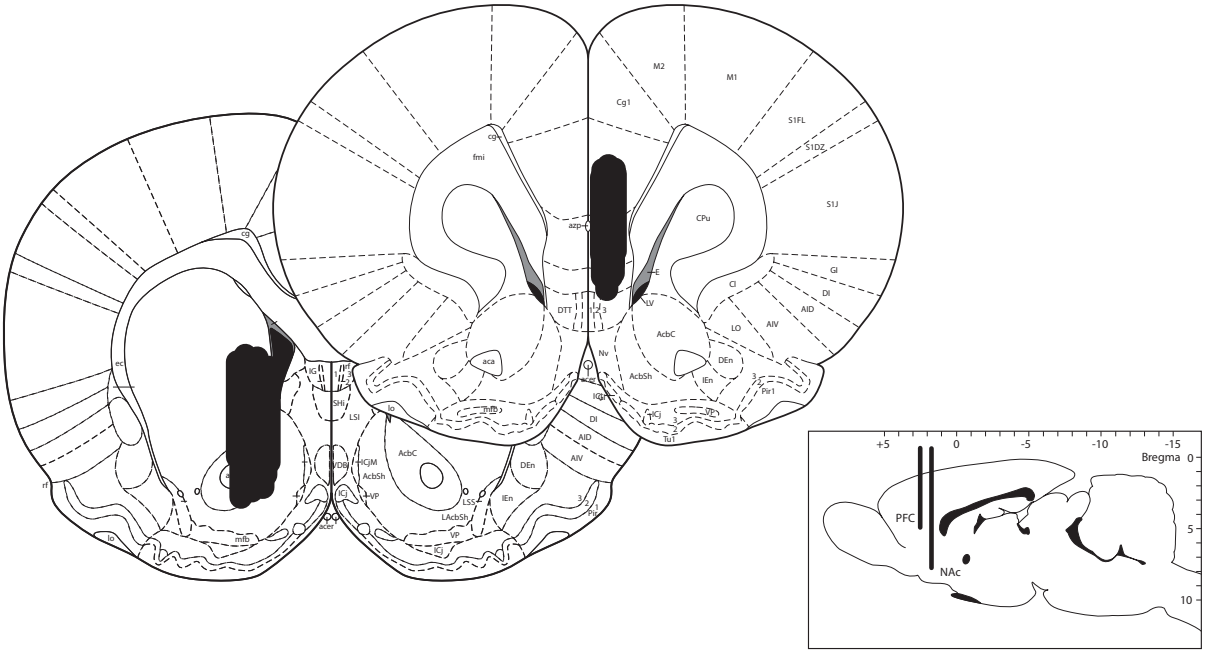


Supplemental Figure S1

Individual probe placements for dual-probe microdialysis in mPFC (shown in front) and NAc (shown in back). Insert shows target coordinates in a sagittal view. Atlas from Paxinos and Watson 2005.

Paxinos, G. and Watson, C. (2005). The Rat Brain in Stereotaxic Coordinates. *Compact 6th Edition, Academic Press, New York*, 400.

Supplemental Methods

Behavioral studies: cocaine self-administration

Apparatus. Rat operant conditioning chambers (21 cm x 29.5 cm x 24.5 cm; MED Associates (Georgia, VT) were used. Each chamber was equipped with three retractable response levers placed 3 cm above the grid floor, two "reinforcer" levers (subsequently "left" and "right" levers) on one wall and a third "observer" lever centered on the opposite wall. A steel cup between the reinforcer levers, 2 cm above the floor, served as a receptacle for the delivery and consumption of liquid food reinforcers. There was a three-light array (red, yellow, green; MED Associates ENV-222M) above the right lever to signal the availability of food reinforcers, and an identical array with one additional yellow light above the left lever to signal the available cocaine dose. There was a white light above the observer lever (ENV-229M). Each chamber was enclosed in a sound-attenuating cubicle (MED Associates ENV-221M) equipped with a house light, an exhaust fan, and two syringe pumps (3.3 rpm, model PHM-100) for the delivery of liquid food and intravenous cocaine, respectively. Cocaine was delivered using a single-channel fluid swivel (MS-1, Lomir Biomedical, Malone, NY) mounted on a balance arm, which allowed rats free movement.

Operant training and surgery. Training and testing occurred five days a week, Monday through Friday. Total training time was typically 12-16 weeks, including post-surgery recovery period.

Food training. Rats were first allowed to acquire lever pressing with liquid food (75 µl of vanilla flavor Ensure® nutrition drink diluted to 32% in water, Abbott Laboratories, Abbott, IL) reinforcing responding under an FR 1 schedule of reinforcement, in daily 2-h sessions. The triple cue light above the right lever was illuminated when food reinforcers were available, and turned off at reinforcer delivery. Responses on the other levers (cues off) were recorded but had no scheduled consequences. Once at least 50 reinforcers were earned within one 2-h session and 90% or more of total responses were emitted on the food-reinforced (right) lever, the response requirement was gradually increased to FR 5. When rats again earned at least 50 reinforcers, a chain schedule was introduced in which one response on the observer lever initiated an FR 5 timeout 20 s schedule on the right lever (see Thomsen et al., 2013 for additional details). When rats again earned at least 50 food reinforcers per session for five consecutive sessions (training criteria), catheters were implanted.

Surgery and catheter maintenance. Rats were anesthetized with an isoflurane/oxygen vapor mixture and implanted with chronic indwelling jugular vein catheters (see step by step protocol in Thomsen and Caine, 2005). A catheter (IVSA28, Camcaths, UK) was inserted 3.7 cm into the external jugular vein and anchored to the vein with silk suture. The catheter ran subcutaneously to the midscapular region where the base was located. Single doses of analgesic (ketoprofen 5 mg/kg) and antibiotic (amikacin 10 mg/kg) were administered subcutaneously immediately before surgery. Rats were allowed ≈7 days of recovery before being given access to intravenous cocaine. During this period, a prophylactic dose of antibiotic (30-40 mg/kg cefazolin) was administered through the catheter once daily. Thereafter, catheters were flushed daily with sterile saline containing 3 USP U/0.1 ml heparin. Catheter patency was verified daily by withdrawing and immediately re-infusing a few microliters of blood through the catheter (just enough for visual detection of blood); if blood could not be withdrawn, catheter patency was tested by administering 0.05-0.1 ml of a ketamine-midazolam mixture (15 + 0.75 mg/ml) through the catheter and observing prominent signs of sedation within 3 seconds of infusion. Only data collected with demonstrated patent catheters were included.

Cocaine Self-Administration Training. Rats acquired cocaine self-administration in once-daily 2-h sessions, responses on the left lever reinforced under an FR 1 FR 1 timeout 20 s chain schedule. Sessions started with an automated noncontingent "priming" cocaine infusion. Then, the white stimulus light over the observer lever was illuminated; one response on the observer lever turned off the cue

light and simultaneously turned on the full cue-light array flashing over the left reinforcer lever and extended the left lever. Completion of the response requirement on the left lever produced an i.v. infusion of 1.0 mg/kg cocaine and initiated a 20 s timeout period (including drug infusion time), during which cue lights were off and responses had no scheduled consequences. Correct dosing was ensured by adjusting the infusion duration, using 5.6 mg/ml cocaine (e.g., 1.0 mg/kg for a 320 g rat was delivered over 3.2 s in 56 µl). The response requirement gradually increased to FR 1 FR 5. Then, training continued until cocaine self-administration behavior was stable, criteria: three consecutive sessions with at least 10 mg/kg cocaine self-administered per 2-h session and at least 90% of left+right lever responses emitted on the drug-reinforced lever. Then, the session was divided into five 20-min components of cocaine availability (1.0 mg/kg/infusion), with 2-min inter-component timeout periods, keeping the same schedule of reinforcement. This schedule was used until behavior was again stable, criteria: at least 10 mg/kg cocaine self-administered per session and at least one reinforcer earned per component. The cocaine dose was then changed to 0.32 mg/kg/infusion for at least one session to observe increased rates of responding.

*Cocaine vs. Food Choice Training***.** Daily sessions consisted of five 20-min components separated by 2-min timeout periods. Each component started with an automated noncontingent delivery of each reinforcer (cocaine, then food) available in that component, and illumination of the observer lever cue light to signify that responses had scheduled consequences. Responding was reinforced under an FR 1 concurrent FR 5 FR 5 chain schedule: One response on the observer lever resulted in retraction of the observer lever and associated cue light going off, extension of the left and right levers, and illumination of the cue lights associated with the left and right levers to signify reinforcer availability. Responses on the right lever were reinforced with liquid food, responses on the left lever were reinforced with cocaine infusions. Cocaine dose increased for successive components: 0, 0.056, 0.18, 0.56, 1.0 mg/kg/infusion, achieved by varying the infusion time, adjusted to each rat's bodyweight. The food reinforcer available remained fixed across components. Cocaine dose available was signaled by flashing of the light cues over the left lever: cues off for 0, green flashing for 0.056, green+yellow flashing for 0.18, green+yellow+red flashing for 0.56, green+yellow+red+yellow flashing for 1.0 mg/kg/infusion. Cues turned off at reinforcer delivery. 15 total reinforcers were available per component, completion of the response requirement on the left lever during availability of the zero cocaine dose counted as one reinforcer. If all 15 reinforcers were earned in less than 20 min, all stimulus lights were extinguished, and responding had no scheduled consequences for the remainder of the 20-min component. Choice training continued until behavior was stable, criteria: three consecutive sessions with 1) at least 5 reinforcers per component in components 1-4 and at least one reinforcer earned in component 5, and 2) the cocaine dose that produced at least 80% cocaine choice on any given day must be within one-half log unit of the three-day mean.

Microdialysis

Relative glutamate concentrations were detected by a C18 reversed-phase column 3.5 µm Zorbax 300SB C18 analytical column (4.6 mm × 150 mm id, Agilent Technology Inc., USA) and gradient fluorescence HPLC detection. The dialysate was pre-column derivatized with *o*-phthalaldehyde (Merck, Darmstadt, Germany). Mobile phase A consisted of 70 mM Na_2HPO_4 in 50 µM of EDTA 0.15% (v/v) tetrahydrofurane (Sigma-Aldrich) and 20% (v/v) methanol (Sigma-Aldrich); pH was adjusted to 7.2 with 0.1 M acetic acid. Mobile phase B was 100% methanol. The gradient elution was performed at room temperature using the following conditions: 0 min 15% B (flow rate of pump B: 0.65 mL/min), 2.00 min 22% B (flow rate of B: 0.65 mL/min) for A+B after 15 min 10% B mobile phase. The excitation and emission wavelengths were 350 nm and 450 nm, respectively. Known standards were used to identify and quantify the glutamate peaks in the HPLC chromatogram. The HPLC system consisted of a Shimadzu chromatograph (LC-20AD, Tokyo, Japan) equipped with an autosampler, a degasser (DGU-20A3), a gradient pump (LC-20AD), a fluorescence detector model RF-

20Axs. Characteristic retention times for glutamate were 4.9 min. The peak areas of glutamate were integrated with the calibration curve of known standards.

Data analysis

Behavior. The same subset of time points (baseline, treatment day, and 1, 7, 14, 21, 28 days post-treatment) as shown in the figures were analyzed. Numbers of cocaine injections and food reinforcers taken and cocaine injections as percent choice over total reinforcers were recorded for each component, and were analyzed for each VU0364572 dose by two-way ANOVA with cocaine dose and time after treatment as repeated-measures factors. Significant effects of time or cocaine by time interactions were followed by simple effects analysis on each time point vs. baseline, by two-way ANOVA (factors: cocaine dose, time). Session-wide cocaine intake (mg/kg), food intake (ml), and total percent cocaine choices were calculated and were analyzed for each VU0364572 dose by one-way ANOVA with time after treatment as repeated-measures factor. Significance at individual cocaine doses or time points was determined by two-tailed paired-sample t-test corrected for false discovery rate (Benjamini-Hochberg, 5% false discovery rate limit). Repeated-measures analyses were Huynh-Feldt-corrected for any violations of the sphericity assumption.

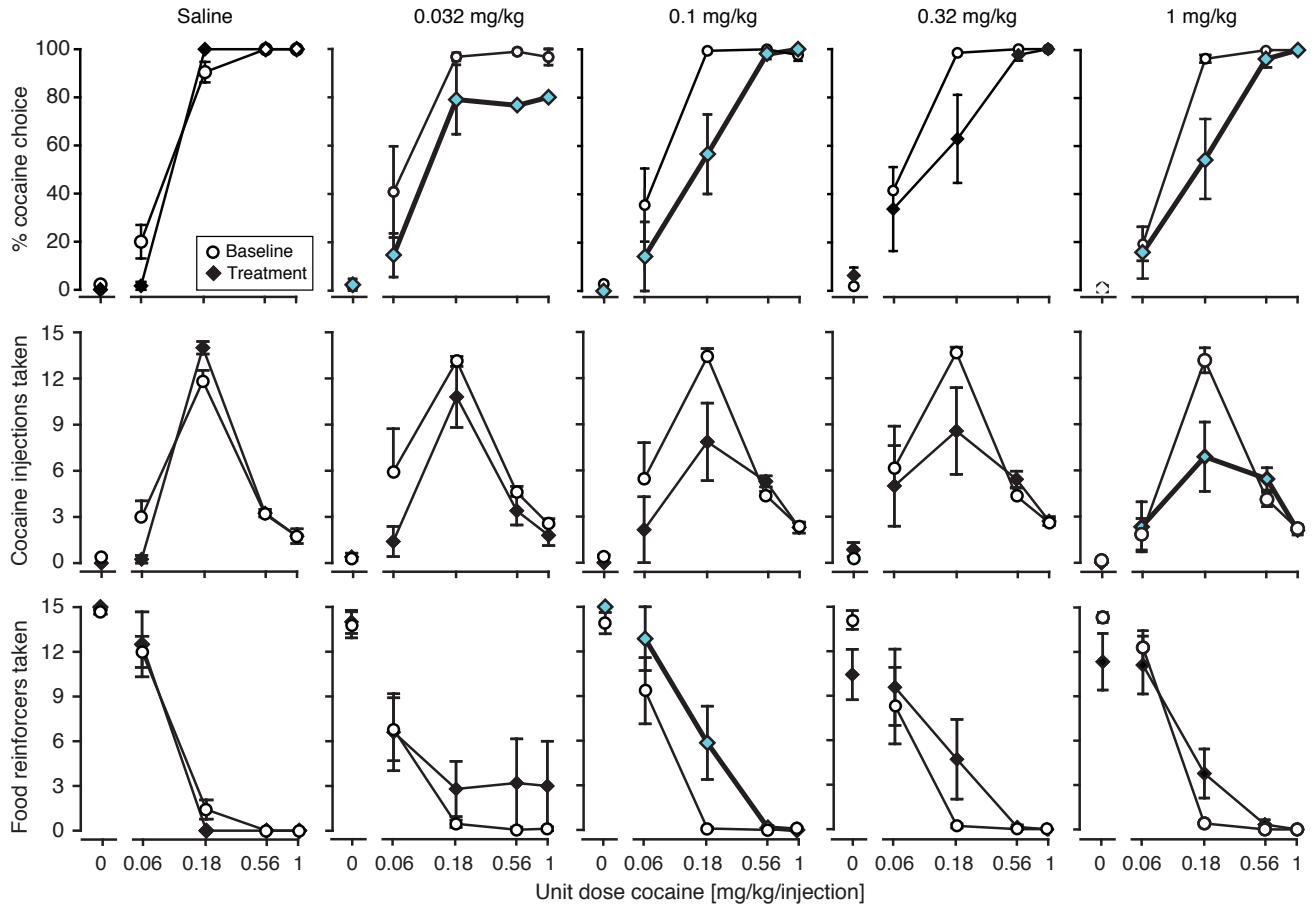
Microdialysis. Effects of treatment history were evaluated for each brain region and neurotransmitter separately, by three-way ANOVA with cocaine and VU0364572 as between-subjects factors and time after cocaine injection (0-100min) as repeated-measures factor. Only pre-planned effects and interactions considered biologically meaningful were followed by further analysis, as follows. Where there were significant effects of both cocaine history and VU0364572 history, or a significant cocaine by VU0364572 interaction, data were further analyzed by two-way ANOVA in cocaine-naïve rats and cocaine-experienced rats separately with VU0364572 history and time as factors, followed by false discovery rate-corrected unpaired-sample t-test. Interactions with the time factor were not investigated further as their biological relevance is difficult to interpret. In addition, area under the curve (AUC) for increase in neurotransmitter levels above baseline 0-100 min after cocaine administration was calculated (trapezoid method) and analyzed by two-way ANOVA with cocaine and VU0364572 history as factors, followed by false discovery rate-corrected unpaired-sample t-test. Repeated-measures analyses were Huynh-Feldt-corrected for any violations of the sphericity assumption. The effect of acute cocaine administration on the test day was evaluated as a pre-planned analysis by one-way repeated measures ANOVA in each treatment group and for each brain region and neurotransmitter, and was indicated by a significant change in neurotransmitter level as a function of time. All ANOVA outcomes are reported in Table S2

Dopamine uptake. Uptake was analyzed by three-way ANOVA with cocaine and VU0364572 as between-subjects factor and dopamine concentration as a repeated-measures factor (Huynh-Feldt corrected). The fitted values for V_{max} and K_M were compared by two-way ANOVA with cocaine and VU0364572 as factors.

Level of significance was set at α=0.05. Data are presented as group means ± standard error of the mean (s.e.m.) unless noted otherwise (individual data). Power analyses were performed using G*power 2 (Faul et al. 2007). Behavior: a priori analysis based on effects of xanomeline in the choice assay (Thomsen et al. 2014) yielded a required n=4-6. Microdialysis: post-hoc analysis yielded a power of >0.99 for all four analyses, dopamine and glutamate in mPFC and NAc. Dopamine uptake: a priori analysis based on our previous studies in mice yielded a minimum n=3; however variation was larger than expected. Post-hoc power analysis showed low power in the cocaine-experienced groups, we therefore repeated the experiment in a second cohort, cocaine-experienced only.

Faul F, Erdfelder E, Lang AG, Buchner A (2007): G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 39:175-191.

Thomsen M, Fulton BS, Caine SB (2014): Acute and chronic effects of the M1/M4-preferring muscarinic agonist xanomeline on cocaine vs. food choice in rats. *Psychopharmacology (Berl)*. 231:469-479.



Supplemental Fig. S2

All VU0364572 doses tested produced a small apparent reallocation of behavior away from cocaine self-administration towards food taking. The shift in choice allocation reached significance at 0.032 mg/kg [$F(1,36)=5.24$, $p=0.03$], 0.1 mg/kg [$F(1,45)=4.72$, $p=0.04$], and 1 mg/kg [$F(1,72)=5.58$, $p=0.02$], the latter with a significant cocaine dose by treatment interaction [$F(1,72)=3.71$, $p=0.008$].

Effects on cocaine injections taken only showed a significant cocaine dose by treatment interaction at the highest dose, 1 mg/kg [$F(1,72)=3.88$, $p=0.007$]. The interaction and lack of main effect of treatment reflect a shift in cocaine taking from lower doses towards higher doses of cocaine, consistent with functional antagonism of cocaine reinforcement. However, the relatively short half-life of VU0364572 (46 min in rats; Lebois et al. 2011) may also contribute to the lack of effect at higher cocaine doses: VU0364572 levels would have been lower towards the end of the 108-min session.

Effects on food appeared biphasic, with a non-significant increase at the lowest dose, reaching a significant increase at 0.1 mg/kg [$F(1,45)=5.62$, $p=0.02$]; then mixed decrease/increase at the higher doses, with an initial suppression of food-taking early in the session followed by an increase in later components.

Significant effects are indicated by blue symbols and bold lines; no comparisons reached significance post-hoc for individual cocaine doses. Group sizes: from left to right n=4, n=5, n=7, n=7, n=9. Saline administration had no significant effect on percent choice allocation, cocaine injections taken, or food reinforcers taken.

Lebois EP, Digby GJ, Sheffler DJ, Melancon BJ, Tarr JC, Cho HP, et al. (2011): Development of a highly selective, orally bioavailable and CNS penetrant M1 agonist derived from the MLPCN probe ML071. *Bioorg Med Chem Lett.* 21:6451-6455.

Supplemental Table S1
Total cocaine and food reinforcer intake per session after acute treatment with VU0364572

Dose VU0364572	Cocaine intake [mg/kg/session]	Food reinforcer intake [ml/session]	Total cocaine choice [% choices/session]
Saline			
Baseline	5.82±0.26	2.11±0.10	41.70±2.34
Treatment day	6.10±0.45	2.06±0.16	41.44±2.16
0.032 mg/kg			
Baseline	7.81±0.70	1.60±0.16	54.83±5.35
Treatment day	5.73±1.46	2.22±0.48	39.58±7.41
0.1 mg/kg			
Baseline	7.58±0.51	1.68±0.18	54.18±4.76
Treatment day	5.80±1.19	2.36±0.68	44.11±11.7
0.32 mg/kg			
Baseline	7.86±0.63	1.68±0.16	53.55±4.97
Treatment day	7.58±0.91	1.86±0.37	46.76±10.1
1.0 mg/kg			
Baseline	6.89±0.61	1.95±0.11	45.96±1.93
Treatment day	6.53±0.54	1.99±0.27	39.62±5.84

No dose of VU0364572 significantly affected cocaine intake (mg/kg/session) or food reinforcer intake (ml/session) on the treatment day, relative to baseline. Values are group means ± s.e.m. Group sizes as in Fig. S1.

Supplemental Table S2. Full list of ANOVA outcomes.

Cocaine self-administration

Table S2A. Cocaine dose-response functions, % cocaine choice

	Cocaine dose	Time	Interaction
Saline			
Overall	F(4,102)=182.2, p<0.0001	F(6,102)=0.56, p=0.75	F(24,102)=0.89, p=0.51
VU0364572 1 mg/kg:			
Overall	F(4,226)=70.4, p<0.0001	F(6,226)=8.22, p=0.0001	F(24,226)=1.68, p=0.18
Baseline vs.:			
Treatment day	F(4,72)=95.6, p<0.0001	F(1,72)=5.58, p=0.02	F(4,72)=3.71, p=0.03
1 day	F(4,72)=97.1, p<0.0001	F(1,72)=4.52, p=0.04	F(4,72)=2.06, p=0.12
7 days	F(4,68)=63.0, p<0.0001	F(1,68)=9.44, p=0.003	F(4,68)=3.19, p=0.03
14 days	F(4,66)=48.8, p<0.0001	F(1,66)=26.9, p<0.0001	F(4,66)=4.01, p=0.02
21 days	F(4,51)=21.9, p<0.0001	F(1,51)=22.9, p<0.0001	F(4,51)=6.19, p=0.003
28 days	F(4,57)=28.7, p<0.0001	F(1,57)=46.7, p<0.0001	F(4,57)=7.83, p=0.0001

Table S2B. Cocaine dose-response functions, cocaine reinforcers

	Cocaine dose	Time	Interaction
Saline			
Overall	F(4,102)=83.1, p<0.0001	F(6,102)=0.63, p=0.67	F(24,102)=0.95, p=0.48
VU0364572 1 mg/kg:			
Overall	F(4,232)=29.7, p<0.0001	F(6,232)=5.38, p=0.0007	F(24,232)=2.69, p=0.003
Baseline vs.:			
Treatment day	F(4,72)=26.4, p<0.0001	F(1,72)=2.28, p=0.14	F(4,72)=3.88, p=0.03
1 day	F(4,72)=48.7, p<0.0001	F(1,72)=2.69, p=0.11	F(4,72)=1.99, p=0.16
7 days	F(4,72)=29.3, p<0.0001	F(1,72)=14.1, p=0.0004	F(4,72)=7.51, p=0.003
14 days	F(4,67)=27.9, p<0.0001	F(1,67)=15.7, p=0.0002	F(4,67)=7.04, p=0.001
21 days	F(4,52)=17.9, p<0.0001	F(1,52)=16.2, p=0.0002	F(4,52)=8.68, p=0.0001
28 days	F(4,57)=24.4, p<0.0001	F(1,57)=52.9, p<0.0001	F(4,57)=27.2, p<0.0001

Table S2C. Cocaine dose-response functions, food reinforcers

	Cocaine dose	Time	Interaction
Saline			
Overall	F(4,102)=86.1, p<0.0001	F(6,102)=1.11, p=0.36	F(24,102)=0.75, p=0.79
VU0364572 1 mg/kg:			
Overall	F(4,232)=44.9, p<0.0001	F(6,232)=6.87, p<0.0001	F(24,232)=1.16, p=0.34
Baseline vs.:			
Treatment day	F(4,72)=63.2, p<0.0001	F(1,72)=0.09, p=0.88	F(4,72)=2.09, p=0.09
1 day	F(4,72)=61.2, p<0.0001	F(1,72)=0.02, p=0.90	F(4,72)=2.54, p=0.0500
7 days	F(4,72)=35.1, p<0.0001	F(1,72)=0.75, p=0.39	F(4,72)=3.23, p=0.09
14 days	F(4,67)=33.9, p<0.0001	F(1,67)=8.55, p=0.005	F(4,67)=2.90, p=0.03
21 days	F(4,52)=17.5, p<0.0001	F(1,52)=11.5, p=0.001	F(4,52)=5.59, p=0.004
28 days	F(4,57)=24.6, p<0.0001	F(1,57)=32.0, p<0.0001	F(4,57)=6.63, p=0.002

Table S2D. Session-total choice and intakes, effect of time over consecutive sessions

	Saline	VU0364572 1 mg/kg:
Cocaine choice	F(6,18)=1.02, p=0.44	F(6,40)=3.08, p=0.049
Cocaine intake	F(6,18)=1.02, p=0.43	F(6,40)=3.25, p=0.044
Food intake	F(6,18)=0.76, p=0.61	F(6,40)=2.83, p=0.041

Microdialysis

Table S2E. Three-way ANOVA with cocaine experience, VU0364572 experience, and time after acute cocaine injection

mPFC dopamine	
Cocaine experience	F(1,20)=10.1, p=0.005
VU0364572 experience	F(1,20)=6.64, p=0.02
Cocaine by VU0364572 interaction	F(1,20)=26.1, p=0.0001
Time after acute cocaine	F(5,98)=1.30, p=0.28
Time by cocaine experience interaction	F(5,98)=2.57, p=0.03
Time by VU0364572 experience interaction	F(5,98)=2.00, p=0.09
3-way interaction	F(5,98)=4.30, p=0.001
Follow-up 2-way analyses, cocaine-naive	
VU0364572 experience	F(1,10)=1.78, p=0.049
Time after acute cocaine	F(5,48)=0.24, p=0.91
Time by VU0364572 experience interaction	F(5,48)=1.09, p=0.37
Follow-up 2-way analyses, cocaine-experienced	
VU0364572 experience	F(1,10)=10.4, p=0.002
Time after acute cocaine	F(5,50)=3.39, p=0.02
Time by VU0364572 experience interaction	F(5,50)=4.89, p=0.003
NAc dopamine	
Cocaine experience	F(1,20)=9,66, p=0.006
VU0364572 experience	F(1,20)=43.2, p<0.0001
Cocaine by VU0364572 interaction	F(1,20)=6.29, p=0.02
Time after acute cocaine	F(5,100)=20.2, p<0.0001
Time by cocaine experience interaction	F(5,100)=1.48, p=0.20
Time by VU0364572 experience interaction	F(5,100)=5.44, p=0002
3-way interaction	F(5,100)=1.95, p=0.09
Follow-up 2-way analyses, cocaine-naive	
VU0364572 experience	F(1,10)=62.9, p<0.0001
Time after acute cocaine	F(5,50)=8.39, p=0.0004
Time by VU0364572 experience interaction	F(5,50)=9.70, p=0.0001

Follow-up 2-way analyses, cocaine-experienced	
VU0364572 experience	F(1,10)=4.56, p=0.03
Time after acute cocaine	F(5,50)=12.04, p<0.0001
Time by VU0364572 experience interaction	F(5,50)=0.68, p=0.64
mPFC glutamate	
Cocaine experience	F(1,20)=23.5, p=0.0001
VU0364572 experience	F(1,20)=0.64, p=0.43
Cocaine by VU0364572 interaction	F(1,20)=0.02, p=0.90
Time after acute cocaine	F(5,100)=1.38, p=0.26
Time by cocaine experience interaction	F(5,100)=3.62, p=0.02
Time by VU0364572 experience interaction	F(5,100)=0.65, p=0.59
3-way interaction	F(5,100)=0.25, p=0.86
NAc glutamate	
Cocaine experience	F(1,20)=3.10, p=0.09
VU0364572 experience	F(1,20)=7.39, p=0.01
Cocaine by VU0364572 interaction	F(1,20)=0.73, p=0.40
Time after acute cocaine	F(5,100)=4.20, p=0.002
Time by cocaine experience interaction	F(5,100)=2.16, p=0.07
Time by VU0364572 experience interaction	F(5,100)=4.22, p=0.002
3-way interaction	F(5,100)=2.10, p=0.07

Table S2F. Pre-planned one-way ANOVA, effect of time in each group – "Did acute cocaine injection affect the neurotransmitter level in this group?"

Cocaine-naïve vehicle-treated	
mPFC dopamine	F(7,35)=2.09, p=0.08
NAc dopamine	F(7,35)=17.65, p=0.0001
mPFC glutamate	F(7,35)=2.10, p=0.10
NAc glutamate	F(7,35)=5.91, p=0.0001
Cocaine-experience vehicle-treated	
mPFC dopamine	F(7,35)=10.96, p=0.0006
NAc dopamine	F(7,35)=10.72, p<0.0001
mPFC glutamate	F(7,35)=2.14, p=0.17
NAc glutamate	F(7,35)=2.59, p<0.10
Cocaine-naïve VU0364572-treated	
mPFC dopamine	F(7,33)=0.56, p=0.59
NAc dopamine	F(7,35)=1.05, p=0.41
mPFC glutamate	F(7,35)=1.27, p=0.32
NAc glutamate	F(7,35)=4.07, p=0.04

Cocaine-experience VU0364572-treated	
mPFC dopamine	F(7,35)=0.43, p=0.62
NAc dopamine	F(7,35)=6.98, p=0.002
mPFC glutamate	F(7,35)=4.18, p=0.02
NAc glutamate	F(7,35)=1.32, p=0.30

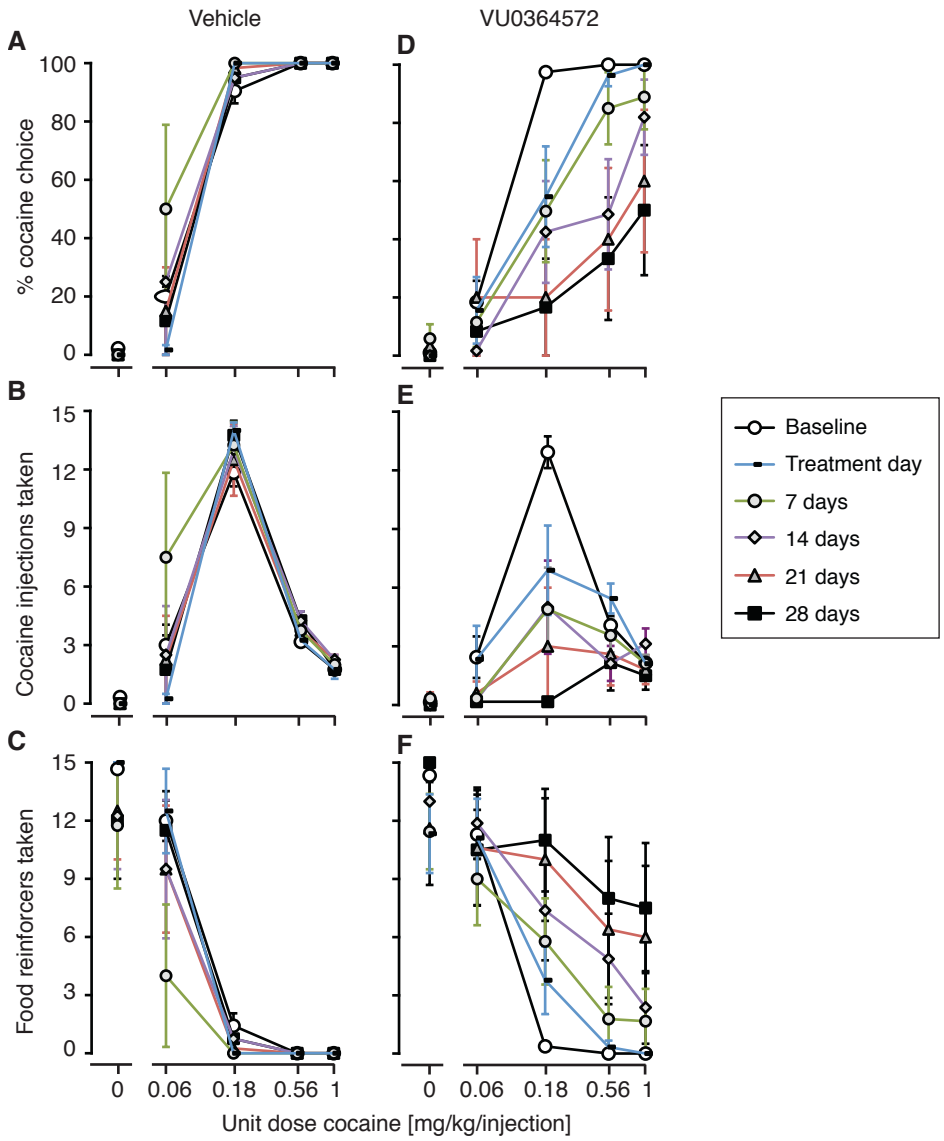
Table S2G. Total area under the curve of increase over baseline, 0-100 min after acute cocaine injection

	Cocaine experience	VU0364572 vs. vehicle	Interaction
mPFC dopamine	F(1,20)=8.66, p=0.008	F(1,20)=6.25, p=0.02	F(1,20)=11.6, p=0.003
NAc dopamine	F(1,20)=9.54, p=0.006	F(1,20)=41.4, p<0.0001	F(1,20)=7.24, p=0.01
mPFC glutamate	F(1,20)=20.9, p=0.0002	F(1,20)=0.39, p=0.54	F(1,20)=0.03, p=0.86
NAc glutamate	F(1,20)=3.20, p=0.08	F(1,20)=7.37, p=0.01	F(1,20)=0.87, p=0.36

Dopamine uptake

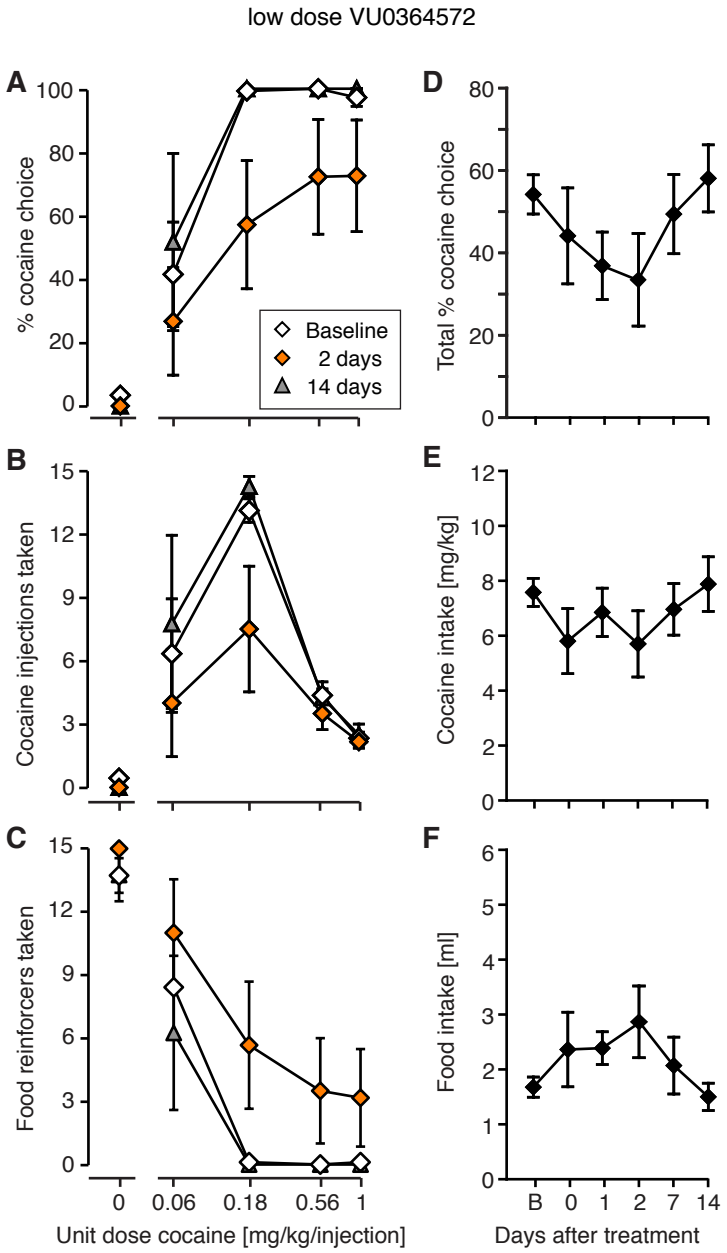
Table S2H. Effects of cocaine experience, VU0364572 experience, and dopamine concentration

Cocaine experience	F(1,12)=0.05, p=0.82
VU0364572 experience	F(1,12)=0.56, p=0.47
Cocaine by VU0364572 interaction	F(1,12)=0.48, p=0.50
Dopamine concentration	F(5,60)=59.2, p<0.0001
Dopamine by cocaine interaction	F(5,60)=1.18, p=0.33
Dopamine by VU0364572 interaction	F(5,60)=0.82, p=0.46
3-way interaction	F(5,60)=0.14, p=0.88



Supplemental Fig. S3

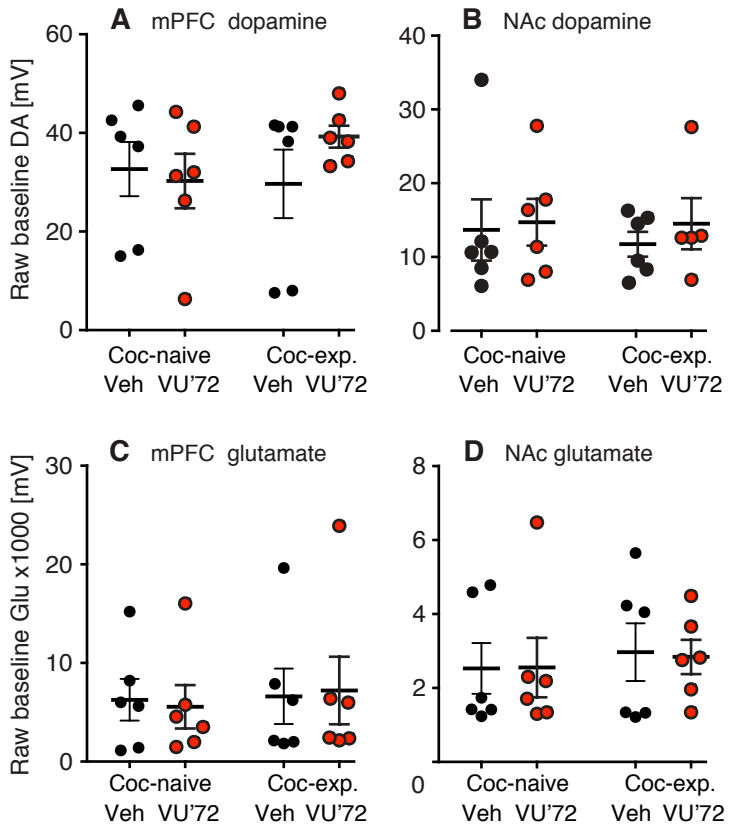
Dose-response functions for cocaine vs. food choice allocation (A,D), cocaine self-administration (B,E), and food taking (C,F) in saline-treated rats (A-C, n=4) and in rats treated with 1 mg/kg VU0364572 (D-F; baseline through 7 days: n=9, 14 days: n=8, after 14 days: n=6). This is the same as Figure 2, with the addition of dose-response functions for the intervening time points, showing the progressive shift from cocaine-taking behavior toward food-taking behavior in the VU0364572-treated rats.



Supplemental Figure S4

Cocaine self-administration behavior was transiently reduced after administration of a lower dose of VU0364572, 0.1 mg/kg (n=6). This was reflected by a near-significant effect of time on %cocaine choice [$F(2,84)=2.94$, $p=0.08$] over the first two days after VU364572 administration. On day 2 post-treatment (orange diamonds), cocaine choice allocation (A, [$F(1,45)=10.1$, $p=0.003$]) and cocaine injections taken (B, [$F(1,45)=4.61$, $p=0.04$]) were reduced and food reinforcers taken were increased (C, [$F(1,45)=9.06$, $p=0.004$]), relative to baseline (open diamonds). After two weeks, behavior had returned to baseline levels (A-C, grey triangles, n=5). Some rats were tested for a full four weeks post-treatment, and did not show further suppression of cocaine taking (n=3, data not shown).

Reallocation of cocaine-taking behavior towards food-taking behavior peaked 1-2 days after treatment, and returned to baseline after 1-2 weeks (D-F). However, analysis of session-wide choice allocation (D), cocaine intake (E), and food intake (F) over two weeks did not reach statistical significance. B: baseline.

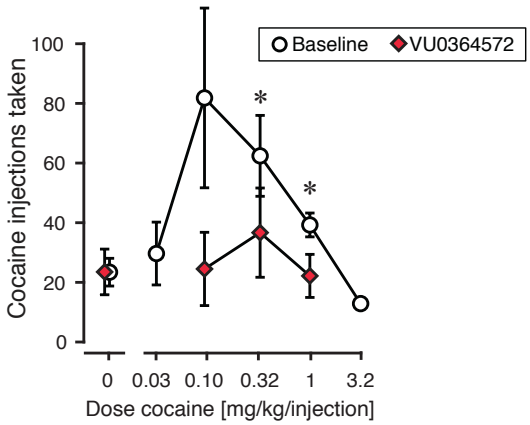


Supplemental Fig. S5

Basal level from the microdialysis study, mean of three consecutive 20-min samples.
Data are the raw measurement area under the curve of the dopamine (A, B) or glutamate (C, D) peak for mPFC (A,C) and NAc (B,D). Values are shown as group means ± s.e.m. and as individual values. One value was excluded as an extreme outlier in the NAc dopamine group.

ANOVA outcomes:

	Cocaine experience	VU0364572 vs. vehicle	Interaction
mPFC dopamine	$F(1,20)=0.32$, $p=0.58$	$F(1,20)=0.45$, $p=0.52$	$F(1,20)=1.26$, $p=0.27$
NAc dopamine	$F(1,19)=0.11$, $p=0.75$	$F(1,19)=0.35$, $p=0.56$	$F(1,19)=0.07$, $p=0.79$
mPFC glutamate	$F(1,20)=0.06$, $p=0.81$	$F(1,20)<0.01$, $p=0.98$	$F(1,20)=0.06$, $p=0.71$
NAc glutamate	$F(1,20)=0.27$, $p=0.61$	$F(1,20)<0.01$, $p=0.94$	$F(1,20)=0.01$, $p=0.91$



Supplemental Fig. S6

VU0364572 1 mg/kg pretreatment decreased intravenous cocaine self-administration acutely in a single-reinforcer assay (main effect of pretreatment p=0.003, Huynh-Feldt-corrected), in male Swiss Webster mice (Taconic Farms, Germantown, NY). * $p<0.05$ vs. baseline, false discovery rate corrected two-tailed paired-sample t-test; n=6.

Modular mouse operant conditioning chambers (MED Associates) were used, equipped with two nose-poke holes 10 mm above the grid floor, each hole with a yellow cue light. Responding in the right hole were reinforced under an FR 1 timeout 20 s schedule of reinforcement, responses in the left hole were recorded but had no other scheduled consequences. Sessions started with an automatic noncontingent delivery of the reinforcer available in the session. The mice were initially trained with liquid food (undiluted Ensure®, vanilla): mice were placed in the operant conditioning chamber daily for 2-h sessions, 5 days/week. The mice were allowed at least 3 consecutive sessions to acquire responding, and as long as needed until criteria were met (never more than 5 sessions; criteria: at least 20 reinforcers per session and at least 70% responses in the reinforced hole) Then, water was substituted for at least three sessions and until responding was extinguished to <50% of each mouse's food-maintained responding level. A jugular-vein catheter (CamCaths mivsa model) was then implanted under oxygen/sevoflorane vapor anesthesia. Step-by-step protocol of the surgical procedure is available in (Thomsen & Caine, 2005). In brief, a catheter (Silastic tubing 0.2 mm inner diameter, 0.4 mm outer diameter) was inserted 1.2 cm into the jugular vein and anchored to the vein with silk suture. The catheter ran subcutaneously to the base located above the midscapular region. Mice were allowed 7 days recovery, during which 0.02 ml of 0.9% saline containing heparin (30 USP units/ml) and antibiotic (cefazoline, 67 mg/ml) was infused daily through the catheter. Subsequently catheters were flushed with heparin-containing saline immediately before and after self-administration sessions. Catheter patency was confirmed before initiation of cocaine self-administration and after completion of each dose-effect determination by the infusion of 0.02-0.03 ml ketamine-midazolam mixture (15 + 0.75 mg/ml). Loss of muscle tone and clear signs of anesthesia within 3 s of infusion indicated catheter patency. Only data collected with demonstrated patent catheters are included. After recovery, mice were allowed to self-administer 1.0 mg/kg/infusion cocaine in daily 3-hr sessions, 5 days/week, using the same FR 1 schedule and "acquisition" criteria as for food. Saline was then substituted until extinction criteria were met (<50% of cocaine responding). Cocaine dose-effect functions were determined in each mouse with cocaine varied over successive sessions according to a Latin square design (saline, 0.03, 0.10, 0.32, 1.0, 3.2 mg/kg/infusion). Then the dose-effect function (saline, 0.10, 0.32, 1.0 mg/kg/infusion) was determined again with each session preceded 30 min earlier by IP administration 1 mg/kg VU0364572.