Supporting Information

Liu et al. 10.1073/pnas.1207531110

SI Materials and Methods

Surgery and Postoperative Maintenance. Animals were deeply anesthetized with ketamine (30 mg/kg i.p.) and xylazine (5 mg/kg i.p.). A catheter was implanted in an external jugular vein and passed s.c. to the top of the back where it exited into a connector. Catheters were flushed daily with 0.2 mL of an ampicillin solution (0.1 g/mL) containing heparin (300 IU/mL) to maintain patency. All animals had free access to water but were restricted to 15–20 g of food each day to maintain body weight at ∼370 g.

Apparatus. Training and testing sessions were conducted in Plexiglas operant chambers (34 × 23 × 329 cm; length × width × height) placed inside sound-attenuating cubicles. Each chamber was equipped with a response lever, lever light, drinking trough, and a syringe pump for sucrose or cocaine delivery.

Cocaine SA. We used distinct olfactory odors as conditional stimuli for both practical and theoretical reasons. First, we needed to provide readily distinguishable discriminative stimuli in both the awake behaving and anesthetized animal. Further, given the hedonic primacy of odor perception (1), odors have long been known to act as unconditional stimuli in conditioning experiments, whereas experience and familiarity significantly enhance odor quality discrimination (2, 3).

Odor Discrimination Training. During the intersession interval, animals were returned to their home cages, and the operant chambers were ventilated. On S− sessions, animals were exposed to an S− odor cue, and lever presses were reinforced with an infusion of saline. The S− odor was either lemon or vanilla scent, whichever was not assigned to the animal as their S+.

Sucrose and Housing Control Groups. These animals were exposed to identical SA pretraining, odor discrimination training and testing, and LgA training as the abovementioned cocaine group with the following exceptions: (*i*) each reinforced lever press was followed by delivery of a sucrose solution into a drinking well (0.2 mL of 32% sucrose over 10 s), and (*ii*) the number of sucrose infusions was matched to the daily number of cocaine infusions earned by a rat in the cocaine group.

Statistical Analysis. Because of poor image quality, one control animal was omitted from the olfactory bulb analysis. Data were not obtained from two cocaine SA rats because of errors in or complications during scan preparation. The cocaine and sucrose groups therefore differed in number. As a consequence, the average number of reinforcers earned by the sucrose and cocaine groups is not identical, given the behavioral matching procedure experimental design. However, statistical comparisons confirmed that there was no significant difference in the average reinforcement exposure between the cocaine and sucrose groups (*Results*).

Animal Preparation and Physiological Measurements. On imaging test days, rats were anesthetized with 2% (vol/vol) isoflurane in a 1:1 mixture of O_2:air, and glycopyrrolate (0.5 mg/kg, s.c.), a peripherally acting muscarinic antagonist, was administered to prevent airway blockade. Both femoral veins and one femoral artery were catheterized with PE-50 tubing for drug delivery and monitoring arterial blood gases and blood pressure, respectively. The wound area and incision were infiltrated with the local anesthetic Marcaine and closed. Last, rats were intubated and immediately transferred to a customized animal holder and placed on artificial ventilation (Rodent Ventilator, Model 683; Harvard Apparatus). Delivery of isoflurane was terminated, and an i.v. infusion of propofol (35 mg/kg/h) was initiated to maintain a stable anesthetic level throughout MRI data acquisition. The rat head was secured with a bite bar and ear bars for positioning within the center of the magnet. Core body temperature was maintained at 37.0 ± 0.5 °C with a circulating water heating pad. The neuromuscular blocker, pancuronium bromide, was administered continually (loading dose, 2.0 mg/kg followed by continuous infusion at 2.0 mg/kg/h) via a dedicated i.v. line to ensure the absence of motion artifacts. End tidal CO_2 and O_2, heart rate, blood pressure, and temperature were monitored continuously. Arterial blood gases were sampled intermittently and maintained within normal physiological limits (pCO_2, 35–45 mmHg; pO_2, >110 mmHg).

SI Results

Self-Administration Behavior. ***Acquisition.*** A three-way ANOVA revealed significant main effects of reward (cocaine vs. sucrose) ($F_{(1,24)} = 18.23$; $P < 0.001$), day ($F_{(13,312)} = 12.94$; $P < 0.001$), and cue (S+ vs. S−; $F_{(1,24)} = 80.63$; $P < 0.001$). There were also significant cue × reward ($F_{(1,24)} = 11.80$; $P < 0.01$) and day × cue interactions ($F_{(13,312)} = 16.01$; $P < 0.001$), but no three-way interaction ($F_{(13,312)} = 1.51$; not significant), indicating that both groups learned to discriminate the two odors.

Based on the above, secondary analyses demonstrated a significant effect of day ($F_{(13,143)} = 4.03$; $P < 0.001$), cue ($F_{(1,11)} = 66.41$; $P < 0.001$), and a day × reward interaction ($F_{(13,143)} = 7.91$; $P < 0.001$) in the cocaine self-administration (SA) group. Post hoc analysis revealed that the number of infusions during the S− session significantly decreased starting at the third session ($P < 0.001$) and continued to decrease thereafter until reaching a stable level at session 11 ($P < 0.01$). In contrast, the number of infusions during the S+ session remained stable during the 14 d of training (Fig. S1).

Similarly, the sucrose SA group showed a significant effect of day ($F_{(13,169)} = 11.35$; $P < 0.001$), cue ($F_{(1,13)} = 17.82$; $P < 0.001$), and a day × reward interaction ($F_{(13,169)} = 10.05$; $P < 0.001$). Post hoc analysis revealed that the number of infusions during the S− session significantly decreased from the third session ($P < 0.05$) and continued to decrease thereafter until stabilizing on day 8 ($P < 0.01$). Once again, the number of reinforcers during the S+ session remained stable during the 14 d of training (Fig. S1). As designed, the sucrose and cocaine SA rats were matched for number of rewards.

Discrimination test session. A two-way ANOVA with reward and cue as factors revealed a significant effect of cue ($F_{(1,24)} = 57.24$; $P < 0.001$) but not reward ($F_{(1,24)} = 0.37$; not significant) or reward × cue interaction ($F_{(1,24)} = 0.09$; not significant). Thus, for both cocaine and sucrose SA animals, the number of total presses was significantly higher for the S+ than for the S− odor in the discrimination test session ($P < 0.001$; Fig. S2).

Long-access training. As expected, cocaine intake gradually escalated from 56 to 90 infusions/d ($F_{(19,209)} = 15.34$; $P < 0.001$). The rate of cocaine infusions significantly increased from the fourth SA session ($P < 0.01$) and then maintained throughout the 20-d LgA training ($P < 0.01$; Fig. S3).

A repeated measures ANOVA for the S- sessions with DAY (last day of initial training phase and 8 d of S- sessions during the LgA phase) and REWARD (cocaine vs. sucrose) revealed a significant effect of REWARD ($F_{(1,24)} = 11.06$; $P < 0.01$), but no DAY x REWARD interaction ($F_{(8,192)} = 0.56$; NS), demonstrating that both cocaine- and sucrose-SA animals maintained the odor discrimination across the long-access (LgA) phase. Moreover, the escalation in responding during S+ sessions did not reflect a general increase in operant responding.

Odor Information Processing. To demonstrate that rats were indeed able to sense and process odors in this anesthetized preparation, we first analyzed the cerebal blood volume response in all three groups in a single ANOVA model. This 3 (groups) × 2 (scents) ANOVA revealed a significant main effect of scent in the olfactory bulb, with greater functional MRI (fMRI) response to the lemon vs. vanilla odor. In addition, a group × scent interaction was found in the insula (Fig. S4*A*), showing a different pattern of response in SA groups compared with naïve animals (Fig. S4*B*). To account for potential neuronal responding associated with olfactory stimuli novelty and training procedures (the housing control group had never experienced any odors before imaging), we compared the response from both self-administering groups separately for S+ (lemon vs. vanilla) and S− (lemon vs. vanilla), independent of their associative properties, and compared that in naïve animals (lemon vs. vanilla). Individual two-sample *t* tests showed similar patterns of scent effects in the olfactory bulb in both SA groups (with a larger response to the lemon compared with the vanilla odor) regardless of learning experiences (S+ or S−; Fig. S5). This response pattern was similar in the naïve control group, suggesting minimal effect of prior odor exposure on neuronal responding.

1. Schiffman SS (1974) Contributions to the physicochemical dimensions of odor: A psychophysical approach. *Ann N Y Acad Sci* 237(0):164–183.
2. Jehl C, Royet JP, Holley A (1995) Odor discrimination and recognition memory as a function of familiarization. *Percept Psychophys* 57(7):1002–1011.
3. Rabin MD (1988) Experience facilitates olfactory quality discrimination. *Percept Psychophys* 44(6):532–540.

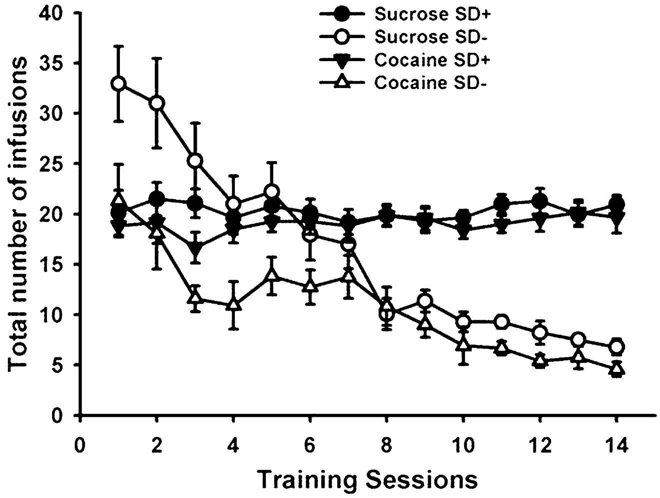

Fig. S1. Number of presses during S+ and S− training sessions in cocaine and sucrose SA groups. The total number of reinforced responses for the S+ and unreinforced S− cue is plotted as a function of training day.

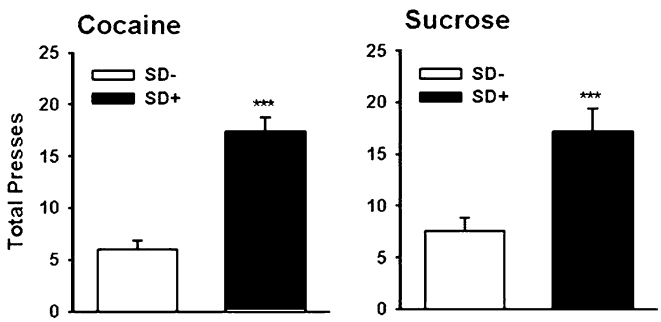

Fig. S2. Average number of presses during the odor discrimination test sessions by the cocaine (*Left*) and sucrose (*Right*) SA groups. In each graph, average number of total presses made by each group is plotted for the S− and S+ sessions. ***Significant difference between S+ and S− sessions.

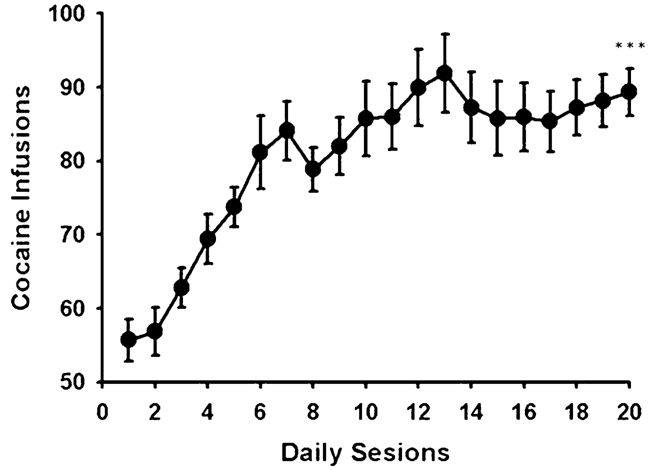

Fig. S3. Escalation of cocaine intake during the LgA phase. Average number of cocaine infusions earned during the 20 LgA cocaine SA sessions is plotted as a function of LgA session. ***Significant difference between day 3 and days 4–14.

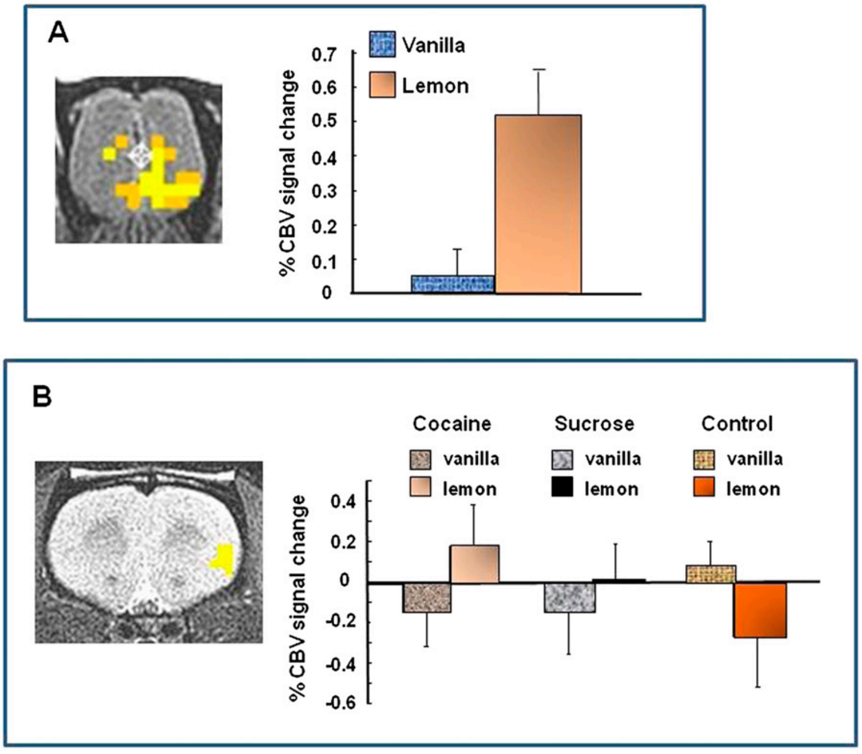

Fig. S4. Brain responses to odor stimuli. Statistical maps and fMRI signal changes in (*A*) olfactory bulb (bregma +7.64; 42 voxels) and (*B*) insula associated with lemon or vanilla scent presentation. Images in *A* and *B* represent percent regional cerebral blood volume (rCBV) signal changes during lemon or vanilla scent presentation compared with the air epochs plotted onto anatomical images. Data were analyzed using a 3 (groups: cocaine, sucrose, and control) × 2 (scent: lemon and vanilla) ANOVA. Main effect of scent was observed in olfactory bulb (*A*). Group × scent interaction was seen in insula (12 voxels; bregma +1.64) (*B*). Both SA groups showed a similar pattern of responding to lemon and vanilla odors, which was different from the control group.

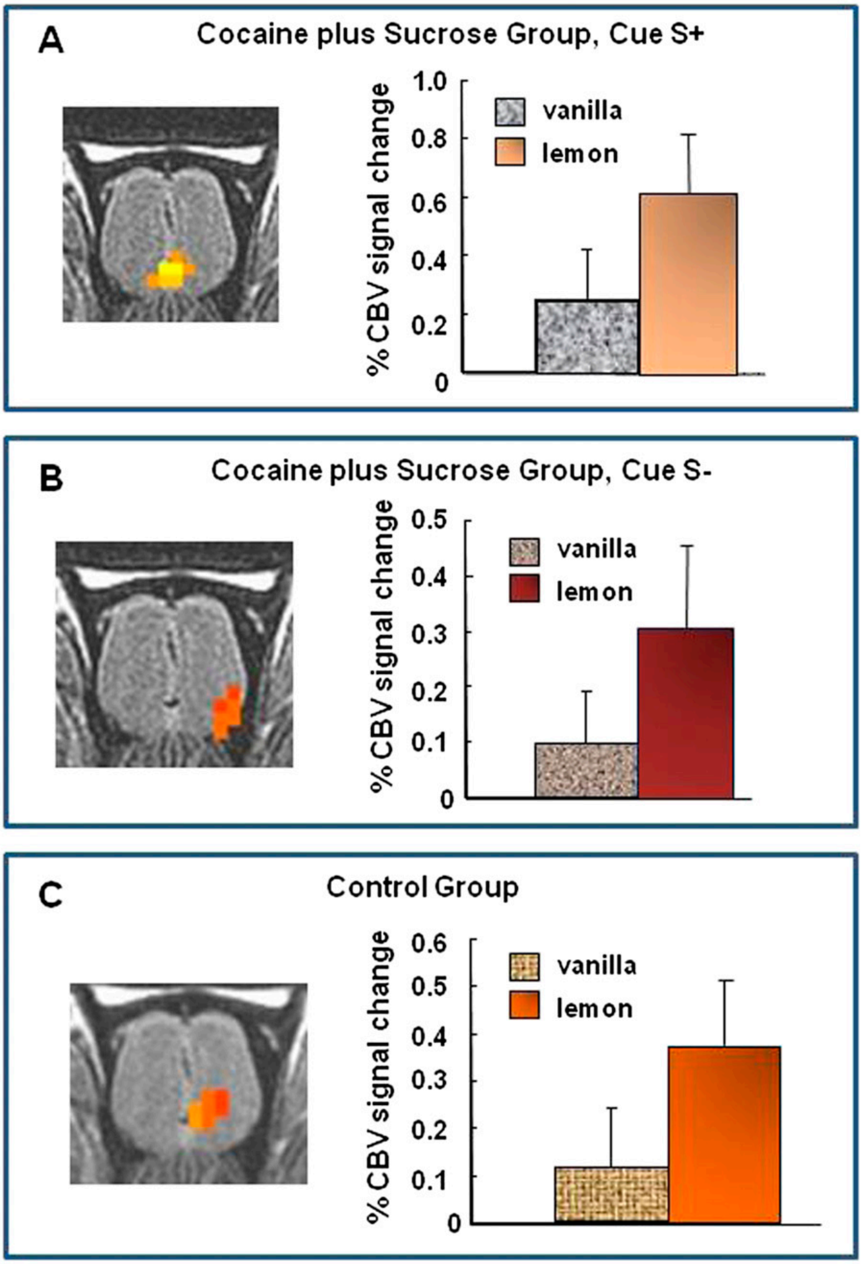

Fig. S5. Odor discrimination in olfactory bulb as a function of learning. Statistical maps of scent effect and fMRI signal changes in SA-trained animals under (*A*) S+ (vanilla vs. lemon; 8 voxels, Bregma +8.64∼+9.64 mm) and (*B*) S− (vanilla vs. lemon) conditions (8 voxels, Bregma +8.64∼+9.64 mm), and (*C*) naïve animals (vanilla vs. lemon; 11 voxels, Bregma +8.64∼+9.64 mm).

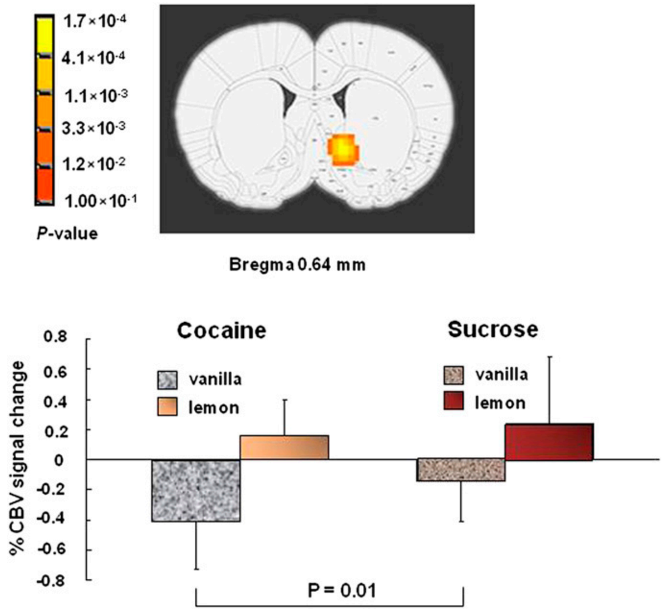

Fig. S6. Percent fMRI signal change extracted from the nucleus accumbens (NAc) learning effect in each of the four subgroups of animals.

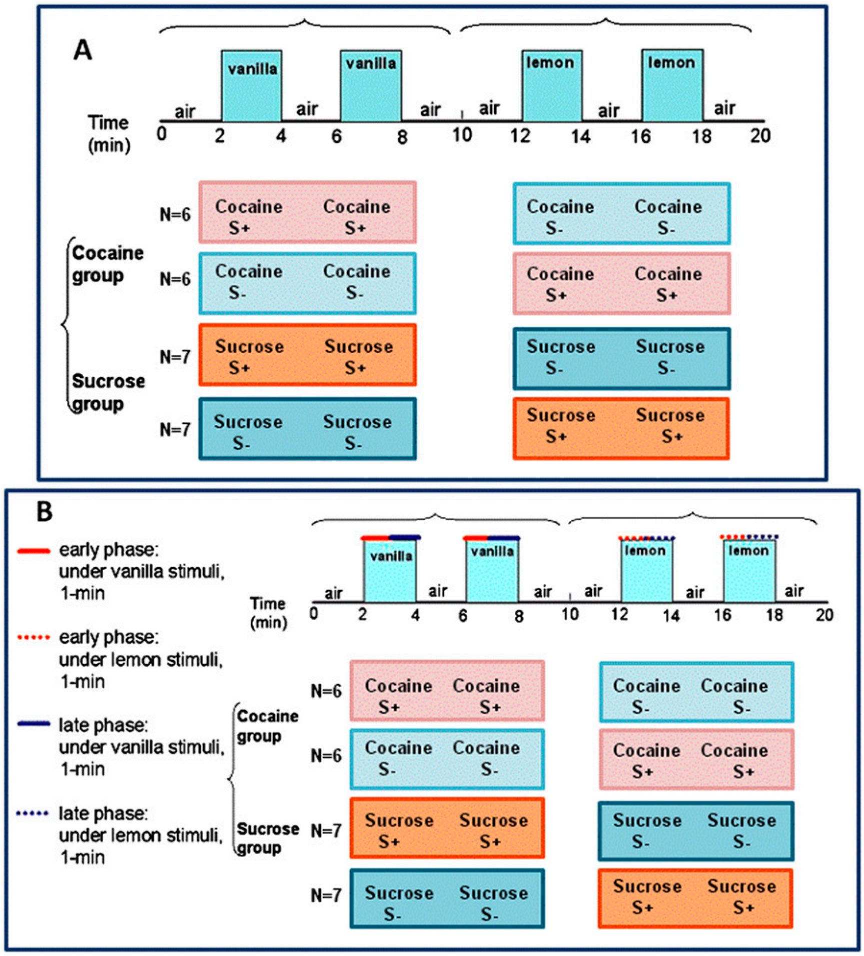

Fig. S7. Experimental design for the fMRI study with olfactory cues presentation in rats with a history of cocaine or sucrose SA and data analysis schema. (*A*) Overall 2-min epoch analysis. Linear mixed-modeling (LME) with effects of learning (S+ vs. S−) and group (cocaine vs. sucrose) was performed for the entire 2-min olfactory stimuli presentation period. (*B*) Data analysis schema dividing the 2-min odor presentation period into early (first minute) and late (second minute) phases. Early or late phase under the same repeated odorant stimuli (vanilla or lemon) was averaged for each animal. LME modeling with effects of learning (S+ vs. S−) and group (cocaine vs. sucrose) was performed separately for early and late phases. S+, olfactory stimuli associated with cocaine or sucrose SA; S−, olfactory stimuli used in discrimination testing during the no-reward condition (see *Experimental Paradigm* for details).

Table S1. Spatiotemporal cue responding

LME analysis	Brain region	rCBV signal change (%)			
Early phase: LEARNING effect		CS+		CS−	
	Insula	−0.62 ± 0.67		0.49 ± 0.67	
	NAc	−0.36 ± 0.25		0.21 ± 0.22	
	Dorsolateral striatum	−0.30 ± 0.16		0.01 ± 0.27	
Early phase: GROUP effect		Cocaine		Sucrose	
	NAc	−0.36 ± 0.54		0.01 ± 0.58	
Early phase: LEARNING × GROUP interaction		Cocaine CS+	Cocaine CS−	Sucrose CS+	Sucrose CS−
	Dorsomedial striatum	−0.26 ± 0.26	0.15 ± 0.32	0.14 ± 0.22	−0.06 ± 0.24
	Central striatum	−0.26 ± 0.21	0.06 ±0.23	0.08 ± 0.30	−0.03 ± 0.25
Late phase: GROUP effect		Cocaine		Sucrose	
	mPFC	−0.30 ± 0.31		0.1 ± 0.30	
	Insula	−0.26 ± 0.24		0.1 ± 0.31	
	Dorsolateral striatum	−0.20 ± 0.21		0.04 ± 0.26	
	Amygdala	0.47 ± 0.31		−0.23 ± 0.54	

LME, linear mixed effects modeling; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; rCBV, regional cerebral blood volume.