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Neural correlates of variations in event processing during learning in central nucleus of amygdala

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SUMMARY

Attention or variations in event processing help drive learning. Lesion studies have implicated the central nucleus of the amygdala (CeA) in this process, particularly when expected rewards are omitted. However, lesion studies cannot specify how information processing in CeA supports such learning. To address these questions, we recorded CeA neurons in rats performing a task in which rewards were delivered or omitted unexpectedly. We found that activity in CeA neurons increased selectively at the time of omission and declined again with learning. Increased firing correlated with CeA-inactivation sensitive measures of attention. Notably CeA neurons did not fire to the cues or in response to unexpected rewards. These results indicate that CeA contributes to learning in response to reward omission due to a specific role in signaling actual omission rather than a more general involvement in signaling expectancies, errors, or reward value.

INTRODUCTION

Studies evaluating the role of the amygdala in associative learning have identified the central nucleus of the amygdala (CeA) as a critical contributor to the processing of violations to event expectancies (Bucci and Macleod, 2007; Holland and Gallagher, 1993a, 1993b). In intact rats, alteration of predictive relationships between conditioned stimuli (CSs) or between CSs and reward enhances processing of those events, as reflected in increases in their ability to participate in new learning (Holland and Gallagher, 1993b; Wilson, 1992). For example, in unblocking experiments, learning about a new CS is enhanced if an

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additional reward is presented or an expected one is omitted when the new cue is introduced. Rats with lesions of CeA fail to show enhanced learning when expected events are omitted or when reward value is suddenly shifted downward, but these rats appear to learn normally after unexpected upshifts in reward (Holland, 2006; Holland and Gallagher, 1993a, 1993b; Holland and Kenmuir, 2005).

Holland and Gallagher (1993b, 1999) proposed that upon omission of an expected reward or other event, CeA might act by enhancing the gain in sensory or attentional systems involved in processing CSs, through its influence on the basal forebrain cholinergic system. Similarly, Holland and Kenmuir (2005) suggested that after the omission of an expected reward, CeA might in some circumstances enhance the value of remaining rewards, perhaps through its influence on midbrain dopamine reward systems. However, these suggestions did not provide *a priori* accounts for why CeA function was critical only for processing changes observed after the unexpected omission of an event and not those occurring after the unexpected presentation of an event. Furthermore, these accounts did not specify the precise nature of coding in CeA neurons critical to those processes. For example, CeA neurons might encode prediction errors directly or they might provide information about reward expectancies or value necessary for the computation of prediction errors in downstream targets such as the midbrain dopamine neurons.

To address these questions, we recorded single unit activity in CeA of rats engaged in a simple choice task in which we shifted the value of expected rewards up or down by changing their number or timing. The shifts in reward value were comparable to those used in previous tasks (see Holland and Kenmuir, 2005 for use of a related variation on unblocking). We found that firing in many CeA neurons increased in response to omission of an expected reward, but firing in CeA neurons did not change in response to presentation of an unexpected reward. Furthermore, encoding of reward value or more general prediction errors was not observed in these or any other population of CeA neurons. Omission-responsive activity correlated with orienting responses thought to be related to attention, and these behavioral responses were sensitive to inactivation of CeA. These data directly support the proposal that CeA plays a very specific role in signaling variations in event processing when rewards become worse than expected, and further suggest that this reflects signaling of actual reward omission.

RESULTS

CeA neurons were recorded in a choice task. On each trial, rats responded to one of two adjacent wells after sampling an odor at a central port. Rats were trained to respond to three different odor cues: one that signaled reward in the right well (forced-choice), a second that signaled reward in the left well (forced-choice), and a third that signaled reward in either well (free-choice). Rats were fully trained on this task prior to recording. Subsequently, during recording sessions, we manipulated the value of the available reward by changing either the timing (short versus long) or the size (big versus small) of the reward in each well across four blocks. This design resulted in upshifts and downshifts in expected reward at several transition points between blocks (figure 1A). Upshifts occurred when new, more valuable rewards were introduced in blocks 2^{sh}, 3^{bg}, and 4^{bg}. Downshifts occurred when these rewards were suddenly omitted in blocks 2^{lo} and 4^{sm}.

As illustrated in figure 1B, rats reliably changed their behavior in response to these value manipulations. On forced choice trials, rats responded significantly faster (ANOVA, main effect of value, $F(1, 92) = 98.71$, $p < 0.0001$) and more accurately (ANOVA, main effect of value, $F(1, 92) = 182.11$, $p < 0.0001$) after sampling the odor cues associated with high value rewards. Further, on free choice trials, rats chose high value rewards significantly

more often than they chose low value rewards (ANOVA, main effect of value $F(1, 92) = 542.71$, $p < 0.0001$).

In addition, we observed changes in orienting to the odor port during trial initiation after shifts in reward. Rats were faster to orient to the port after light onset at the beginning of trial blocks in which a value shift occurred than at the end of the previous block when reward value had been learned (figure 1C; ($F (1, 278) = 7.4$, $p < 0.008$), and the speed of orienting slowed again as rats learned the value of the rewards within these blocks ($F (1, 278) = 96.23$, $p < 0.0001$). Notably orienting speed became faster after a change in reward regardless of the direction of the shift (figure 1C, inset; upshift, $F (1, 185) = 83.20$, $p < 0.0001$, downshift, $F (1, 185) = 30.41$, $p < 0.0001$) and became progressively faster across the first several trials following a shift (figure 1C). This pattern of increases in orienting following both increments and decrements in reward has been frequently observed in behavioral studies (Kaye and Pearce, 1984; Pearce et al., 1988; Swan and Pearce, 1988) and is predicted by theories that relate variations in cue processing to surprise (Pearce and Hall, 1980).

We recorded 266 single units in the CeA in seven rats over 93 behavioral sessions. Recording locations are illustrated in figure 2A. Consistent with the hypothesis that CeA is critical for signaling changes in reward value, many neurons tended to fire significantly more at the time of reward omission (blocks 2^{lo} and 4^{sm}) than at baseline. This is illustrated by the single unit example shown in figure 2B (left); activity actually increased when a reward was omitted at the transition from immediate (1^{sh}) to delayed reward (2^{lo}). Activity in the same neuron did not change when a reward was added (2B center; transition from delayed (1^{lo}) to immediate reward (2^{sh})), nor did it change when there was no overt shift in reward (2B right; transition from immediate reward (2^{sh}) to small reward (3^{sm})).

Omission-responsive neurons, such as the one shown in figure 2B, comprised a significant proportion of the population. The distribution of the contrast scores comparing firing to omission versus baseline (1 s pre-trial) in each neuron was shifted significantly above zero (figure 2C; $p < 0.001$, $\mu = 0.022$), and analysis of activity in each neuron showed that 9% of the population (25 neurons) significantly increased firing to the omission of an expected reward in blocks 2^{lo} and 4^{sm}. This proportion was significantly larger than that expected by chance ($X^2 = 10.77$, $p < 0.01$). By contrast, significantly fewer neurons ($n = 12$, 4%, $X^2 = 4.91$, $p < 0.05$) showed the opposite effect, a proportion that did not differ from what would be expected by chance ($X^2 = 1.36$, $p = 0.24$). The average activity of these 25 omission-responsive neurons is shown in figure 3. Increased firing was evident in blocks 2^{lo} and 4^{sm} when expected rewards were omitted (figure 3A). Activity in these neurons increased sharply at the start of these blocks, when reward was first omitted, and then declined, with learning. By contrast, activity in these neurons showed little change when a new reward was introduced in blocks 2^{sh}, 3^{bg}, and 4^{bg} (figure 3B) or when there was no overt shift in reward value in block 1^{sh}, 1^{lo}, 3^{sm} (figure 3C).

Notably, some omission-responsive neurons also fired to reward (64% vs. pre-trial baseline, see supplemental figure S1A). However while there was a significant increase in firing at the time of reward, the omission-related activity was significantly higher (figure 4A), and the distribution of contrast scores comparing firing to reward and reward omission was shifted significantly above zero (figure 4A inset; wilcoxon t-test; $p < 0.001$, $\mu = 0.058$). Higher firing on omission suggests that this activity did not reflect memory for reward. Consistent with this idea, reward-related firing in these neurons did not vary with the value of the reward. This is illustrated in figure 4B, which plots activity in this population during delivery of high and low value rewards after learning, and also by the inset showing the contrast scores, which compare activity during the delivery of low and high value rewards

after learning. The distribution of these contrast scores was not shifted significantly away from zero (figure 4B inset; wilcoxon t-test: $p = 0.679$, $\mu = 0.072$).

Firing at the time of reward omission in these neurons also changed with learning, consistent with the proposal that the observed signal reflected violations of reward expectations. This is illustrated in figure 5A, which plots activity in the omission-responsive neurons during the first five versus the last five trials in the downshift blocks (2^{lo} and 4^{sm}), corresponding to the times at which we see maximal differences in behavior (orienting and learning). Activity in the omission-responsive population was significantly higher early in these blocks, when reward was omitted unexpectedly, than at the end, when the value of the reward had been learned, and the distribution of contrast scores comparing firing at the time of reward omission early versus late in the downshift blocks was shifted significantly above zero (figure 5A inset; wilcoxon t-test; $p < 0.05$, $\mu = 0.064$). Thus, these neurons fired more to omission than at baseline, and this phasic response was strongest when omission was fully unexpected, at the start of the block.

By contrast, this population of neurons did not exhibit changes in firing to upshifts in reward value. This is illustrated in figure 5B, which plots activity in the omission-responsive neurons during the first five and last five trials of blocks in which reward was increased or delivered unexpectedly (2^{sh}, 3^{bg}, and 4^{bg}). These blocks were often concurrent with omission blocks, and yet there was no difference in activity early versus late at the time of reward delivery (figure 5B inset: wilcoxon t-test; $p = 0.75$, $u = -0.013$). Activity in the omission-responsive population also did not change across blocks in which there was no overt shift in reward value (1^{sh}, 1^{lo}, and 3^{sm}; figure 5C: wilcoxon t-test: $p = 0.28$, $u = -0.045$).

We next looked at whether changes in firing on reward omission in CeA might be related to changes in behavior on these trials. As described earlier (figure 1C), we observed changes in the latencies of orienting responses to the odor port after shifts in reward. The direction and pattern of these changes were consistent with those predicted by theoretical accounts relating prediction errors to attention to or processing of conditioned stimuli (Pearce and Hall, 1980).

Consistent with the idea that the omission signal might be relevant to such increased processing, activity in the omission-responsive CeA neurons was closely related to changes in the rats' latency to respond at the odor port following illumination of a panel light signaling the start of each trial. This is illustrated in figure 6A, which plots the difference in firing in each omission-responsive neuron in the first versus the last 5 trials of a downshift block, in relation to the orienting response on each following trial (i.e., how fast the rat initiated the trial immediately after the omission of reward that generated the neural data). As indicated by the significant inverse correlation ($r = -0.321$, $p = 0.023$), a stronger neural response to omission of an expected reward was correlated with faster orienting on the next trial, as predicted by the Pearce-Hall model. Notably, there was no correlation between firing in the omission-responsive neurons on upshift trials and latency to respond on the next trial (figure 6B, $r = 0.066$, $p = 0.650$).

To further investigate the relationship between the signaling of omission by CeA neurons and changes in orienting, we conducted an additional experiment in which we inactivated CeA during performance of the same choice task used for recording. Eight rats with bilateral guide cannulae targeting CeA were trained to a point at which their behavior was similar to that of the rats used for recording. Infusion sites are illustrated in figure 7A. Rats performed the choice task after bilateral infusions of NBQX, a competitive AMPA receptor antagonist,

or PBS vehicle. Eight rats contributed to a total of thirty-four behavioral sessions after infusion, with each rat receiving on average two saline and two NBQX infusions.

Consistent with the correlation between neural activity and behavior, as well as the reported effects of CeA-lesions on conditioned orienting behavior, inactivation of CeA disrupted changes in orienting responses after shifts in reward value. This effect was specific to orienting after downshifts in reward value (figure 7B); there was no effect of inactivation on faster orienting caused by reward upshifts (figure 7C). Accordingly a 3-factor ANOVA (treatment X shift type X phase) revealed a significant treatment x shift x phase interaction ($F(1, 33) = 5.37$, $p = 0.027$), and posthoc comparisons showed that while rats oriented to the odor port faster after upshifts on both saline and NBQX days (planned comparison early versus late saline: $p = 0.005$, NBQX: $p = 0.05$), they showed faster orienting after downshifts only on saline days (planned comparison early vs. late saline $p = 0.042$, NBQX: $p = 0.23$).

Lastly, we compared activity in the omission-responsive neurons in CeA to reward-evoked firing in the basolateral amygdala and midbrain dopamine neurons at the time of reward in this task, reported previously (Roesch et al., 2010; Roesch et al., 2007). For this comparison, we plotted the change in firing in the omission-responsive CeA neurons to reward or reward omission across the first and last 10 trials in the downshift and upshift blocks. As expected from analyses presented earlier, activity remained unchanged in response to increased reward in the upshift blocks, while firing increased immediately at the start of the downshift block in response to the unexpected decrement in reward value. This impression was confirmed by a two factor repeated measures ANOVA (shift X phase) that revealed significant main effects of shift ($F(1, 49) = 12.67$, $p < 0.001$) and phase ($F(1, 49) = 8.78$, $p < 0.005$) and by posthoc comparisons that showed that firing in omission responsive neurons was significantly greater immediately after downshifts in reward value than after learning (planned comparison early versus late downshift: $p < 0.05$).

In addition, the comparison with reward-evoked activity in basolateral amygdala (figure 8B, top) and midbrain dopamine neurons (figure 8C, top) served to highlight several unique features of the error signal in CeA. While reward-evoked activity in these two regions complied closely with predictions of modified Pearce-Hall models (figure 8B, bottom) and Rescorla-Wagner (figure 8C, bottom) respectively, activity in CeA (figure 8A, top) failed to match key features of either model. Particularly relevant in this regard was that the CeA signal lacked two key features characteristic of a Pearce-Hall signal present in basolateral amygdala: increases in firing to both upshifts and downshifts in reward and a progressive increase at the start of a block reflecting the integration of the Pearce-Hall signal across trials. As a result, in order to accurately model the data from CeA, it was necessary to modify the Pearce-Hall model so that the attentional signal only increased when actual reward was less than expected and to set the constant determining the contribution of prior trials to zero (figure 8A, bottom). As a result, the CeA signal becomes more similar in some regards to that in the midbrain dopamine neurons. This has important implications for the relationships among these different error signaling systems.

DISCUSSION

In this report we have demonstrated that CeA neurons signal omission of an expected reward in a temporally-precise fashion. Activity in these neurons was correlated with faster orienting to the odor port after unexpected decrements in reward value, and that faster orienting was selectively abolished by CeA inactivation. Notably, cue-directed orienting similar to that observed here has been found to habituate with repeated confirmation of cue-reward expectancies, but re-emerges when those learned stimulus relationships are violated

(Kaye and Pearce, 1984; Pearce et al., 1988; Swan and Pearce, 1988). Such behaviors have been hypothesized to reflect increased attention to and processing of those cues, which are learned about more rapidly after predictive relationships are violated. At the same time, we did not find CeA neurons that responded more to the surprising delivery of reward than to the expected delivery of that reward.

These results are broadly consistent with lesion studies showing that CeA is critical for allocating attention for increased processing of events after downshifts but not upshifts in reward value (Holland and Gallagher, 1993b). Our results provide a potential neural mechanism to account for this behavioral finding in the activity of the omission responsive population, while at the same time ruling out several prominent alternative hypotheses for the role of CeA. For example, it is unlikely that CeA provides some downstream region information about value or expectancies necessary to compute prediction errors (e.g., (Lee et al., 2006), because we found little CeA coding of value or expectancies at the time of cue or reward presentation (supplemental figure S1). Furthermore, our data do not support the possibility that CeA plays a more general role signaling any change to reward value. We failed to find any evidence of general signaling of either a signed or unsigned errors in CeA neurons. This was true both in the omission-responsive population and also in a more generally reward-responsive population (supplemental figure S1B, and S2), neither of which showed differential firing to reward based on whether or not it was expected. Indeed a straightforward analysis for prediction errors, similar to that applied to characterize activity in midbrain dopamine neurons in this task (Roesch et al., 2007), failed to find evidence of general error signaling (see supplemental materials). We also did not see a CeA signal indicating increased processing of remaining rewards on omission trials (Holland and Kenmuir, 2005). This suggests that CeA does not signal enhanced value of remaining rewards on omission trials. Instead CeA appears to provide a very specific signal, reporting when an expected reward is omitted.

Both our observations of the effects of CeA inactivation on orienting to the odor port and the results of previous lesion and inactivation studies show that CeA function is essential for surprise-induced enhancements in cue associability. Thus, the lack of cue-evoked activity in the present study is especially interesting, because it suggests that the CeA is not itself coding increased cue processing or supporting behavior associated with that processing, but rather is driving downstream variations in cue processing by providing a signal for unconditioned stimulus (US) omission. This assertion is consistent with the results of inactivation studies, which showed that CeA function is critical only at the time of the surprising omission of important events, and not when that enhanced cue processing is expressed in faster learning (Holland and Gallagher, 2006). Although CeA is critical to changes that occur when surprising event omission is processed, it is not an essential site of storage of information acquired at that time. By contrast, Holland and Gallagher (2006) found the opposite pattern for basal forebrain neurons, whose normal function was critical when the enhanced learning was eventually expressed, but not at the time of the surprise on which that enhancement depends. Notably, other experiments have shown that omission-induced enhancements in cue processing depend on communication between CeA and basal forebrain cholinergic neurons, which in turn project to the posterior parietal cortex (Bucci et al., 1998; Chiba et al., 1995; Han et al., 1999; Lee et al., 2008; Lee et al., 2006) Thus, those regions seem likely candidates for representing enhanced attention to cues after CeA-dependent processing of the omission of important events.

Neuronal activity in the omission-responsive CeA population contrasts with both the signed and unsigned error signals that have been observed previously in other brain regions of rats performing this task (Roesch et al., 2010; Roesch et al., 2007). For example, we have previously reported that activity in basolateral amygdala signals errors at the time of reward

in this task. Our results supported two other reports showing that activity in basolateral amygdala was modulated by reward expectancy and omission (Belova et al., 2007; Tye et al., 2010). Given the substantial communication between basolateral amygdala and CeA (Pare et al., 2004), it would seem reasonable at first glance to suppose that error signals in the two regions might be related. However the signal reported here differs in important ways from that in the basolateral amygdala (Figure 8B; Roesch et al., 2010). There we found a unidirectional error signal that tracked closely with predictions of the Pearce-Hall theory of attention in associative learning (Pearce and Hall, 1980). In Pearce and Hall's theory, attention to a stimulus is adjusted proportionally to the absolute value of the reward prediction error, decreasing when rewards are well-predicted and increasing when rewards are unexpectedly increased or decreased in value. Signaling in basolateral amygdala increased in response to both increases and decreases in reward value and also integrated across trials, matching closely with an extended version of the Pearce-Hall theory (Pearce J.M., 1982). By contrast, the signal in CeA increased selectively for omission of expected reward (figure 8A). Furthermore, whereas in CeA changes in firing were confined to the time of reward omission, in basolateral amygdala increased firing to a less valuable reward encompassed the entire reward period. Thus, CeA neurons provide a much more specific and temporally accurate account of reward omission than do basolateral amygdala neurons.

Of course, basolateral amygdala could provide a general Pearce-Hall-like unsigned error signal (Belova et al., 2007; Roesch et al., 2010; Tye et al., 2010) to CeA, which could then extract a more specialized signal for further processing. However, learning supported by CeA in response to omission of important events is typically not affected by damage to basolateral amygdala (Holland et al., 2001). It is also difficult to imagine how the signal in basolateral amygdala, which integrates across trials, could be transformed into the signal in CeA, which does not. Such evidence suggests at a minimum that the signal in CeA is not derived from or serially dependent on the signal in basolateral amygdala.

Indeed the timecourse of the signal in CeA and its general features have more in common with activity in the midbrain dopamine neurons. Although dopamine neurons show decreases rather than increases in firing in response to reward omission (Figure 8C; Roesch et al., 2007), the timecourse of the change is similar in the two areas. In addition, the specificity of the change in firing to the precise time of omission is mirrored by firing in dopamine neurons, which shows a high degree of temporal specificity. Notably, CeA receives strong projections from the midbrain dopamine neurons most closely associated with signaling of simple bidirectional reward prediction errors (Pitkanen, 2000; Swanson, 1982; Wallace et al., 1992), and communication between CeA and the midbrain has been shown to be essential for enhancement of learning after omission of expected events (Lee et al., 2008; Lee et al., 2006). Negative prediction errors signaled by midbrain dopamine neurons may be conveyed to CeA, which might then activate basal forebrain cholinergic neurons and other attention-related systems for increases in attention after omission of expected events.

All of these findings indicate, perhaps unsurprisingly, that the theoretical accounts developed by Rescorla and Wagner (Rescorla and Wagner, 1972), Pearce and Hall (Pearce and Hall, 1980) and others become more complex when they are implemented by neural circuitry. This is already evident for temporal difference reinforcement learning in the growing number of studies linking signaling of simple bidirectional reward prediction errors to neural signaling in a variety of brain regions (Hollerman and Schultz, 1998; Hong and Hikosaka, 2008; Matsumoto and Hikosaka, 2007; Montague et al., 1996; Schultz et al., 1997; Schultz and Dickinson, 2000; Waelti et al., 2001). It seems likely that this general mechanism is implemented by a variety of neural circuits, some working in concert with connected regions and others acting more independently. The data presented here suggest a

similar situation likely exists for instantiation of the elegant account of error-driven variations in event processing developed by Pearce and Hall (Pearce J.M., 1982; Pearce and Hall, 1980). Future work is necessary to dissociate the contributions of these various error signaling mechanisms both to downstream neural processing and the subsequent expression of attention and learning.

EXPERIMENTAL PROCEDURES

This research was conducted at the University of Maryland School of Medicine in accordance with university and the National Institutes of Health guidelines. Seven adult male Long-Evans rats were used for recording and eight rats for inactivation (obtained at 175–200g from Charles River Laboratories, Wilmington, MA).

Surgical Procedures

For recording, a drivable electrode bundle was chronically implanted in the left hemisphere at 2.3 mm posterior to bregma, 4.0 mm laterally, and 6.95 mm ventral to the surface of the brain for recording in CeA. This electrode bundle was composed of ten, 25-μm diameter FeNiCr wires (Stablohm 675, California Fine Wire, Grover Beach, CA) in a 27-gauge thin wall cannula (Small Parts, Miami Lakes, FL). Immediately prior to implantation, these wires were freshly cut with surgical scissors to extend ~1 mm beyond the cannula and electroplated with platinum (H_2PtCl_6, Aldrich, Milwaukee, WI) to an impedance of ~300 kOhms. After recording, the electrode bundle was advanced in 40-μm increments to acquire activity from new neurons for the following day. In a given session, neural activity was acquired from neurons in CeA. For inactivation, infusion cannulae (23G; Plastics One inc., Roanoke, VA) were implanted bilaterally in CeA (2.3 mm posterior to bregma, 4.0 mm lateral, and 6.0 mm ventral from skull surface). Actual infusions were made at 2.3 mm posterior, 4.0 mm lateral, and 8.0 mm ventral in CeA), in order to allow infusion of inactivating agents NBQX (1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium salt hydrate from Sigma Aldrich, St. Louis, MO) or phosphate buffered saline (PBS) vehicle prior to test sessions.

Behavioral Apparatus and Training Procedures

Recording and inactivation sessions were conducted in aluminum chambers approximately 18 inch on each side, with sloping walls narrowing to an area of 12 inch by 12 inch at the bottom. A central odor port was located above the two fluid wells. Two lights were located above the panel. The odor port was connected to an air flow dilution olfactometer to allow the rapid delivery of olfactory cues. Trials were signaled by illumination of the panel lights inside the box. When these lights were on, a nosepoke into the odor port resulted in delivery of the odor cue to a small hemicylinder behind this opening. One of three odors was delivered to the port on each trial, in a pseudorandom order. At odor offset, the rat had 3 s to make a response at one of the two fluid wells located below the port. One odor instructed the rat to go to the left to get a reward, a second odor instructed the rat to go to the right to get a reward, and a third odor indicated that the rat could obtain a reward at either well. Odors were presented in a pseudorandom sequence so that the free-choice odor was presented on 7/20 trials and the left/right odors were presented in equal numbers. In addition, the same odor was not presented any more than three consecutive trials. Once the rats were trained to perform this basic task, we introduced blocks in which we independently manipulated the size of the reward and the delay preceding reward delivery. For recording, one well was randomly designated as short and the other long at the start of the session. In the second block of trials these contingencies were switched. The length of the delay under long conditions was determined by the following procedure. The side designated as long started off as 1 s and increased by 1 s every time that side was chosen until it became 3 s. If the rat

continued to choose that side, the length of the delay increased by 1 s to a maximum of 7 s. If the rat chose the side designated as long on fewer than 8 out of the previous 10 choice trials then the delay was reduced by 1 s to a minimum of 3 s. The reward delay for long forced-choice trials was yoked to the delay in free-choice trials. In the third block of trials, delay was held constant (500 ms) and the reward size was manipulated by presenting additional boli, such that responding at one well resulted in big reward and the other small reward. In the final block the size contingencies were switched. During training, rats were maintained on water restriction. After each session, the rats were given ad lib access to water for 10–30 min depending on the fluid intake of each rat during the session.

Single-unit recording

Procedures were the same as described previously (Roesch et al., 2007). Wires were screened for activity daily. If single units were not detected the rat was removed and the electrode assembly was advanced 40 or 80 μm. Otherwise active wires were selected to be recorded, a session was conducted, and the electrode was advanced at the end of the session. Neural activity was recorded using two identical Plexon Multichannel Acquisition Processor systems (Dallas, TX), interfaced with odor discrimination training chambers. Signals from the electrode wires were amplified 20X by an op-amp headstage (Plexon Inc, HST/8o50-G20-GR), located on the electrode array. Immediately outside the training chamber, the signals were passed through a differential pre-amplifier (Plexon Inc, PBX2/16sp-r-G50/16fp-G50), where the single unit signals were amplified 50X and filtered at 150–9000 Hz. The single unit signals were then sent to the Multichannel Acquisition Processor box, where they were further filtered at 250–8000 Hz, digitized at 40 kHz and amplified at 1–32X. Waveforms (>2.5:1 signal-to-noise) were extracted from active channels and recorded to disk by an associated workstation with event timestamps from the behavior computer. Waveforms were not inverted before data analysis.

Inactivation Procedures

On each test day, cannulated rats (n = 8) received bilateral infusions of either the inactivating agent NBQX or the vehicle PBS immediately prior to performance in the choice task. NBQX is a competitive AMPA receptor antagonist, which blocks excitatory post-synaptic potentials. Infusion procedures were identical to those used previously (Roesch et al., 2010) except that the CeA was targeted in this study. Briefly, dummy cannulae were removed and 30G injector cannulae extending 2.0 mm beyond the end of the guide cannulae were inserted. Each injector cannula was connected with PE20 tubing (Thermo Fisher Scientific, Inc., Waltham, MA) to a Hamilton syringe (Hamilton, Reno, NV) placed in an infusion pump (Orion M361, Thermo Fisher Scientific, Inc., Waltham, MA). Volume and concentration of NBQX were based on prior work by Holland and colleagues (McDannald et al., 2005). Each infusion consisted of 4 μg NBQX (Sigma, St Louis, MO). The drug was dissolved in 0.2 μl PBS and infused at a flow rate of 0.2 μl/min. At the end of each infusion, the injector cannulae were left in place for another two to three minutes to allow diffusion of the drugs away from the injector. Approximately 10 min after removal of the injector cannulae rats performed the choice task. The order of infusions was counterbalanced such that each NBQX session had a corresponding vehicle session for comparison. Rats received reminder training between pairs of inactivation and vehicle sessions.

Data Analysis

Units were sorted off-line using software from Plexon Inc. For this analysis, files were first imported into Offline Sorter where waveforms on each channel were sorted using a template-matching algorithm. Sorted files were then processed in Neuroexplorer to extract these unit time stamps and relevant event markers. These data were subsequently analyzed using statistical routines in Matlab (Natick, MA) to examine activity during designated

behavioral epochs. The baseline epoch was determined as the one second period prior to light onset. The reward epoch was defined as the one second period from onset of reward delivery (0–1000 ms). The omission epoch was defined as the one second period from onset of reward omission (delay: 500 ms from well entry + 1000ms, size: 1000 ms from well entry + 1000 ms i.e. omission of second drop). Wilcoxon t-tests were used to determine significant shifts from zero in distribution plots. T-tests or ANOVAs were used to measure within cell differences in firing rate. Pearson Chi-square tests ($p < 0.05$) were used to compare the proportions of neurons. Behavioral data from inactivation sessions was processed and analyzed using Matlab. Reaction times were calculated as the time elapsed between beam breaks for different events (i.e. odor guided response reaction time (figure 1B) is the time elapsed between odor offset until the rat unpoked from odor port; orienting reaction time (figure 1C and 7B/C) is the time lapsed from light onset until the rat nose poked into the odor port). Repeated measure ANOVAs were used to measure within subjects differences in behavior in Statistica (Statsoft, Tulsa, OK).

Histology

Following testing, rats were given an overdose of isoflurane and prepared for perfusion. Immediately prior to perfusion, the final electrode position was marked by passage of a 15-μA current through each microwire for approximately 10 s to create a small iron deposit. The rats were then perfused intracardially with 0.9% saline followed by 4% formaldehyde followed by 100 ml of 3% potassium ferrocyanide in perfusate (for recording only) to visualize the iron deposit. Brains were removed from the skulls and stored in a 30% sucrose/4% formaldehyde/3% potassium ferrocyanide solution (for recording only) for several days until sectioning. The brains were sectioned on a freezing microtome, and coronal sections (40 μm) collected through CeA. Sections were mounted on glass slides, stained with thionin, and coverslipped with DPX. Electrode and cannulae placements were verified under a light microscope and drawn onto plates adapted from the atlases of Paxinos and Watson sixth edition (2009).

Modeling

Simulations of the neural signal in CeA were based on an adaption of the original Pearce-Hall model in which only negative prediction errors (reward was worse than expected) result in attentional increments. The parameter used was $S = 0.2$. Simulations of the unsigned neural signal in ABL were based on the extended version of the Pearce and Hall model (Pearce J.M., 1982) with parameters $\gamma = 0.6$, $S = 0.1$. Simulations of the bidirectional neural signal in VTA were based on the Rescorla-Wagner model (Rescorla and Wagner, 1972), with $\alpha = 0.2$. These standard parameters were used to simulate activity across a series of theoretical training trials, and the output was rescaled to approximate the range of the neural data. Importantly, the critical features of the shape of the curves were not dependent on these parameters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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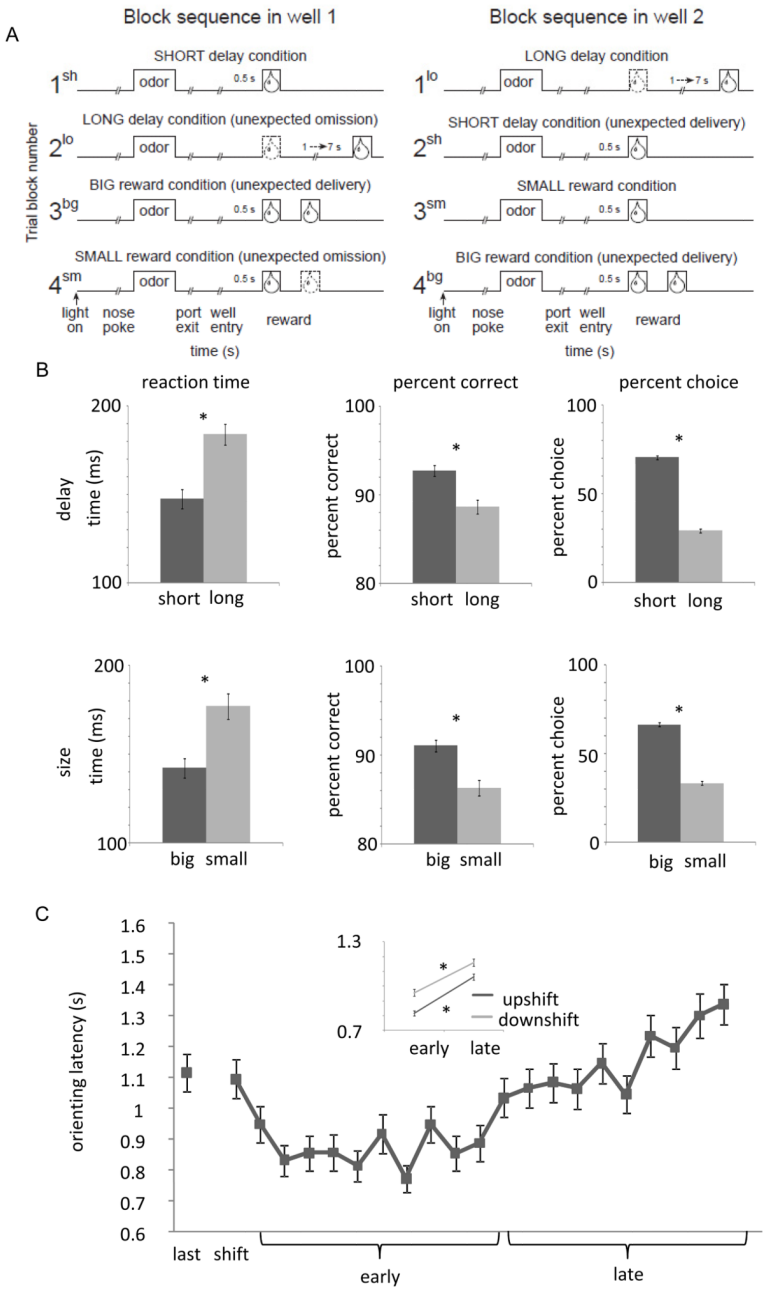

Figure 1.

Behavioral performance during recording in CeA. A. Choice task block sequence. In the beginning of the session reward available at one well was presented at a short delay (500 ms) and the other well after a long delay (1–7 s) (counterbalanced across days). In block two, reward contingencies were switched, such that the well that was previously short delay becomes long, resulting in a surprising omission of expected reward at 500 ms (downshift, 2^{lo}). Concurrently, a surprising reward delivery occurs at the well that was previously associated with long delay that is now designated as short delay (upshift, 2^{sh}). In the third block delay to reward is held constant and reward size is manipulated. Importantly, while big reward (one bolus at 500 ms and another at 1 s) is surprisingly better than delayed reward (one bolus at 1–7 s) (upshift, 3^{bg}), small reward (one bolus at 500 ms) is identical to

reward delivered at a short delay (one bolus at 500 ms; no shift). In the fourth block the size contingencies are switched, such that small reward becomes big (upshift, 4^{bg}) and big becomes small (downshift, 4^{sm}). B. Behavior during choice task performance in recording sessions. (Top) Impact of delay length on reaction time (left) and percent correct (center) during forced trials, and percent choice (right) during free choice trials. (Bottom) Impact of reward size on reaction time (left) and percent correct (center) during forced trials, and percent choice (right) during free choice trials. C. Impact of surprising value shifts ($2^{sh/lo}$, $3^{bg/sm}$, $4^{bg/sm}$) on orienting latency during recording sessions. 'Last' indicates the last trial of the previous block. 'Shift' indicates the first trial in a block just before the rats have experienced a value shift. Inset shows change in orienting latencies across shift blocks as rats learn about upshifts and downshifts in reward value. Error bars represent standard error of the mean.

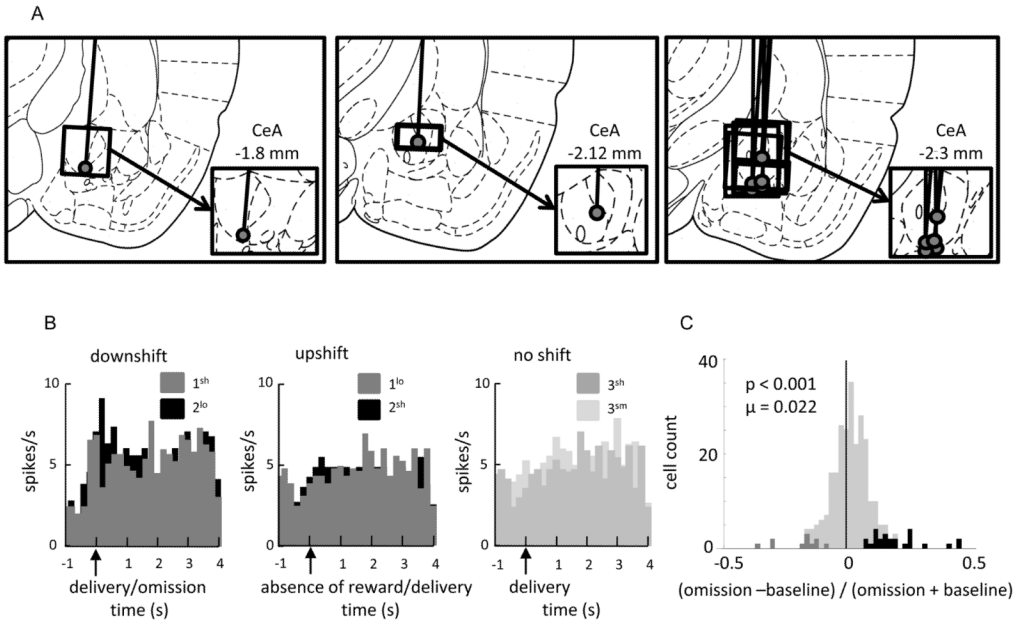

Figure 2.

Effect of reward omission on neural activity in CeA. A. Location of recording electrodes. Gray dots represent final electrode placement, boxes represent approximate extent of recording sites, and black lines indicate center of electrode track. Plates adapted from the atlases of Paxinos and Watson (2009). B. Activity from a single CeA omission responsive neuron to downshift (left: activity from immediate reward block (1^{sh}) and delayed reward block (2^{lo}) aligned on reward delivery or omission respectively; center: activity from delayed reward block (1^{lo}) and immediate reward block (2^{sh}) aligned on when immediate reward is absent (1^{lo}) or present (2^{sh}) respectively; right: when no shift in reward value occurs (activity from delayed reward block (2^{sh}) to small size block (3^{sm}) aligned on reward delivery). C. Index of CeA neural activity to reward omission over baseline. Distribution of contrast scores for all neurons recorded in CeA comparing activity at the time of reward omission (2^{lo} / 4^{sm}) versus baseline activity. Black bars indicate single units that were defined as omission responsive (showed significantly greater firing to reward omission than to baseline). Darker gray bars indicate single units that were significantly suppressed during reward omission (count not different than would be expected by chance). Lighter gray bars indicate non-selective cells (omission vs. baseline) in CeA. For a waveform analysis of all 266 neurons recorded in CeA, see figure S3.

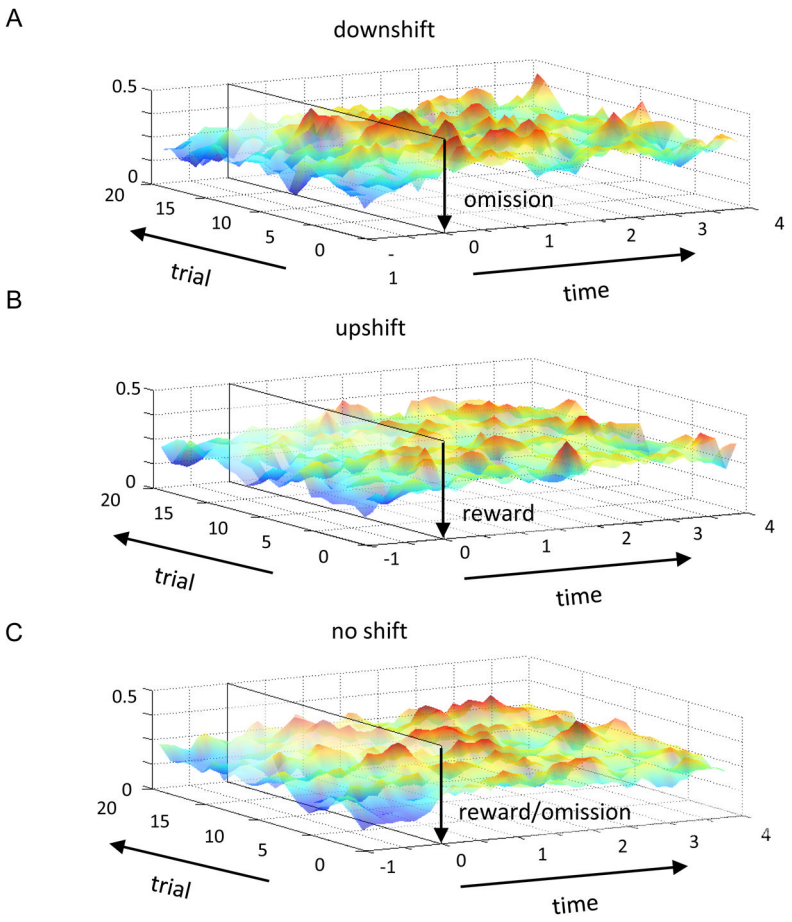

Figure 3.

A. Population heat plot representing neural activity in CeA omission-responsive neurons at the time of reward omission (2^{lo} / 4^{sm}). Average activity is shown for the first and last 10 trials in these two blocks. Activity is shown, aligned on reward omission, which is 500 ms after well entry in the 2^{lo} block and 1000 ms after well entry in the 4^{sm} block. B. Population heat plot representing neural activity in CeA omission-responsive neurons at the time of reward delivery (2^{sh} / 3^{bg} / 4^{bg}). Average activity is shown for the first and last 10 trials in these three blocks. Activity is shown, aligned on reward delivery, which is 500 ms after well entry in the 2^{sh} block and 1000 ms after well entry in the 3^{bg} and 4^{bg} blocks. C. Population heat plot representing neural activity in CeA omission-responsive neurons during blocks in which there was no overt shift in reward value (1^{sh} / 1^{lo} / 3^{sm}). Average activity is shown for the first and last 10 trials in these three blocks. Activity is shown, aligned on reward delivery or omission, which is 500 ms after well entry in the 1^{sh} and 1^{lo} blocks and 1000 ms after well entry in the 3^{sm} block. For activity in the omission responsive population aligned on cue and reward delivery across the entire task, see figure S1A. For activity in a CeA reward responsive population, see figure S1B.

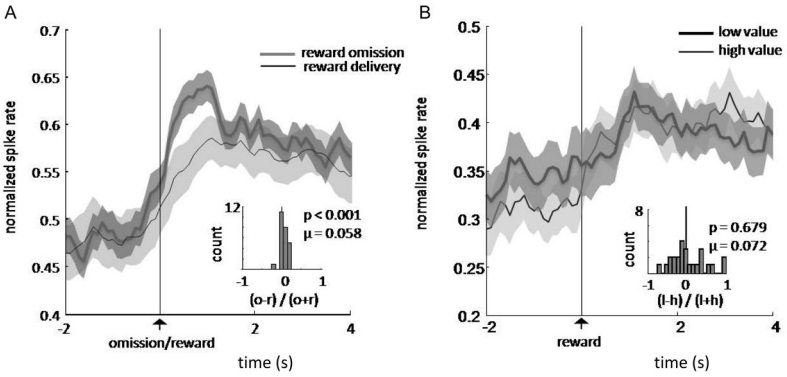

Figure 4.

Effect of reward on activity in omission-responsive CeA neurons. A. Neural activity in CeA in response to reward delivery and omission. Impact of reward delivery or omission on activity in omission responsive population of neurons. Curves represent the normalized population firing rate (normalized to the maximum firing rate for each individual neuron) as a function of time across reward ($1^{sh/lo}$, $2^{sh/lo}$, $3^{bg/sm}$, $4^{bg/sm}$) or omission blocks (2^{lo} / 4^{sm}). Activity aligned on reward omission or delivery. Inset shows the distribution of contrast scores for omission responsive neurons comparing activity at the time of reward omission versus activity at the time of reward delivery (o = omission, r = reward). B. Impact of reward value on activity in omission responsive population of neurons. Curves represent the normalized population response as a function of time after rats had learned (last five trials in a block) about high value (1^{sh}, 2^{sh}, 3^{bg}, 4^{bg}) and low value (1^{lo}, 2^{lo}, 3^{sm}, 4^{sm}) reward conditions. Activity aligned on reward delivery. Inset shows the distribution of contrast scores for activity after learning during delivery of low value rewards versus activity during delivery of high value rewards (l = low value, h = high value). Error bars represent standard error of the mean.

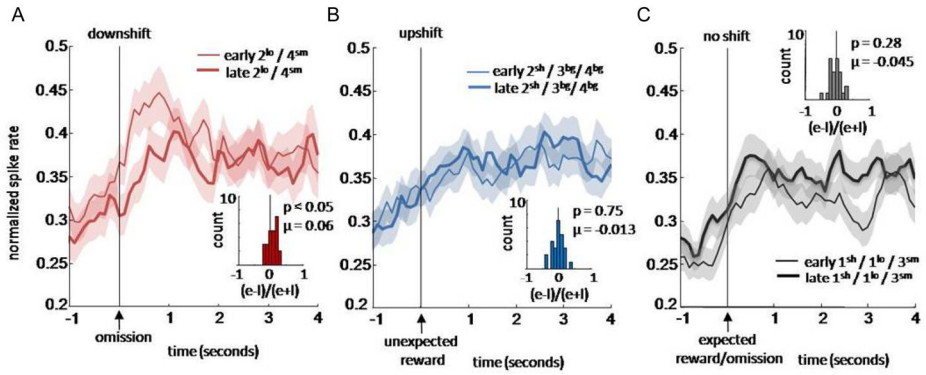

Figure 5.

Effect of learning on omission and reward related activity in omission-responsive CeA neurons. A. Curves represent the normalized population firing rate (normalized to the maximum firing rate for each individual neuron) as a function of time during the first five and last five trials presented within downshift blocks (2^{lo} and 4^{sm}). Activity aligned on reward omission. Inset shows the distribution of contrast scores for activity e = early (first five trials) versus l = late (last five trials) for downshift condition (2^{lo} and 4^{sm}). B. Curves represent the normalized population firing rate as a function of time during the first five and last five trials presented within upshift blocks (2^{sh},3^{bg}, and 4^{bg}). Activity aligned on reward delivery. Inset shows the distribution of contrast scores for activity early versus late during upshift blocks (2^{sh},3^{bg}, and 4^{bg}). C. Curves represent the normalized population firing rate as a function of time during the first five and last five trials presented within non-shift blocks (1^{sh}, 1^{lo} and 3^{sm}). Activity aligned on reward delivery for high value or omission for low value conditions. Inset shows the distribution of contrast scores for activity early versus late during blocks with no shift in reward value (1^{sh}, 1^{lo} and 3^{sm}). Error bars represent standard error of the mean. For the effect of learning on omission and reward related activity in CeA reward responsive neurons, see figure S2.

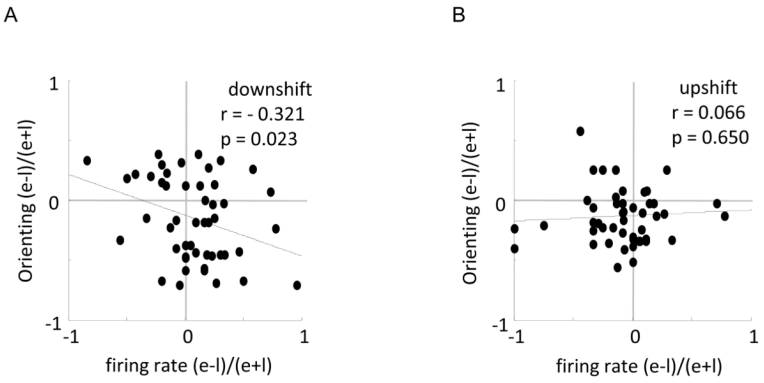

Figure 6.

Correlations between signaling of reward omission by CeA neurons and shifts in orienting behavior. Plots show normalized orienting latencies on trial after a shift as a function of normalized firing rate in omission responsive neurons on trial of a shift. A. Correlation between contrast indices for firing rate on trials of a downshift versus orienting latency on trials after a downshift. B. Correlation between contrast indices for firing rate on trials of an upshift versus orienting latency on trials after an upshift. e = early = first five trials, l = late = last five trials.

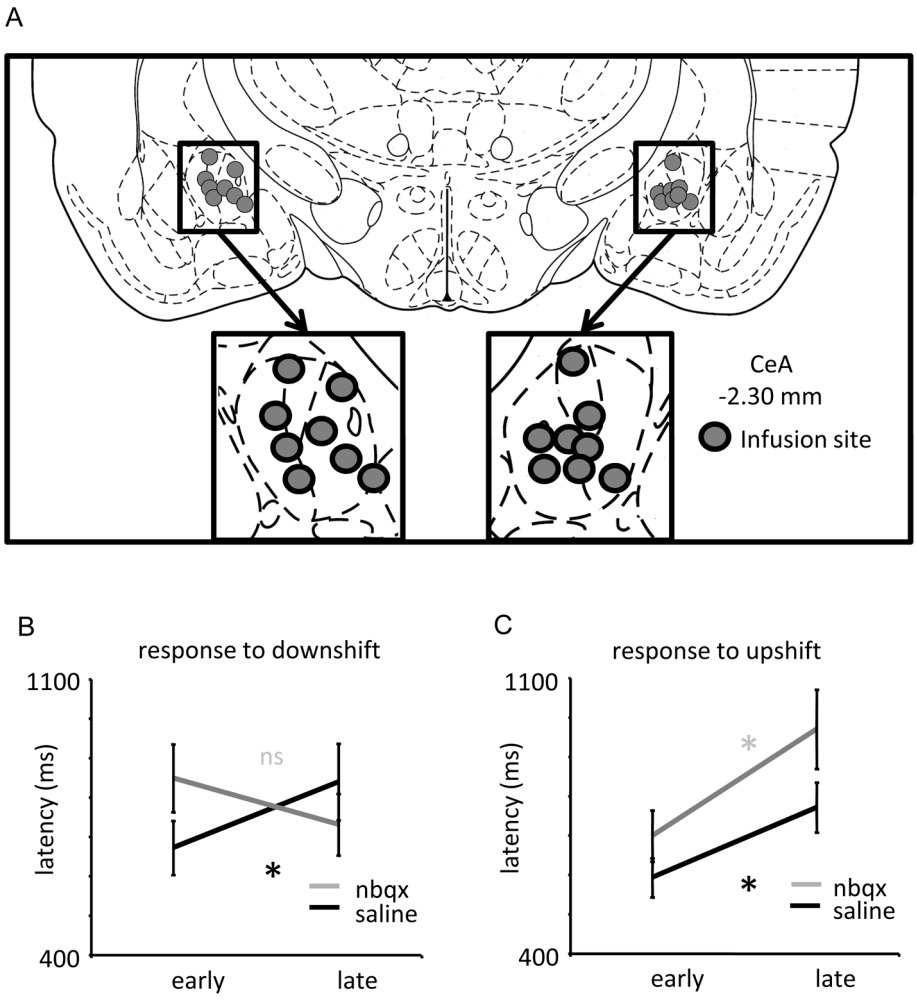

Figure 7.

Effect of CeA inactivation on orienting behavior. A. Location of infusion sites. Gray dots represent the placement of needle tips inserted into bilateral cannulae targeting central nucleus for infusion of vehicle or inactivating agents. Plates adapted from the atlases of Paxinos and Watson (2009). B. Impact of surprising downshift in reward value on orienting latency. C. Impact of surprising upshift in reward value on orienting latency. Early = first three trials, late = last three trials after a downshift.

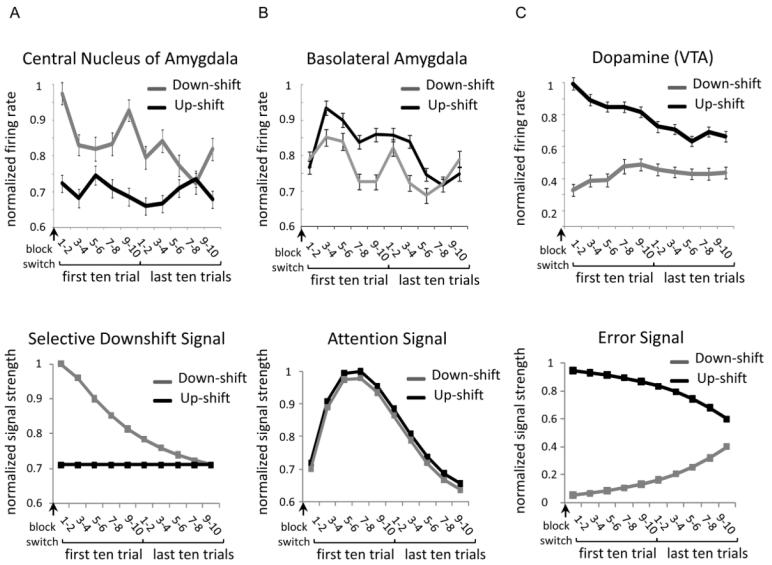

Figure 8.

Time course of neural activity in CeA, ABL, and VTA dopamine neurons (DA) in response to changes in reward value that occur after a block switch. A. (Top) Average firing of CeA omission responsive neurons in response to reward upshifts (block; 2^{sh}, 3^{bg}, 4^{bg}) and downshifts (gray; 2^{lo}, 4^{sm}) normalized to the maximum. (Bottom) Signal predicted by an adapted Pearce-Hall model which only permits signal increases selectively for downshifts in reward value, and does not integrate over trials ($\alpha = |\lambda - \Sigma V|$, ($\lambda - \Sigma V < 0$)). Simulation of this predicted signal in response to unexpected reward delivery (black) and omission (gray). B. (Top) Average firing of ABL reward responsive neurons in response to reward upshifts (block; 2^{sh}, 3^{bg}, 4^{bg}) and downshifts (gray; 2^{lo}, 4^{sm}) normalized to the maximum. (Bottom) Signal predicted by the Pearce-Hall model after unexpected reward delivery (black) and omission (gray) ($\alpha = \gamma(|\lambda - \Sigma V)| + (1-\gamma)\alpha$)C. (top) Average firing of VTA DA neurons in response to reward upshifts (block; 2^{sh}, 3^{bg}, 4^{bg}) and downshifts (gray; 2^{lo}, 4^{sm}) normalized to the maximum. (Bottom) Signal predicted by the Rescorla-Wagner model after unexpected reward delivery (black) and omission (gray) $\alpha (\lambda - \Sigma V)$.Neural activity averaged in two trial blocks. Error bars represent standard error of the mean.