Glutamatergic Biomarkers for Cocaine Addiction: A Longitudinal Study Using MR Spectroscopy and mGluR5 PET in Self-Administering Rats

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Cocaine addiction is a disorder that still lacks diagnostic biomarkers or effective pharmacotherapy. We present findings on a rat model of cocaine self-administration that was followed up longitudinally using the metabotropic glutamate receptor type 5 (mGluR5) tracer ^{18}F-3-fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile (^{18}F-FPEB) PET, proton MR spectroscopy (^{1}H-MRS), and behavioral tests. **Methods:** Forty-two Wistar rats were scanned with ^{18}F-FPEB PET and ^{1}H-MRS before and after sucrose or intravenous cocaine self-administration, during withdrawal, and during relapse. All animals performed a rodent Iowa Gambling Task (rIGT) at baseline to evaluate decision making. Baseline values were used in a mixed model to assess associations with later cocaine use, and follow-up measurements were compared with the values before drug exposure. **Results:** Preexposure rIGT scores were significantly related to both cocaine and sucrose use during the drug-exposure phase. However, only cocaine self-administration induced a decrease in ^{18}F-FPEB binding. This decrease was most pronounced bilaterally in the hippocampus, where mGluR5 availability correlated with the amount of cocaine used during relapse. Compared with the sucrose group, a larger decrease was observed in the hippocampo–prefrontal cortex pathway. Preexposure glutamate and glycine concentrations in the prefrontal cortex were significantly associated with cocaine use during the drug-exposure phase. Moreover, prefrontal glutamate exhibited a distinct, reversible decrease when animals had access to cocaine but not sucrose. **Conclusion:** Baseline values of prefrontal glutamate and glycine are associated with future cocaine use. Furthermore, baseline rIGT scores are associated with both sucrose and cocaine. Finally, both glutamate concentration and mGluR5 availability decrease during exposure to cocaine.

Key Words: cocaine; ^{18}F-FPEB; ^{1}H-MRS; rodent Iowa gambling task; prefrontal cortex

To date, therapeutic options for drug-use disorders remain limited despite intensive research to unravel their complex neurobiology (*1*). This complexity is partly due to the dynamic changes during different phases of these disorders. For example, neurologic control mechanisms that modulate drug-induced release of dopamine become progressively more prominent when the brain is repeatedly exposed. The increased role of these inhibitory mechanisms can ultimately result in phenotypical traits associated with cocaine abuse, such as poor decision making and impulsivity. For example, cocaine users are known to perform worse on the Iowa Gambling Task, a widely used measure of decision making (*2*). These controlling mechanisms are thought to be in place mainly as a result of the glutamatergic system, with the prefrontal cortex as its predominant enforcer (*3*). In this context, the extrasynaptic metabotropic glutamate receptors have gained considerable interest (*4*). These receptors, in particular metabotropic glutamate receptor types 2 and 5 (mGluR5), have a modulatory role that is disturbed in several psychiatric disorders, including addiction (*5*). In cocaine addiction, mGluR5 knockout mice have been used to show that the absence of this receptor influences conditioned locomotor activity and the signaling cascade involved in the propensity to self-administer (*6,7*).

Several drugs have been designed to modulate the glutamatergic system, aiming at an antiaddictive effect (*8*). However, most were plagued by toxicity and side effects or lacked convincing results in clinical trials (*9*). It has been hypothesized that the disappointing results are due to a lack of insight into the dynamics of addiction and to the absence of suitable biomarkers to guide pharmacopsychologic therapy (*10*). Because of these issues, there has risen a demand to elucidate the role of the glutamatergic system during different phases of addiction, such as drug exposure, withdrawal, and relapse (*11*).

In this context, we conducted a longitudinal experiment on a cocaine self-administration rat model. This protocol commenced before the exposure to cocaine and followed each animal through its initial drug exposure, but also through withdrawal and relapse. In every phase, animals were characterized using ^{18}F-3-fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile (^{18}F-FPEB) PET for mGluR5 imaging, as well as noninvasive proton MR spectroscopy (^{1}H-MRS) for glutamate and glycine concentration measurement. Additionally,

at baseline a behavioral test for decision making, namely the rodent Iowa Gambling Task (rIGT; Med Associates), was performed. This information was used to study whether there are baseline markers associated with future drug use. We also assessed where and how these potential biomarkers changed during the different phases of the addictive process.

MATERIALS AND METHODS

Animals

Cocaine-exposure experiments were performed in duplicate, within a total of 36 animals originating from these 2 identical and independent experiments 8 wk apart. In both experiments, 18 adult (110–115 d old) male Wistar rats (Janvier Laboratories) weighing on average (±SD) 500 ± 40 g were used. Additionally, a matched control group was used to control for cocaine-independent effects. Because sucrose induces homogeneous self-administration behavior, we anticipated a low level of variance in the control group. Therefore, a smaller number of animals, that is, 6, were included in this group. On arrival at our facility, all rats were housed individually under an inversed 12-h light–dark cycle. With free access to water and 20 ± 1 g of food (Ssniff) per day, a stable body weight was maintained. At least 7 d before the self-administration sessions, the rats had a polyurethane 22-gauge catheter (Instech Laboratories) inserted through the femoral vein in the cranial direction for 7 cm. A vascular access button was mounted between the shoulder blades using a polyethylene terephthalate mesh. The research protocol was approved by the Animal Ethics Committees of the University of Leuven (P156/2013) and was performed in accordance with the guidelines of the European Ethics Committee (decree 86/609/EEC).

Self-Administration

Nine experimental test chambers (Med Associates) equipped with a solid floor and 2 levers were used for self-administration experiments. The rats were trained to use the levers with sucrose pellets (45 mg, TestDiet) as a reward in 10 training sessions until 100 responses were achieved within 1 h. After the baseline week, the rats could self-administer cocaine hydrochloride (0.3 mg/kg/infusion) by pressing the active lever 1 time for 3 h per day for 14 consecutive days. Cocaine, dissolved in 0.9% NaCl, was administered intravenously via a pump infusing 0.05 mL/infusion over 2 s. Each administration resulted in a 15-s time-out to prevent lethal doses. This drug-exposure phase was followed by a forced withdrawal period and a subsequent regained access for 7 d, mimicking a relapse (Fig. 1). Catheter patency was tested using a 4 mg/kg dose of propofol (Propovet; Abott) before the first self-administration session and whenever animals showed a more than 20% decrease in lever presses within 3 d. Control animals followed the same experimental phases but continued to receive sucrose pellets as a reward.

rIGT

Decision making was assessed with the rIGT during the baseline phase and after catheter implantation. In this test, animals can poke their nose into any of 5 holes, each with its own reward–punishment profile (profile = chance of success, reward size [number of pellets], time-out duration [s]), as described by Zeeb et al. (12). More specifically, the largest reward size could be obtained by solely choosing the (profile = 0.8, 2, 10) response hole, whereas the riskier choice of the (profile = 0.4, 4, 40) option resulted in the smallest reward size. The outcome, decision-making score, was calculated according to the following formula:

$$Score = \frac{\#\ pellets\ rewarded}{\#\ nose\text{-}pokes} - 2,$$

with the "2" being the score of optimal decision making.
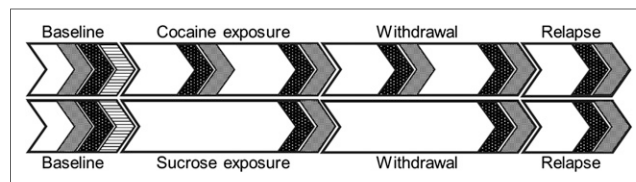
FIGURE 1. Schematic overview of experimental design for cocaine group (top) and control group (bottom). From left to right, each set of patterns represents rIGT, ^{18}F-FPEB PET, and ^{1}H-MRS.

Small-Animal PET Imaging

PET imaging was always performed at approximately the same time during the dark cycle on a lutetium oxyorthosilicate detector–based small-animal tomograph (Focus 220; Siemens Medical Solutions). During imaging, the rats were kept under 2.0% isoflurane anesthesia, and their body temperature was maintained with a heating pad. Scans were obtained at baseline ($n = 33$), during drug-exposure weeks 1 and 2 ($n = 24$ and 25, respectively), during withdrawal weeks 1 and 2 ($n = 15$ and $= 21$, respectively), and during relapse ($n = 22$), as a function of the available amount of radiotracer produced and its specific activity. (The control animals were scanned only at baseline, during drug-exposure week 2, during withdrawal week 2, and during relapse.) On average, 18.5 ± 3.6 MBq (specific activity range, 80–301 GBq/μmol; mass dose range, 1.03–5.64 μg) were injected into the tail vein using an infusion needle set. For each 60-min dynamic scan, 3 animals were placed in the PET scanner. They simultaneously received an ^{18}F-FPEB injection, upon which the emission scan and subsequent ^{57}Co-attenuation scan were started.

Nondisplaceable binding (BP_{ND}) parametric images were calculated, upon spatial normalization in the Paxinos stereotactic space, with the previously validated simplified reference tissue model, using the cerebellum as a reference region (13). A volume-of-interest–based analysis was performed to assess the value of baseline measurements. In contrast, changes in ^{18}F-FPEB BP_{ND} between different phases were assessed voxelwise with Statistical Parametric Mapping, version 12 (SPM) (14). For the SPM analysis, data were analyzed with a flexible factorial design (Tables 1 and 2) and a cluster extent threshold of 200. Significance thresholds were set at a familywise error–corrected P value of less than 0.00001 for the comparison between the cocaine and sucrose groups. For the longitudinal evaluation of the cocaine group, we applied a familywise error–corrected P value of less than 0.005 for phases with drug exposure, whereas an uncorrected P value of less than 0.0005 was used for both withdrawal weeks. In addition, a voxel-based correlation analysis between the median self-administration and ^{18}F-FPEB BP_{ND}, or prefrontal glutamate concentration, was performed.

^{1}H-MRS

^{1}H-MRS was performed on a subset of 18 randomly selected animals from the cocaine group, which were scanned each week, as well as all 6 animals in the sucrose group. All MR images and spectra were acquired with a 9.4 T Biospec small-animal MR scanner (Bruker Biospin) equipped with a horizontal-bore magnet and an actively shielded gradient set of 600 mT/m using a 7-cm linearly polarized resonator for transmitting and an actively decoupled dedicated rat surface coil for receiving (Rapid Biomedical). For the placement of ^{1}H-MRS voxels, 2-dimensional T2-weighted MR images were acquired. Spectroscopy voxels were manually placed over 2 a priori–selected regions: the left nucleus accumbens (2.5 × 2.5 × 2.5 mm) medioventral to the caudate putamen, and the prefrontal cortex (2 × 3 × 2.5 mm) caudad to the rhinal fissure and craniodorsal to the corpus callosum (Supplemental Fig. 1; supplemental materials are available at

TABLE 1
SPM Results for Clusters of Difference in mGluR5 Availability Between Cocaine and Sucrose Group

Parameter	Threshold (P)	Cluster level P_{Corr}	K_E	ΔS (%)	ΔC (%)	Voxel level T	P_{Corr}	Coordinate x	y	z	Anatomic structure
Baseline > DE2	0.00001 (FWE)	0.0001	1,631	3	−14	7.90	0.0001	2.1	−0.9	5.2	CPU L, NAC L, AI L, OC L
		0.0001	780	−0	−15	7.82	0.0001	−2.3	−0.8	5.0	CPU R, NAC R
		0.0001	397	−3	−16	6.85	0.0001	−5.5	−1.0	6.5	AI L, OC L
		0.0001	439	−2	−15	6.69	0.0001	−5.2	−6.8	6.7	Hippocampus R (CA1, S)
		0.0001	234	0	−13	6.34	0.0001	−5.2	−5.8	7.2	Hippocampus L (CA1, S)
Baseline > W2	0.00001 (FWE)	0.0001	502	0	−15	7.67	0.0001	−2.3	−0.8	5.0	CPU L, NAC L
		0.0001	701	5	−14	7.65	0.0001	2.1	−0.5	4.7	CPU R, NAC R
Baseline > relapse	0.00001 (FWE)	0.0001	2,478	−0	−24	9.67	0.0001	−2.3	−0.8	5.0	CPU L, NAC L, AI L, OC L
		0.0001	2,892	0	−13	9.34	0.0001	2.1	−0.7	5.1	CPU R, NAC R, AI R, OC R
		0.0001	387	−5	−15	6.89	0.0001	5.2	−6.8	6.7	Hippocampus R (CA1, S)
		0.0001	387	−7	−13	6.69	0.0001	−6.0	−6.0	6.0	Hippocampus L (CA1, S, DG)

DE2 = drug-exposure week 2; W2 = withdrawal week 2; FWE = familywise error–corrected; K_E = cluster extent; ΔS = percentage change between respective phases for sucrose group; ΔC = percentage change between respective phases for cocaine group; T = T-score value; CPU = striatum; NAC = nucleus accumbens; AI = agranular insular cortex; OC = orbital cortex; S = subiculum; DG = dentate gyrus.

http://jnm.snmjournals.org). Localized Fastmap shimming was performed before the PRESS sequence (320 averages; echo time/repetition time, 20 ms/1.8 s; acquisition duration, 9 min 38 s). Analyses were performed with jMRUI, version 5.2, on total creatine (creatine plus phosphocreatine), γ-aminobutyrate, glucose, glutamate, glutamine, glycine, lactate, N-acetylaspartate, and taurine.

Statistical Analysis

All statistical analyses were performed using SAS JMP Pro, version 12.1 (SAS Institute Inc.). By design, the reported data of animals exposed to cocaine were pooled from 2 independent experiments to minimize the number of false-positive results. Moreover, effects were withheld when significant for the pooled data and either significant in both experiments separately or significant in one experiment and consistent in the other (i.e., the estimate of a significant effect in one experiment included the estimate of the other experiment within its 95% confidence interval). Therefore, no additional multiple-test comparison was applied to avoid false-negatives. This approach was not adopted for the reported SPM results because of the nature of the SPM output.

The ability of baseline measurements to explain variability in drug use during the drug-exposure phase was assessed with linear mixed models, including a random effect per animal and animal × day. To visualize these results, we divided the animals into 3 groups based on the baseline value of the variable of interest such that each group included approximately one third of the total number of animals. Self-administration results were then plotted for these 3 groups. Differences between phases were evaluated with a Kruskal–Wallis test,

TABLE 2
Longitudinal Analysis of Effect of Cocaine Self-Administration on mGluR5 Availability

Parameter	Threshold (P)	Cluster level P_{Corr}	K_E	Decrease (%)	Voxel level T	P_{Corr}	Coordinate x	y	z	Anatomic structure
Baseline > DE1	0.005 (FWE)	0.0001	1,470	37 ± 6	5.95	0.01	3.2	−6.2	6.6	Hippocampus R (DG)
Baseline > DE2	0.005 (FWE)	0.0003	533	20 ± 8	6.90	0.0001	−0.4	−5.4	2.4	Prefrontal cortex (FAC, PLC)
		0.0001	20,986	25 ± 8	6.68	0.0001	2.2	−4.8	5.6	Hippocampus BL, CPU (S, DG)
Baseline > relapse	0.005 (FWE)	0.0001	14,223	19 ± 7	9.50	0.0001	3.8	−6.4	6.8	Hippocampus R (DG)
		0.0003	539	12 ± 7	9.04	0.0001	−0.2	−5.8	2.4	Prefrontal cortex (FAC, PLC)
		0.0001	4,108	14 ± 8	8.35	0.0001	−6.0	−5.2	7.2	Hippocampus L (CA1)
Baseline > W1	0.0005 (Unc)	0.003	2,330	19 ± 6	4.18	0.02	0.6	−1.2	4.4	Hippocampus BL (FoHC)
Baseline > W2	0.0005 (Unc)	0.002	2,866	14 ± 5	4.62	0.004	−0.4	−0.4	2.0	Cingulate cortex (A2)

DE1/2 = drug-exposure week 1/2; W1/2 = withdrawal week 1/2; FWE = familywise error–corrected; Unc = uncorrected; K_E = cluster extent; T = t-score value; DG = dentate gyrus; FAC = frontal association cortex; PLC = prelimbic cortex; S = subiculum; FoHC = fimbriae of hippocampus; A2 = region A2 of cingulate cortex.
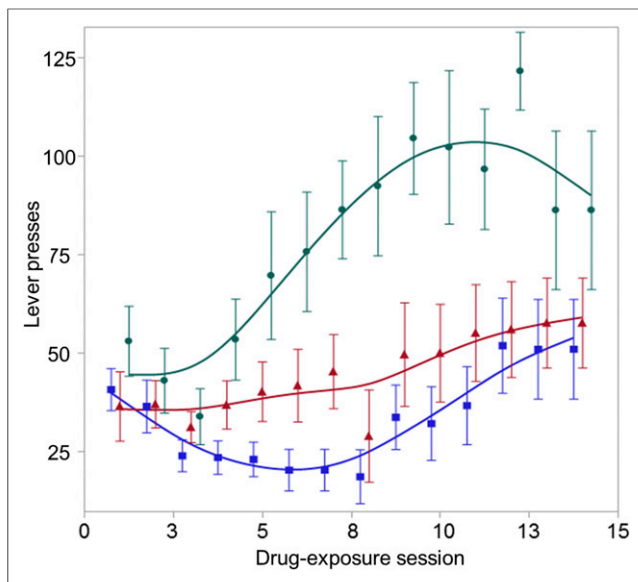
FIGURE 2. Number of lever presses during the self-administration phase. Cocaine was associated with preexposure score of decision making in rIGT. Animals were grouped according to low (blue, 0–0.15), average (red, 0.15–0.35), and high (green, 0.35–0.60) levels of decision-making score, with each category containing one third of observations.

which, if significant, was followed by a Wilcoxon signed-rank comparison. Significance was defined at the 95% confidence level. The reported values are mean ± SE of the mean.

RESULTS

Self-Administration

All catheters remained patent during the experiment. Animals readily self-administered cocaine during the first and second drug-exposure weeks (median number of lever presses per 3 h, 34 and 60, respectively; interquartile range, 17–53 and 19–100, respectively). Moreover, compared with the first and second drug-exposure weeks, the relapse week showed a significant increase in cocaine intake (median, 89; interquartile range, 57–116; average, 25 ± 2 [z = 9.53, P < 0.0001]). Sucrose intake by the control group showed a different profile, with significantly higher intake during the first

self-administration week (median, 134; interquartile range, 103–180 [z = 5.55, P < 0.0001]) but no difference between the second self-administration and relapse weeks (median, 89 and 88, respectively; interquartile range, 74–123 and 69–104, respectively). Median inactive lever presses remained low throughout both the first and the second drug-exposure weeks (median, 1 and 0.5, respectively; interquartile range, 0–4 and 0–4, respectively), as well as during the relapse week (median, 1; interquartile range, 0–4).

rIGT

The rats performed the rIGT actively, with on average 164 ± 10 responses in 30 min. The mean decision-making score was 0.09 ± 0.31. The rats thus obtained 50 ± 3 rewards consisting of 98 ± 6 pellets in total. Mixed-model analysis of the baseline decision-making score showed a significant association with the increase in lever presses during the drug-exposure phase ($F_{3,78} = 9.19$, $P = 0.001$) (Fig. 2). Indeed, an increase in decision-making score with 1 SE (0.31) increased the predicted number of lever presses by 36 ± 8. Interestingly, a positive association was also found in the sucrose group ($F_{2,6} = 15.71$, $P = 0.005$), with an increase of 66 ± 26 in the predicted number of lever presses for the same increase in decision-making score.

Small-Animal PET Imaging

Baseline volume-of-interest–based BP_{ND} was used in a mixed model to assess for a potential association with substance use. However, significant results were not found for either the cocaine group or the sucrose group.

Compared with sucrose self-administration, cocaine induced a more pronounced decrease between baseline and the drug-exposure phase bilaterally in the nucleus accumbens, agranular insular cortex, and orbital cortex. Additionally, the *cornu ammonis* subfield 1 (CA1) and subiculum regions of the hippocampi showed a significantly larger bilateral decrease (Table 1). During withdrawal, these differences became insignificant, whereas during relapse the same regions again significantly differed between the two groups (Fig. 3). In all phases, a significantly lower BP_{ND} for the sucrose group was found bilaterally in the striatum. Because this difference was already present at baseline, it was not evaluated in the light of this comparison.

Voxel-based analysis of the cocaine group demonstrated decreased ^{18}F-FPEB BP_{ND} bilaterally in the hippocampus during the second drug-exposure week and during the relapse phase, as compared with baseline (Table 2). A smaller cluster of decrease was observed during the first and second withdrawal weeks (Fig. 4). During the first week of drug exposure, FPEB BP_{ND} decreased only in the right hippocampus. Another region that showed a decrease in mGluR5 BP_{ND} in comparison to baseline was the prefrontal cortex during the second drug-exposure week and during relapse. Lastly, a bilateral decrease of 14% ± 5% was observed in the cingulate cortex during the second week of withdrawal.

Voxel-based correlation analysis showed a positive correlation (z = 3.55, P < 0.001) between the median number of lever presses for cocaine, but not sucrose,
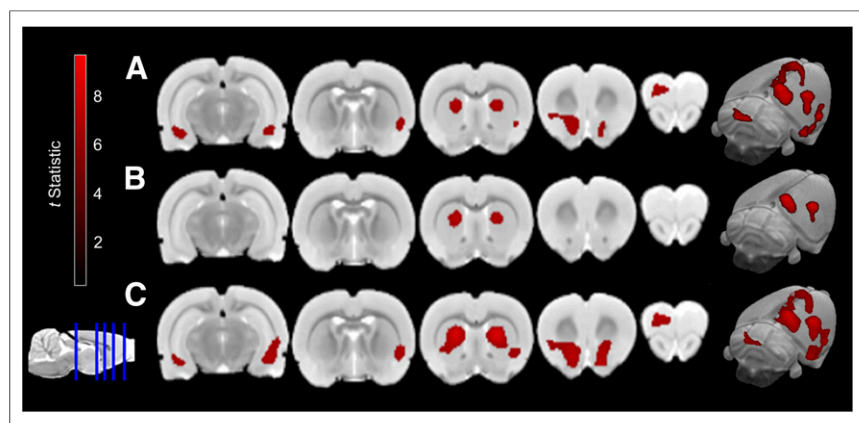
FIGURE 3. Significant SPM clusters (P < 0.00001, familywise error–corrected) locate significant differences between rats self-administering sucrose or cocaine during drug exposure (A), withdrawal (B), and relapse (C).
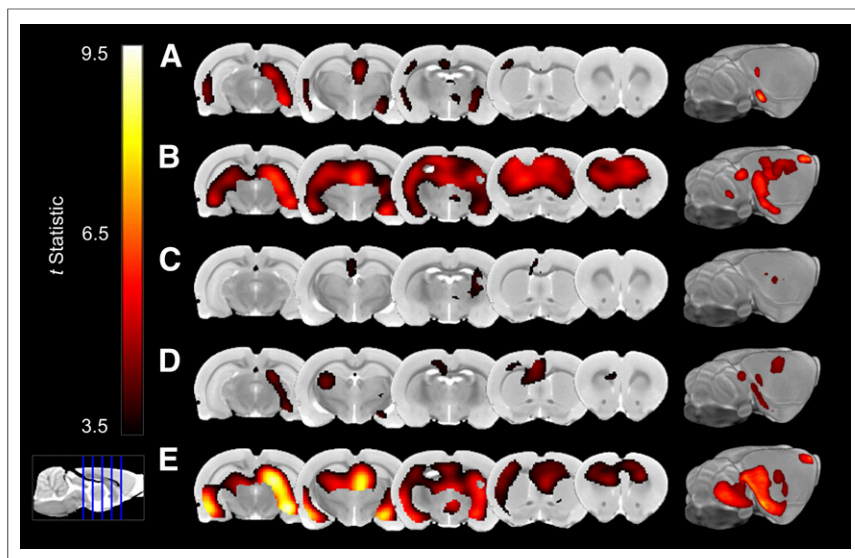
FIGURE 4. Significant SPM clusters ($P < 0.0005$, uncorrected) locate decrease in mGluR5 BP_{ND} of cocaine group during drug-exposure week 1 (A), drug-exposure week 2 (B), withdrawal week 1 (C), withdrawal week 2 (D), and relapse (E). Most important decreases are bilaterally in hippocampus during weeks of drug exposure. Normalization can be observed during both withdrawal weeks (C and D).
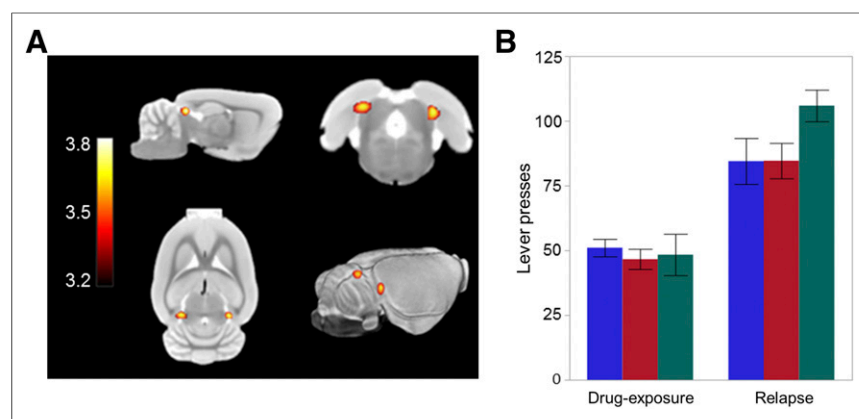
and ^{18}F-FPEB BP_{ND} in the left and right subicula of the hippocampus (respectively: cluster extent, 281 and 234; $x = -3.2$ and 3.2; $y = 8.0$ and 8.2; $z = 2.8$ and 3.2; $P_{Cluster\ Level} =$ <0.0001 and <0.0001). Classification of animals based on ^{18}F-FPEB BP_{ND} in this cluster suggested that this effect was most important during the relapse phase (Fig. 5).

^{1}H-MRS

All measured metabolites were in line with previously reported ranges for healthy rats (*15*). Interestingly, mixed models showed that prefrontal glutamate ($F_{2,36} = 8.98$, $P < 0.001$) and glycine ($F_{2,36} = 17.41$, $P < 0.001$) had a significant association with cocaine use during the drug-exposure phase (Fig. 6). On the one hand, an animal with a 1 mmol/L higher prefrontal glutamate concentration was related to, on average, 14.9 ± 4.1 more lever presses per session. On the other hand, an increase of 1 mmol/L in prefrontal glycine was related to an overall increase of 13.6 ± 2.6 lever presses. The prefrontal glutamate concentration was the only metabolite for which a significant effect of cocaine exposure was found. In particular, a decrease was observed when animals had access to cocaine during the first week of drug exposure (1.46 ± 0.53 mmol/L, $z = 2.74$, $P = 0.006$) and during the relapse phase (1.37 ± 0.54 mmol/L, $z = 2.54$, $P = 0.011$), compared with baseline. The first and second withdrawal weeks were also found to differ significantly from the same 2 phases—that is, the first week of drug exposure (0.93 ± 0.56, $z = 2.15$, $P < 0.031$, and 1.01 ± 0.65, $z = 2.46$, $P < 0.013$, respectively) and the relapse phase (0.85 ± 0.56, $z = 1.96$, $P = 0.049$, and 0.93 ± 0.66, $z = 2.32$, $P = 0.020$, respectively) (Fig. 7). No significant differences in any metabolites were found among the different phases, nor were there any significant associations with baseline measurements for the nucleus accumbens voxel and the sucrose group.

A possible relation among the reported significant baseline measurements was investigated using a nonparametric correlation analysis. No significant correlation was found between the rIGT and prefrontal glutamate or glycine concentrations. However, between the latter two, a significant correlation was found ($\rho = 0.77$; $P < 0.0001$). Finally, since prefrontal concentrations of glutamate showed significant changes between the different phases, we also investigated the relationship between glutamate and mGluR5 BP_{ND} in the prefrontal cortex, as measured with ^{18}F-FPEB PET. However, no significant association was found with the voxel-by-voxel analysis.


FIGURE 5. (A) Clusters of ^{18}F-FPEB binding that positively correlated with quantity of cocaine use in left and right subiculum. (B) Classification of rats based on ^{18}F-FPEB BP_{ND} in this cluster suggests that this association was most apparent during relapse phase. Observations are categorized by low (blue, 1.1–2.5), average (red, 2.5–3.2), or high (green, 3.3–4.4) ^{18}F-FPEB binding potential, with each group containing approximately one third of all observations. Error bars represent SEM.

DISCUSSION

In a longitudinal rat model of cocaine self-administration, we showed that baseline measurements of the rIGT, prefrontal glycine, and glutamate were associated with future cocaine use as expressed by the number of lever presses. Furthermore, mimicking of withdrawal and relapse after the initial drug exposure induced a distinct cocaine-dependent pattern in prefrontal glutamate concentration. A similar pattern was found in mGluR5 availability, explicitly for the hippocampus, where it was associated with the level of cocaine intake during the drug-exposure and relapse phases. Compared with sucrose self-administration, bilateral decreases were found in the hippocampus, nucleus accumbens, insular cortex, and orbital cortex during drug-exposure phases.
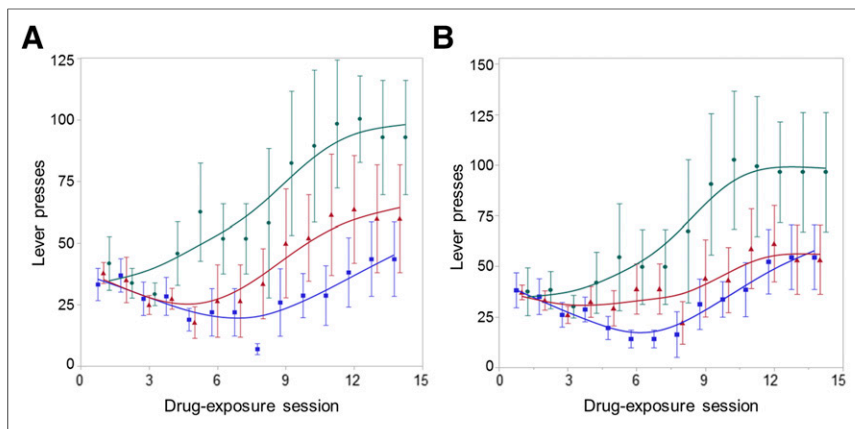
FIGURE 6. (A) Number of lever presses during the self-administration phase. Cocaine was associated with level of prefrontal glycine concentration (mmol/L). For visualization purposes, rats are categorized by their prefrontal glycine concentration in low (blue, 0.20–0.45), average (red, 0.45–0.55), and high (green, 0.55–1.00) groups. Each group contained one third of all observations. (B) Cocaine use during drug-exposure phase was associated with prefrontal glutamate concentration. Rats are categorized by their prefrontal glutamate concentration in low (blue, 4.3–5.5), average (red, 5.5–6.8), and high (green, 7.2–13.5) groups, with one third of observations in each group. Error bars represent SEM.

Patients with substance-use disorders typically show impaired decision-making abilities, as they experience greater difficulty in evaluating the negative consequences of a choice (16). A long-standing question in addiction research is whether this character trait is preexisting or induced by the drug use and resulting addiction (17). Clinical studies have not provided definitive answers, because most were performed only after addiction had been established. We here show that rats with poor decision-making skills, of which specific aspects were assessed with the rIGT, had higher future intake of either sugar or cocaine (12). This finding might indicate an inverse relation between decision making and reward salience that transcends reward type (18).
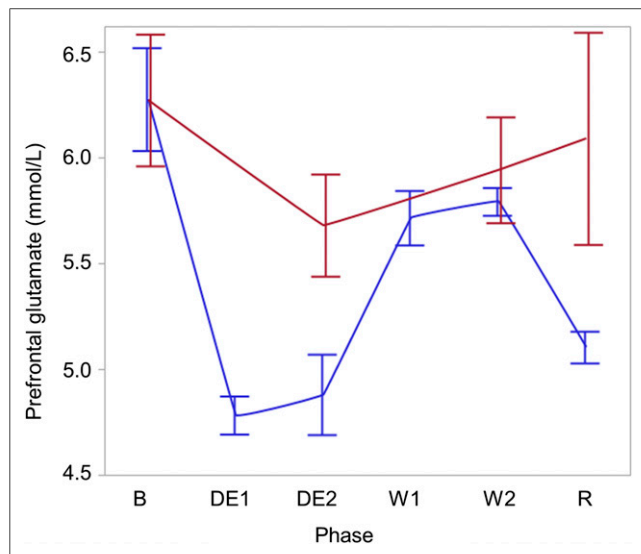
FIGURE 7. Prefrontal glutamate levels as measured with in vivo ^{1}H-MRS show strong, but reversible, effect of exposure to cocaine (blue) but not to sucrose (red). B = baseline; DE1 = drug-exposure week 1; DE2 = drug-exposure week 2; R = regained access; W1 = withdrawal week 1; W2 = withdrawal week 2.

We report that, compared with sucrose, cocaine self-administration induced a significant decrease in mGluR5 availability in the brain regions involved in the hippocampo-prefrontal cortex pathway in the rat (19). This pathway originates in the CA1 and subiculum and is projected to the orbitofrontal cortex, and both sites have projections toward the nucleus accumbens. This pathway has been associated with several memory processes, including goal-oriented reward learning (20). The robust difference in mGluR5 availability in these regions between sucrose and cocaine self-administration is remarkable and could be relevant to the ongoing debate on whether food rewards should be considered on a par with addictive substances (21). Although sucrose can be an incentive as strong as, or stronger than, cocaine, sucrose does not directly interfere with the dopaminergic system (22). Hence, one could hypothesize that the observed neurobiologic changes are due to unnaturally high synaptic dopamine levels elicited by cocaine rather than to the inherently pleasurable effects of both.

To our knowledge, this was the first study to assess mGluR5 availability in all phases of addiction. We found a decrease in mGluR5 availability after exposure to cocaine, particularly in the hippocampus, although other regions, such as the prefrontal cortex, were also implicated. This finding is in line with literature reports showing decreased mGluR5 binding in cocaine-dependent patients (23). Withdrawal from cocaine induced normalization of mGluR5 availability, although localized smaller decreases remained. This normalization is similar to that observed for prefrontal glutamate concentration and indicates a general downregulation of the glutamatergic system during drug exposure.

The cocaine-induced decreases were most explicit in the hippocampus—a finding that can be considered a corroboration of the hypothesis that addiction is, in large part, a pathologically strong learning process (24). Our data here suggest that the subiculum plays a specific role in this pathway, as we showed that mGluR5 densities in this region correlate with a rat's cocaine intake. The suggestion has already been made that the subiculum is involved in the formation of drug-associated memories, as lesions in this region reduce cocaine use in rats (25). This suggestion is in line with the existing hypothesis that addictive behavior arising from the center of the nucleus accumbens is heavily influenced by glutamatergic input from the subiculum (26). Therefore, mGluR5 in the subiculum might be involved in the formation of drug-associated memories, which later induce craving.

In the prefrontal cortex, which is one of the main effectors of the mesolimbic reward system, lower glutamate levels have been measured with ^{1}H-MRS in human chronic users of cocaine (27). Lower extracellular basal glutamate levels have also been reported in rats after methamphetamine self-administration (28). Here, we confirm this decrease in extracellular basal glutamate levels in rats exposed to cocaine, as measured with ^{1}H-MRS, and show that normalization occurs during withdrawal. Our findings

complement the data of Hermann et al., who showed that drug exposure leads to an increase in prefrontal glutamate lasting up to 60 h after exposure, followed by a progressive decrease to a level lower than that at baseline (29). Additionally, in humans a similar normalization is observed during alcohol abstinence (30). Indeed, Meshul et al. reported a significant decrease after 2 d of withdrawal that was no longer present after 14 d (31). However, cross-sectional results alone can be misleading. For example, based on O'Neill et al., the decrease in glutamate could be considered a direct effect of withdrawal, whereas we show here that the glutamate decrease is a remnant of earlier exposure to cocaine (32). This finding emphasizes the need for longitudinal experiments in addiction research to study the temporal dynamics of biologic processes.

Glycine is an obligatory coagonist of glutamate at the N-methyl-D-aspartate receptor (33), which plays an important role in the dopamine-elevating properties of drugs (34). Indeed, several rat studies have shown that antagonists of glycine can reduce the addictive properties of cocaine (35). However, to our knowledge, we are the first to report that glycine levels before drug exposure can help explain future cocaine use in rats. A clinical trial with the glycine transporter 1 inhibitor Org 25935 was performed on patients with alcohol dependence but was abandoned when evidence of efficacy was lacking (36). However, because no complete dose–response study was performed in that trial, its true value is difficult to evaluate. In schizophrenia, in which dysregulation of the glutamatergic system is also believed to be fundamental, glycine has been found to be correlated with the severity of symptoms (37). Therefore, we believe glycine could be an interesting target deserving future studies into the pathophysiology and therapy of cocaine addiction.

CONCLUSION

We report the temporal dynamics of several important mediators of cocaine self-administration in rats before and after drug exposure. Specifically, prefrontal glycine and glutamate are found to be interesting biomarkers of vulnerability for cocaine use. Furthermore, the influence of cocaine on mGluR5 availability and prefrontal glutamate concentrations is different from the influence of sucrose. Finally, poor decision making in rats is associated with increased future cocaine self-administration. We hope that our study can provide a reference for longitudinal setups and can guide the interpretation of cross-sectional studies.

DISCLOSURE

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