

Cocaine-induced decision-making deficits are mediated by miscoding in basolateral amygdala

Thomas A Stalnaker¹, Matthew R Roesch¹, Theresa M Franz¹, Donna J Calu², Teghpal Singh² & Geoffrey Schoenbaum^{1,3,4}

Addicts and drug-experienced animals have decision-making deficits in reversal-learning tasks and more complex ‘gambling’ variants. Here we show evidence that these deficits are mediated by persistent encoding of outdated associative information in the basolateral amygdala. Cue-selective neurons in the basolateral amygdala, recorded in cocaine-treated rats, failed to change cue preference during reversal learning. Further, the presence of these neurons was critical to the expression of the reversal-learning deficit in the cocaine-treated rats.

Addicts make poor decisions. These deficits have been modeled in addicts and drug-experienced animals using reversal-learning tasks and more complex ‘gambling’ variants. In these settings, subjects first learn to associate different cues with different probabilities of reward and punishment, and then the meanings of the cues are reversed. After this reversal, addicts and animals exposed to psychostimulants have difficulty learning to stop responding to previously rewarded cues^{1–4}. Similar reversal deficits caused by damage to orbito-frontal cortex are mediated by miscoding of associative information in the basolateral

amygdala^{5,6}. Here we test whether miscoding of associative information in the basolateral amygdala (ABL) also mediates cocaine-induced reversal deficits in rats.

In the first experiment (Fig. 1, Experiment 1 and **Supplementary Methods** online for procedures), neural activity was recorded in ABL in rats previously exposed to saline or cocaine (30 mg per kg of body weight, intraperitoneal \times 14 d, locomotor data in **Supplementary Results** and **Supplementary Fig. 1** online). Recording began \sim 4 weeks after drug exposure and was conducted in a different room and boxes than those used for drug exposure. We recorded 118 neurons in saline-treated rats ($n = 4$) and 228 neurons in cocaine-treated rats ($n = 3$) during acquisition and reversal of a series of novel two-odor go, no-go discrimination problems. In each problem, one odor predicted the delivery of sucrose, and another odor predicted the delivery of quinine; after learning, these predictive relationships were reversed (see **Supplementary Methods**). Recording locations (Fig. 2a,b) and baseline firing rates (see **Supplementary Results** and **Supplementary Fig. 2** online) were similar between groups, and as expected, rats exposed to cocaine were slower to learn the reversals (Fig. 1b; detailed analysis available in **Supplementary Results** and **Supplementary Fig. 3** online).

To assess encoding of the odor-outcome associations, we analyzed neural activity to the sucrose- and quinine-predictive cues during learning and after reversal (description and additional analyses of other time periods available in **Supplementary Methods** and **Supplementary Results**). We found little effect of cocaine on the development of activity to either cue during learning (Table 1, Fig. 2a,b and **Supplementary Results**). However, cocaine-treatment markedly reduced the changes in cue-evoked activity that are normally observed across reversal. In controls, 48% of the cue-selective neurons evident during learning switched their cue preference after reversal, and only a single neuron maintained the same preference (Table 1). These

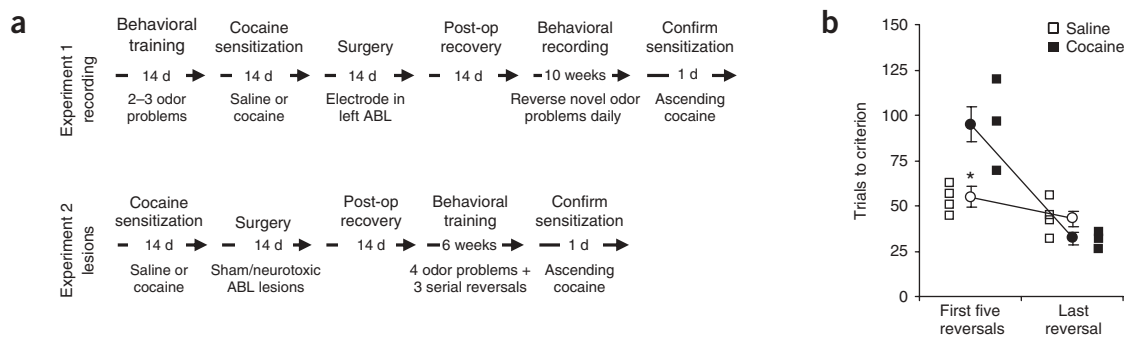


Figure 1 Experimental design and effect of cocaine exposure on reversal learning during recording. **(a)** Experimental timelines. Animal testing procedures were approved by the Institutional Animal Care and Use Committee at the University of Maryland School of Medicine. **(b)** Individual and group trials to criterion after reversal during the initial and final recording sessions (* $P < 0.05$). Error bars represent s.e.m.

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proportions are nearly identical to those in two prior studies of neural activity in ABL in this task^{6,7}, in which 55% (10/18 cue-selective ABL neurons in 4 rats) and 53% (34/64 cue-selective ABL neurons in 3 rats) switched cue preference after reversal (see **Supplementary Results** and **Supplementary Fig. 4** online for comparison of individual rats). Thus, the effect of reversal on cue-evoked activity in controls in the current experiment reflects a highly reliable and reproducible result. Indeed, similar results have been reported in primates⁸.

Flexibility of cue-evoked activity in ABL is evident in the population responses (**Fig. 2a**), which reversed cue preference during reversal learning, tracking the outcomes predicted by the odor cues rather than their sensory features. This is also evident in the ‘cue-selectivity index’, calculated for each neuron in each population as $(\text{firing}_{\text{odor1}} - \text{firing}_{\text{odor2}}) / (\text{firing}_{\text{odor1}} + \text{firing}_{\text{odor2}})$ (**Fig. 2a**, scatterplot). These indices were inversely correlated across reversal ($r = -0.68, P = 0.00001$), indicating that the neurons fired according to the outcomes predicted by the cues and not to the cues’ specific sensory features.

By contrast, cue-evoked activity in the cocaine-treated rats was significantly less likely to reverse and significantly more likely to persist to the same odor across reversal (**Table 1**; $\chi^2 = 11.98, P = 0.0005$, and $\chi^2 = 10.1, P = 0.0015$, respectively). There were no differences between animals within groups in the proportion of neurons that reversed (P values = 0.27–0.97), indicating that this effect was not carried by any one animal in the cocaine-treated group (see **Supplementary Results** and **Supplementary Fig. 4** for comparison of individual rats).

Inflexibility of cue-evoked activity in ABL in cocaine-treated rats is evident in the population responses (**Fig. 2b**) and in the cue-selectivity indices (**Fig. 2b**, scatterplot), which showed a highly significant positive correlation across reversal ($r = 0.46, P = 0.0004$). Because these neurons developed cue-selective firing during initial learning (see **Supplementary Results** for details), this positive correlation indicates that these neurons continued to fire on the basis of the pre-reversal significance of the cues. This effect was particularly apparent in neurons that were selective for the sucrose-predictive odor before reversal;

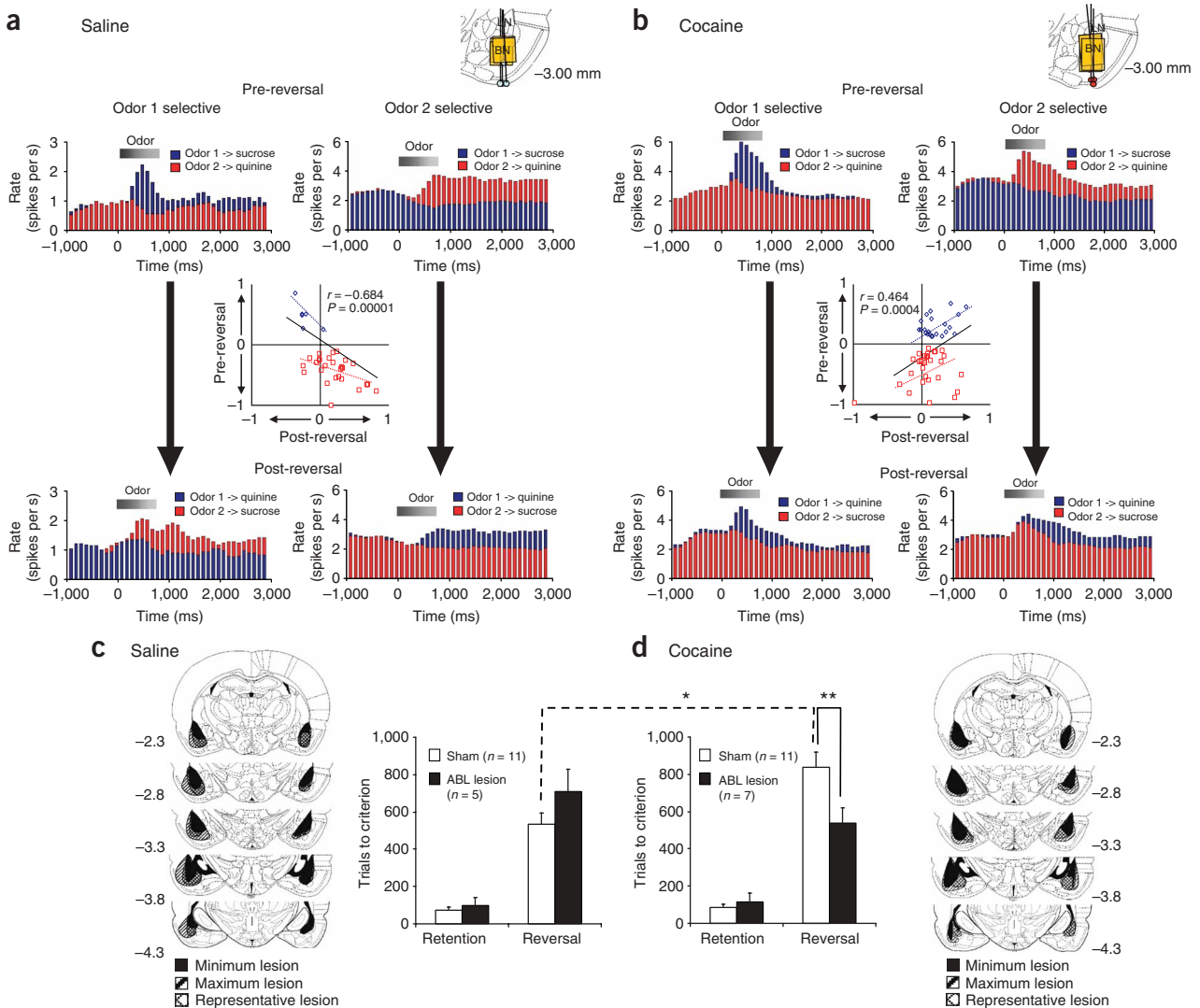


Figure 2 Cocaine-induced decision-making deficits are mediated by miscoding in basolateral amygdala. (**a,b**) Population histograms show average activity in neurons selective for each odor cue after learning and after reversal. Scatterplots show selectivity indices calculated from cue-evoked firing before and after reversal for each neuron. (**c,d**) Drawings show size and extent of lesions; bar graphs show average trials to criterion for retention and reversal. Planned comparisons after three-factor ANOVA: * $P = 0.0046$, ** $P = 0.013$. No other comparisons, planned or unplanned, were significant. Error bars represent s.e.m.

Table 1 Effect of reversal on cue-evoked activity in ABL in cocaine- and saline-treated rats

Pre-reversal	Post-reversal		
	Same odor	Opposite odor	Nonselective
All cue-selective neurons			
Saline (<i>n</i> = 33)	1 (3%)	16 (48%)	16 (48%)
Cocaine (<i>n</i> = 55)	15* (27%)	8* (15%)	32 (58%)
Odor 1 + selective neurons			
Saline (<i>n</i> = 6)	0 (0%)	4 (67%)	2 (33%)
Cocaine (<i>n</i> = 23)	13* (57%)	0* (0%)	10 (43%)
Odor 2- selective neurons			
Saline (<i>n</i> = 27)	1 (4%)	12 (44%)	14 (52%)
Cocaine (<i>n</i> = 32)	2 (6%)	8 (25%)	22 (69%)

Values indicate the number of cue-selective neurons (with percentages in parentheses) that were selective for the same odor cue versus the opposite odor cue (or were nonselective) after reversal. Odor 1 is the sucrose-predictive odor before reversal, and Odor 2 is the quinine-predictive odor cue before reversal. Thus, although many neurons reversed their cue-selectivity across reversal in saline-treated rats, very few neurons did so in cocaine-treated rats. * indicates difference from saline-treated controls at $P = 0.01$ or better by χ^2 (no others were significant at $P < 0.05$ or better).

however, inflexible encoding was also apparent in neurons selective for the quinine-predictive odor (see **Supplementary Results** for analyses by outcome).

Although ABL is not normally critical for reversal learning^{9,10}, persistent encoding of outdated associative information in ABL could slow reversal learning by interfering with normal processing in other structures^{1,2,10}. We have demonstrated such a mechanism for reversal deficits caused by orbito-frontal cortex lesions^{5,6}. To test whether drug-induced reversal deficits might be mediated by a similar mechanism, we tested the effects of ABL lesions on the reversal deficit caused by cocaine (see **Fig. 1a**, Experiment 2 and **Supplementary Methods** for procedures). Groups included saline/sham = 11, saline/lesion = 5, cocaine/sham = 11 and cocaine/lesion = 7. Lesion size (**Fig. 2c,d**) and sensitization to cocaine did not differ between relevant groups (locomotor data available in **Supplementary Results** and **Supplementary Fig. 5** online). Rats were trained 4 weeks after drug exposure on four discriminations followed by three reversals of the final problem. There was no effect of lesions or cocaine on learning (see **Supplementary Results**). As expected, cocaine impaired reversal learning in rats with sham lesions, but this reversal deficit was completely abolished by ABL lesions (**Fig. 2c,d** and **Supplementary Results**). Improved reversal learning was not a result of a general blockade of cocaine's effects by ABL lesions, as lesioned rats showed normal locomotor sensitization at the end of reversal training (see **Supplementary Results** and **Supplementary Fig. 5**). The failure of ABL lesions to alter performance in normal rats also suggests that lesions did not alter rats' normal learning strategy. Nor did ABL lesions independently facilitate reversal learning; lesioned controls performed no better than shams on the reversals

(**Fig. 2c**). Instead, cocaine created a pathological role for ABL in reversal learning.

The observation that cocaine exposure affects decision-making by diminishing the flexibility of associative encoding in ABL suggests a mechanism whereby drug-associated cues may persistently affect behavior, even after extinction and in the face of adverse consequences. Thus in addicts and animal models, inflexible associative representations in ABL might contribute to relapse and compulsive drug-seeking. Indeed, ABL neurons fire strongly to drug-associated cues¹¹, and ABL is critical to cue-induced relapse^{12–14}. Manipulations designed to disrupt reconsolidation of memories in ABL reduce cue-evoked drug-seeking¹⁴. Inflexible encoding in ABL may be related to pathological changes in prefrontal areas, as has been suggested in other neuropsychiatric disorders¹⁵ (see **Supplementary Discussion** online).

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

T.A.S. and G.S. conceived the experiments; T.A.S., M.R.R. and D.J.C. carried out the recording work; T.A.S. and T.S. carried out the lesion work; and T.M.F. assisted with electrode construction, surgeries and histology. The data were analyzed by T.A.S. and G.S., who also cowrote the manuscript with assistance from each of the other team members.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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COCAINE-INDUCED DECISION-MAKING DEFICITS ARE MEDIATED BY MISCODING IN BASOLATERAL AMYGDALA

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SUPPLEMENTARY METHODS

Subjects: Male Long-Evans rats (275-400g; Charles River Labs, Wilmington, MA) were tested at the University of Maryland in accordance with University and NIH guidelines.

Cocaine exposure: Cocaine-exposure procedures were identical to those we have used previously^{1,2}. Rats received 14 daily i.p. injections of 30 mg/kg cocaine HCl or saline vehicle (NIDA, Bethesda, MD). This treatment regimen is similar to that used by Robinson and Kolb and colleagues to demonstrate effects of drug exposure on dendritic structure in corticolimbic areas³⁻⁵. It is also the same regimen we have used to show changes in reversal learning and other behaviors and in neural correlates of reversal learning in OFC in past studies^{1,2,6-8}. Locomotor activity was monitored during a 1 hour period after each injection (Coulbourn Instruments). At the end of each experiment (i.e. after all odor discrimination testing and/or neural recording was completed), all rats received ascending doses of cocaine (0.9% saline and 7.5, 15.0, 30.0 mg/kg cocaine HCl i.p.). Activity was again monitored for an hour after each injection.

Note that in the lesion study, some of the control rats in the second experiment did not receive saline injections due to IACUC requests to combine control groups for this and a parallel experiment⁹ in order to minimize pain and suffering to the animals. These seven rats received similar handling and exposure to the testing environment as the saline-treated rats, only they did not receive i.p. injections. As we will describe below, these rats did not differ statistically from saline-treated controls, and so these

groups were combined in the lesion study. However, as our statistical analyses below will show, none of the effects we report require these rats to be included.

Surgery and histology: Surgical and histological procedures were identical to those we have used previously^{10,11}. For recording, a driveable bundle of 10 25-um FeNiCr wires, cut and platinum plated to 300 kOhms, was implanted dorsal to ABL in the left hemisphere at 2.8 mm posterior to bregma, 5.0 mm laterally, and 6.7 mm ventral to the surface of the brain. Lesions of ABL, including the lateral, basal and accessory basal nuclei, were made using intra-cerebral infusions of *N*-methyl-D-aspartic acid (NMDA, Sigma, St Louis, MO; 12.5 µg/µl) in saline vehicle. Bilateral infusions of 0.1 µl and 0.2 µl were made at 8.7 mm and at 8.4 mm ventral to the skull surface, respectively, both at a site 2.7 mm posterior to bregma and 5.0 mm lateral to midline, using a 26-gauge cannula attached by a length of plastic tubing to a microsyringe (Hamilton, Reno, NV) mounted on a syringe pump (Sage Instruments, Boston, MA). Controls received infusions of saline vehicle alone. At the end of each study, the rats were euthanized with an overdose of isoflurane, perfused, and the brains were removed from the skulls and processed for histology using standard techniques. Lesions and electrode placements were verified under a light microscope.

Odor discrimination training: Training and analytical procedures were identical to those we have used previously^{1,2,10,11}. Discrimination training was conducted in aluminum chambers approximately 18" on each side with sloping walls narrowing to an area of 12" x 12" at the bottom (n.b. these chambers differed from those in which sensitization occurred). An odor port and fluid well were located on a panel in the right wall of each chamber below two panel lights; the odor port was connected to an air flow dilution olfactometer to allow the rapid delivery of olfactory cues. Odor

discrimination problems were composed of odor pairs chosen from compounds obtained from International Flavors and Fragrances (New York, NY). On each trial, the rat sampled an odor and then had 3 seconds to make a go response at the fluid well. If a response was made after sampling the odor designated as the 'positive' odor, a 0.05 ml bolus of a 10% sucrose solution was delivered to the well after a variable delay (500-1500 ms). If the same response was made after sampling the 'negative' odor, a 0.05 ml bolus of a 0.02 M quinine solution was delivered after a similar delay. If the rat did not respond within 3 seconds, the trial was counted as a no-go. A behavioral criterion was defined as 18 correct responses (go on positive trials, no-go on negative trials) in a moving block of 20 trials. During training, rats were maintained on water restriction.

For the recording experiment, rats were shaped to perform the discrimination task prior to sensitization and surgery to implant the electrode arrays. None of the odor problems used during this initial training was reused during subsequent recording. Recording began 4 weeks after the end of sensitization (2 weeks post-op) and continued for approximately 2 months. Neural data were collected in each session as the rats acquired new discrimination problems and reversals of those problems.

For the lesion experiment, the rats did not receive any experience in the task before sensitization/lesions. Training began 4 weeks after the end of sensitization (2 weeks post-op) with a series of 4 two-odor discrimination problems (D1-D4). Once the first four odor problems (D1-D4) were acquired, the rats were required to learn three serial reversals of the final odor problem (D4). The problem was first presented using the same contingencies that were employed in initial training (S1+/S2-). Rats were required to meet criterion on this problem again (18/20 correct), show 80% performance over the next 60 trials, and then the problem was reversed. Training on the reversed

problem (S1-/S2+) continued over multiple sessions until the rat met the behavioral criterion again (18/20 correct). This process of retention and subsequent reversal was repeated twice more on subsequent days using the same procedures. Behavioral data were analyzed by ANOVA with repeated measures and planned contrasts as noted below (Statistica, Statsoft, Tulsa OK).

Single-unit recording and data analysis: Recording and analytical procedures are similar to those we have used previously^{2,10}. Wires were screened daily, and active wires were selected for recording. The electrode was advanced 40 or 80 μm between each session. Neural activity was recorded differentially using MAP systems (Plexon, Dallas, TX), interfaced with odor discrimination training chambers described above. Technical details are available in prior reports. Waveforms were recorded to disk by an associated workstation with event timestamps. Units were sorted using Offline Sorter (Plexon, Dallas, TX) combined with notes regarding the waveforms made during the session. Sorted files were processed in Neuroexplorer to extract unit timestamps and relevant event markers. These data were subsequently analyzed using statistical routines (Matlab, Natick, MA) to examine firing activity (spikes/second) during sampling of the two odor cues (from 50 ms after odor onset to 50 ms after odor offset). This neural measure or its derivatives was analyzed using ANOVA, Pearson X_2 tests, and correlation coefficients ($p < 0.05$) (n.b. the results were also checked with non-parametric tests).

SUPPLEMENTARY RESULTS

Experiment 1: Recording

Sensitization: Rats in the cocaine-treated group received a daily injection of cocaine (30 mg/kg i.p.) for 14 days; rats in the control group received a similar volume of saline i.p.. Figure S1A shows locomotor activity, monitored during the hour after each injection, for the saline- and cocaine-treated groups across these 14 treatment sessions and a single pre-treatment session. Locomotor activity increased significantly in rats treated with cocaine relative to their pre-exposure levels and in comparison to activity in saline-treated controls. A 2-factor ANOVA (treatment X day) revealed significant main effects of treatment ($F_{1,5}=21.6$, $p=0.006$) and day ($F_{14,70}=4.05$, $p=0.0004$) and a significant interaction ($F_{14,70}=3.96$, $p<0.0001$). Approximately 12 weeks later, at the end of the recording phase of the experiment, all rats were challenged with ascending doses of cocaine to test for locomotor sensitization. As illustrated in Figure S1B, rats with a history of cocaine exposure exhibited greater locomotor activity than saline-treated controls to saline and the lowest dose of cocaine, consistent with a persistent sensitizing effect of cocaine in these rats. A 2-factor ANOVA (treatment X dose) revealed a significant interaction ($F_{3,15}=11.8$, $p=0.0003$); subsequent contrast testing showed that the cocaine-treated rats were significantly more active after saline injections ($p<0.05$).

Behavior: Previously we have reported that cocaine-treatment causes deficits in odor discrimination and reversal learning, extending months after drug exposure. In our original experiment, cocaine-treated rats learned the original discriminations but in the process failed to modify their response latencies during learning, particularly on negative trials. These rats also required many more trials than controls to learn a pair of serial reversals. Here we observed a similar pattern of deficits during recording, mitigated somewhat by the substantial practice all rats received on the reversal task.

The cocaine-treated rats again failed to modify their latencies to respond at the fluid well after sampling the sucrose- and quinine-predicting odor cues during learning. In saline-treated rats, these response latencies changed as they learned which outcome was predicted by each odor; the rats made faster responses after sampling the positive odor, as they came to expect the appetitive sucrose outcome, and slower responses after sampling the negative odor, as they came to expect the aversive quinine outcome. Cocaine-treated rats failed to slow their responses after sampling the quinine-predicting odors, consistent with a particular inability to signal the quinine outcome when making the decision to respond. Consistent with this interpretation, a 2-factor ANOVA comparing the change in latency during learning for positive versus negative trials revealed a significant interaction between treatment and phase ($F_{3, 156}=3.49$, $p=0.017$), as well as significant main effects of both factors (treatment, $F_{1, 52}=12.6$, $p=0.0008$; phase, $F_{3, 156}=9.76$, $p<0.0001$). Subsequent analyses revealed a significant interaction between treatment and the change in response latency during learning on negative (Figure S2B; $F_{1, 52}=3.98$, $p=0.05$) but not positive trials (Figure S2A; $F_{3, 156}=0.09$, $p=0.96$), and pairwise comparisons of the data shown in the figure indicated that normal rats differed significantly from cocaine-treated rats on negative trials late in the pre-criterion phase and also during the reversal phase ($p < 0.05$). Thus, even though the cocaine-treated rats were able ultimately to withhold responding on negative trials like saline-treated controls, as revealed in their normal go, no-go behavior during learning (Figure S2C; $F_{1, 52}=0.14$, $p=0.70$), cocaine-treated rats failed to change the speed or vigor of responding to reflect the motivational value of the expected outcome.

During subsequent reversal learning, cocaine-treated rats also exhibited a mild impairment, as described in the main text. This was evident in their overall reversal

scores shown in Figure S2C, which showed a non-significant trend towards impairment, and also in the average performance of individual rats. However because the recording procedure provided the rats with practice training on 20-30 discrimination problems, including 5-10 reversals, we did not observe a significant overall deficit. This practice effect was evident when we compared the performance of the rats on the first several reversal problems versus their performance on the last problem. This analysis, illustrated in the inset in Figure S2C, revealed that while the performance of both the controls and the cocaine-treated rats improved across the recording sessions, the cocaine-treated rats were initially much worse and improved more during recording. Consistent with this, a 2-factor ANOVA of performance on these problems revealed main effects of treatment ($F_{1,5}=7.59$, $p=0.04$) and reversal number ($F_{5,25}=4.07$, $p=0.007$) and also an interaction between these factors ($F_{5,25}=2.80$, $p=0.038$); subsequent analyses showed that both groups improved significantly across training and that cocaine-treated rats performed significantly worse than controls at the beginning but not at the end of training (Figure 1 in main text or Figure S2C, inset; $p < 0.05$). Notably, a similar improvement was not apparent in the response latency changes, nor did we discern any normalization of the encoding changes we have described above by the end of the experiment. This is consistent with what we have argued previously based on OFC lesion studies, in which similar improvement is evident with practice, which is that other brain regions become more efficient at mediating the reversal behavior. Part of this increased efficiency may be the ability to tune out or ignore aberrant input from ABL in these rats.

For our neural data analyses, each session was divided into a pre-criterion phase, a post-criterion phase and a reversal phase. In addition, the pre-criterion phase was also

divided into an early block of trials at the beginning of the session, consisting of trials before the rat's sixth error, and a late block of trials, consisting of the remaining trials before the rat met the behavioral criterion. The average number of trials in each phase for these sessions is shown in Table S1; it did not differ between groups.

Table S1: Average Number of Trials in Each Performance Block

	Pre-Criterion		Post-Criterion	Reversal
	Early	Late		
Saline (n = 22):	13	37	85	126
Cocaine (n = 32):	13	34	86	136

Note: ANOVA indicated no significant differences at $p < 0.05$

Distribution of baseline firing rates: As in prior studies, neural data were acquired primarily (>95%) from regular spiking cells. The distribution of the average firing rates for the neurons in each group is given in Figure S3. Neurons recorded in cocaine-treated rats had a slightly higher overall baseline firing rate ($F_{1,344}=4.66$, $p=0.03$); however this difference did not reflect a general shift in the distribution of the baseline firing rates but rather was primarily due to the inclusion of 4 neurons in the cocaine-treated group with very high firing rates. When these neurons were removed, the averages did not differ ($F_{1,340}=2.70$, $p=0.10$) nor did the results we have reported.

Changes in cue-evoked activity during learning: We found little effect of cocaine-treatment on the development of cue-evoked activity in ABL during learning. Both saline- and cocaine-treated rats had substantial numbers of cue-selective neurons in ABL after learning, accounting for 28% or 33/118 neurons in the saline-treated group and 24% or 55/228 neurons in the cocaine-treated group ($X_2 = 0.61$, $p = 0.43$), and cue-selectivity in these neurons developed as the rats learned the significance of the odor cues. Thus while 27% of the neurons in each group (9/33 and 15/55) were cue-selective in advance of criterion performance, only 1 neuron in controls and 3 neurons in cocaine-

treated rats exhibited this cue-selective response at the start of the session ($X_2 = 0.28$, $p = 0.6$). The only significant effect of cocaine-treatment was that more of these neurons responded to the positive odor cue in cocaine-treated rats (23/55) than in saline-treated controls (6/33; $X_2 = 5.22$, $p = 0.022$). Similarly cocaine treatment also had little effect on the development of cue-selectivity after reversal in neurons that had been non-selective prior to reversal. Such novel cue-selective correlates were observed in 13% or 16/118 neurons in the saline-treated group and 15% or 34/228 neurons in the cocaine-treated group ($X_2 = 0.12$, $p = 0.73$). Indeed the overall proportions of cue-selective neurons did not differ between groups either before (33/118 for saline vs 55/228 for cocaine; $X_2 = 0.61$, $p = 0.43$) or after reversal (33/118 for saline vs 57/228 for cocaine; $X_2 = 0.36$, $p = 0.55$). From these data, it appeared that cocaine-treatment did not alter the development of associative correlates in ABL.

Changes in cue-evoked activity during reversal by outcome: Encoding of outdated associative information was particularly apparent in cocaine-treated rats in neurons that were selective for the positive odor cue before reversal. None of these neurons reversed cue-selectivity, and the majority of them continued to fire to the same odor after reversal, despite the fact that it predicted quinine (Table 1, main text). As a result, this population, shown in Figure 1D in the main text (“Odor 1 Selective”), responded for the same odor cue before and after reversal, and comparison of the cue-selectivity indices before and after reversal for this population, shown in blue in the scatter-plot in Figure 1D in the main text, revealed a highly significant positive correlation in these measures ($r = 0.65$, $p = 0.0007$ versus $r = -0.70$, $p = 0.10$ for this population in saline-treated rats).

However, inflexible encoding was also apparent in cocaine-treated rats in neurons selective for the negative odor cue. Although this was not evident in the individual neurons (Table 1, main text), it was clear in the population response, shown in Figure 1D in the main text (“Odor 2 Selective”). While this population did respond to the quinine-predicting cue after reversal, it also continued to show a phasic response to the odor cue that had predicted quinine prior to reversal. In accordance with this description, the cue-selectivity indices of these neurons, shown in red in the scatter-plot in Figure 1D in the main text, were not correlated across reversal ($r = 0.22$, $p = 0.23$). By contrast, the same population in controls, illustrated in red in the scatter-plot in Figure 1C in the main text, exhibited a highly significant inverse correlation ($r = -0.48$, $p = 0.011$).

Changes in cue-evoked activity during reversal by individual animal: Figure S4 shows the proportion of cue-selective neurons in each rat that reversed cue preference during reversal learning. As the figure shows, the proportion was uniformly higher in saline-treated controls than in cocaine-treated rats in the current study. As described in the main text, cue-selective activity that had developed during learning in the cocaine-treated rats was significantly less likely to reverse ($X_2 = 11.98$, $p = 0.0005$) and significantly more likely to persist to the same odor ($X_2 = 10.1$, $p = 0.0015$). Furthermore, there were no differences between animals within groups in the proportion of neurons that reversed (p 's = 0.27-0.97), indicating this effect was not carried by any one animal in the cocaine-treated group. Moreover, as illustrated in Figure S4, this is also true if data from the present report is compared to results from an earlier study in which we examined reversal of encoding in ABL in normal rats and rats with ipsilateral OFC lesions. Neural data from control rats in the current study are statistically

indistinguishable from that in control rats in this previous report, and neural data from cocaine-treated rats in the current study are similar to that from OFC-lesioned rats from this previous report. These results support the reliability of our control data here and also of the aberrant encoding observed in the 3 cocaine-treated rats.

Analyses of neural activity in other time periods during the trial: To identify whether neural correlates of these associative representations in ABL were affected by prior cocaine-treatment in the current study, we compared neural activity during odor sampling, beginning with trials in the post-criterion phase. The main text presents an analysis of these neurons as a group. However we have also previously divided neurons with differential activity during this cue-sampling period according to whether they also fired in anticipation of the appropriate outcome earlier in training. Thus here we also analyzed neural activity during learning after responses were made, as the rats waited in the fluid well for sucrose or quinine. Activity during this 500-1500 ms delay period was compared during pre-criterion trials, when substantial numbers of errors were made on negative trials. As in previous studies, we found that substantial numbers of ABL neurons fired differentially in anticipation of either the sucrose or the quinine outcome. These data are shown in Table S2. There was no effect of cocaine-treatment on the development of selective neural activity during this period of the trial, either in the absolute number of neurons with this correlate or in the relative numbers firing in anticipation of the differently valenced outcomes.

Table S2: Outcome-Expectant Neurons in ABL in Saline- and Cocaine-Treated

Rats

	Sucrose-Expectant	Quinine-Expectant	Totals
Saline (n = 118):	3 (2)	16 (7)	19 (9)
Cocaine (n = 228):	7 (2)	30 (7)	37 (9*)

*Note: numbers in parentheses show the number of neurons that went on to become selective for the appropriate odor cue after learning; χ^2 tests indicated no significant differences at $p < 0.05$, although the comparison marked by * approached significance at $p = 0.08$.*

However there did appear to be an effect of cocaine-treatment on whether or not these outcome-expectant neurons went on to become selective for the odor cues that predicted their preferred outcome (Table S2; in parentheses). In saline-treated rats, we found that 9 of the 19 outcome-expectant neurons became selective for the odor cue that predicted the neurons preferred outcome, which was a proportion greater than chance, given the prevalence of the cue-selective and outcome-expectant correlates in the population (chance = 3 cells, $\chi^2 = 4.38$, $p = 0.036$). By contrast, in rats previously treated with cocaine, only 9 of 37 outcome-expectant neurons developed selective firing for the appropriate odor cue. This proportion did not differ from chance (chance = 5 cells, $\chi^2 = 2.33$, $p = 0.127$), and, despite the relatively small sample size, the comparison with controls approached significance ($\chi^2 = 3.06$, $p = 0.08$). Although this result is inconclusive, it suggests that cocaine may have a selective effect on the development of these neural correlates during learning (n.b. although sample size precludes an analysis by valence, the effect was evenly distributed; see Table S2).

Experiment 2: Lesions

Sensitization: Rats in the cocaine-treated group received a daily injection of cocaine (30 mg/kg i.p.) for 14 days; rats in the control group received a similar volume of saline i.p. Figure S5A shows locomotor activity, monitored during the hour after each injection, for the saline- and cocaine-treated groups across these 14 treatment sessions and a single pre-treatment session; data is shown separately for rats with lesions or shams, even though these groups were matched for activity levels prior to

surgery. Locomotor activity increased significantly in rats treated with cocaine relative to their pre-exposure levels and in comparison to activity in saline-treated controls; as expected, there were no effects of lesions. A 3-factor ANOVA (treatment X lesion X day) revealed significant main effects of treatment ($F_{1,23}=29.9$, $p<0.0001$) and day ($F_{14,322}=8.68$, $p<0.0001$) and a significant interaction between these two factors ($F_{14,322}=9.02$, $p<0.0001$); there was no main effect nor any interactions with lesion (F 's <0.34 , p 's >0.93). Approximately 12 weeks later, at the end of the reversal testing phase of the experiment, all rats were challenged with ascending doses of cocaine to test for sensitization. As illustrated in Figure S5B, rats with a history of cocaine exposure exhibited greater locomotor activity than saline-treated controls to saline and the lowest dose of cocaine, consistent with a persistent sensitizing effect of cocaine in these rats. A 2-factor ANOVA (treatment X lesion X dose) revealed a significant interaction between treatment and dose ($F_{3,69}=17.9$, $p<0.0001$). Again there was no main effect nor any interactions with lesion (F 's <1.05 , p 's >0.37); subsequent contrast testing showed that the cocaine-treated rats were significantly more active after saline, 7.5 or 15 mg of cocaine ($p<0.05$).

Lesions: Five out of six rats from the saline-treated lesioned group and seven out of twelve rats from the cocaine-treated lesioned group were judged to have acceptable lesions. One rat was excluded due to collateral damage, and five were excluded because little or no damage was detected in one hemisphere. Lesions from the remaining rats are illustrated in Figure 1E and 1F of the main text. There were no differences in lesion size between groups (average lesion: saline-treated: 79%, range 68-92%; cocaine-treated 87%, range 78-95%; $F_{1,10} = 2.6$, $p = 0.14$).

Behavior on the initial discriminations: Prior to the reversal testing reported in the main text, these rats were trained on four 2-odor go, no-go discriminations (the final problem was subsequently used for the reversals). Performance on these problems is shown in Table S-3. As noted in the main text, there were no effects of cocaine or lesion on acquisition of these problems. A 3-factor ANOVA (cocaine X lesion X odor problem) revealed no main effects of cocaine ($F_{1,30} = 1.64$, $p = 0.21$) or lesion ($F_{1,30} = 2.87$, $p = 0.10$) and no significant interactions between cocaine or lesion and any other factor (F 's < 2.1 , p 's > 0.11). There was a significant main effect of odor problem ($F_{3,90} = 60.0$, $p < 0.000001$), reflecting the increasing facility with which all the groups reached criterion on the four successive discriminations.

Table S-3: Effect of Cocaine and ABL Lesions on Odor Discrimination Learning

Group	D1 (shaping)	D2	D3	D4
Saline-Sham:	304±72	92±17	93±15	33±4
Saline-Lesion:	269±56	52±14	26±3	31±6
Cocaine-Sham:	443±52	70±15	62±13	32±3
Cocaine-Lesion:	340±77	71±12	44±9	35±9

Note: values show average trials-to-criterion ± SEM on 4 different discrimination problems (D1-D4)

Behavior on the reversals: As noted in the text, cocaine impaired reversal learning in rats with sham lesions, and this reversal deficit was not present in cocaine-treated rats that had ABL lesions; these rats learned reversals at the same rate as intact saline-treated rats. This improvement was not due to a general blockade of the effects of cocaine exposure by ABL lesions, since ABL lesioned rats showed normal locomotor sensitization when they were exposed to ascending doses of cocaine at the completing of reversal training. Nor did ABL lesions independently facilitate reversal learning; lesioned controls performed no better than shams on the reversals. Consistent with this interpretation, a 3-factor ANOVA (cocaine X lesion X retention/reversal) showed no

significant main effects of cocaine ($F_{1,30} = 1.0$, $p = 0.32$) or lesion ($F_{1,30} = 0.16$, $p = 0.69$) but a highly significant interaction between the two factors ($F_{1,30} = 8.3$, $p = 0.0074$). Furthermore, there was a significant interaction between cocaine, lesion, and retention/reversal ($F_{1,30} = 6.3$, $p = 0.018$). Planned contrasts revealed significant differences between the performance of saline- and cocaine-treated shams (*; saline-sham = 534 TTC; cocaine-sham = 839 TTC; $p = 0.0046$) and cocaine-treated shams and cocaine-treated lesioned rats (**; cocaine-sham = 839 TTC; cocaine-lesions = 540 TTC; $p = 0.013$) but not between saline-treated shams and cocaine-treated lesioned rats ($p = 0.96$). No other comparisons were significant.

Behavior of the saline- and non-saline treated controls: As noted in the methods, some of the control rats in the second experiment did not receive saline injections due to IACUC requirements to combine control groups in this and a parallel study⁹. They received similar handling and exposure to the training environment only they did not receive i.p. injections. Their performance on the initial discriminations and the reversals did not differ from saline-treated controls (F 's < 2.5 , p 's > 0.14), thus they were collapsed to create the single control group here. Furthermore none of the statistical effects reported here or in the main text requires that these rats be included. In particular, when these uninjected controls are excluded, the 3-factor ANOVA (cocaine X lesion X retention/reversal) still showed no significant main effects of cocaine ($F_{1,23} = 2.1$, $p = 0.16$) or lesion ($F_{1,23} = 0.1$, $p = 0.79$) but a highly significant interaction between the two factors ($F_{1,23} = 9.2$, $p = 0.0059$). Furthermore, there remained a significant interaction between cocaine, lesion, and retention/reversal ($F_{1,23} = 7.0$, $p = 0.014$). Planned contrasts revealed significant differences between the performance of saline- and cocaine-treated shams (saline-sham = 409 TTC; cocaine-

sham = 839 TTC; $p = 0.0063$) and cocaine-treated shams and cocaine-treated lesioned rats (cocaine-sham = 839 TTC; cocaine-lesions = 540 TTC; $p = 0.013$) but not between saline-treated shams and cocaine-treated lesioned rats ($p = 0.4$). No other comparisons were significant.

SUPPLEMENTARY DISCUSSION

Our results are consistent with the hypothesis that inflexible encoding in ABL in cocaine-treated rats causes impaired reversal learning. According to this proposal, signalling of the outdated associative information by ABL causes slower reversal learning by interfering with processing in other regions, such as striatum or temporal lobe structures, that normally set the rate of reversal. Importantly, this proposal does not address the role of ABL in normal reversal learning. Indeed, as noted above, the flexibility of encoding in ABL does not appear to determine the rate of reversal learning in intact animals¹¹; rather, cocaine-exposure causes a pathological condition in which ABL assumes a critical role in determining this rate.

In addition, it is important to note that lesions of ABL in otherwise intact rats did not cause a general facilitation of reversal learning, and neither did ABL lesions have any effect on the acquisition of the initial discriminations. This argues against an account by which ABL lesions corrected the reversal impairment by an effect on initial acquisition of the discriminations or by independently improving cognitive flexibility. The absence of effects of ABL lesions on reversal learning is consistent with our own prior work and other reports that have used fiber-sparing lesion techniques to examine the role of amygdala in reversal learning¹¹⁻¹³. The simplest explanation of these results is that changes in associative encoding in ABL during reversal learning are normally

faster than those in other regions (at least in paradigms similar to ours). As a result, removing ABL has little effect in otherwise normal rats. However, in some pathological conditions – such as after chronic exposure to cocaine or damage to OFC - reversal of associative encoding in ABL becomes impaired, rendering it slow enough to retard the rate of behavioral reversal.

Of course we do not know how specific this effect is to our particular behavioral and drug setting; we can only speculate. As we have noted, we see chronic, long-lasting reversal deficits not only after passive exposure to cocaine but also in rats trained to self-administer the drug for several hours a day^{1,14}. This suggests that factors accompanying passive drug exposure – such as stress and bolus dosing – are not the underlying cause of the effect. Further there are some parallels in the literature between behavioral effects of chronic cocaine and other drugs, particularly other psychostimulants. For example, deficits in flexible behavior have been observed after exposure to various drugs in a variety of settings, suggesting that the behavioral effect may be expressed outside the reversal task^{1,6,14-24}. Finally the mechanism described here also appears to hold for OFC-lesioned rats inasmuch as OFC-dependent reversal deficits reflect inflexible encoding in ABL and are abolished by ABL lesions^{9,10}. These data suggest that the mechanism – inflexible encoding in ABL – may serve as a proximal cause for inflexible decision making in a variety of settings.

The observation that cocaine exposure affects decision-making by diminishing the flexibility of associative encoding in ABL suggests a mechanism whereby drug-associated cues might come to control behavior, even after extinction and in the face of adverse consequences. Thus in addicts and in animal models of addiction, inflexible associative representations might contribute to cue-evoked relapse and apparently

compulsive drug-seeking behavior²⁵⁻²⁷. Indeed, ABL neurons fire strongly to drug-associated cues during self-administration²⁸, and ABL has been shown to be critical to cue-induced relapse in animal models²⁹⁻³². Furthermore, pharmacological manipulations of ABL designed to disrupt reconsolidation of memories stored in ABL reduces cue-evoked drug-seeking behavior³³. Disrupting reconsolidation of memories evoked by drug-associated cues is particularly interesting as it is thought to permanently abolish previously acquired associative representations much like ABL lesions used here. Interestingly, cocaine withdrawal has been shown to enhance long-term potentiation in ABL³⁴; an imbalance between long-term potentiation and long-term depression, which is reportedly blocked by psychostimulants elsewhere³⁵, might enhance initial learning but cause an inability to subsequently alter representations. Effects of addictive drugs on flexibility of encoding in amygdala would also provide a final common pathway by which co-morbidity between drug addiction and anxiety disorders, also thought to reflect inflexible encoding in amygdala, might be expressed^{36,37}.

Another potential cause of inflexible neural correlates in ABL in cocaine-treated rats might be drug-induced dysfunction in prefrontal areas. Medial prefrontal cortex undergoes marked changes after drug exposure³⁸. The infralimbic region within medial prefrontal cortex has been linked to modulating expression of fear memories encoded in amygdala³⁶. Drug-induced dysfunction in this circuit may promote the inflexible encoding in ABL observed here. Another possible prefrontal substrate is the orbitofrontal cortex (OFC)³⁹⁻⁴⁴. Abnormal metabolic responses in OFC have been linked to cue-induced drug craving in addicts⁴⁴⁻⁴⁷, and addicts and drug-experienced animals are impaired on a number of OFC-dependent tasks^{1,6,8,18,19,22,23,48,49}, including

reversal learning^{11,50-53}. We have suggested that OFC lesions cause reversal deficits due to the loss of outcome signalling from OFC in lesioned animals⁵⁴. As a result, OFC-lesioned animals lack the ability to generate normal error signals in the face of unexpected outcomes, thereby causing slower associative changes in areas such as ABL¹⁰. We have recently reported that OFC neurons in cocaine-treated rats fail to signal expected outcomes during cue-sampling in this task². The loss of this signalling function after cocaine exposure may cause inflexible encoding in ABL.

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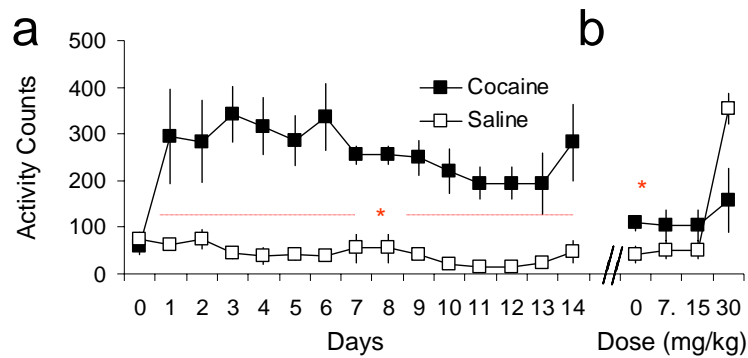


Figure S-1: Effect of cocaine on locomotor activity. Locomotor activity in saline- (white squares) and cocaine-treated (black squares) rats during the 2-weeks of cocaine exposure conducted approximately a month before the recording phase (A) and during dose-response testing after the recording phase (B). Average activity counts are shown for each 1 hour session, which followed each cocaine or saline injection. Counts are measured in 5 minute blocks and averaged across the session. Cocaine-treated rats exhibited increased locomotor activity before and after recording, consistent with sensitization. (*, significant difference at $p < 0.05$ or better)

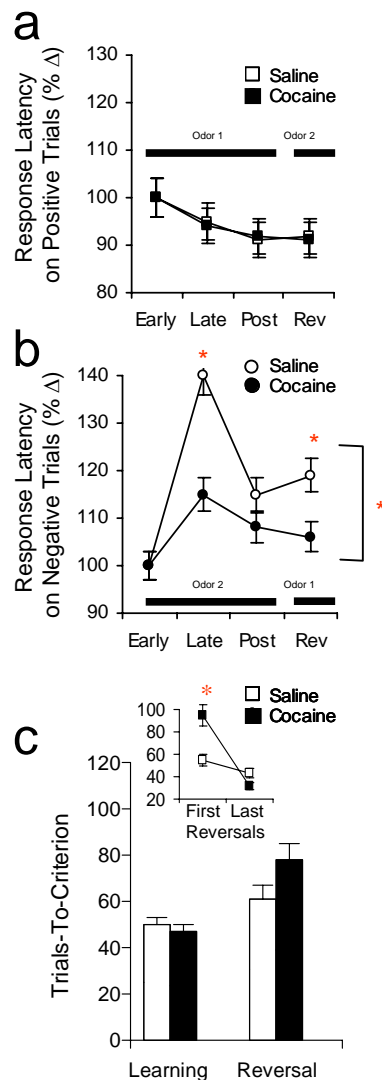


Figure S-2: Behavioral performance in saline- and cocaine-treated rats during recording sessions. A, B. Latencies to enter the fluid well when a decision was made to respond on positive (A) and negative (B) trials. Response latencies were calculated as the time from leaving the odor port until entry into the fluid well. Data are shown separately for trials early and late in the pre-criterion phase and post-criterion, represented as the % change from the average latency in the early pre-criterion trials. Data from no-go trials are not included. Cocaine-treated rats failed to develop the normal increase in latency to respond on negative trials during learning and also after reversal. C. Trials required to attain the go, no-go performance criterion of 18 correct responses in a moving block of 20 trials, during initial learning and after reversal. For reversal, data is also shown from the first 5 reversal sessions completed by each rat compared to the last reversal session (inset). Cocaine-treated rats exhibited a non-significant trend towards poor reversal performance over all recording sessions, and they were significantly impaired relative to controls in the first 5 reversals. (*, significant difference at $p < 0.05$ or better)

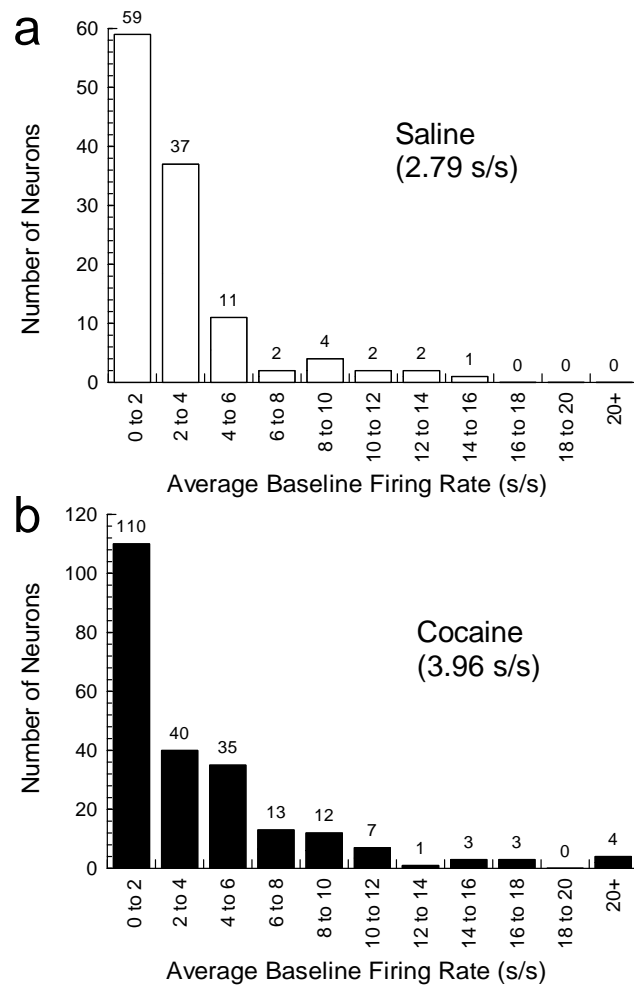


Figure S-3: Average baseline firing rate and distribution of baseline firing rates for neurons recorded in saline- and cocaine-treated rats.

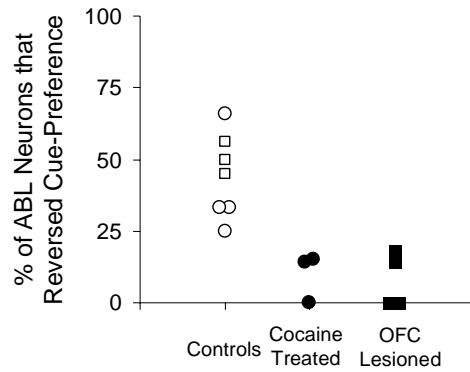


Figure S-4: Effect of cocaine treatment on the percentage of cue-selective ABL neurons that normally reverse cue-preference during reversal learning. Shown are data from individual rats, to demonstrate the reliability of the effect across rats. The percentage of reversing neurons was uniformly low in cocaine-treated rats (black circles) compared to controls from the current (white circles) or a previous study of ABL encoding in this exact same task (gray circles). Interestingly the likelihood of reversal of encoding after cocaine is similar to that in OFC-lesioned rat in this prior report (Saddoris, et al. *Neuron*. 2005).

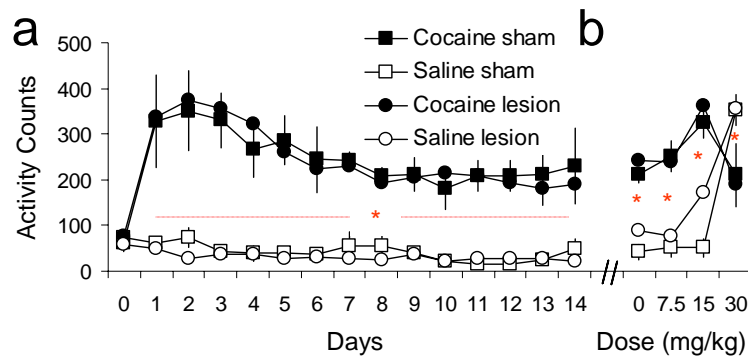


Figure S-5: Effect of cocaine and ABL lesions on locomotor activity. Locomotor activity in saline- (white squares = sham; white circles = lesioned) and cocaine-treated (black squares = sham; black circles = lesioned) rats during the 2-weeks of cocaine exposure conducted approximately a month before behavioral testing (A) and during dose-response testing after behavioral testing (B). Average activity counts are shown for each 1 hour session, which followed each cocaine or saline injection. Counts are measured in 5 minute blocks and averaged across the session. Cocaine-treated rats exhibited increased locomotor activity before and after recording, consistent with sensitization. There were no effects of lesions. (*, significant difference at $p < 0.05$ or better)