SUPPLEMENTAL ONLINE MATERIALS

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Supplemental Methods and Materials

Food Training

Food training was undertaken to expedite the acquisition of lever responding for un-signaled intravenous cocaine infusions at a later stage of the experiment. Rats were acclimated to handling for at least three consecutive days prior to food training. Food training occurred in operant-conditioning chambers (26 × 27 × 27 cm; Coulbourn Instruments, Allentown, PA) equipped with two levers. During the food-training session, each response on a designated lever (active lever) resulted in food reinforcement (45 mg pellets; Bio-Serv, Flemington, NJ) under a continuous reinforcement schedule. Presses on a second lever (inactive lever) did not result in food reinforcement. Training continued until the rats obtained at least 100 food pellets during a session (mean ± SEM = 1.38 ± 0.63 sessions). During food training, the rats had no access to the contextual stimuli that were used during subsequent cocaine self-administration training.

Stereotaxic and Intravenous Catheter Surgery Procedures

Rats were anesthetized using ketamine and xylazine (100.0 mg/kg and 5.0 mg/kg, i.p., respectively) 24 h after they reached the acquisition criterion for food training. Virus microinfusions were aimed at the DH (-3.4 mm AP, +/- 3.97 mm ML, -3.5 mm DV from bregma; 15°) and delivered through 5-µl Hamilton syringes fitted with 26-gauge needles, using a nanoinfusion pump mounted on the stereotaxic instrument. The needles remained at the injection site for 2 min before and for 10 minutes after each infusion. Immediately after, optic fibers were aimed at the dCA3 (-3.4 mm

AP, +/- 3.1 mm ML, -3.58 mm DV; 0°) then secured to the skull using stainless-steel screws and dental acrylic.

Up to three days after the stereotaxic surgery, rats were anesthetized using ketamine and xylazine (100 mg/kg and 5 mg/kg, i.p., respectively). Catheters were constructed in-house and implanted into the right jugular vein as described previously[22]. The catheter tubing ran under the skin and exited posterior to the rat's shoulder blades. The rats were given at least five days for post-operative recovery. The catheters were flushed daily with 0.1 ml of a cefazolin antibiotic solution (10.0 mg/ml), followed by 0.1 ml of heparinized saline (70 U/ml) to maintain catheter patency. Catheter patency was tested using propofol (1 mg/0.1 ml), which induces rapid loss of muscle tone when administered intravenously.

Post-operative Analgesia

Rats received a bacon-flavored placebo tablet (5g tablet; Bio-Serv) 24 h prior to the first surgery to minimize neophobia. Immediately after each surgery and for at least 48 h thereafter, the rats received access to bacon-flavored Rimadyl® MD tablets (2 mg carprofen/5g tablet/day; Bio-Serv) for post-operative analgesia.

Training Contexts

The operant conditioning chambers were modified to construct two distinct environmental contexts in separate behavioral testing rooms. The two contexts were used for drug self-administration and extinction training in a counterbalanced manner across rats in each treatment group. Context 1 contained a continuous red light, intermittent tone (78 dB, 10 Hz, 2 sec on, 2 sec off), a pine-scented air freshener, and wire mesh flooring (26 cm x 27 cm). Context 2 contained an intermittent white light stimulus above the inactive lever, a continuous tone stimulus

(78 dB, 2kHz, 2 sec on, 4 sec off), a vanilla-scented air freshener, and steel grid flooring bisected by a slanted ceramic tile (19 cm x 27 cm).

Optogenetics Acclimation Procedure

Immediately after extinction sessions 5, 6, and 7, rats were acclimated to the optogenetic procedure. To this end, rats were placed into novel, black metal chambers (15 x 11 x 20"). Their indwelling optic fibers were connected to fiber optic patch cables (Thor Labs). The other end of the patch cables was connected to an optical communicator (Doric Lenses, Quebec, CAN) that was suspended above the chamber. The rats remained in the chambers for 1 h without laser-light stimulation.

Brain Histology and Immunohistochemistry

Rats were overdosed with ketamine and xylazine (300/15 mg/kg, i.p., respectively) then transcardially perfused with 1X ice-cold phosphate-buffered saline (PBS, 100 ml) then 4% formaldehyde (100 ml; Sigma). Brains were dissected out, fixed using 4% formaldehyde at 4 C° for 24 h, and cryoprotected with 30% sucrose/0.1% sodium azide solution at 4 C°. The brains were stored at -80 C° until they were cut into 30-µm coronal sections.

c-Fos expression was assessed in the dCA3 (experiments 1-4) and dCA1 (experiments 1-2) in tissue collected at test. Free-floating brain sections were first blocked in 5% normal donkey and goat serum in 0.5% phosphate-buffered saline (PBS) with 3% Triton X for 30 min. The sections were then incubated with mouse anti-c-Fos (1:500; sc-271243, Santa Cruz Biotechnology, Dallas, TX) and chicken anti-GFP (1:2000; GFP697986, Aves Labs, Tigard, OR) primary antibodies at room temperature (RT) for 24 h. Next, the sections were washed in PBS and then incubated with a secondary antibody solution containing donkey anti-mouse-cy3 (1:500; 715-165-150, Jackson

ImmunoResearch, West Grove, PA) and goat anti-chicken-488 (1:1000; A11039, ThermoFisher Scientific, Waltham, MA) secondary antibodies at RT for 2 h. AT last, the sections were stained with DAPI for 5 minutes (1:1000; DAPI solution, 62248, ThermoFisher Scientific). Brain sections were mounted on glass slides, preserved with ProLong Diamond antifade mountant (ThermoFisher Scientific, Waltham, MA), and coverslipped. Three images of the dCA3, ventral to the tip of the optic fiber, were obtained using a 10x objective for each rat (Leica DMI6000 B, Leica, Wetzlar, Germany). Uniform background subtraction and contrast enhancement were applied in ImageJ. c-Fos-IR cell body density (cell bodies/mm^2) was quantified ventral to the optic fiber tract, within the SL and SP cell layers of the dCA3 and in three regions of the SP dCA1 (as shown on **Figs. 1K** and **2K**), using a custom macro in ImageJ. Cell density values were averaged across the three images and across the three regions for SP dCA3. Researchers performing cell counts were blinded to the rats' group assignment.

In experiment 5, c-Fos expression was assessed in tissue collected after memory reactivation and the 1-h optogenetic manipulation (i.e., during cocaine-memory reconsolidation), and c-Fos expression was characterized in the SL and SP, and specifically in CaMKII-IR cell populations in the SP and in GAD67-IR cell populations in the SP and SL. Brain sections were blocked in 5% normal donkey and goat serum in 0.5% phosphate-buffered saline (PBS) with 3% Triton X for 30 min. Separate sets of brain sections were then incubated with mouse anti-GAD67 (1:1000; MAB5406, Sigma-Aldrich, St. Louis, MO) or mouse anti-CaMKII (1:200; sc5306, Santa Cruz Biotechnology) primary antibodies at RT for 24 h. Next, the brain sections were incubated with AffiniPure fab fragment rabbit-anti-mouse IgG (1:100; 315-007-003, Jackson ImmunoResearch, West Grove, PA) at RT for 30 minutes to prevent cross-binding during c-Fos staining. Brain sections were then incubated with donkey anti-rabbit-680 (1:500; 926-68023, Li-Cor, Lincoln, NE) secondary antibody at RT for 2 h. Next, both sets of brain sections were incubated with mouse anti-c-Fos (1:500; sc-271243, Santa Cruz Biotechnology, Dallas, TX) primary antibody then

donkey anti-mouse-cy3 (1:500; 715-165-150, Jackson ImmunoResearch) secondary antibody. Brain sections were mounted on glass slides, preserved with ProLong Diamond antifade mountant (ThermoFisher Scientific), and coverslipped. Two confocal z-stack images were obtained using a 20x objective (14 steps, z-step size 0.35 µm; Leica SP8; Leica, Wetzlar, Germany) for each brain section. After uniform background subtraction and contrast enhancement, GAD67+c-Fos-IR and CaMKII+c-Fos-IR cell body density (cell bodies/mm^2) was visualized in the dCA3, ventral to the optic fiber tract. C-Fos-IR cell bodies were quantified, and cell density values were averaged over two z-stack max projection images using a custom macro in ImageJ. Double-labeled cells were hand counted in each step, and density values were averaged over the two z-stack images. Researchers performing cell counts were blinded to the rats' group assignment.

Statistical Data Analysis

For each experiment, potential pre-existing treatment group differences in drug infusions and lever presses during the training sessions were analyzed using mixed-factorial analyses of variance (ANOVAs) with treatment (Light-ON, Light-OFF) as between-subjects factor and time (day) as within-subject factor. Potential pre-existing group differences in lever presses during the memory-reactivation session were analyzed using independent-samples *t*-tests. Treatment effects on lever presses upon first re-exposure to the extinction and cocaine-paired contexts at test were assessed using mixed-factorial ANOVAs with treatment as between-subjects factor and context (cocaine-paired, extinction) and time (20-min intervals) as within-subject factors, where appropriate. Significant interaction and time main effects were further investigated using *post hoc* Tukey's or Bonferroni tests (adjusted α = 0.01). Treatment effects on c-Fos expression were examined using independent-samples *t*-tests. Alpha was set at 0.05.

Table S1. Behavioral History in Experiments 1-5

Experiment 1													
		Treatment Main Effects				Time Main Effects				Treatment x Time			
Phase	Measure	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*
Self-Administration[a]	Active Lever	*F*	1,15	3.69	0.07	*F*	9,135	0.45	0.9	*F*	9,135	1.56	0.13
	Inactive Lever	*F*	1,15	0.14	0.71	*F*	9,135	1.79	0.1	*F*	9,135	2.67	0.01[d]
	Cocaine Infusions	*F*	1,15	3.12	0.1	*F*	9,135	18.84	<0.001	*F*	9,135	1.89	0.06
Extinction[b]	Active Lever	*F*	1,15	1.46	0.25	*F*	6,90	35.39	<0.001	*F*	6,90	2.97	0.01[e]
	Inactive Lever	*F*	1,15	0.18	0.68	*F*	6,90	7.03	<0.001	*F*	6,90	0.4	0.9
Memory Reactivation[c]	Active Lever	*t*	15	-0.2	0.85								
	Inactive Lever	*t*	15	1.22	0.24								
Experiment 2													
		Treatment Main Effects				Time Main Effects				Treatment x Time			
Phase	Measure	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*
Self-Administration[a]	Active Lever	*F*	1,14	0.1	0.76	*F*	9,126	1.28	0.26	*F*	9,126	0.77	0.65
	Inactive Lever	*F*	1,14	0.18	0.68	*F*	9,126	1.16	0.33	*F*	9,126	0.79	0.63
	Cocaine Infusions	*F*	1,14	0.52	0.48	*F*	9,126	8.5	<0.001	*F*	9,126	0.38	0.94
Extinction[b]	Active Lever	*F*	1,14	0.02	0.88	*F*	6,84	28.14	<0.001	*F*	6,84	1.53	0.18
	Inactive Lever	*F*	1,14	3.68	0.08	*F*	6,84	9.03	<0.001	*F*	6,84	0.88	0.51
Memory Reactivation[c]	Active Lever	*t*	14	0.3	0.77								
	Inactive Lever	*t*	14	0.11	0.91								
Experiment 3													
		Treatment Main Effects				Time Main Effects				Treatment x Time			
Phase	Measure	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*
Self-Administration[a]	Active Lever	*F*	1,12	1.03	0.33	*F*	9,108	5.33	<0.001	*F*	9,108	2.21	0.03[d]
	Inactive Lever	*F*	1,12	0.11	0.75	*F*	9,108	1.15	0.34	*F*	9,108	0.86	0.56
	Cocaine Infusions	*F*	1,12	9.54	0.01	*F*	9,108	51.51	<0.001	*F*	9,108	1.28	0.26
Extinction[b]	Active Lever	*F*	1,12	0.8	0.39	*F*	6,72	17.94	<0.001	*F*	6,72	0.82	0.56
	Inactive Lever	*F*	1,12	0.27	0.61	*F*	6,72	3.84	<0.001	*F*	6,72	0.32	0.93
No-Memory Reactivation[c]	Active Lever												
	Inactive Lever												
Experiment 4													
		Treatment Main Effects				Time Main Effects				Treatment x Time			
Phase	Measure	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*
Self-Administration[a]	Active Lever	*F*	1,14	0.21	0.65	*F*	9,126	0.54	0.84	*F*	9,126	0.73	0.68
	Inactive Lever	*F*	1,14	0.46	0.51	*F*	9,126	1.81	0.07	*F*	9,126	0.9	0.53
	Cocaine Infusions	*F*	1,14	2.15	0.17	*F*	9,126	9.39	<0.001	*F*	9,126	1.23	0.28
Extinction[b]	Active Lever	*F*	1,14	0.74	0.4	*F*	6,84	9.94	<0.001	*F*	6,84	0.27	0.96
	Inactive Lever	*F*	1,14	10	0.01	*F*	6,84	2.45	0.03	*F*	6,84	0.59	0.74
Memory Reactivation[c]	Active Lever	*t*	14	0.41	0.69								
	Inactive Lever	*t*	14	-1.1	0.31								
Experiment 5													
		Treatment Main Effects				Time Main Effects				Treatment x Time			
Phase	Measure	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*	Test	*df*	Statistic	*p*
Self-Administration[a]	Active Lever	*F*	1, 9	0.41	0.54	*F*	9,81	1.97	0.05	*F*	9,81	0.52	0.85
	Inactive Lever	*F*	1,9	0.86	0.38	*F*	9,81	1.47	0.17	*F*	9,81	1.32	0.24
	Cocaine Infusions	*F*	1.9	1.48	0.26	*F*	9,81	12.8	<0.001	*F*	9,81	0.14	1
Extinction[b]	Active Lever	*F*	1,9	1.35	0.28	*F*	6,54	20.13	<0.001	*F*	6.54	1.03	0.41
	Inactive Lever	*F*	1,9	0.97	0.35	*F*	6.54	3.59	0.01	*F*	6.54	1.35	0.1
Memory Reactivation[c]	Active Lever	*t*	9	-1	0.35								
	Inactive Lever	*t*	9	0.41	0.69								

Data were analyzed using [a]2 x 10 mixed-factorial ANOVA, [b]2 x 7 mixed-factorial ANOVA, or [c]independent samples *t*-test. [d]Tukey *post-hoc* tests did not indicate subsequent treatment group effects ($p > 0.05$) at any time point despite a significant interaction effect. [e]Subsequent Laser-ON group > subsequent laser-OFF group on extinction day 1, Tukey's test, $p < 0.05$

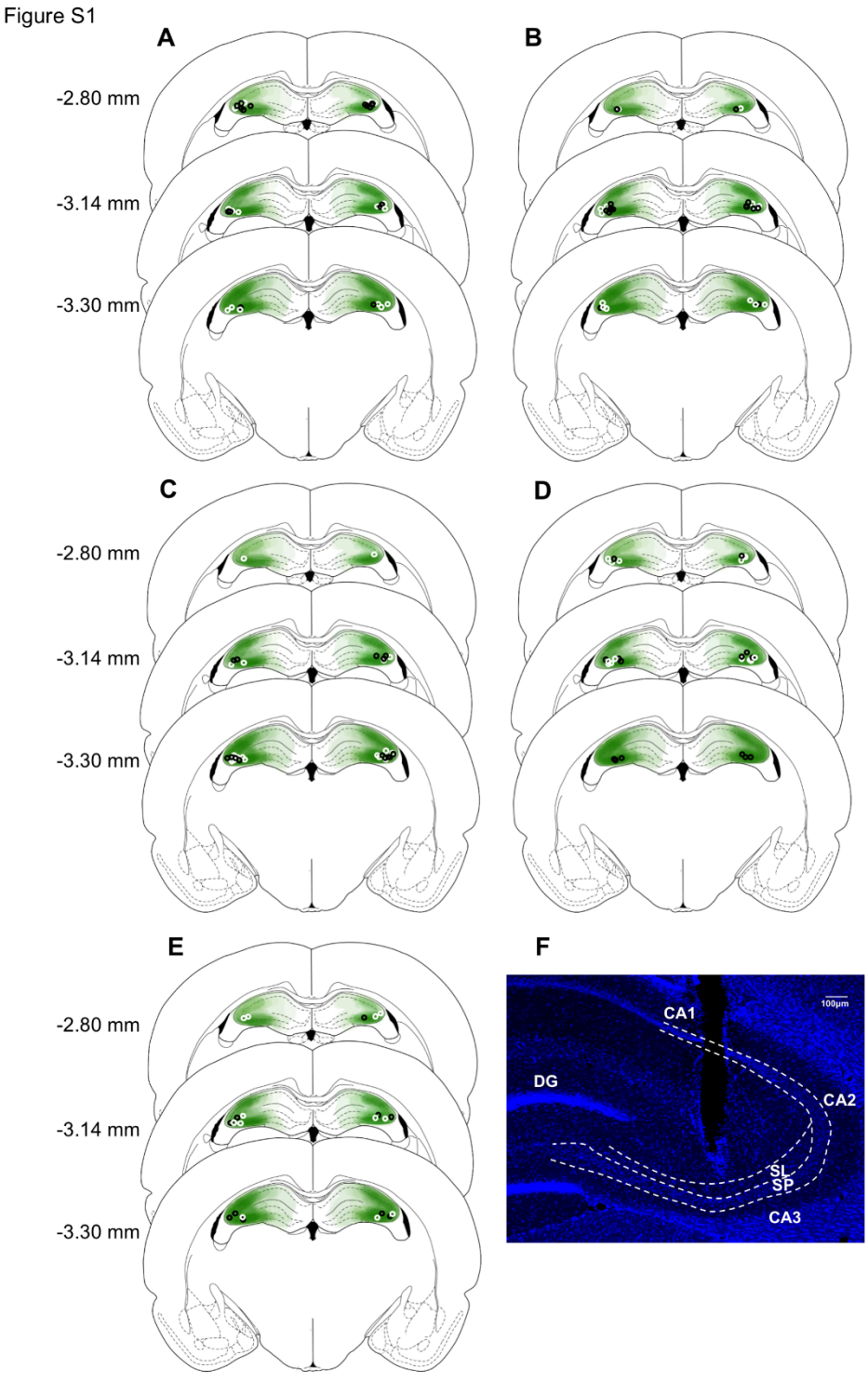

Figure S1. Schematic illustration of eNpHR3.0-eYFP expression in experiments 1 and 3- 5 (green shading in **A**, **C**, **D** and **E**, respectively), eYFP expression in experiment 2 (green shading in **B**),

and the most ventral point of optic fiber-tracts for all rats in the Laser-OFF (*black symbols*) and Laser-ON (*white symbols*) groups in each experiment. The numbers next to the schematics represent the AP distance relative to bregma. (**F**) Representative 5x photomicrograph of a DAPI-stained DH section showing an optic-fiber track in relation to the stratum lucidum (SL) and stratum pyramidale (SP) cell layers of the dCA3 and the SP cell layer of the dCA1. SP and SL boundaries were identified based on differences in cell density[25].

Figure S2

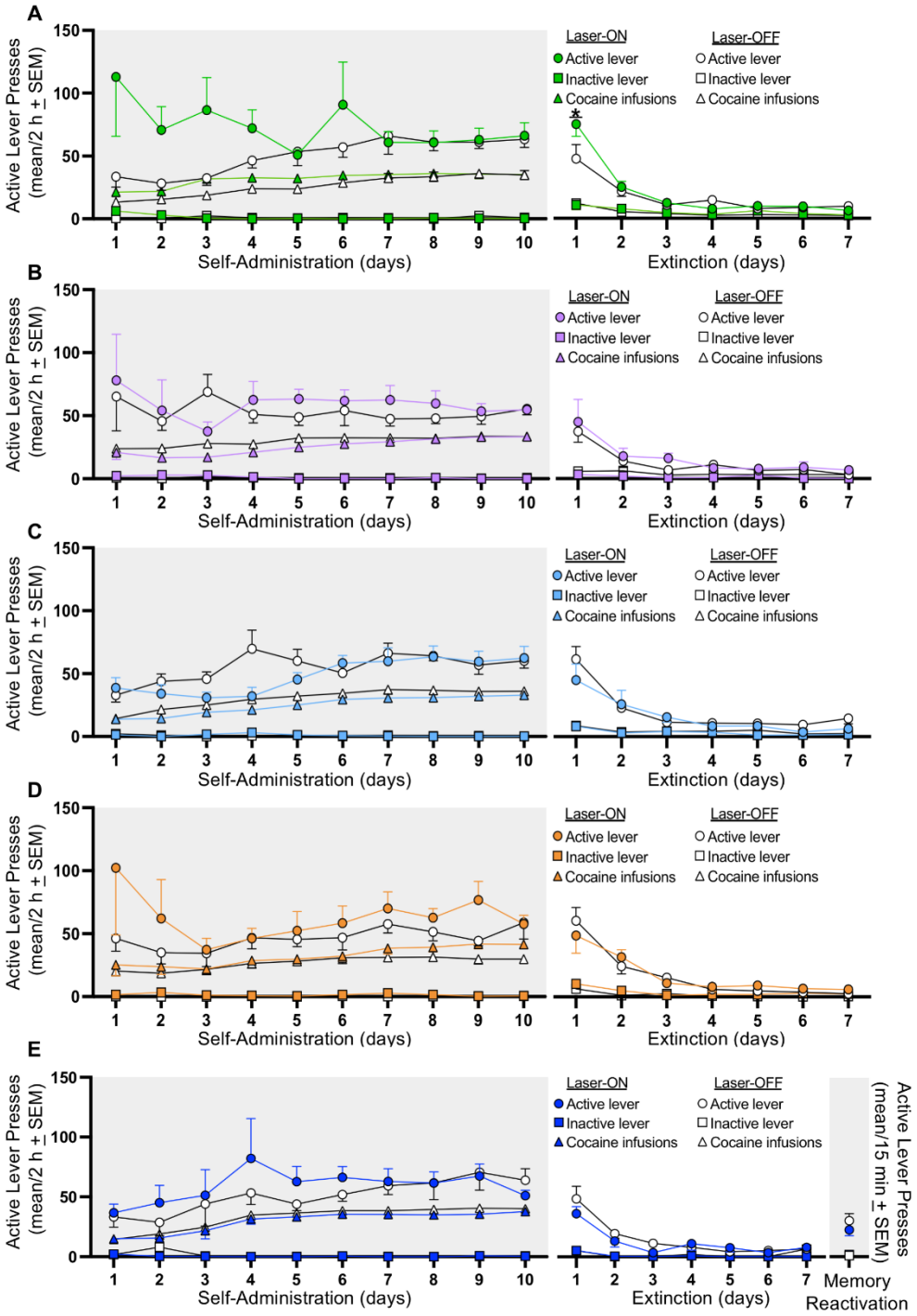

Figure S2. Behavioral history of the subsequent treatment groups in experiments 1-5. Active- and inactive-lever presses and cocaine infusions (mean/2 h ± SEM) during cocaine self-administration training (last 10 days) and extinction training (7 days) in (**A**) experiment 1, (**B**) experiment 2, (**C**)

experiment 3, (**D**) experiment 4. Active- and inactive-lever presses and cocaine infusions during cocaine self-administration training (last 10 days; mean/2h session ± SEM), extinction training (7 days; mean/2h session ± SEM), and upon exposure to the previously cocaine-paired context during the memory reactivation session (mean/15min session ± SEM) in (**E**) experiment 5. The corresponding statistics are reported in Table S1. The subsequent Laser-ON group exhibited more active-lever presses during the first extinction session in experiment 1 than the subsequent Laser-OFF group (*ANOVA subsequent treatment group x time interaction, Tukey test, $p < 0.05$).