

Cocaine-induced Impulsive Choices Are Accompanied by Impaired Delay-dependent Anticipatory Activity in Basolateral Amygdala

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Abstract

■ Addicts and drug-experienced animals have decision-making deficits in delayed reinforcement choice task, in which they prefer small immediate rewards over large delayed rewards. Here, we show evidence that this deficit is accompanied by changed coding of delay length in the basolateral amygdala (BLA). A subset of neurons in BLA demonstrated delay-dependent anticipatory activity (either increase or decrease as a function of delay to reward) in naive rats. After 30 days of withdrawal from chronic cocaine treatment (30 mg/kg/day for 10 days ip), the proportion of delay-dependent anticipatory neurons reduced,

whereas delay-dependent activity in response to elapsed delay after reward delivery increased, both in the proportion of delay-dependent neurons and in the extent of delay dependence. Cocaine exposure increased, instead of decreased, BLA neuronal expectation for different reward magnitudes. These results indicate that BLA is critical for representing and maintaining the information of delayed reward before its delivery, and cocaine exposure may affect decision-making by impairing perception of delay instead of the ability to assess the differences in reward size. ■

INTRODUCTION

The overall value of a reward is determined by its size, delay, and probability (Cardinal, 2006). Incentive value and frequency of selection of the larger reward decline monotonically as a function of the delay to that reward (Cardinal et al., 2003). Inability of an individual to choose a large, delayed reward in preference to a small, immediate reward is an impulsive choice (Ainslie, 1975) and has been implicated as one of the core features of drug addiction—drug addicts often choose immediate reward of consuming drugs over delayed benefits associated with abstinence (Paine, Dringenberg, & Olmstead, 2003). This deficit has been modeled in addicts and drug-experienced animals using delayed-reinforcement choice tasks. In these settings, when given a choice between a small immediate reward and a large delayed reward, both animals chronically exposed to cocaine and drug addicts discount delayed rewards faster than nondrug controls (Dandy & Gatch, 2009; Kirby & Petry, 2004; Coffey, Gudleski, Saladin, & Brady, 2003; Paine et al., 2003; Kirby, Petry, & Bickel, 1999; Madden, Petry, Badger, & Bickel, 1997).

Earlier studies suggested that basolateral amygdala (BLA) is involved in impulse control. Lesions to BLA in rats result in increased impulsive choice behavior similar to that observed in rats following cocaine exposure (Winstanley, Theobald, Cardinal, & Robbins, 2004). In addition, BLA

neurons are involved in the processing of reward and reward-related cues (Belova, Paton, Morrison, & Salzman, 2007; Fuchs, Feltenstein, & See, 2006; Carelli, Williams, & Hollander, 2003; Schoenbaum, Chiba, & Gallagher, 1998, 1999). Accordingly, we speculated that chronic cocaine exposure may cause long-lasting changes in the representation of reward in a circuit including BLA. Here, we test this hypothesis by examining neuronal activity from BLA in rats (naive vs. cocaine exposed) performing a delayed-reinforcement choice task. It is not clear whether cocaine-induced decision deficits reflect selective impairments in discounting functions, changes in evaluation of reward size, or both. As shown in a previous article, BLA neurons fire selectively during the delay period that precedes the actual delivery of the reward (Schoenbaum et al., 1998). In addition, BLA neurons represent reward size (Belova, Paton, & Salzman, 2008). Therefore, we focused on the representation of delay length and reward size in BLA and its changes induced by chronic cocaine exposure.

METHODS

Subjects

Sixteen male Sprague–Dawley rats (2–3 months old, 250–300 g) were randomly assigned to cocaine ($n = 8$) or saline groups ($n = 8$). The protocols of all experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH

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Apparatus

Training and testing took place in operant chambers (40 cm × 20 cm × 40 cm) equipped with two retractable levers. The levers were 4 cm wide, 5 cm from the plexiglass floor, 10 cm apart, and equally distant to the sidewall. A nose poke and an extended liquid receptacle, which were 2 and 4 cm from the floor, respectively, were located between the two levers. Liquid reward (30 μ l of water per drop) was delivered by a pump. There was a 3-W cue light above the nose poke. The house light was 6 W. The chamber was equipped with a video camera. Two computers were located in an adjacent room, one of which was used to control the operant equipment and to collect data, and the other was used for video recording.

Behavioral Testing

Daily behavioral testing included an active choice session and a forced choice session. The active choice task was adapted from a delayed reinforcement choice task described by Cardinal, Pennicott, Sugathapala, Robbins, and Everitt (2001) and Evenden and Ryan (1996). Animals progressed through several stages of training before undergoing surgical implantation of microelectrode arrays into the brain regions of interest and subsequent recording of the neuronal activity.

Subjects first learned to nose poke to trigger presentation of the levers and to press the lever for water reward, and then they were trained to perform an active choice task until a stable behavioral performance was achieved. Each training session consisted of six blocks. Within each block, the first two trials were forced choice (only one randomly chosen lever was presented); the remaining trials were free choice (both levers were presented). Each block continued until 25 free choice trials were completed. The onset of the house light and the cue light signaled the beginning of each trial, after which the rat had to nose poke within 10 sec to trigger lever presentation. Upon a successful nose poke, the cue light was switched off and both levers were introduced into the chamber after a 1.5-sec delay. Animals were required to respond on either lever within 10 sec. Responding on one lever always provided a drop of water (30 μ l) after a delay of 2 sec (designated small immediate reward). Responding on the other lever produced two drops of water (60 μ l) that were delivered 0.2 sec apart, after a predetermined delay (2–20 sec; designated large delayed reward). Assignment of immediate and delayed levers was counterbalanced. When one of the levers was pressed, both levers retracted. The house light turned off 8 sec after the reward was delivered, and the chamber entered into the intertrial state (i.e., complete darkness period) until the start of next trial. The dura-

tion of each successful trial equaled the delay to large reward of this trial plus 35 sec. The delay to large reward remained constant within each block and increased from 2 to 4, 8, 16, 18, and 20 sec across blocks for eight rats and from 2 to 4, 8, 12, 16, and then 20 sec for the remaining eight rats (Figure 1). If the rat failed to nose poke or press the lever within the designated time, the chamber turned to darkness for 5 sec before a new trial began.

The forced choice session occurred right after the active choice session and consisted of about 100 trials. On each forced choice trial, one of two levers was presented at random. The reward contingency of the immediate lever was still a drop of water with a 2-sec delay. Responding on the delayed lever produced two drops of water with a delay of 2, 4, or 8 sec in a pseudorandom order. All other parameters were identical to that in the active choice session.

After recording the behavior and neuronal activity during baseline sessions (naive condition), the cocaine group received daily intraperitoneal injections of 30 mg/kg cocaine HCl in 0.9% saline at a volume of 2 ml/kg for 10 consecutive days. The saline group received an identical schedule of injection of 0.9% saline vehicle (2 ml/kg). The injection was given 1 hr following daily test sessions in the home cages. Thirty days after the last injection, behavior and neuronal activity were examined (Figure 1). To make certain that we did not record the same neurons multiple times, for each rat, neuronal activity was recorded only during one session before and after cocaine treatment.

Surgery

Intracranial electrodes were implanted when performance criterion was reached (see Data Analysis). Rats were anesthetized with sodium pentobarbital (50 mg/kg ip) and stereotaxically implanted with arrays of eight stainless steel Teflon-insulated microwires (50- μ m diameter, Biographics, Winston-Salem, NC), directed to the BLA (stereotaxic coordinates: AP -2.8 , ML ± 4.7 L, and DV -7.8). Animals received penicillin (16,000 U im) after surgery to prevent infection. The final electrode position was marked by passing current (10–20 ADC current, 10–20 sec duration, anode at the electrode) through each electrode. Animals were perfused with 5% potassium ferricyanide–4% paraformaldehyde to develop the Prussian blue deposits. Coronal sections (40 μ m) were prepared. The location of electrode tips was determined.

Electrophysiological Recordings

Neuronal activity was collected by the microwires and passed from the headset assemblies to a preamplifier via two lightweight cables and a commutator. Signals were sampled at 50 kHz and filtered (0.5 and 5 kHz, 6 dB cutoff) and then sent to a multichannel spike-sorting device. Neuronal spikes were monitored on a computer and picked up by setting proper parameters for amplitude

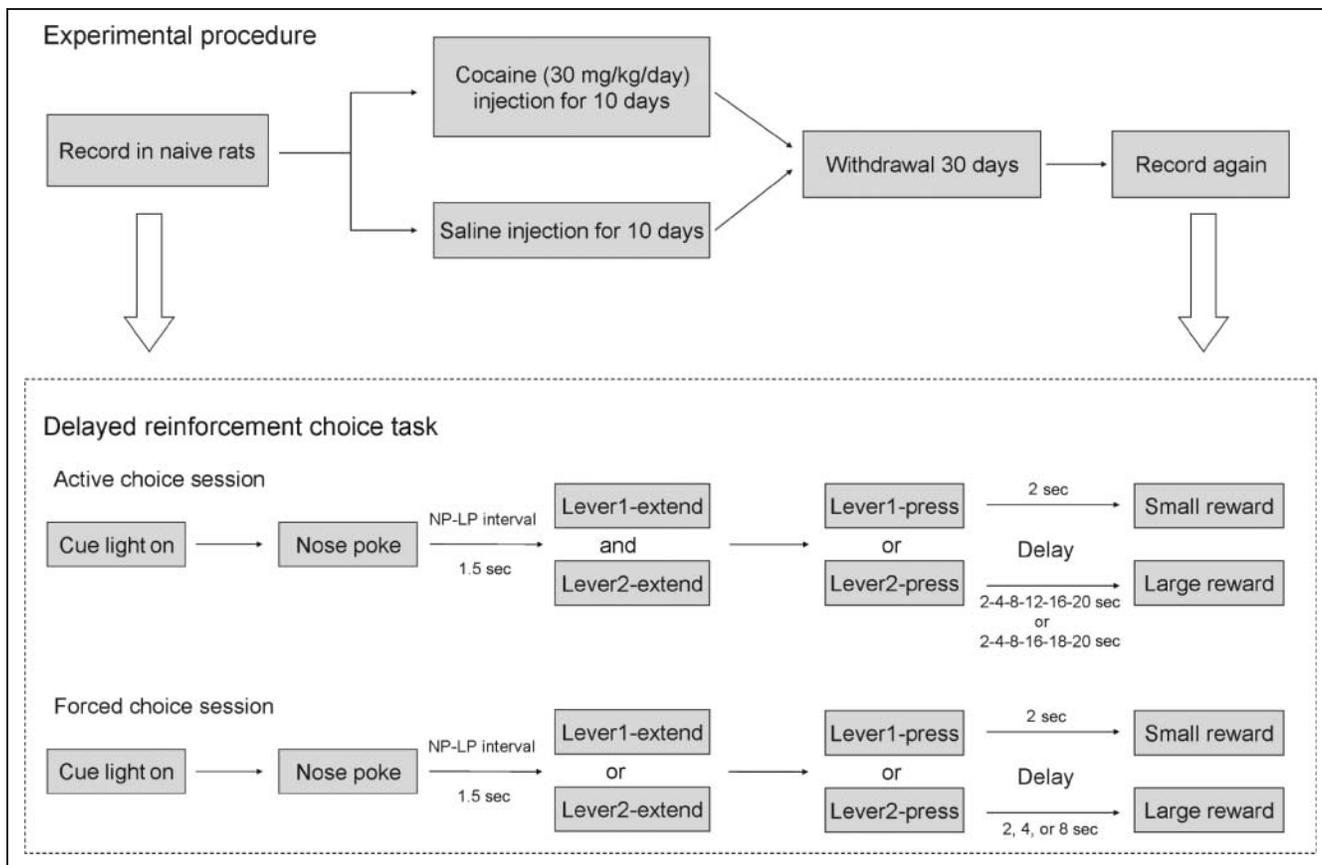


Figure 1. Schematic representation of the experimental procedure. On the recording days, behavior and BLA neuronal activity were recorded simultaneously during active choice session and forced choice session. During the active choice session, the two levers were presented simultaneously. Only one of the two levers was presented in the forced choice session. NP = nose poke; LP = lever press.

and duration with a PC-based software Magnet (Biographics). Units of $>3:1$ signal-to-noise ratio were isolated. The time stamps of the spike activities were saved into a database for off-line analysis with a PC-based program (STRANGER, Biographics Inc., Winston-Salem, NC). Auto-correlograms were constructed for each unit; single units were identified by well-defined refractory periods. Cross-correlograms were constructed for units on the same wire. If two units exhibited a common refractory period and waveforms, the waveforms were combined.

Data Analysis

For behavioral experiments, the primary measure of interest was the percentage of trials in which the animal chose the large delayed rewards. This percentage was calculated separately for each delay upon each session. To determine whether animals had successfully acquired the task, data from seven consecutive sessions in each subject were analyzed by repeated measures ANOVA using Day and Delay as the independent variables. If the effect of Delay was significant at the $p < .05$ level but there was no main effect of Day, subjects were judged to have reliably acquired the task (Winstanley et al., 2004). For each group, the behavioral effect of the intervention (cocaine or saline) was

assessed by comparing the performance of the subjects before and after the treatment. Bonferroni posttest was used for pairwise comparison.

We also examined RT and indifference point. RTs were defined as the time it took for the rat to press the lever after the lever was extended. The indifference point (D_{50}) was calculated as the delay to reinforcement corresponding to

$$\%B = 50\%; D_{50} = d_i + (d_j - d_i) \times [(\%B \text{ at } d_j - 50) / (\%B \text{ at } d_j - \%B \text{ at } d_i)],$$

in which B is the delay lever, d_i is the delay when less than 50% responses were made, and d_j is the delay when more than 50% delay lever responses were made (Paine et al., 2003).

The neuronal data from the sessions that yielded the behavioral data were analyzed. Bin counts for each trial (10 msec bin size) were calculated using the NeuroExplorer (Plexon, Dallas, TX), and the results were exported into Matlab. Neural response within 500 msec after cue light onset or reward delivery was evaluated using a sliding window averaging technique, in which a 30-msec window was moved in 10-msec increments across the entire period. The bin counts of each window were compared with the baseline (a 500-msec control window before cue light

onset) using Student's *t* test. The neuron was considered cue or reward responsive only when the difference reached a level of $p < .05$ in more than three consecutive steps.

It has been suggested that the subjective value of a reward decreases hyperbolically with increasing delay (Mazur, 1984). We tested this hypothesis by examining how firing rate varied as a function of the delay. Delay-dependent neurons were selected according to the following criteria:

- (1) excitation or inhibition in the observed time window,
- (2) monotonic change of firing rate as a function of the delay when reward size was held constant in the active choice session,
- (3) no delay-dependent anticipatory activity in the forced choice session, and
- (4) no change of firing rate on the forced choice trials as the session proceeded. Activity in BLA neurons is affected by satiety state (de Araujo et al., 2006). Because delays were presented in ascending order in the active choice session, the observed ascending or descending activity could be because of increased satiation as the session proceeds instead of increased delay. Considering that similar total number of large rewards was chosen in the active choice and the forced choice session, we arranged the forced choice trials toward the delayed lever. Neuronal firing rate in the forced trials was calculated in the early, mid, and late phases of the forced choice session. The delay-dependent neurons should not show difference in firing across the three phases.

Our experiments showed that the large reward was chosen in $\geq 50\%$ of the trials in the first three blocks (delay at 2 sec, 4 sec, and 8 sec, respectively). In the last three blocks, the large reward was chosen in $< 50\%$ of the trials. To ensure that enough trials were available for analysis, we compared the neuronal activity in response to the large rewards delivered after a delay of 2 sec (large-2s), 4 sec (large-4s), or 8 sec (large-8s). Neural responses across different delay lengths were compared in each sliding window (one-way ANOVA). The difference was considered significant when it reached a level of $p < .05$ in more than three consecutive steps. An average firing rate in the consecutive significant steps was calculated for the following analyses.

We computed each neuron's delay index to reflect the impact of delay length on neuronal activity. Delay index was defined as the response amplitude (absolute value of the firing rate difference from baseline) difference between 2-sec trials and 8-sec trials normalized to the sum of those two amplitudes (delay index = [short - long]/[short + long]). A delay index between -1 and 0 signifies a stronger response during 8-sec trials whereas a value between 0 and 1 signifies a stronger response during 2-sec trials.

To observe population response trend across the delays, mean firing rate of each neuron on the large-2s, large-4s, or large-8s trials was normalized to the mean value on the large-2s trials for each delay (normalized firing rate = [mean firing rate on the large-2s, large-4s, or large-8s trials]/[mean firing rate on the large-2s trials]).

Reward-dependent neurons were verified by comparing firing rates for constant delay (2 sec) but varying reward size. Specifically, the firing rate in response to the large rewards in the first block (large-2s) was compared with that in response to the small rewards in the last block (small-2s). For each neuron, the mean firing rate on the small-2s trials was normalized to the mean value on the large-2s trials. Reward index (reward index = [large - small]/[large + small]) was also computed on each responsive neuron. Positive index implies that the neuron fires more strongly for large rewards, and negative value implies that the neuron fires more strongly for small rewards.

Conventional statistical procedures (noted in Results) were carried out with Matlab or Prism to assess significance. Results are presented as mean \pm SEM.

RESULTS

Chronic Cocaine Exposure Increases Delay Discount

Two of the 16 rats died during the course of cocaine treatment. The remaining 14 rats were used for behavioral experiments. Before cocaine or saline treatment, rats displayed a choice pattern typical of delayed reinforcement paradigm: preference for the large reward with short delay and shift towards the small reward with increasing delay to the large reward (Figure 2A and B, two-way ANOVA, Treatment \times Delay; main effect of Delay, $F(5, 84) = 32.08$, $p < .001$ and $F(5, 72) = 48.01$, $p < .001$ for the saline and the cocaine groups, respectively). Chronic cocaine treatment dramatically decreased the preference for the large reward (Figure 2B, main effect of Treatment, $F(1, 72) = 20.68$, $p < .001$). In contrast, chronic saline treatment did not affect choice behavior (Figure 2A, main effect of Treatment, $F(1, 84) = 0.95$, $p > .05$).

Because different delays were implemented for different rats in the last three blocks of the active choice session, percent choice of large reward was compared among the first three delays. Before treatment, there was no difference between the saline and cocaine groups (Figure 2C, dashed gray line vs. dashed black line; two-way ANOVA, Group \times Delay, group effect, $F(1, 42) = 2.27$, $p > .05$), suggesting that the different last three delays did not lead to different behavior in the first three blocks. A comparison between pre- and post-treatment revealed that cocaine exposure dramatically decreased the preference for the large reward (dashed black line vs. solid black line; two-way ANOVA, Treatment \times Delay; treatment effect, $F(1, 36) = 5.32$, $p < .05$). In contrast, chronic saline treatment did not affect choice behavior (dashed gray line vs. solid gray line; treatment effect, $F(1, 42) = 1.21$, $p > .05$).

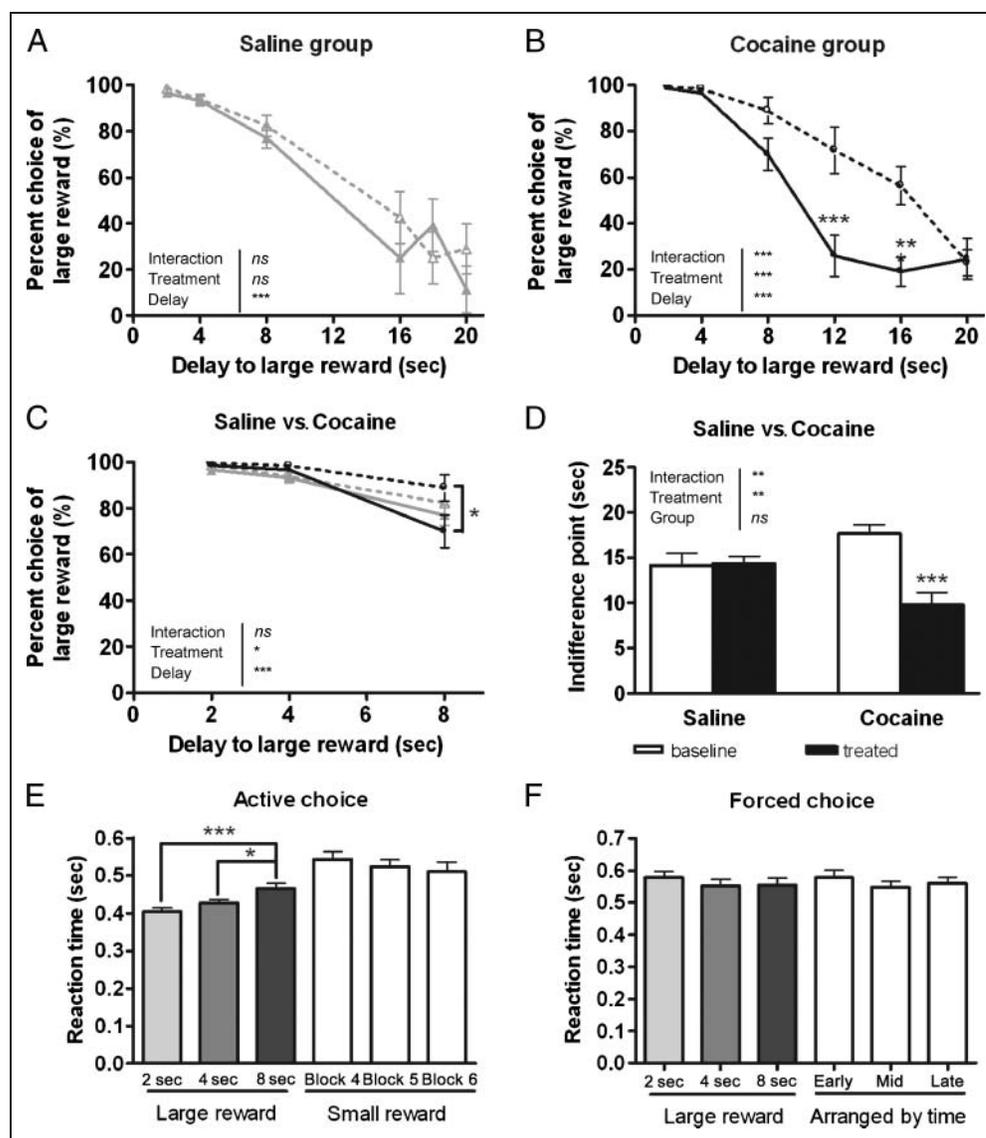
Indifference point demonstrated that when the delay increased to longer than 14.27 ± 0.73 sec, the rats tended to make more responses on the immediate lever. The baseline indifference point did not differ between the saline

and cocaine groups (14.16 ± 1.35 vs. 17.66 ± 0.97 , $t(14) = 2.28$, $p > .05$). A 2×2 (Group \times Treatment) ANOVA revealed significant interaction between the two variables (Figure 2D, $F(1, 26) = 12.62$, $p < .01$): Cocaine exposure decreased the mean indifference point relative to the baseline condition (9.80 ± 1.36 vs. 17.66 ± 0.97 , $t(12) = 4.74$, $p < .001$), but no difference was found between pre- and post-saline treatment (14.16 ± 1.35 vs. 14.33 ± 0.80 , $t(14) = 0.11$, $p > .05$).

In addition, the rats also exhibited significantly shorter RTs on trials toward the lever associated with shorter delay than longer delay (Figure 2E, one-way ANOVA, $F(2, 864) = 7.67$, $p < .001$; Tukey's multiple comparison test: 2 sec vs. 8 sec, $p < .0001$, 4 sec vs. 8 sec, $p < .05$). For the unpredicted rewards in the forced choice session, no difference was found in RTs (Figure 2F, $F(2, 1091) =$

0.43 , $p > .05$). These data further confirmed that the rats' behavior was influenced by delay length. In the active choice session, no difference was found on trials toward the small immediate lever across the last three blocks (Figure 2E, $F(2, 404) = 0.57$, $p > .001$). There was also no difference across the early, mid, and late phases of the forced choice session (Figure 2F, $F(2, 1091) = 0.56$, $p > .05$), confirming that the rats' behavior was not influenced by satiation. Cocaine treatment significantly decreased RTs on the large-2s (t test, $t(309) = 2.57$, $p < .05$) and the large-4s trials (t test, $t(299) = 3.28$, $p < .01$) but not on the large-8s trials (t test, $t(249) = 0.83$, $p > .05$; Supplementary Figure 1B), indicating that cocaine exposure increased incentive to the large reward, which was counteracted by increased delay discounting on the 8-sec trials.

Figure 2. Effects of cocaine treatment on performance of a delayed reinforcement choice task. (A) Percent choice of large reward for the saline group ($n = 8$). Saline treatment had no effect on their choices. (B) Percent choice of large reward for the cocaine group ($n = 6$). The rats chose the delayed reward significantly less often than they did before cocaine treatment. (C) Comparison among the first three delays. The baseline did not differ between the two groups. Cocaine exposure induced significant impulsive choice. (D) Comparison of indifference point. Chronic cocaine treatment significantly decreased the indifference point. The results of a two-way ANOVA are shown in the corner of each panel. The results of post hoc tests are shown between the curves or above the bars. $*p < .05$, $**p < .01$, $***p < .001$. (E) RTs in the active choice session. The left three bars show RTs toward large delayed rewards in Blocks 1–3 (delay = 2, 4, or 8 sec, respectively); the right three bars show RTs toward small immediate rewards (delay = 2 sec) in Blocks 4–6. The results of post hoc tests are shown above the bars. $*p < .05$, $***p < .001$. (F) RTs in the forced choice session. The left three bars show RTs toward large delayed rewards (delay = 2, 4, or 8 sec); the right three bars show RTs toward large delayed rewards arranged in time order.



BLA Neurons Encode Reward Delay and Size in Naive and Saline-treated Rats

We recorded 113 BLA neurons before the two treatments (naive condition, 65 from the saline group and 48 from the cocaine group), 72 neurons after saline treatment, and 37 after cocaine treatment. Baseline firing rate of each neuron was calculated. The median baseline firing rate of all the neurons was 8.0 Hz. The 25 and 75 percentiles were 4.8 and 14.75 Hz, respectively. Because the proportion of responsive neurons was not significantly different between the naive condition and the saline-treated condition (see Results, no difference was found between the saline- and cocaine-group before treatment section), we pooled these data ($n = 185$).

Subpopulations of neurons were found modulated during each of the task events (cue onset, nose poke, lever press, reward presentation). From video analysis, the rats started each trial with a nose poke after the illumination of the cue light and then immediately turned their upper body left or right to wait for the extension of the desired lever. The rats maintained a posture with upper limbs raised in front of the place where the desired lever would be extended until a lever-press response occurred. They usually did not change direction after they turned their bodies after nose poke. Thus, we suppose that the decision might have been made before or during nose poke. In this article, we focused on the neural activity during the initial event on each trial, that is, the illumination of the cue light to see if the neural response reflects size or delay length of future rewards. We also examined BLA neuronal activity in response to delivered reward to see effects of elapsed delay length and reward size on post-reward activity. The learning curve showed that the rats chose the large reward with a similar rate across the trials within each block (Supplementary Figure 2), indicating that the rats learned to expect the reward from the beginning of each block. Thus, we treated all the trials within each block as essentially after learning.

BLA Activity to Cue Light Was Affected by Anticipated Delay Length

Most of the BLA neurons (82 of 185) were excited by cue onset, with a peak latency at 115.0 ± 44.8 msec. A small proportion of BLA neurons (12 of 185) were inhibited by the cue, with a trough latency at 112.2 ± 15.1 msec. Figure 3A shows a representative neuron that was activated during cue presentation. The activity was clearly dependent on the length of the anticipated delay, reaching a peak at 140 msec. The response was strongest when the predicted delay was 2 sec and weakest at 8 sec. We termed this response profile as “short-preferring.” Twenty-six of the 185 neurons (14.1%) demonstrated short-preferring. Figure 3D shows the mean population firing rate. A repeated measures ANOVA showed a significant decrease in firing rate as the delay increased ($F(2, 50) = 123.3$,

$p < .0001$). In 16 other neurons (8.6%), the activity increased with delay, which we referred to as “long-preferring” (Table 1). Their mean population firing rate is shown in Figure 3E, with the highest firing rate on 8-sec trials ($F(2, 47) = 24.01, p < .0001$).

Delay index distribution for all neurons is shown in Figure 3F. The mean value of delay index was 0.36 ± 0.03 for the short-preferring neurons (short > long; $n = 26$) and -0.36 ± 0.04 for the long-preferring neurons (short < long; $n = 16$). To rule out that delay-dependent response was because of change in satiety state, these neurons were chosen only when no time-dependent response was found across the early, mid, and late phases of the forced choice session. Distribution of time index (time index = [late – early]/[late + early]) is concentrated around zero (Figure 3G, mean = 0.02, SEM = 0.01).

BLA Activity to Cue Light Was Affected by Anticipated Reward Size

Thirty-five neurons (18.9%) fired more for large rewards than for small rewards during cue presentation (Figure 3B and C, examples). Fewer neurons (13, 7.0%; chi-square test, 7.0% vs. 18.9%, $X^2 = 11.59, p < .001$) fired more for small rewards than for large rewards (Table 1). Mean population firing rate of the large-preferring and small-preferring neurons are shown in Figure 3H (paired t test, $t(34) = 14.68, p < .0001$) and Figure 3I ($t(12) = 2.34, p < .05$).

The mean reward index was 0.38 ± 0.04 ($n = 35$) for the large-preferring neurons and -0.30 ± 0.06 ($n = 13$) for the small-preferring neurons. Distribution of reward index during the cue epoch (Figure 3J) was shifted above zero (one sample t test, $t(82) = 3.63, p < .001$), indicating that, as a population, neurons fire more strongly in anticipation of the large reward than the small reward. In the forced choice session, no significant anticipatory response depending on reward size was found on these neurons (Figure 3K, mean = 0.01, SEM = 0.01).

Co-coding of Anticipated Delay Length and Reward Size in BLA

On the basis of the above-mentioned responses of individual neurons to delay length and reward size, we divided the neurons into three subgroups. One subgroup (21, 11.3%) responded to the trial-initiating cue in a manner that is dependent on the upcoming delay to reward (Figure 3A). The second subset of neurons (27, 14.6%) responded differentially to the cue depending on reward size (Figure 3B). These two subgroups were termed as delay dependent and reward dependent, respectively. A third subgroup (21, 11.3%) encoded both delay and reward size (co-coding). An overwhelming majority of these neurons (20 of 21, 95.3%) fired more strongly in anticipation of both large rewards and short-delay rewards (Figure 3C, example), suggesting that delay and reward size are

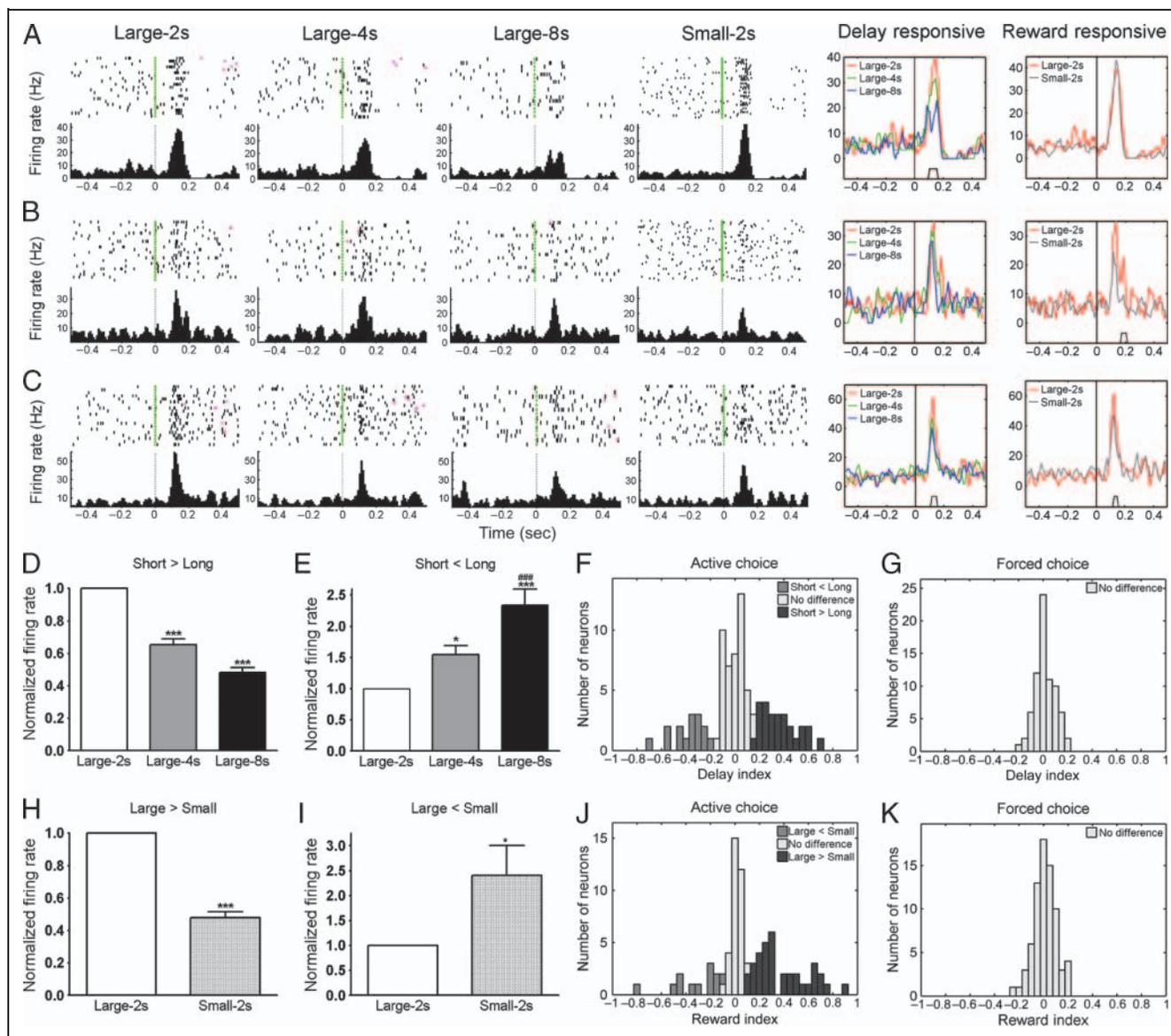


Figure 3. Anticipatory delay dependence and reward dependence during cue presentation. (A) A representative delay-dependent neuron. This neuron was short-prefering. Reward size did not affect the firing rate. (B) A representative reward-dependent neuron. This neuron fired more frequently in anticipation of the large reward than the small reward. The response was not dependent upon the delay length. (C) A representative neuron that was both delay and reward dependent. In A, B, and C, rasters and peristimulation time histograms (PSTHs) are shown on the left. Each row of dots in the rasters represents an individual trial. Rasters are aligned on the onset of cue light (0 msec, vertical line). The light green symbols in rasters indicate the onset time of cue light. The pink symbols indicate nose poke. Plots on the right side compared the firing rates across different delays and reward sizes by sliding a 30-msec window with a step of 10 msec. One-way ANOVA was used to compare the bin counts of each window. The trapezoid marker along the x axis indicates the statistical significance ($p < .05$ in more than three consecutive steps). (D) Normalized firing rate of the short-prefering neurons. (E) Normalized firing rate of the long-prefering neurons. (F) Distribution of delay index in the active choice session. (G) Distribution of time index in the forced choice session. (H) Normalized firing rate of the large-prefering neurons. (I) Normalized firing rate of the small-prefering neurons. (J) Distribution of reward index in the active choice session. (K) Distribution of reward index in the forced choice session. In D and E, one-way repeated measures ANOVAs were used. The results of Bonferroni post-tests are shown above the bars. $*p < .05$, $***p < .001$, compared with the value on the large-2s trial. $###p < .001$, compared with the value on the large-4s trial. In H and I, the results of paired t tests are shown above the bars. $*p < .05$, $***p < .001$.

encoded using a common currency in these neurons. Different from the short-prefering neurons, most (15 of 16, 93.8%) of the long-prefering neurons was not affected by the anticipated reward size (Table 1). Although delay index and reward index of the 21 co-coding neurons was not significantly correlated (Supplementary Figure 3A, Pearson's correlation, C1, $p > .05$, $r^2 = .069$). A significant positive correlation was found in all the respond-

ing neurons (Supplementary Figure 3A, C2, $p < .0001$, $r^2 = .174$).

BLA Response to Reward Presentation Was Affected by Elapsed Delay Length

Forty-seven neurons were excited by reward presentation, with a peak latency of 71.8 ± 3.4 msec. No neuron was

inhibited during this period. Figure 4A shows a representative neuron that was activated with a sharp peak after a latency of 95 msec. The excitation was highest on 8-sec trials. Neurons that fired more strongly after a long delay (25, 13.5%) significantly outnumbered those firing more strongly after a short delay (6, 3.2%; Table 1; 13.5% vs. 3.2%, $\chi^2 = 12.71$, $p < .001$). As the delay increased, the firing rate decreased significantly in the short-preferring neurons (Figure 4D, $F(2, 10) = 22.28$, $p < .001$) but increased in the long-preferring neurons (Figure 4E, $F(2, 48) = 33.67$, $p < .0001$). Delay index distribution for the post-reward epoch (Figure 4F) was shifted below zero ($t(44) = 3.29$; $p < .01$), indicating that as a population, magnitude of reward-related activity increased with delay. The mean delay index was -0.38 ± 0.03 and 0.43 ± 0.05 for the long-preferring and short-preferring neurons, respectively. Figure 4G shows the distribution of time index of these neurons in the forced choice session (mean = 0.01, $SEM = 0.01$).

BLA Response to Reward Presentation Was Affected by Reward Size

Twenty neurons (10.8%) displayed stronger activity on large-2s trials than on small-2s trials (Figure 4H, mean population firing rate). Fewer (Table 1; 9, 5.4%; 5.4% vs. 10.8%, $\chi^2 = 4.53$, $p < .05$) neurons were small-preferring (Figure 4B and C, examples; Figure 4I, mean population firing rate). The mean reward index was 0.31 ± 0.04 and -0.35 ± 0.05 for the large- and small-preferring neurons, respectively (Figure 4J). Unlike the anticipatory response during cue presentation, 28 neurons displayed large-preferring (mean = 0.38, $SEM = 0.03$) and 4 displayed small-preferring (mean = -0.45 , $SEM = 0.05$) for the presented reward in the forced choice session (Figure 4K). This is important to make sure that the reward-dependent response in the active choice session was not simply because of a drift in the baseline for both rewards induced by satiation, instead, when the large and small rewards

Table 1. Counts of Neurons Modulated by Delay Length or Reward Size during the Cue Epoch and the Reward Epoch in the Naive and Saline-treated Rats

	<i>Large < Small</i>	<i>Large > Small</i>	<i>None</i>	<i>Sum</i>
<i>Cue</i>				
Short > Long	0	20 (10.8%)	6 (3.2%)	26 (14.0%)
		12.1 ± 1.6 Hz	7.6 ± 1.6 Hz	11.1 ± 1.3 Hz
Short < Long	0	1 (0.5%)	15 (8.1%)*	16 (8.6%)
		21.5 Hz	14.4 ± 2.5 Hz	14.9 ± 2.4 Hz
None	13 (7.0%)	14 (7.6%)	116 (62.8%)	143 (77.4%)
	7.7 ± 1.4 Hz	14.4 ± 4.0 Hz	11.1 ± 0.8 Hz	11.1 ± 0.8 Hz
Sum	13 (7.0%)	35 (18.9%)**	137 (74.1%)	185 (100%)
	7.7 ± 1.4 Hz	13.3 ± 1.8 Hz	11.3 ± 0.7 Hz	11.4 ± 0.7 Hz
<i>Reward</i>				
Short > Long	0	6 (3.2%)	0	6 (3.2%)
		5.0 ± 0.5 Hz		5.0 ± 0.5 Hz
Short < Long	6 (3.2%)	4 (2.2%)	15 (8.1%)**	25 (13.5%)**
	10.4 ± 1.8 Hz	5.9 ± 1.1 Hz	9.0 ± 1.1 Hz	7.6 ± 0.8 Hz
None	3 (1.6%)	10 (5.4%)	141 (76.2%)	154 (83.3%)
	11.1 ± 3.4 Hz	7.7 ± 1.9 Hz	12.4 ± 0.8 Hz	12.1 ± 0.8 Hz
Sum	9 (4.9%)	20 (10.8%)*	156 (84.3%)	185 (100%)
	7.0 ± 1.4 Hz	8.1 ± 1.1 Hz	12.1 ± 0.8 Hz	11.4 ± 0.7 Hz

Short > Long or Short < Long = firing rate significantly higher for short than for long delay conditions or vice versa; Large > Small or Large < Small = firing rate significantly higher for large than for small reward conditions or vice versa. Chi-square test was used to compare the proportion between the short-preferring and the long-preferring neurons or between the large-preferring and the small-preferring neurons. The shading refers to the mean baseline firing rates of subpopulations (mean \pm SEM).

* $p < .05$.

** $p < .001$.

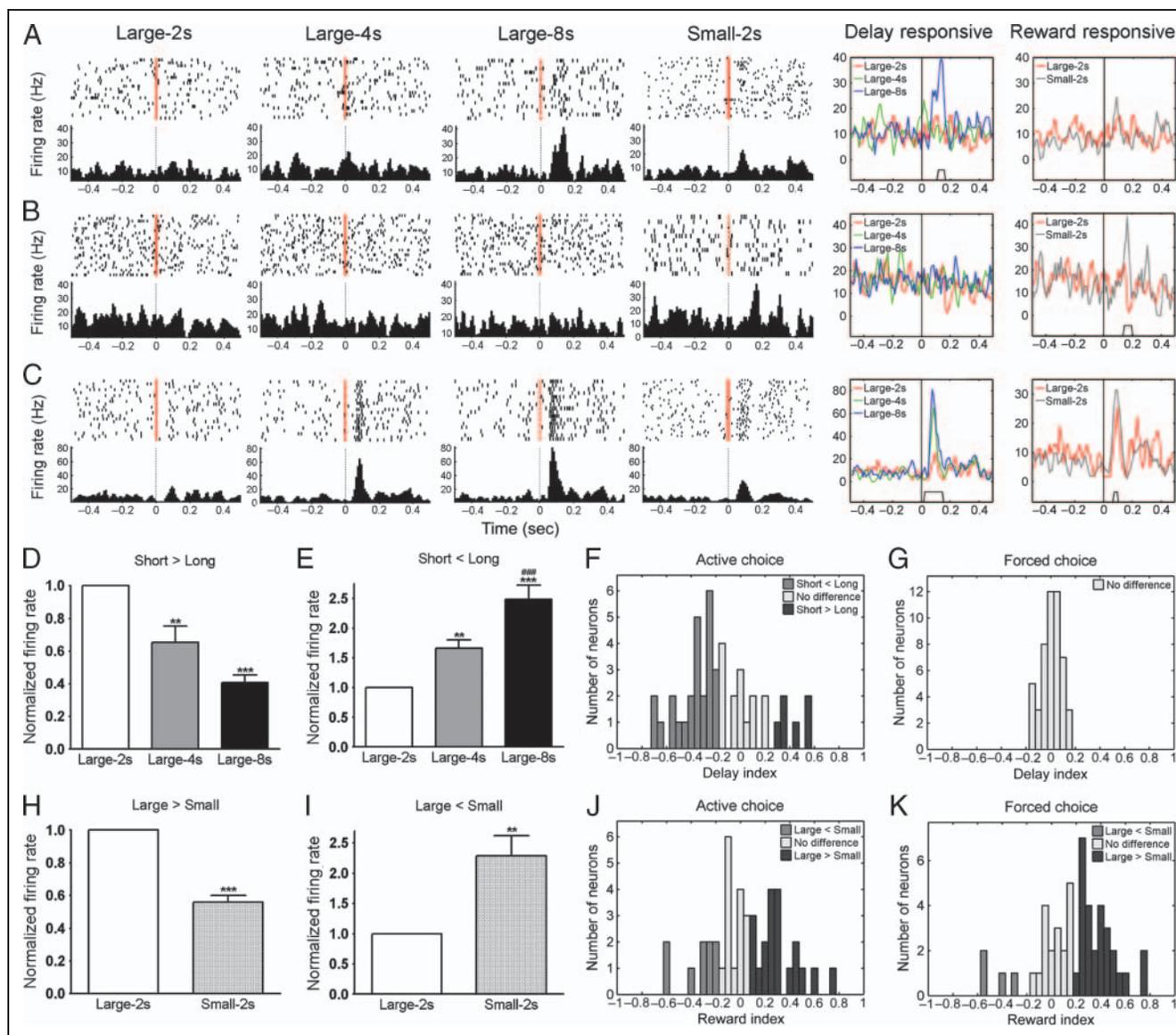


Figure 4. Delay dependence and reward dependence in response to reward delivery. (A) A representative delay-dependent neuron. (B) A representative reward-dependent neuron. (C) A representative neuron that encoded both reward size and delay. In A, B and C, rasters are aligned on the delivery of reward (0 msec, vertical line). The red symbols in rasters indicate the delivery of reward. (D) Normalized firing rate of the short-preferring neurons. (E) Normalized firing rate of the long-preferring neurons. (F) Distribution of delay index in the active choice session. (G) Distribution of delay index in the forced choice session. (H) Normalized firing rate of the large-preferring neurons. (I) Normalized firing rate of the small-preferring neurons. (J) Distribution of reward index in the active choice session. (K) Distribution of reward index in the forced choice session. In D and E, one-way repeated measures ANOVAs were used. The results of Bonferroni post-tests are shown above the bars. $**p < .01$, $***p < .001$, compared with the value on the large-2s trial. $###p < .001$, compared with the value on the large-4s trial. In H and I, the results of paired t tests are shown above the bars. $**p < .01$, $***p < .001$.

were given randomly, reward-dependent response was also observed on these neurons.

Co-coding of Elapsed Delay Length and Reward Size in BLA

All six neurons that fired more strongly after a short delay also fired more strongly after a large reward (Table 1). The ten neurons that fired more strongly in response to a long delay fired differentially to reward size (six small-

preferring and four large-preferring). Delay index and reward index of these 16 co-coding neurons was positively correlated (Supplementary Figure 3B, C1, $p < .0001$, $r^2 = .791$). Similarly, for all the responsive neurons, a significant positive correlation was also found (Supplementary Figure 3B, C2, $p < .0001$, $r^2 = .580$). Thus, for the overall population of co-coding neurons, time-discounted activity covaried with activity related to reward size. We conclude that, as a population, BLA neurons responded similarly to a more desirable reward

either in the form of a short delay or in the form of a large reward.

Relation between Anticipatory Activity and Post-reward Activity

A significant difference was observed when proportions of the long-preferring and short-preferring neurons were compared between the cue epoch and the reward epoch (Figure 5A, 61.9% vs. 19.4%, $X^2 = 13.12$, $p < .01$). During the cue epoch, more neurons fired more strongly in anticipation of the short-delay reward ($n = 26$) than the long-delay reward ($n = 16$; Table 1). In contrast, more neurons fired more strongly for the long-delay reward ($n = 25$) than the short-delay reward ($n = 6$) after reward was delivered. Thus, we can conclude that there was an inversion of a delay-dependent effect between pre- and post-reward. The inversion also occurred in individual neurons. An example is shown in Figure 5B, which is the same neuron in Figures 3A

and 4C. This neuron fired more in anticipation of the reward with a short delay but fired less after the short delay reward was actually delivered. Eight of the 12 neurons (66.7%) that were delay dependent during both cue and reward presentation changed from short-preferring during the cue epoch to long-preferring during the reward epoch.

For reward both delivered after 2 sec, the proportion of small- versus large-preferring neurons did not differ between the cue epoch and the reward epoch (27.1% vs. 31.0%, $X^2 = 0.14$, $p > .05$): large-preferring neurons dominated during the cue epoch (18.9% vs. 7.0%, $X^2 = 11.59$, $p < .001$) and the reward epoch (10.8% vs. 4.9%, $X^2 = 4.53$, $p < .05$; Figure 5C).

Relation between Active Choice Activity and Forced Choice Activity

Average neuronal responses to cue and reward presentation in the active choice session and forced choice session

Figure 5. Relationship between neuronal activity during presentation of the cue light and the actual reward. (A) An inversion of the delay-dependent effect was found after reward delivery. During cue presentation, more neurons fired more frequently in anticipation of the reward delivered after a short delay (short-preferring). After reward was presented, more neurons fired more frequently in response to an elapsed long delay (long-preferring). (B) A representative neuron that switched from short-preferring during the cue epoch to long-preferring during the reward epoch. The trapezoid marker along the x axis indicates the statistical significance (one-way ANOVA, $p < .05$ in more than three consecutive steps). (C) Sustained large-preferring throughout a trial. More neurons fired more for the large rewards, during both the cue epoch and the reward epoch. (D) Significantly, more neurons were responsive to the cue light onset under the active choice condition than the forced choice condition. More neurons were excited in response to an unpredicted reward after the reward was delivered.

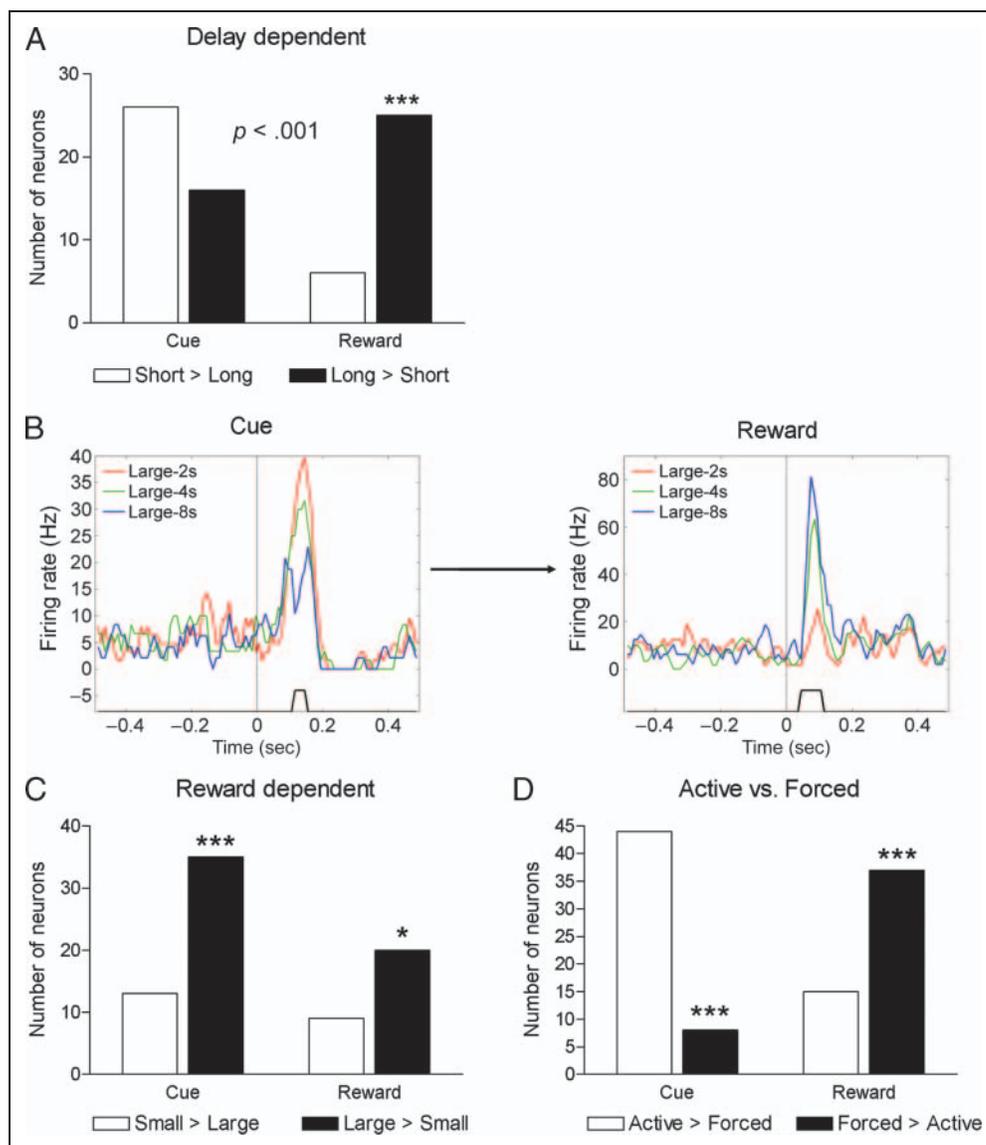
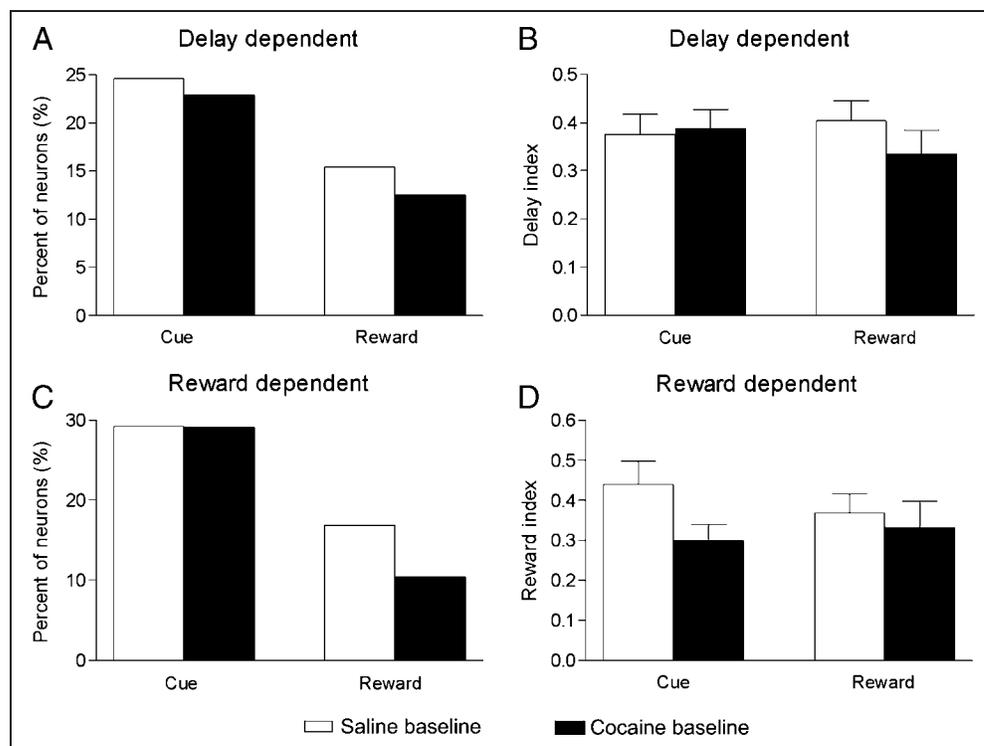


Figure 6. Comparison of baseline delay-dependent and reward-dependent activity. (A) The proportion of delay-dependent neurons did not differ between the groups. (B) No group difference in the delay index. (C) The proportion of reward-dependent neurons did not differ between the groups. (D) The reward index did not differ between the two groups.



were calculated in spite of the delay or size. Comparison between the responses under the active and forced conditions further proved the effect of expectation on neuronal response. We counted the number of neurons that showed increased or decreased activity in the forced session in relative to the active session. The response induced by the unpredicted rewards in the forced session was stronger than that induced by the predicted ones in the active choice session (20.0% vs. 8.1%, $X^2 = 10.83$, $p < .001$; Figure 5D). Fewer neurons demonstrated predicting activity during cue presentation in the forced choice session (4.3 vs. 23.8%, $X^2 = 29.0$, $p < .0001$).

Cocaine Exposure Decreased Delay-dependent Anticipatory Activity but Increased Delay-dependent Activity in Response to the Elapsed Delay

No Difference Was Found between the Saline and Cocaine Groups before Treatment

The baseline did not differ between the two groups in either the delay-dependent proportion (Figure 6A; cue, 24.6% vs. 22.9%, $X^2 = 0.04$, $p > .05$; reward, 15.4% vs. 12.5%, $X^2 = 0.19$, $p > .05$) or the delay index (Figure 6B; cue, $t(24) = 0.20$, $p > .05$; reward, $t(14) = 1.03$, $p > .05$). Similarly, the reward-dependent proportion (Figure 6C; cue, 29.2% vs. 29.2%, $X^2 = 0.00$, $p > .05$; reward, 16.9% vs. 10.4%, $X^2 = 0.96$, $p > .05$) and the reward index (Figure 6D; cue, $t(31) = 1.90$, $p > .05$;

reward, $t(14) = 0.45$, $p > .05$) did not differ between the two groups.

Cocaine Exposure Decreased BLA Delay-dependent Anticipatory Activity

After cocaine exposure, only 2 of the 37 (5.4%) neurons demonstrated delay-dependent activity in response to the cue light. The number of delay-dependent anticipatory neurons after cocaine treatment was significantly smaller than the number under the naive condition (26 of 113, 23.0%; chi-square with Yates' correction, 5.4% vs. 23.0%, $X^2 = 5.69$, $p < .05$) and the saline-treated condition (16 of 72, 22.2%; 5.4% vs. 22.2%, $X^2 = 5.01$, $p < .05$; see Supplementary Figure 4 for statistical power analysis). No significant difference was found between the saline-treated condition and the naive condition (22.2% vs. 23.0%, $X^2 = 0.02$, $p > .05$; Table 2 and Figure 7A).

Cocaine Exposure Increased BLA Delay-dependent Activity to Elapsed Delay

On the contrary to the anticipatory activity, after chronic cocaine exposure, more neurons demonstrated delay-dependent activity to elapsed delay. Fifteen of the 37 neurons (40.5%) showed delay-dependent responses during the reward epoch. This percentage was significantly greater than that in the naive condition (16 of 113, 14.2%; 40.5% vs. 14.2%, $X^2 = 11.83$, $p < .001$) and the saline-treated condition (15 of 72, 20.8%; 40.5% vs. 20.8%, $X^2 = 5.76$, $p < .05$).

The measure was not statistically different in rats receiving saline versus naive rats (20.8% vs. 14.2%, $X^2 = 0.24$, $p > .05$; Table 2 and Figure 7A).

Cocaine exposure also increased the absolute value of the delay index (0.51 ± 0.04 vs. 0.40 ± 0.04 in naive rats, $t(30) = 2.07$, $p < .05$; vs. 0.38 ± 0.04 in saline-treated rats, $t(27) = 2.28$, $p < .05$). Saline treatment did not change this measure significantly (0.38 ± 0.04 vs. 0.40 ± 0.04 , $t(29) = 0.36$, $p > .05$; Figure 7B).

BLA Reward-dependent Activity to Cue Light Did Not Decrease after Cocaine Exposure

As shown in Table 2, after cocaine exposure, 15 of the 37 recorded neurons (40.5%) showed reward-dependent anticipation. This percentage was significantly higher than the saline-treated condition (15 of 72, 20.8%; 40.5% vs. 20.8%, $X^2 = 4.76$, $p < .05$), although it was not significantly different from that under the naive condition (33 of 113, 29.2%; 40.5 vs. 29.2%, $X^2 = 1.65$, $p > .05$; Figure 7C). Similar to the naive condition, most of the 15 neurons (14 of 15, 93.3%) that displayed reward-dependent anticipatory

activity were large-preferring (14 vs. 1 small-preferring; 37.8% vs. 2.7%, $X^2 = 12.04$, $p < .001$).

Cocaine exposure did not affect reward index (Figure 7D), 0.28 ± 0.05 after exposure vs. 0.32 ± 0.05 before exposure; $t(45) = 1.47$, $p > .05$; vs. 0.38 ± 0.04 in saline-treated rats; $t(27) = 0.48$, $p > .05$).

More Neurons Became Sensitive to Size of Presented Reward after Cocaine Exposure

More neurons (14 of 37, 37.8%) were reward dependent to the delivered reward in the cocaine-treated rats than the naive rats (17 of 113, 15.0%; 37.8% vs. 15.0%, $X^2 = 8.83$, $p < .01$) and the saline-treated rats (14 of 72, 19.4%; 37.8% vs. 19.4%, $X^2 = 4.33$, $p < .05$; Table 2 and Figure 7C).

The magnitude of reward-dependent activity in each neuron was not affected by cocaine exposure. The mean absolute reward index of the cocaine-treated group (0.38 ± 0.06) did not differ from that in the naive rats (0.36 ± 0.04 ; $t(28) = 0.40$; $p > .05$) or the saline-treated rats (0.28 ± 0.05 ; $t(25) = 1.46$; $p > .05$; Figure 7D).

Table 2. Counts of Neurons Modulated by Delay Length or Reward Size in the Cocaine-treated Rats

	Large < Small	Large > Small	None	Sum
<i>Cue</i>				
Short > Long	0	1 (2.7%)	1 (2.7%)	2 (5.4%)
		5.4 Hz	4.8 Hz	5.1 ± 0.3 Hz
Short < Long	0	0	0	0
None	1 (2.7%)	13 (35.1%)	21 (56.8%)	35 (94.6%)
	7.5 Hz	6.4 ± 0.5 Hz	6.8 ± 0.8 Hz	7.0 ± 0.6 Hz
Sum	1 (2.7%)	14 (37.8%)**	22 (59.5%)	37 (100%)
	7.5 Hz	6.3 ± 0.4 Hz	6.7 ± 0.8 Hz	6.9 ± 0.5 Hz
<i>Reward</i>				
Short > Long	0	5 (13.5%)	0	5 (13.5%)
		8.5 ± 2.3 Hz		8.5 ± 2.3 Hz
Short < Long	2 (5.4%)	4 (10.8%)	4 (10.8%)	10 (27.0%)
	8.0 ± 1.2 Hz	6.7 ± 2.0 Hz	5.7 ± 1.4 Hz	6.5 ± 0.9 Hz
None	1 (2.7%)	2 (5.4%)	19 (51.4%)	22 (59.5%)
	4.8 Hz	4.7 ± 2.7 Hz	7.0 ± 0.7 Hz	6.7 ± 0.6 Hz
Sum	3 (8.1%)	11 (29.7%)*	23 (62.2%)	37 (100%)
	6.9 ± 1.2 Hz	7.1 ± 1.3 Hz	6.8 ± 0.6 Hz	6.9 ± 0.5 Hz

Short > Long or Short < Long = firing rate significantly greater for short than for long delay conditions or vice versa; Large > Small or Large < Small = firing rate significantly greater for large than for small reward conditions or vice versa. Chi-square tests were used to compare the proportions between the short-preferring and the long-preferring neurons or between the large-preferring and the small-preferring neurons. The shading refers to the mean baseline firing rates of the different subpopulations (mean \pm SEM).

* $p < .05$.

** $p < .001$.

Figure 7. Effects of cocaine exposure on BLA delay dependence and reward dependence. (A) Withdrawal from chronic cocaine treatment decreased the number of delay-dependent anticipatory neurons but increased the number of delay-dependent neurons to the elapsed delay. (B) The drug experience increased the delay index during reward presentation. (C) The drug experience increased the number of reward-dependent neurons during cue presentation and reward presentation. (D) The drug experience did not affect the reward index of the reward-dependent neurons. * $p < .05$, ** $p < .01$, compared with the naive condition. # $p < .05$, compared with the saline-treated condition.

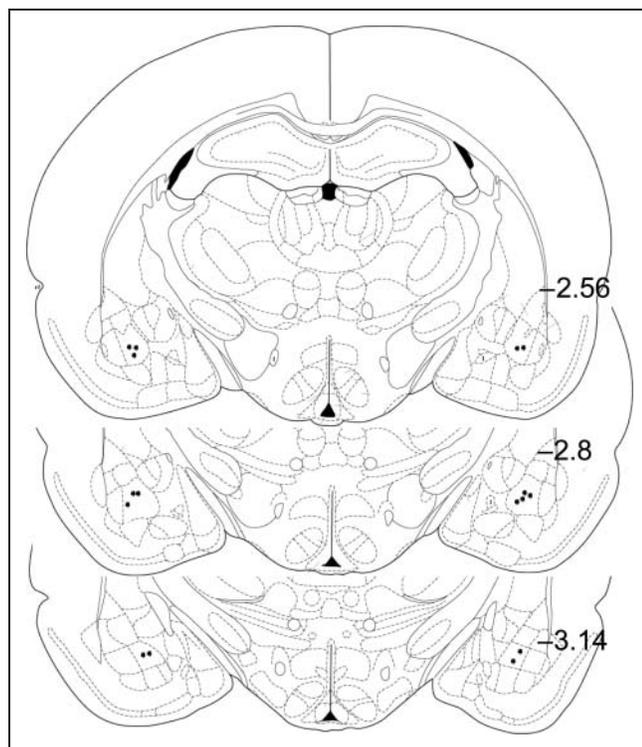
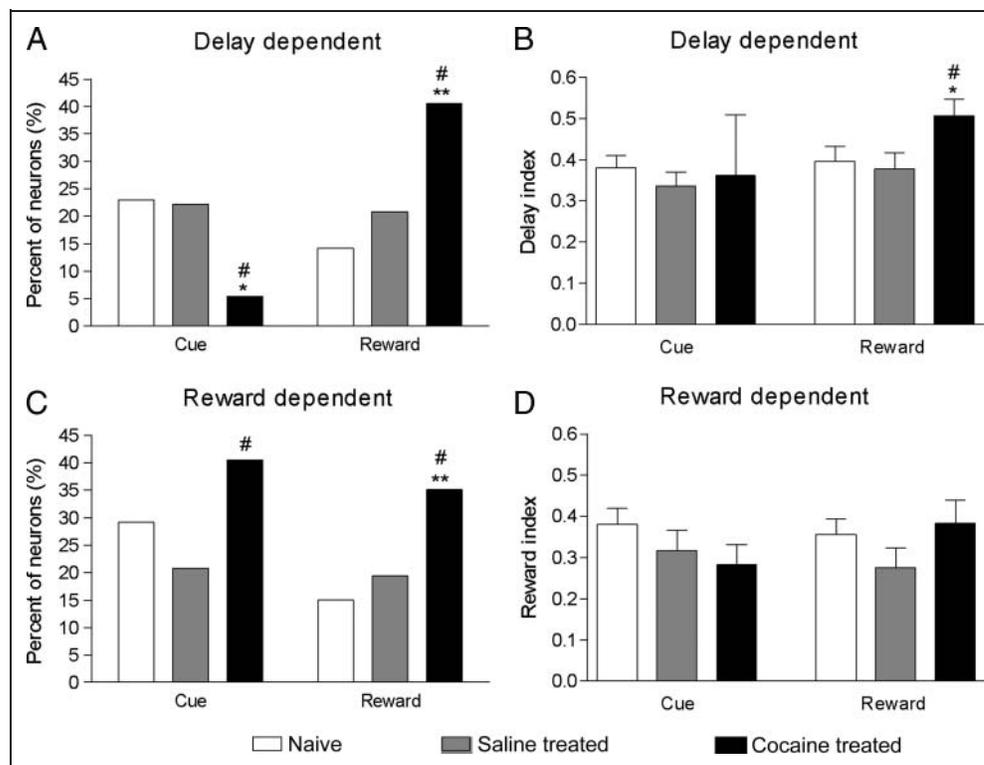


Figure 8. Histological location of recording sites in BLA revealed by potassium ferricyanide staining of iron deposited by current applied to recording microwires.

Cocaine Exposure Decreased Delay-dependent Activity during Nose Poke–Lever Press Interval and Post-lever Press Period

BLA neurons also demonstrated significant delay dependence in response to nose poke, lever press, the interval between them, and the interval following lever press. Cocaine exposure decreased the percentage of BLA delay-dependent neurons during the nose poke–lever press interval and the post-lever press interval (Supplementary Figure 5).

Histological Localization of Recording Sites

Potassium ferricyanide staining revealed recording sites as blue dots in BLA. The locations of recording sites included in this report were depicted in Figure 8.

DISCUSSION

We monitored the effects of withdrawal from cocaine on behavior and BLA neuronal activity in rats performing a delay discounting task. Consistent with previous reports (Dandy & Gatch, 2009; Simon, Mendez, & Setlow, 2007), cocaine exposure decreased responding toward the delayed reward and decreased the indifference point. Such effects lasted at least for 30 days. Such behavioral changes were accompanied by neuronal activity in BLA in a manner dependent on reward delay. Briefly, BLA neuronal activity was modulated monotonically by delay to reward, both in anticipation of and in response to

reward delivery. More neurons fired more strongly for an expected short-delay reward before its delivery, whereas the response to the short-delay reward was weaker than a long-delay reward after its delivery. Chronic cocaine exposure decreased delay-dependent anticipatory activity but increased delay- and reward-size-dependent activity during reward presentation. These results implicate that cocaine-induced impulsive choice may result from BLA-mediated failure to represent information about rewards across delays.

BLA Activity Reflects Anticipated and Elapsed Time-discounted Value

Earlier reports have shown that different populations of amygdala neurons are involved in conditional learning. One population fired more to cues paired with rewards, and another one fired more to cues paired with punishments (Belova et al., 2007, 2008; Paton, Belova, Morrison, & Salzman, 2006; Schoenbaum et al., 1999). Amygdala neurons also respond differently to conditioned stimuli associated with different magnitudes of rewards (Belova et al., 2008). However, whether amygdala neurons encode delay length to rewards remains unknown. Our present study demonstrated that BLA neurons are sensitive to both delay and reward size. Certain BLA neurons fired more strongly in anticipation of both large rewards and short-delay rewards (Table 1), suggesting that features of a reward that determines its “desirability,” namely delay and size, could be encoded in the same neurons. This kind of common subjective metric of time-to-reward and reward amount is very important to provide information about reward incentive properties to guide future choice behavior (Weller, Levin, Shiv, & Bechara, 2007; Balleine & Killcross, 2006; O’Doherty, 2004).

Several confounding factors must be considered while interpreting the observed delay- and reward-dependent activity. First, the cue-evoked activity may have been contaminated by the following event of nose poke. Consistent with previous reports (Belova et al., 2007; Paton et al., 2006; Sugase-Miyamoto & Richmond, 2005), our observed BLA neurons were excited at short latency (~100–200 msec) by cues and they demonstrated early, discriminative responses to cue. On most trials, the latency of cue-evoked response was shorter than the latency to nose poke (mean = 1.96 sec, *SEM* = 0.06 sec), rendering motion artifact an unlikely cause for the cue-evoked selective response. Second, the delay- or size-dependent response may have resulted from possible confound of satiation (de Araujo et al., 2006). This factor was controlled in our study by including only those neurons nonresponsive to satiety, namely, the neurons responded similarly in early, mid, and late phases of the forced choice session (see Methods). In addition, the rats did not exhibit significantly different RTs in these three phases (Figure 2E) and the rats also did not exhibit different RTs on the trials toward the small reward across the last three blocks (Figure 2F), indicating

that the rats’ behavior was not significantly influenced by satiation in our experiments. Third, as the 2-sec large reward in the first block and 2-sec small reward in the final block were presented with different alternative choice, respectively, the observed neural response to reward size could reflect a relative relationship that existed only within a given block. We controlled this factor by comparing neural activity between the preferable rewards of these two blocks, instead of between the preferable reward in one block and the unpreferable reward in the other block. But it could still be argued that the relative value of the 2-sec large reward and the 2-sec small reward in their blocks could be different.

We observed that some BLA neurons were excited and produced a brief, short-latency burst of action potentials within 200 msec after reward delivery. These neuronal responses are similar to the short-duration response reported in rodent BLA by Fontanini, Grossman, Figueroa, and Katz (2009). As reported in previous studies (Belova et al., 2007, 2008; Paton et al., 2006; Schoenbaum et al., 1999), separate populations of BLA neurons preferentially respond to positive and negative values, respectively. We also observed that the BLA neurons showed strongest response to the most rewarding (short-delay reward) and weakest to the least rewarding reward (long-delay reward) or vice versa. Notably, the long-preferring neurons outnumbered the short-preferring neurons after reward presentation. Before reward delivery, however, the short-preferring neurons outnumbered the long-preferring neurons (Figure 5A). This phenomenon could be explained by effects of expectation on neuronal response, as reported previously, that responses of neurons in amygdala and midbrain to reward are strongest when the reward is unanticipated (Kufahl et al., 2008; Belova et al., 2007; Roesch, Calu, & Schoenbaum, 2007; Schultz, Dayan, & Montague, 1997), and when a reward is expected, the response shifts from the reward itself to the stimulus (Schultz, 2001). After repeated training, the rats have learned to predict reward timing. Shorter delay is easier to predict than longer delay. Therefore, the neurons fire more in anticipation of short delay than long delay. After the reward is presented, the neurons fire more in response to the less-predicted long-delay reward than the better-predicted short-delay reward. Additionally, decreased response during cue presentation and increased response during reward presentation in the forced choice session (Figure 5D) further support the effect of expectation on neuronal response. Consistent with the role of amygdala in moment-to-moment tracking of state value (Belova et al., 2008), the preference for the large reward over the small reward did not change between pre- and post-reward when the delay was held constant (Figure 5C).

Cocaine Exposure Decreases Anticipatory Representation of Delay to Reward

Our behavioral results are consistent with the notion that drugs of abuse can increase impulsive choice behavior.

Faster reward discount has been documented in alcoholics (Vuchinich & Simpson, 1998), opiate addicts (Kirby & Petry, 2004; Giordano et al., 2002; Kirby et al., 1999; Madden, Bickel, & Jacobs, 1999), cocaine addicts (Heil, Johnson, Higgins, & Bickel, 2006; Bornovalova, Daughters, Hernandez, Richards, & Lejuez, 2005; Kirby & Petry, 2004; Coffey et al., 2003), and cigarette smokers (Bickel, Odum, & Madden, 1999). This evidence indicates that drug addicts have a poor waiting capacity to attain rewards such that the “subjective value” of large delayed rewards diminished in comparison with small immediate rewards.

The above interpretations about the BLA neurons’ responses in the normal rats help us to explain the underlying mechanisms of cocaine-induced impulsive choice. After cocaine withdrawal, delay-dependent anticipatory responses significantly decreased (Figure 7A), indicating that cocaine exposure may impair BLA ability to expect a reward based on the evaluation of waiting time. This result is consistent with the reduced anticipatory responding (entries into the food trough) during the delays before reward delivery (Simon et al., 2007). Attenuated delay perception weakens the learning between responding on the “delayed” lever with delivery of the delayed reward, leading to apparently impulsive responding for immediate reward. When the rats chose the large delayed reward, the reward was less predictable because of lack of delay information, which in turn induced overreaction to the reward when they were actually delivered (Figure 7A and B). Our finding that BLA neurons became less sensitive to delay length of the upcoming reward after cocaine exposure is consistent with recent reports that chronic cocaine-exposed animals are less sensitive to the value of expected outcomes (Stalnaker, Roesch, Calu, et al., 2007; Stalnaker, Roesch, Franz, et al., 2007; Schoenbaum, Saddoris, Ramus, Shaham, & Setlow, 2004; Jentsch, Olsson, De La, & Taylor, 2002).

A previous study showed that rats with lesions to the BLA prefer larger reward if no delay is involved (Winstanley et al., 2004). Results from the current study confirmed this finding: Cocaine-exposed rats consistently selected the large reward over the small reward with the same delay. Thus, the impulsive choice in cocaine-exposed rats was not likely because of an inability to assess the differences in magnitude between the large and the small reward. As a matter of fact, we found that more neurons became sensitive to reward size after cocaine exposure (Figure 7C) and the cocaine-exposed rats exhibited significantly shorter RTs for large rewards relative to pretreatment (Supplementary Figure 1B), indicating the rats valued large rewards more highly. This finding seems at odds with previous studies (Stalnaker, Roesch, Calu, et al., 2007; Stalnaker, Roesch, Franz, et al., 2007; Schoenbaum et al., 2004; Jentsch et al., 2002), showing that animals exposed to psychomotor stimulants are insensitive to the value of expected outcomes. But as shown by Stalnaker, Roesch, Franz, et al. (2007), cocaine-induced deficits in reversal learning tasks are because BLA neurons remain selective to the sucrose-predictive cue even after reversal, which is actually consis-

tent with our finding that BLA neurons stuck to responding to the large reward after cocaine exposure. This could be the underlying neural substances of the increased behavioral sensitivity to reward size reported by Roesch, Takahashi, Gugs, Bissonette, and Schoenbaum (2007). Hypersensitivity to size tends to counteract the effect of decreased delay perception and limit impulsive choice by making the rats more motivated to respond for the large reward. Cocaine exposure only increased the percentage of reward-dependent neurons during the reward period but not the reward index (Figure 7C and D), whereas both measures (percentage and delay index) in delay-dependent neurons were increased (Figure 7A and B). Thus, we speculate that the change of delay representation won over size and, therefore, produced a net result of enhanced delay discounting and lack of difference in RT between pre- and post-cocaine exposure on 8-sec trials (Supplementary Figure 1B).

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