment 2, lidocaine was infused bilaterally to suppress conduction down CeA axons. The rationale for selecting lidocaine (see §4.1 for discussion) emerged from reports that intra-amygdalar pretreatment with that anesthetic attenuated amnesia produced by subsequent intra-amygdalar infusion of anisomycin, perhaps by preventing disruptive release of monoamines that can be produced by such infusions (Canal et al., 2007; Sadowski et al., 2011). In Experiment 3, fluorescent muscimol (FCM) was infused bilaterally to achieve greater specificity of dysfunction than lidocaine infusion by sparing conductance down fibers of passage. A supplementary experiment (Supplementary Material) assessed the spread of FCM at various post-infusion intervals.

2. Methods

2.1. Subjects

Male Long-Evans rats (Charles River Laboratories, Raleigh, NC) were used in this study: 96 in Experiment 1, 48 in Experiment 2, and 64 in Experiment 3. Rats weighed 300–325 g upon arrival to the laboratory vivarium, and were given about a week of free access to food and water prior to surgery. Surgery was followed by 10–14 days of recovery before behavioral training. During the recovery period, the rats were handled for at least 2 min each day. After recovery, they were food restricted to reach and subsequently maintain 85% of their free feeding weights throughout the course of the study. Rats were individually housed in a colony room with a 12:12 hr light-dark cycle. The care and experimental treatment of rats was conducted according to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*, and protocols were approved by the Johns Hopkins University Animal Care and Use Committee.

2.2. Apparatus

The behavioral training apparatus consisted of four separate chambers (22.9 X 20.3 X 20.3 cm). Each chamber had aluminum front and back walls, clear acrylic sides and top, and a floor of stainless steel rods (0.48 cm in diameter spaced 1.9 cm apart). A recessed food cup was located in the center of the front wall 2 cm above the floor, and was fitted with phototransistors to detect head entries. Two 45-mg sucrose pellets (Formula 5TUT, Test Diets, St. Louis, MO) delivered to the food cup served as the reinforcer. The light conditioned stimulus (CS) was generated by illumination of a 6-W panel lamp with a translucent covering, mounted 15 cm directly above the food cup. A 1500-Hz, 80-dB tone CS was presented via a speaker mounted on the inside wall of a sound-attenuating box that surrounded each chamber.

2.3. Surgical procedures

Rats were anesthetized with 2–3% isoflurane mixed with oxygen and placed into the stereotaxic apparatus (Kopf Model 902, Tujunga, CA). After incision and craniotomy, four 1/8" self-tapping mounting screws were installed into the skull. Then, the dura was punctured with a 27-gauge needle and 26-gauge guide cannulae (PlasticsOne, Roanoke, VA), with stainless steel tubing cut to extend 8.5mm below the 8.0mm long pedestal, were implanted dorsal to each CeA at –2.4mm posterior and ± 4.3 mm lateral to bregma, to a depth of 5.9 mm below the skull surface. Cannulae were held in place with dental acrylic and fitted with obturators that were cut to match the length of the guide. Once the acrylic set, the incision was closed with surgical staples and topical antibiotic ointment was applied to the wound edges. Then, all rats received subcutaneous 0.02 mg/kg injections of sterile buprenorphine HCl (Sigma, St. Louis, MO) to ameliorate pain.

2.4. Behavioral training procedures

Table 1 provides an outline of the behavioral training procedures. Hungry rats were first given 10 sucrose pellets in their home cages to familiarize them with the reinforcer. Each training session in each phase of the experiments was 64 min in duration and included 16 trials, distributed across random intertrial intervals, which averaged 4 min (range = 2 to 6 min). One session was given each day at approximately the same time. The rats were first trained to eat sucrose pellets from the recessed food cups in a single session, which included 16 unsignaled reinforcer deliveries. Then, to establish a strong light-tone association, all rats received trials consisting of a 10-s light→10-s tone serial compound. In each session of this “expectancy” phase, half of the 16 trials had the light→tone compound reinforced with sucrose pellets and the other half were non-reinforced. Trial order in each session was randomly determined. After 10 sessions of expectancy training, rats were allocated into performance-matched shift and consistent groups, and given 2 surprise phase sessions. During each surprise session, light→tone prediction error was induced for the shift rats by omitting the tone on the 8 nonreinforced trials, while consistent rats had their light→tone expectancies confirmed through continuation of the expectancy protocol. Finally, in each of the 5 sessions in the test phase, all rats received 16 presentations of the light CS alone followed immediately by sucrose pellet reinforcement. If induction of a prediction error in

the shift rats enhanced the associability of the light cue, then those rats would acquire conditioning more rapidly in this test than rats in the consistent condition.

2.5. Drugs and infusion procedures

In Experiment 1, anisomycin (Sigma) was infused to inhibit translation in CeA. Anisomycin was dissolved into HCl at a concentration of 125 µg/µL in 0.9% saline vehicle and the pH was adjusted to 7.2 (Nader et al., 2000). In Experiment 2, 2% lidocaine hydrochloride solution (Vedco, St. Joseph, MO) was infused to disrupt the propagation of action potentials down CeA axons. In Experiment 3, the GABA-A agonist muscimol (Beaumont et al., 1978) conjugated to the BODIPY® TMR-X fluorophore through covalent amide bonding (Thermo Fisher, Waltham, MA) was infused to reversibly inactivate CeA neurons while minimizing perturbation of transmission down fibers of passage. The FCM fluorophore has excitation and emission peaks at 543 and 572 nm, respectively, and is highly lipophilic (Allen et al., 2008). FCM was dissolved into 0.9% saline vehicle at a concentration of 0.5 µg/µL.

In each experiment, rats had their obturators removed and reinserted after each of the expectancy sessions, to familiarize them with manipulation of their headsets. Two 33-gauge injector cannulae (0.2mm O.D., 0.1mm I.D.) that extended 2.0mm below the tip of the guide were connected by PE50 tubing to separate 10-µL Hamilton syringes in a multiple-syringe pump (KD Scientific, Holliston, MA). In Experiments 1 and 2, the pump simultaneously administered 0.2 µL of infusate over one minute. In Experiment 3, 0.5 µL of infusate was delivered over two minutes. This dose was determined after a pilot fear conditioning experiment with other rats found it to be sufficient to disrupt the expression of freezing behavior following bilateral infusions into CeA, whereas 0.2-µL infusions were considerably less effective. After infusion, the injector was left in place for an additional minute. After removal of the injectors, the obturators were reinserted. Rats in the “immediate” drug treatment received infusions of anisomycin (Experiment 1), lidocaine (Experiment 2), or FCM (Experiment 3) immediately after the end of each surprise session, whereas rats in the “delayed” condition received saline-only infusions at these times. The delayed rats also received anisomycin, lidocaine, or FCM infusions, but at 24 h after each surprise session. Rats in the immediate condition received saline-only infusions at these (24-hr delay) times. Thus, each rat received two anisomycin (Experiment 1), lidocaine (Experiment 2), or FCM (Experiment 3) infusions and two saline infusions in the surprise phase, but only the rats in the immediate drug treatment received the anisomycin, lidocaine, or FCM at a time when it was likely to interfere with CeA-dependent consolidation of memories acquired during the surprise sessions.

2.6 Behavioral measure and statistical analysis

The response measure was the percentage of time within a trial measurement epoch in which a rat's head was in the food cup, as assessed by interruption of the infrared photobeam. Trial epochs were defined as a 5 s stimulus-free pre-CS period (immediately prior to the light CS), the first 5 s of the light CS, the second 5 s of the light CS, the first 5 s of the tone CS, the last 5 s of the tone CS, and the 5 s initiated by reinforcer delivery. Conditioned food cup responding was assessed during the latter half of CS presentations because in that epoch,

food cup CRs are more frequent and less contaminated by conditioned orienting behaviors (Holland, 1977).

Responding during the pre-CS, light, and tone (when applicable) epochs were each analyzed with separate analyses of variance (ANOVAs) with behavioral condition (shift or consistent) and drug treatment (immediate or delayed: anisomycin in Experiment 1; lidocaine in Experiment 2; FCM in Experiment 3) as between-subject variables, and repeated measures on the within-subjects variable of session blocks (1–5). The Greenhouse-Geisser procedure was used to correct for violations of sphericity; where relevant we provide an estimate of sphericity, ϵ . The critical analyses were those of the test data in each experiment. The effect of surprise on the light's associability would be revealed as a significant effect of behavioral condition (greater responding in the shift rats than in the consistent rats), and modulation of that effect by post-surprise drug infusions would be indicated by a significant behavioral condition X drug treatment interaction. In addition, the ANOVAs on test phase data were supplemented by planned contrasts to evaluate the hypotheses that behavioral treatment groups differed within each drug treatment (shift-immediate vs. consistent-immediate and shift-delayed vs. consistent-delayed) and that drug treatment groups differed within each behavioral condition (shift-immediate vs. shift-delayed; consistent-immediate vs. consistent-delayed).

2.7. Histological procedures

All rats except for those allocated to the supplemental FCM spread study ($n = 29$ from Experiment 3; see supplementary materials) were deeply anesthetized and perfused intracardially with 0.9% saline followed by 3.7% formalin solution after completion of behavioral testing. After removal of the headset, brains were removed and stored at 4°C in 3.7% formalin 12% sucrose solution. These brains were sliced on a freezing microtome and 40- μ m coronal sections were taken in series. To confirm cannula tip placements in the bilateral CeA, every third section was mounted on glass slides, dehydrated in ascending concentrations of alcohol, defatted in xylene, and stained with thionin. Slides were coverslipped using Permount thinned with xylene, and examined with a light microscope.

Twenty-nine rats from Experiment 3 were used to evaluate the spread of FCM over time. The procedures and results of this analysis are provided as Supplementary Materials.

3. Results

3.1. Histological results

Of the 144 rats acquired for the study, the data from 43 were excluded. In Experiment 1, 3 of the 96 rats were excluded because their headsets detached, three rats were removed due to infectious brain lesion, one ataxic rat was euthanized, 6 rats were excluded for missed cannula placement, and one rat died during surgery. Notably, in 17 additional rats, neurotoxic lesions of CeA were observed in at least one hemisphere; these rats were excluded (see Morris et al., 2006, for a detailed description of such damage noted after anisomycin infusion into hippocampus). In Experiment 2, 4 of the 48 rats were excluded after their headsets detached, 4 rats were excluded for missed cannula placement, and one rat

died during surgery. In Experiment 3, 8 of the 64 rats were euthanized during the surprise phase as part of the supplemental spread study, 2 rats were euthanized after their headsets detached, and 4 rats were excluded for missed cannula placement.

Assessments of cannula tip placements confirmed that the site of injection for all rats whose data were included for behavioral analyses was within the CeA (Figure 1). In Experiment 1 (anisomycin), the final numbers of rats in the shift-immediate, shift-delayed, consistent-immediate, and consistent-delayed conditions were 16, 16, 15, and 15, respectively. In Experiment 2 (lidocaine), those sample sizes were 8, 10, 11, and 10, respectively. In Experiment 3 (FCM), those sample sizes were 12, 12, 13, and 13, respectively.

The results of an analysis of the spread of FCM over time are described in the Supplementary materials. This analysis indicated that the FCM infusion procedures used were appropriate: FCM spread was pervasive within the CeA and minimal to adjoining BLA, although the spread included the intervening intercalated cells in rats with more lateral cannula placements. However, more remote spreading along fiber tracts (e.g., stria terminalis, internal and intermediate capsules) was evident in some rats. Because this spread was observed in only a small sample of rats and was generally unilateral, we do not think that such spread played an important role in our results.

3.2. Behavioral results

Figure 2 depicts food-cup responding during pre-CS, light, and tone measurement epochs across all three phases (expectancy, surprise, and test) of Experiments 1 (Figures 2A and 2D), 2 (Figures 2B and 2E) and 3 (Figures 2C and 2F).

3.2.1. Expectancy phase—In this phase of the experiments, which occurred prior to any drug treatments, all rats received light→tone trials, reinforced with food on half of those trials. In all 3 experiments, all groups of rats acquired comparably high levels of food-cup responding to the tone while exhibiting minimal responding to the light (leftmost portion of each panel of Figure 2). For each experiment, subsequent behavioral condition (shift vs. consistent) x subsequent drug treatment (immediate vs. delayed) x two-session block (1–5) ANOVAs were performed on data from each measurement epoch (pre-CS, light, and tone). No significant effects or interactions ($ps > 0.074$) were observed for any variable in any of the three experiments aside from significant effects of block on responding during the light ($ps < .001$) and tone ($ps < .001$) in all three experiments, and a significant effect of block on pre-CS responding in Experiment 3 ($\eta^2 = 0.94$, $F_{4, 232} = 2.47$, $p = .049$). Additional subsequent behavioral condition x subsequent drug treatment ANOVAs of performance over the final two sessions of the expectancy phase for each experiment found that in Experiment 3, the effect of subsequent drug treatment on responding to the light was significant ($F_{1, 58} = 4.70$, $p = 0.034$). Notably, the slightly greater responding by “immediate” rats (presumably a sampling error) worked against observation of a drug effect in later experimental stages. No other effects ($ps > .099$) or interactions were significant ($ps > .183$) for those analyses. Thus, within each experiment, rats in all groups entered the surprise phase with similar levels of responding.

3.2.2. Surprise phase—The bars in each panel of Figure 2 show food-cup response means ($\pm$ s.e.m.) collapsed across the two surprise phase sessions, in which the tone stimulus was unexpectedly omitted on nonreinforced trials in the shift rats. Recall that drug treatments were administered immediately *after* each of these sessions and so would not be expected to alter responding in this phase. For each experiment, data from each of the three measurement epochs were subjected to behavioral condition $\times$ drug treatment ANOVAs. No effects or interactions were significant for any response measure in any of the three experiments ($ps > .145$), although in Experiment 1 the main effect of drug treatment on responding during the light was marginally significant ($F_{1, 58} = 3.45, p = .068$). Thus, within each experiment, rats in all groups began the test phase after showing similar levels of responding to both the light and tone during the surprise phase.

3.2.3. Test phase—The rightmost portion of each panel of Figure 2 shows the primary data of this study, the acquisition of food-cup responding to the light during the test phase. Immediate, but not delayed, post-surprise session infusions of anisomycin in Experiment 1 (Figures 2A and 2D), lidocaine in Experiment 2 (Figures 2B and 2E), and FCM in Experiment 3 (Figures 2C and 2F) each eliminated the normally-occurring shift condition advantage in learning about the light.

Separate behavioral condition $\times$ drug treatment $\times$ sessions ANOVAs showed a significant behavioral condition $\times$ drug treatment interaction in each of Experiments 1 ($F_{1, 58} = 5.35, p = .024$), Experiment 2 ($F_{1, 35} = 5.07, p = .031$), and Experiment 3 ($F_{1, 46} = 4.75, p = .035$). Furthermore, individual comparisons showed that in each of the three experiments, rats that received delayed drug infusions exhibited the normal shift advantage, that is, significantly more responding in the shift condition than in the consistent condition ($ps < .040$). By contrast, rats that received immediate drug infusions failed to show the shift advantage ($ps > .339$). Furthermore, immediate drug infusions significantly reduced responding of rats in the shift condition ($ps < .031$) relative to rats in that condition that received delayed drug infusions. By contrast, the effects of immediate and delayed drug infusions did not differ among rats in the consistent condition ($ps < .418$).

None of the effects of drug treatment was attributable to differences in pre-CS responding: there were no significant effects or interactions involving the drug treatment variable. In Experiment 1, a significant main effect of session on pre-CS responding ($\epsilon = 0.83, F_{4, 232} = 3.35, p = .016$) interacted with behavioral condition ($\epsilon = 0.83, F_{4, 232} = 3.09, p = .024$), but no other effects ($ps > .421$) or interactions were significant ($ps > .107$). In Experiment 2, the behavioral condition $\times$ session interaction was significant for pre-CS responding ($\epsilon = 0.868, F_{4, 140} = 2.72, p = .040$) and the effect of session was marginally significant ($\epsilon = 0.868, F_{4, 140} = 2.34, p = .068$), but no other effects ($ps > .135$) or interactions ($ps > .116$) were significant. Finally, in Experiment 3, the effect of session was significant ($\epsilon = 0.749, F_{4, 184} = 14.39, p = .001$), but there were no other significant effects ($ps > .158$) or interactions ($ps > .339$).

4. Discussion

The results of these studies demonstrate the importance of intact CeA function soon after the induction of surprise for enhancements of cue associability assessed in subsequent new learning. Infusions of anisomycin (Experiment 1), lidocaine (Experiment 2), or FCM (Experiment 3) immediately after, but not 24 hr after, surprise phase sessions abolished the enhanced learning observed for shift group rats in the serial prediction task. Because these drugs were administered *after* the surprise sessions, their effects could not be attributed to changes in the detection or perception of the cues or reinforcer, the retrieval of light-tone expectancies, or the registration of negative prediction error when the tone was omitted. Instead, CeA activity was likely critical to the consolidation of altered associability memories within hours after induction of those changes by surprise.

4.1. Role of CeA in consolidation of associability memories

A simple interpretation of the results of Experiment 1, consistent with the logic of most protein synthesis inhibition studies, is that surprise induces a cascade of events that normally culminates in structural change in CeA corresponding to an altered associability memory. However, if CeA itself was a critical site for storage of altered associability memories, then accelerated learning about the light later in testing would presumably require access to that memory at that time. But Holland and Gallagher (2006) found that whereas NBQX infusions into CeA prior to surprise sessions prevented the shift group advantage in the serial prediction task, the same manipulation prior to test phase sessions had no such effect. Although it might be argued that retrieval of an altered associability value stored in CeA was insensitive to the AMPA receptor antagonism achieved in Holland and Gallagher's (2006) study, the results of the present Experiments 2 and 3 suggest an alternative account. It seems more likely that CeA anisomycin infusions in Experiment 1 acted by profoundly altering CeA neural activity, affecting critical post-session processes elsewhere in the brain (e.g., frontal-parietal attention networks; Schiffino et al., 2014a), rather than by preventing structural modification in CeA itself.

Although anisomycin and other nonspecific protein-synthesis inhibitors can interfere with consolidation through translational inhibition (Costa-Mattioli et al., 2009; Davis & Squire, 1984; Dudai, 2004; Kandel, 2001; Sutton & Schuman, 2006), they also cause extensive proteomic alterations that confound most *in vivo* studies (Alberini, 2008; Gold 2006; 2008; Hernandez & Abel, 2008; Routtenberg & Rekart, 2005; Rudy, 2008). For example, anisomycin is capable of rapidly eliciting persistent neurophysiological dysfunction as a potent agonist of p38, ERK, and JNK mitogen-activated protein kinase (MAPK) cascades (Radulovic & Tronson, 2008). These MAPK cascades exert bidirectional control over neuronal excitability (e.g., Costello & Herron, 2004; Poolos et al., 2006; Schrader et al., 2006; Wu et al., 2011) via fast cytoplasmic protein/protein interactions and slower transnuclear mechanisms. Stimulation of MAPK cascades by anisomycin *in vitro* occurs prior to and perhaps independently of translational inhibition (Edwards & Mahadevan, 1992; Mahadevan & Edwards, 1991; Shifrin & Anderson, 1999; Torocsik & Szeberenyi, 2000).

Thus, anisomycin infusion in Experiment 1 may simply have introduced excessive noise into activity of CeA (see Canal et al., 2007; Gold, 2008; Rudy, 2008), altering its usual influence

on other brain systems. In support of that view, in an unrelated conditioning preparation, intra-amygdalar pretreatment with the anesthetic lidocaine attenuated anisomycin-induced amnesia, without affecting consolidation when it (lidocaine) was delivered alone (Sadowski et al., 2011). Those authors suggested that anisomycin produced amnesia primarily by producing irregular or hyperactive neuronal firing, which lidocaine allayed. We first sought to address that confound in Experiment 2 by infusing lidocaine into CeA after surprise sessions, which was expected to reduce “noisy” post-session CeA neural activity without interfering with translational activity induced by cascades that were initiated during the surprise sessions themselves. A null effect of these infusions would have set the stage for a subsequent examination of the effects of lidocaine pretreatment on the effects of anisomycin infusions. However, in Experiment 2, intra-amygdalar infusions of lidocaine alone after surprise sessions prevented surprise-induced enhancements in cue associability, thereby implicating normal post-session CeA neuronal activity as critical to consolidation of associability memories. Notably, Sharma, Nargang, and Dixon (2012) found that intrahippocampal anisomycin infusions profoundly *suppressed* multiunit activity and local field potentials. In Experiment 3, we confirmed and extended the implications of the results of Experiments 1 and 2 by demonstrating comparable effects of post-surprise inactivation of CeA with fluorescent muscimol, a GABA_A agonist, which, unlike lidocaine, spares conductance along fibers of passage. Time-course assessment of FCM spread after CeA infusion suggested that the principal site of drug action was largely constrained to that nucleus.

4.2. CeA modulation of frontal-parietal attention networks

Schiffino et al.’s (2014a) conclusion that PPC served as a storage site for altered associability memories was based in part on the observation that rats that received anisomycin infusions into PPC after surprise sessions in the serial prediction task failed to show the normal shift advantage. Given the present results, one might argue that although this observation revealed the importance of PPC processing within hours after surprise, it provided little evidence for the necessity of protein-synthesis dependent structural change in PPC itself. However, additional evidence supports the claim that PPC stores altered associability memories. First, unlike for CeA, normal performance in the serial prediction task demands intact PPC function at both the time of surprise and the time of expression of enhanced cue associability as faster new learning (Schiffino et al., 2014a). Second, electrophysiological studies revealed neurons in PPC that appeared to directly code alterations in cue associability. Using a successive reward shift discrimination procedure, Schiffino (Holland & Schiffino, 2016; Schiffino, et al., 2014b) identified a population of neurons that selectively increased their firing rates to visual cues that accompanied a shift in reward magnitude. These modified responses to the cues were maintained over time, consistent with a memory representation of associability. Notably, investigations of neuronal activity in CeA in a similar task (Calu et al., 2010) instead found *reward-responsive* neurons that increased their firing rates when cue-reinforcer contingencies were changed. Thus, these neurons were sensitive to prediction error, representing surprise when an unexpected reinforcer was delivered, whereas Schiffino et al.’s (2014b) study showed the modulation of *cue-responsive* neuronal activity by surprise, a more direct correlate of associability.

We have suggested several routes by which CeA might modulate PPC activity. Although PPC does not receive direct projections from CeA, nor from SNc, whose functional connectivity with CeA at the time of surprise is also essential for surprise-induced associability enhancements (Lee et al., 2008), both CeA and SNc project to portions of the subthalamic substantia innominata (SI). Notably, rats with specific lesions of direct magnocellular cholinergic projections from SI to PPC fail to show the shift condition advantage in the serial prediction task (Bucci et al., 1998). Thus, Bucci et al. (1998) and Holland and Gallagher (1999, 2006) suggested that a simple circuit in which CeA and/or SNc influences SI activity, which in turn modulates PPC function, might underlie these associability enhancements. However, because disrupting SI cholinergic innervation of the PPC solely at the time of surprise does not affect performance in the serial prediction task (Holland & Gallagher, 2006; Schiffino & Holland, unpublished observations), other brain regions must mediate the influence of CeA and SNc on the PPC during the initial encoding of enhanced associability. For example, Schiffino et al. (2014a) suggested a potential role for SNc projections to the supragenual portion of the anterior cingulate cortex (Emson & Koob, 1978; Lindvall et al., 1978), which innervates PPC and also connects with adjacent medial agranular cortex, a notable PPC afferent important for directed attention in the rat (Burcham et al., 1997; Reep et al., 1994; Reep & Corwin, 2009;). Notably, considerable research indicates that the anterior cingulate cortex codes prediction errors, including the surprising omission of expected events (Alexander & Brown, 2011; Hayden et al., 2011; Holroyd & Coles, 2002; Rushworth & Behrens, 2008; Totah et al., 2009; but see O'Reilly et al., 2013), and may itself code changes in cue associability (Bryden et al., 2011). Alternately, Schiffino et al. (2014a) suggested a basal ganglia–thalamocortical loop involving projections from SNc to the caudate-putamen, which in turn influences the substantia nigra pars reticularis (SNr), a region that modulates the activity of PPC-projecting thalamic regions such as the lateral posterior and lateral dorsal nuclei (Bucci et al., 1999; Chandler et al., 1992; Deniau & Chevalier, 1992; Kamishina et al., 2009; Reep et al., 1994; Sakai et al., 1998; Sakai & Bruce, 2004). Interestingly, we have found that inducing surprise by omitting an expected food reinforcer produces substantial FOS expression in those thalamic nuclei and in SNr (Holland & Schiffino, 2016; unpublished observations).

4.3. CeA function in associability enhancements

Because transient interference with CeA function at the time of induction of negative prediction errors prevents associability enhancements, but not other aspects of learning that depend on such errors (e.g., Holland, 2016; Holland & Gallagher, 2006), we have assumed that CeA plays a critical role in the recalculation of associability values, likely in concert with other regions, such as SNc (Lee et al., 2008) and PPC (Bucci et al., 1998; Schiffino et al., 2014a). Furthermore, because interference with CeA activity when those altered values are expressed in faster learning has no effect (Holland & Gallagher, 2006), we have assumed that CeA passes those altered values on to other brain regions (e.g., PPC; Schiffino et al., 2014a) for storage and expression. The present results suggest that some variations on that theme may be worth considering. First, it is possible that adjustment of cue associability demands substantial post-session replay of session episodes within attention networks, perhaps encompassing many regions besides CeA and PPC. Second, given that it is possible that the effects of pre-session infusions of NBQX in Holland and Gallagher's (2006) study

lingered beyond the session duration, it is possible that CeA processing of surprise is required *only* for consolidation of changed associability memories elsewhere in the few hours after surprise itself. That is, CeA might not itself alter associability values, or convey associability or prediction error information to other brain regions at the time of surprise. Instead, CeA may serve a more general modulatory effect on consolidation processes (e.g., LeDoux, 2012). Because CeA activity is augmented by surprise (e.g. Calu et al., 2010; Lee et al., 2010), post-session modulation of cue associability by CeA activity would be greatest after surprise-inducing behavioral manipulations. Indeed, aspects of surprise may be considered motivational events. For example, surprise may operate as a psychological stressor if it challenges or invalidates components of a world model that an animal relies heavily upon for survival (Arnsten, 2009; Valentino & Van Bockstaele, 2008). In less dire situations, surprise may simply invigorate an animal by signifying an opportunity to procure novel information and therefore encourage exploration of the environment (Sokolov, 1963). Regardless of the affective valence, registering surprise may increase arousal, which in turn partly determines the efficacy of consolidation. Perhaps surprise-induced alterations in CeA activity influence long-lasting modulation of cue associability through its widespread access to vigilance systems (Gallagher & Holland, 1994), such as cholinergic (Dringenberg and Vanderwolf, 1997; Gozzi et al., 2010; Jones, 2008), orexigenic (Jones, 2008; Sakurai, 2014; Wheeler et al., 2014), and noradrenergic systems (Aston-Jones & Cohen, 2005; Carter et al., 2010; McCall et al., 2015; Sara, 2009; Sara & Bouret, 2012), to enhance arousal (Kapp et al., 1992; Moruzzi & Magoun, 1949; Phelps & LeDoux, 2005). By maintaining or reiterating components of arousal during post-surprise periods, CeA activity might continue to influence consolidation in regions that maintain memories of cue associability or construct attentional priority maps (Bisley & Goldberg, 2010). More temporally precise (e.g., optogenetic, Huff et al., 2013) manipulation of function of CeA and other regions may prove valuable in disentangling those regions' contributions to cue associability changes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Surprising omission of an event enhances future learning about remaining events

These cue salience enhancements depend on function of central amygdala (CeA)

Post-surprise perturbations of CeA activity prevent cue salience enhancements

CeA needed for consolidation of altered cue salience memories elsewhere in brain

Figure 1. Schematic representation of injector cannula tip placements for rats included in Experiment 1 (A), Experiment 2 (B), and Experiment3 (C)

The numbers on the left indicate distance (mm) from bregma along the rostrocaudal axis.

For each placement, a single black dot of 50% opacity was drawn using Adobe Photoshop.

Thus, darker areas indicate greater overlap. Drawings of brain sections (Paxinos & Watson, 1998, p. 29–31) are used by permission of Elsevier.

Figure 2. Conditioned food cup responding

Mean ($\pm$ SEM) food-cup responding during pre-CS, light, and tone measurement epochs sequentially across all three phases (expectancy, surprise, and test) of Experiment 1 (A and D), Experiment 2 (B and E), and Experiment 3 (C and F). The headings over Panels A, B, and C identify the drug that was infused into CeA after each of the two surprise phase sessions. Rats in the Immediate drug condition (D, E, and F) received that drug immediately after each surprise session and vehicle after a 24-hr delay, whereas rats in the Delay drug condition (A, B, and C) received vehicle immediately after each surprise session and drug after a 24-hr delay. Analysis of expectancy and surprise phase data (left and center portions of each panel) showed that rats acquired and maintained comparably high levels of food-cup responding to the tone while exhibiting minimal conditioning to the light. The right side of each panel depicts the acquisition of food-cup responding over the course of the five test phase sessions. In all three experiments, when drug infusions were delayed 24 hr after surprise phase sessions, rats in the shift condition (in which an expected event had been omitted in the surprise phase) showed more rapid learning about the light in test than rats in the consistent condition (A, B, and C). Immediate post-surprise session infusions of anisomycin (D; Experiment 1), lidocaine (E; Experiment 2), and fluorescent muscimol (F; Experiment 3) each eliminated that shift group advantage in learning. Asterisks indicate significant ($ps < .040$) differences in overall test responding between shift and consistent rats; shift rats that received delayed drug infusions (filled circles in A, B, and C) also showed significantly ($ps < .031$) more responding than shift rats that received immediate drug infusions (filled circles in D, E, and F). Critically, the behavioral condition $\times$ drug treatment interaction was significant in all three experiments ($ps < .035$).

Table 1

Outline of Procedures

behavioral condition	expectancy phase	surprise phase	test phase
Shift	light→tone→food light→tone→nothing	light→tone→food light→nothing	light→food
Consistent	light→tone→food light→tone→nothing	light→tone→food light→tone→nothing	light→food

After each of the two sessions in the surprise phase, all rats received infusions of drug and vehicle into the amygdala central nucleus (CeA). Rats with “Immediate” drug treatment received a drug infusion immediately after each session and a vehicle infusion 24 hr later, whereas rats in the “delay” treatment received a vehicle infusion immediately after each session and a drug infusion 24 hr later. The drugs used were anisomycin (Experiment 1), lidocaine (Experiment 2), or fluorescent muscimol (Experiment 3). Among rats in the delayed drug condition, the surprising omission of the tone on nonreinforced trials (bold type) in the shift condition should enhance the associability of the light, allowing it to acquire conditioning more rapidly in the final test phase than rats in the consistent condition.