ARCHIVAL REPORT

Neural Correlates of the Formation and Retention of Cocaine-Induced Stimulus–Reward Associations

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Background: Cocaine can elicit drug-seeking behavior for drug-predicting stimuli, even after a single stimulus-cocaine pairing. Although orbitofrontal cortex is thought to be important during encoding and maintenance of stimulus–reward value, we still lack a comprehensive model of the neural circuitry underlying this cognitive process.

Methods: We studied the conditioned effects of cocaine with monkey functional magnetic resonance imaging and classical conditioning by pairing a visual shape (conditioning stimulus [CS+]) with a noncontingent cocaine infusion; a control stimulus was never paired. We correlated the behavioral preference of the monkey for the CS+, as measured offline, with the activity induced by the CS+ relative to the control stimulus as function of time.

Results: We observed that during formation of stimulus-cocaine associations strong CS+-induced functional magnetic resonance imaging activations emerged in frontal cortex that correlated significantly with behavioral CS+ preference. Afterward, CS+ preference correlated only with activity in early visual cortex. Control experiments suggest that these findings cannot be explained by increased familiarity for the CS+.

Conclusions: Our findings suggest a complex interaction between frontal and occipital cortex during cocaine conditioning. Frontal cortex is important for establishing novel representations of stimulus valence when cocaine is used as reinforcer, whereas early visual cortex is involved in retaining these cocaine-stimulus associations.

Key Words: Cocaine, conditioning, fMRI, macaque, orbitofrontal, reward

The potent effects of drugs of abuse, such as cocaine, are underscored by studies showing that stimuli paired only once with a single cocaine administration can acquire sufficient valence to elicit cocaine-seeking behavior during subsequent nonrewarded stimulus presentations (1). The “incentive salience” theory of addiction (2,3) proposed that, after repeated pairings of a neutral stimulus with an addictive substance, these stimuli acquire incentive motivational properties through a Pavlovian learning process. Most animal studies that investigated how drug-associated cues become endowed with incentive motivational properties have relied on self-administration paradigms (4–8). However, in rat models, Uslaner *et al.* (9) showed that the pairing of a neutral cue with noncontingent cocaine infusions also resulted in approach behavior to subsequent cue presentations, a phenomenon called sign-tracking (10,11). Although the important role of Pavlovian processes in the development of drug seeking and addiction have been recognized (2,9,12–14), the underlying neural circuitry and the time course of the conditioned stimulus effects of cocaine in Pavlovian conditioning settings is unclear, especially for primates.

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We proposed to investigate the neural correlates of this form of learning in macaque monkeys, with functional magnetic resonance imaging (fMRI).

Functional MRI has been extensively used to study human cognition, but ethical considerations have prevented studying the formation of cue-cocaine associations in normal human subjects with no prior history of cocaine use. Although some studies combined cocaine administration and fMRI in humans (15,16), all of them relied on individuals previously using cocaine. Because nonhuman primates are genetically and neuroanatomically closer to humans than any other animal model, they offer a unique opportunity to study reinforcing effects of cocaine, certainly in combination with functional imaging approaches. More specifically, we intended to identify brain regions involved in the formation as well as the retention of cue-cocaine associations.

Methods and Materials

Ethical Considerations

All procedures were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care (Protocol 2003N000338) and the ethical committee of the KU Leuven Medical School and are in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals and European guidelines.

Subjects

Four male (M1, M2, M9, M22) rhesus monkeys (4–6 kg; 3–7 years of age) participated in the experiments. The details of the surgical procedures, training of monkeys, visual stimuli, image acquisition, eye monitoring, and statistical analysis of scan data have been described previously (17,18) and are available in detail in Supplement 1.

Experimental Design

Stimuli were presented in three different types of runs: 1) “fMRI test runs,” displaying both a green control stimulus (CoS) and a red

conditioned stimulus (CS+) in alternating blocks, were designed to probe changes in fMRI brain responses to the observation of the CS+ and CoS during the conditioning process; 2) "conditioning runs with cocaine" consisted of the presentation of CS+ together with a bolus infusion of cocaine (.1 mg/kg); and 3) "free-viewing runs" assessed the behavioral preference of the monkey for the CS+ or CoS by displaying both stimuli simultaneously at different positions on the screen. The monkeys were rewarded with small drops of juice for fixating within a $2 \times 2^\circ$ window during the fMRI test runs and the conditioning runs, exactly as described for the training phase (Supplement 1). During the free-viewing runs, the monkeys received juice rewards for keeping their gaze within the borders of the screen. Juice rewards were delivered at the same rate as during the two other types of runs.

fMRI Test Runs

These fMRI test runs were always carried out inside the scanner and consisted of a central presentation of alternating sequences of 20-sec blocks of: 1) the CoS, 2) the CS+, and 3) fixation point only (Figure 1A). These runs were performed to assess possible changes occurring over time in the neuronal responses throughout the

brain to the presentation of the CS+ or the CoS before, during, and after conditioning the CS+ with cocaine. Total duration of such runs was 8 min. Within a CS+ or CoS block, stimuli randomly alternated among three sizes and positions each second. These test runs were presented during baseline scan sessions, before the conditioning of the red visual shape (CS+) with cocaine (unconditioned stimulus [UCS]) was initiated, as well as at several timepoints during and after the conditioning process. In total, seven scan sessions with these fMRI test runs were performed in both monkeys M1 (total of 18,880 volumes) and M2 (total of 18,240 volumes). For monkey M1, the first five of these seven timepoints correspond to fMRI scan sessions performed after exposure to 0, 21, 51, 63, and 66 cocaine conditioning runs, respectively. No additional conditioning runs were performed for timepoints 6 and 7, because CS+ preference had already reached asymptote. For monkey M2, the first six timepoints correspond to fMRI scan sessions performed after 0, 13, 38, 122, 135, and 146 cocaine conditioning runs, respectively. As for monkey M1, no additional conditioning runs were performed for timepoint 7, because CS+ preference showed no additional increase. Importantly, cocaine was never administered during the fMRI test scans.

Conditioning Runs with Cocaine Reinforcer

The second type of run was the conditioning run, during which the CS+ was paired with cocaine. Such runs took place either inside the scanner or in the fMRI training setup. Initially, the first conditioning sessions of monkeys M1 and M2 consisted of runs including a 5-min baseline of alternating 20-sec blocks of fixation point only and fixation point with the CS+, followed by an IV bolus infusion of .1 mg/kg cocaine solution and an additional 25-min postbolus period during which the alternating fixation-only and CS+ stimulus presentation (20-sec blocks each) was continued. To create a possibly stronger association between the CS+ and the cocaine reward, we slightly modified the conditioning runs in the remainder of the experiments by presenting a 5-min period of fixation point only (hence without the CS+), followed by the IV bolus infusion of .1 mg/kg cocaine solution at the start of a 20-min period of alternating 20-sec blocks of CS+ presentation and fixation only blocks, finally ending with a 5-min period of fixation only (Figure 1B). Total duration of the conditioning runs was 30 min. The cocaine was injected with an automated pump (Harvard Apparatus, Holliston, Massachusetts) through a chronic catheter implanted in the internal jugular vein and accessible through a port attached to the dental acrylic surrounding the MR-compatible head post. We stopped conditioning when behavioral preference for the CS+ reached asymptote. In total, 15 conditioning sessions (6 inside and 9 outside the scanner) were performed with monkey M1, consisting of 66 conditioning runs. Monkey M2 was exposed to 38 conditioning sessions (16 inside and 22 outside the scanner), with a total of 146 runs.

Free-Viewing Runs

To test the behavioral CS+ preference of the monkeys, we applied a free-viewing paradigm after each cocaine conditioning session (inside or outside scanner) and fMRI test session (inside the scanner) during which the CS+ and CoS were simultaneously presented on the screen (Figure 1C). Every 30 sec, CS+ and CoS switched positions relative to each other, a sequence that continued for 5 min. The monkey was rewarded with juice drops for keeping his gaze within the boundaries of the screen. The percent time that the monkey fixated within a $3 \times 9^\circ$ rectangular window around the CS+ or CoS was then calculated (by averaging the data of two separate free-viewing runs collected within the same session). The ratio of the percent times spent viewing the CS+ and CoS

Figure 1. Experimental design. (A) During functional magnetic resonance imaging test runs, monkeys were rewarded with juice for maintaining fixation ($2 \times 2^\circ$ virtual window) upon a white target while an alternating sequence of conditioning stimulus (CS+) (red stimulus) and control stimulus (green stimulus) was presented in the background. Cocaine was never administered in these sessions. (B) During cocaine conditioning runs, fixating monkeys received a bolus infusion of .1 mg/kg of cocaine (unconditioned stimulus) after 5-min fixation-only baseline, followed by a 25-min postinjection period consisting of a 20-min period of alternating CS+ and fixation-only presentation and another 5-min fixation-only period. Monkeys were rewarded with juice for maintaining fixation ($2 \times 2^\circ$ virtual window) upon the white target. (C) During free-viewing runs, behavioral stimulus preference was tested by simultaneously presenting the CS+ and control stimulus on the left or right side of the screen. Monkeys were rewarded with juice for keeping their gaze within the boundaries of the screen.

$(([\%CS+] - \%CoS)/([\%CS+] + \%CoS))$ indicated the degree of preference for the CS+.

Control Experiment

To control for a potential familiarity confound for the CS+ over the CoS, due to the conditioning procedure, we performed a control experiment in two additional animals. Monkeys M9 and M22 were exposed to the same type of conditioning runs as the cocaine monkeys, but cocaine was never administered in these runs. The rationale was that, if possible changes in both behavioral preference and brain response in the cocaine-exposed subjects were due to a mere familiarity bias for the CS+ and not the actual association of the CS+ with the cocaine reinforcer, similar changes should be witnessed in the cocaine-naïve control animals. However, if behavioral preference and functional changes were related to the formation of the stimulus-cocaine association, a mere bias in exposure to the CS+ relative to the CoS, without cocaine administration, would not result in similar behavioral and functional changes. Details of the control experiment can be found in Supplement 1.

Results

With a classical conditioning design, we paired a previously neutral visual stimulus (red abstract line drawing, CS+) with a strong reinforcer (.1 mg/kg IV nonresponse contingent bolus infusion of cocaine hydrochloride [Sigma-Aldrich, St. Louis, Missouri] solution, constituting the UCS). A green abstract line drawing (CoS) served as a visual control and was never presented together with the reinforcer (Figure 1A). Functional MRI sessions (i.e., fMRI test sessions) without cocaine infusions (Figure 1A) were interspersed with conditioning sessions with cocaine (Figure 1B). Differences between fMRI activity evoked by CS+ and CoS in the absence of reinforcer were measured at different stages during the conditioning process. To assess the behavioral CS+ preference of the monkey in a task-unconstrained manner, we applied a free-choice gaze preference paradigm after each conditioning and test session, during which the CS+ and CoS were simultaneously presented on the screen (Figure 1C). More precisely, the ratio of the percent viewing times for CS+ and CoS indicated the relative preference of the monkey for the CS+ and was a measure of the change in "incentive salience" acquired by the CS+ during the conditioning process (Figures 2A and 2B).

During the conditioning process, both monkeys altered their offline gaze preferences significantly over time, developing strong preferences for the CS+ during the free-viewing task (one-way analysis of variance, monkey M1: $F = 36.19$, $p = 1.83 \times 10^{-6}$; monkey M2: $F = 37.05$, $p = 1.64 \times 10^{-6}$). Although M2 never reached a 100% preference for the CS+, as monkey M1 did, the reversal in behavioral preference is remarkable, because monkey M2 had a strong initial bias for the CoS (Figure 2B). To exclude the possibility that these observed changes in the CS+ preferences of monkeys M1 and M2 might be the result of different familiarities with the CS+ and CoS (due to the conditioning procedure; see Methods and Materials) and were unrelated to the formation of the CS+/UCS association, we exposed two additional animals (M9, M22) to a large number of identical condition runs (96 and 75, respectively) but without cocaine delivery. Although this also created a similar familiarity bias for the CS+ over the CoS, as was the case for the cocaine animals, no significant changes in preference (one-way analysis of variance, monkey M9: $F = .80$, $p = .64$; monkey M22: $F = 1.42$, $p = .29$) for the CS+ developed over time in either of these two control subjects (Figures 2C and 2D). Hence, these results argue against a familiarity bias explaining the development of the strong CS+ preference in the cocaine animals.

Figure 2. The effect of nonresponse-contingent cocaine exposure on behavioral preference. (A,B) Individual monkey gaze preferences as a function of total amount of cocaine received during cocaine conditioning sessions, for monkey M1 (A) and M2 (B). Solid circles indicate free-viewing data obtained during seven different cocaine-free functional magnetic resonance imaging test sessions in the scanner. Open circles correspond to free-viewing data obtained during offline cocaine conditioning sessions. Error bars indicate variability over runs. (C,D) Individual monkey gaze preferences as a function of exposure to control conditioning runs (as in Figure 1B) for monkeys M9 (C) and M22 (D). No cocaine was administered during these conditioning runs. Solid circles indicate free-viewing data obtained during four different cocaine-free functional magnetic resonance imaging test sessions (as in Figure 1A) in the scanner. Open circles correspond to free-viewing data obtained during offline, cocaine-free control conditioning sessions. CS+, conditioned stimulus; CoS, control stimulus.

Figure 3. Functional magnetic resonance imaging response before, during, and after conditioning process. (A–F) Right-hemisphere flatmaps (T-score maps, $p < .05$, corrected) displaying differential brain activity (conditioned stimulus [CS+] vs. control stimulus [CoS]) pre-conditioning (timepoint 1; monkey M1: A; monkey M2: D), perconditioning (timepoint 4; monkey M1: B; monkey M2: E), and postconditioning (timepoint 7; monkey M1: C; monkey M2: F). Insets show coronal sections at the level of the ventrolateral prefrontal and orbitofrontal cortex (+25 mm relative to the interaural plane). White dashed lines indicate borders of early visual areas V1, V2, and V3. ar, arcuate sulcus; ci, cingulate sulcus; io, inferior occipital sulcus; ip, intraparietal sulcus; lf, lunate sulcus; p, principal sulcus; st, superior temporal sulcus.

Before cocaine conditioning, the red and green shapes evoked nearly identical fMRI activity throughout the cortex of monkeys M1 and M2 (Figures 3 A and 3D) (see also Figure S1 in Supplement 1 for the left hemisphere). During the initial phase of the conditioning process, both monkeys demonstrated significantly increased fMRI responses in ventral orbitofrontal (VOFC) and ventrolateral prefrontal (VLPFC) cortex (two-tailed t test between timepoint 1 and timepoint 4, monkey M1: VOFC: $t = 2.81$; $p = .031$; VLPFC: $t = 10.06$; $p = .0001$; monkey M2: VOFC: $t = 2.65$; $p = .016$; VLPFC: $t = 4.34$; $p = .0004$) with presentation of the CS+ (as compared with the CoS) as well as in early visual, temporal, and prefrontal regions (Figures 3B and 3E).

To investigate whether these positive changes in differential brain activity (CS+ vs. CoS) were due to a specific increase in activity for the CS+ during the cocaine conditioning process or alternatively could be attributed to a decrease in responses to the CoS, we mapped fMRI responses for both stimuli versus the fixation-only baseline (Figure S2 in Supplement 1). Red and green colors indicate fMRI responses ($p < .001$, uncorrected) for the CS+ and CoS, respectively (compared with fixation-only baseline). Yellow colors indicate regions responding to both CS+ and CoS (compared with fixation-only baseline). Before cocaine-conditioning onset, CS+ and CoS stimuli evoked significant responses (compared with fixation-only baseline) mainly in visual cortex and prefrontal area 45. This prefrontal activation fits with earlier reports of macaque prefrontal responses to two-dimensional shapes (18,19). In both cocaine animals, it was mainly the response to the CS+ (compared with fixation-only) that changed between preconditioning (Figure S2 in Supplement 1: A: monkey M1; C: monkey M2) and perconditioning (Figure S2 in Supplement 1: B: monkey M1; D: monkey M2) in frontal cortex. This suggests that these changes in activity are related to learning about the CS+/cocaine reward association during the conditioning process.

We correlated the progressive changes in the offline free-viewing behavior of the monkey with fMRI activity (CS+ vs. CoS) as a function of time. Figure 4 shows MR signal changes for the CS+ compared with the CoS for monkey M1 (Figure 4A) and M2 (Figure 4B) in VOFC, VLPFC, and early visual cortex (area V2) at all seven timepoints during the conditioning process for which fMRI data during fMRI test runs were collected.

During the initial phase of cocaine conditioning (Figures 4A and 4B) (timepoints 1 to 4), CS+-induced fMRI activity in VOFC and VLPFC correlated strongly with increased gaze preference for the CS+ re-

corded after each test session ($R^2 > .95$, $p < .05$; both monkeys and both regions). Functional MRI activity in frontal eye fields (FEF) mimicked that in VOFC and VLPFC, because it increased significantly during the initial conditioning phase (two-tailed t test between timepoint 1 and timepoint 4; monkey M1: $t = 10.3$; $p = .0001$; monkey M2: $t = 2.58$; $p = .018$). A significant correlation between free-viewing behavior and fMRI responses in FEF, however, was observed only in monkey M2 (M1: $R^2 = .74$, $p = .14$; M2: $R^2 = .97$, $p < .05$) (Figure S3 in Supplement 1).

Interestingly, after an initial peak in frontal cortex activity, fMRI responses to CS+ presentation as compared with CoS dropped significantly to and below baseline activity in monkeys M1 and M2 (two-tailed t test between timepoint 4 and timepoint 7; monkey M1: VOFC: $t = 3.39$; $p = .02$; VLPFC: $t = 8.48$; $p = .0004$; monkey M2: VOFC: $t = 3.94$; $p = .0007$; VLPFC: $t = 5.00$; $p = .0001$), respectively (Figure 4). In contrast, MR responses in visual cortex (area V2) did not drop significantly between perconditioning and postconditioning stages (two-tailed t test between timepoint 4 and timepoint 7; monkey M1: $t = .98$; $p = .37$; monkey M2: $t = 1.21$; $p = .24$) (Figures 4A and 4B). During these later stages in the conditioning process (Figures 4A,B) (timepoints 4–7), the preference of the monkeys for the CS+ correlated significantly with the fMRI activity only in early visual area V2 ($R^2 > .95$, $p < .05$ for both monkeys M1 and M2).

The fMRI data from the fMRI test runs obtained in both control monkeys suggest that the observed increases in MR activity in frontal cortex in our two cocaine animals cannot be explained by a mere familiarity bias for the CS+. Neither of the two control monkeys showed significant ($p < .05$, corrected) responses in frontal activity after exposure to 96 (monkey M9) and 75 (monkey M22) conditioning runs, respectively (Figures S4 and S5 in Supplement 1).

Finally, we also investigated fMRI responses in two subcortical structures, previously implicated in formation of stimulus–reward associations. During initial conditioning stages (between timepoints 1 and 4), MR activity in the nucleus accumbens (NAC) and the amygdala did not change significantly in monkeys M1 and M2 (two-tailed t test between timepoint 1 and timepoint 4; monkey M1: NAC: $t = 1.47$; $p = .19$; amygdala: $t = 1.66$; $p = .15$; monkey M2: NAC: $t = 1.27$; $p = .22$; amygdala: $t = 1.18$; $p = .25$) (Figure S6 in Supplement 1). Functional MRI activity in the NAC during the initial stages of conditioning did not correlate significantly with behavioral CS+ preference (M1: $R^2 = .01$, $p = .89$; M2: $R^2 = .61$, $p = .22$). During later stages, only NAC in monkey M1 showed a negative correlation (M1: $R^2 = .95$, $p < .05$; M2: $R^2 = .64$, $p = .20$). In the amygdala, fMRI

Figure 4. Magnetic resonance (MR) profiles for frontal and early visual regions. (A,B) Bar graph of percent change in functional MR imaging signal, in response to the presentation of conditioned stimulus (CS+) (vs. control stimulus [CoS]), in ventral orbitofrontal cortex (VOFC), ventrolateral prefrontal cortex (VLPFC), and visual area V2 at all seven timepoints along the conditioning process. Timepoints 1, 4, and 7 on the x-axis correspond respectively to flatmaps A, B, and C (monkey M1) and D, E, and F (monkey M2) shown in Figure 3 and Figure S1 in Supplement 1. *Significant correlation between MR response and behavioral CS+ preference over four timepoints. Error bars indicate variability over runs. (C,D) Normalized CS+ preference selectivity index $(([\%CS+] - \%CoS)/([\%CS+] + \%CoS))$ for monkey M1 (C) and monkey M2 (D) for the seven timepoints displayed in A and B.

activity and CS+ preference yielded a significant negative correlation during initial conditioning stages only in monkey M1 ($M1: R^2 = .94, p < .05$; $M2: R^2 = .53, p = .27$), whereas in later stages no significant correlation between functional MR activity and behavior was found in either animal ($M1: R^2 = .89, p = .06$; $M2: R^2 = .08, p = .71$).

These findings suggest that the VOFC and VLPFC play an important role in the initial stimulus–reward association between CS+ and UCS and that, thereafter, this signal is retained in visual cortex. The increased activity observed in visual cortex is possibly a combination of valence and attention-related modulation of activity. However, that the increased activity is restricted to the representation of the stimulus in early visual cortex and that structures typically showing increased activity under selective attention conditions (such as lateral intraparietal area and FEF) did in fact show activation profiles very different from area V2 argues against a purely arousal or attention effect (Figures S3A and S3B in Supplement 1). Importantly, no significant differences in fixation behavior (% fixation, number of saccades, SD of eye position in horizontal or vertical direction) were observed between the CS+ and CoS epochs (Table S1 in Supplement 1).

Discussion

Our data suggest that after initial valence encoding (“formation”) in prefrontal cortex, visual cortex plays a role in retaining stimulus–cocaine associations. Although we were unable to detect fMRI response profiles similar to those of area V2 in any other area, this does not exclude the possibility that other brain regions might be involved in the retention of these formed associations.

It has been suggested that excessive attribution of incentive salience to drug-associated cues contributes to the development of compulsive behavioral disorders and attracts humans to drug-associated stimuli (11,13,20). Therefore, understanding of the mechanisms leading to addiction (21) and relapse also requires understanding the Pavlovian learning processes that initiate incentive motivational properties or “incentive salience” upon previously neutral drug-associated stimuli (2,3,9).

Several studies have suggested that the orbitofrontal cortex (OFC) plays a role in mediating the ability of drug-associated cues to motivate drug-seeking behavior. For instance, an intact OFC is required for cocaine-associated stimuli to act as conditioned reinforcers (22,23) and for Pavlovian conditioned cues to guide instrumental responses (24). More generally, previous research has shown that the OFC plays a major role in learning and reversing the associations between a cue and a primary reinforcer (25–27). Rodents and macaques with OFC lesions are impaired when they have to learn which stimuli are rewarding or when they have to alter their behavior due to a change in stimulus–reward contingencies (28–32).

The proposed role for OFC in signaling the current value of certain visual stimuli (33,34) fits with our data obtained with cocaine conditioning. We observed a strong increase in activity in OFC and VLPFC after a previously neutral visual stimulus (CS+) was conditioned with cocaine rewards. In addition, such changes in the fMRI activity of the frontal lobe along the course of the conditioning process correlated significantly with changes in behavioral CS+ preference.

The robust changes in the preference of both cocaine monkeys for the CS+ after conditioning certainly fit with the idea that drug-associated stimuli serve as “motivational magnets” for the individual (2). Just as importantly, our data show that even the noncontingent pairing of a visual stimulus with a cocaine reward yields strong OFC activations upon subsequent CS+ presentation and leads to robust drug-associated stimulus preference (but see Kearns and Weiss [35]).

Although some rodent models of associative learning suggest a role for OFC in the maintenance of the learned link between a predictive cue and outcome value (36,37), an important function of the OFC is to generate outcome expectancies on the basis of a prediction error signal, which is used to update stimulus–reward associations. Alternatively, it has been suggested that structures outside the OFC might be involved in the actual storage of the acquired stimulus–value associations (38). In this study, we observed that after the initial peak in frontal cortex fMRI activity during conditioning, CS+-induced responses returned back to baseline, although the stimulus preference of the monkey for the CS+ remained high (normalized selectivity approximately .8 in both animals). This suggests that, once the initial stimulus–reward association has been formed, OFC activity might no longer be required to maintain predictions for positively valenced stimuli.

After the initial increased activity in frontal, visual, and temporal areas during the course of the conditioning process, fMRI activity in OFC and VLPFC fell back to or below baseline. Interestingly, at these stages—well beyond the initial emergence of the behavioral CS+

preference—fMRI activity in early visual cortex remained elevated in both monkeys above preconditioning baseline and correlated significantly with the gaze preference of the monkeys for the CS+.

Our data are complementary to a growing body of evidence, with operant as opposed to classical conditioning experiments, that the learned value of a stimulus can influence the magnitude of cortical responses in early sensory areas. For instance, Pleger *et al.* (39) showed that reward can have an influence on stimulus processing in early somatosensory cortex and can even lead to increased somatosensory performance. Shuler and Bear (40) showed that some V1 neurons in rats learning novel stimulus–reward associations can accurately predict the timing of an upcoming reward. Furthermore, reward value can also influence activation levels within early regions of human visual cortex (41–43). The latter studies showed that fMRI activity in early visual cortex is biased in favor of more valuable stimuli. We used a classical rather than an operant conditioning approach, contrary to the aforementioned experiments. Hence, the learned value of a stimulus can bias purely sensory mechanisms that are not involved in planning and execution of actions or in decision making. Neuromodulators such as dopamine (44–46) might play an important role in establishing this link between reward information processed in higher cortical areas and early sensory cortex (47).

Although reinforcer devaluation studies have shown the involvement of human and monkey amygdala in encoding reward value (48,49) and that damage to the amygdala can interfere with conditioned reinforcement learning (50), some evidence argues against a general role played by the amygdala in cue–reward conditioning (51–54). However, it is clear that, in contrast to the amygdala, the OFC displays a much stronger predictive reward signal when the monkey started showing a clear preference for the reward-associated cue—as demonstrated during overt choice behavior (free-viewing task).

Research in rodents pointed to an important role by NAc in the formation of classical stimulus–reward associations (55,56). We did not find consistent increases in MR activity during the conditioning when the CS+ acquired a greater reward value. As shown by Roitman *et al.* (57), the cue–association responses in the NAc of the rat appear quickly, during the first day of the pairing of the CS+ and the UCS; hence, we might have missed observing this fast transient response with the fMRI method. Another possible explanation for this apparent discrepancy is a possible functional shift during cue–reward learning from subcortical regions in rodents to the much larger prefrontal cortex in primates (58).

In conclusion, our data suggest a complex interaction between frontal and occipital cortex during Pavlovian conditioning for which OFC and VLPFC are important for establishing novel representations of stimulus valence with cocaine as the reinforcer, whereas early visual cortex is unexpectedly involved in retaining these associations. Control experiments in the absence of a cocaine reinforcer suggest that these findings cannot be explained by mere increased familiarity for the CS+. Such changes in the representation of cocaine-associated stimuli suggest a more sophisticated function for early visual cortex that goes beyond the processing of simple visual features for perceptual purposes. Future studies should investigate the neurotransmitter and receptors causing these cue-induced changes in fMRI responses and behavior.

Our findings demonstrate that the combination of animal models and fMRI allows preclinical investigations of relevant drug-abuse conditions that are not feasible in humans.

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