Hypocretin Neurotransmission within the Central Amygdala Mediates Escalated Cocaine Self-Administration and Stress-induced Reinstatement in Rats

Supplemental Information

Supplemental Methods

Self-administration

Intravenous self-administration sessions were conducted in standard operant conditioning chambers (Med Associates, St. Albans, VT) as previously described (1). Briefly, rats were trained to press one of the two levers (the active lever) on a fixed-ratio 1 (FR1) schedule of reinforcement (each response resulted in fluid delivery) to obtain 0.1 ml of cocaine (0.50 mg/kg/infusion) in 1 h sessions. Reinforced responses were followed by a 20 s timeout period, in which a cue-light (above the active lever) was turned on and lever presses did not result in additional injections. Food and water were not available to the rats while in the test chambers. After the acquisition of cocaine self-administration, rats were split into two groups matched for baseline cocaine intake determined by the last three sessions of the acquisition phase and were given 1 h (short access; ShA) or 6 h (long access; LgA) of access to FR1 cocaine self-administration. Rats were allowed 14 days of daily escalation sessions, at the end of which ShA rats displayed stable levels of cocaine self-administration, whereas LgA rats displayed an escalation of cocaine intake as repeatedly reported by our laboratory (1–4; Figure S1). For testing under a progressive ratio (PR) schedule of reinforcement, the response requirement began at 1 response/injection and increased according to the following

equation: response/injection = [5 x e $^{(\text{injection number x } 0.2)}$]-5 (e.g., series ratios: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, etc.; 5). When a rat failed to achieve the response requirement within 1 h, the session ended, with a maximum session length of 6 h.

Intracranial Infusions

For intra-CeA testing, on the day before beginning testing, stylets were removed and a mock infusion was conducted (30-gauge needle insertion followed by 10 min waiting period). This permitted acclimation to the mild restraint associated with the infusion and minimized tissue damage associated with insertion of a needle on the day of testing. On the day of testing, a 30-gauge needle was connected to PE20 tubing filled with water, and the needle loaded with vehicle (DMSO) or the HCRT-R1 antagonist, SB-334867, dissolved in vehicle with a 50 nl air bubble separating vehicle/drug from water. The infusion needle was inserted into the cannula, extending 2.0 mm beyond the end. Infusions (250 nl, 125 nl/min) were made 15 min prior to behavioral testing using a microprocessor-controlled pump (KD Scientific; Holliston, MA).

Potential Side Effects of HCRT-R1 Antagonism

In the current studies, there were no apparent signs of sedation or motor impairment following administration of the HCRT-R1 antagonist, SB-334867, at any dose tested. Rats generally appeared active with no discernible behavioral side effects. This is consistent with previous studies demonstrating administration of either SB-334867 or another HCRT-R1 antagonist (SB-408124) at similar doses elicits no significant effect on sleep/wake state (6–8).

Electrophysiological Recordings

Coronal slices (300 µm) were prepared as previously described (9) in an ice-cold high-sucrose solution containing (in mM): sucrose, 206; KCl, 2.5; $CaCl_2$, 0.5; $MgCl_2$, 7; NaH_2PO_4, 1.2; $NaHCO_3$, 26; glucose, 5; and HEPES 5 using a vibrating microtome (Leica VT1000S, Leica Microsystems, Buffalo Grove, IL). Slices were incubated in an oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (aCSF) solution composed of the following (in mM): NaCl, 130; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4$•7H2O, 1.5; $CaCl_2$, 2.0; $NaHCO_3$, 24; and glucose, 10 for 30 min at 35-37 °C, followed by 30 min equilibration at room temperature (21–22 °C). Following equilibration, a single slice was transferred to a recording chamber mounted on the stage of an upright microscope (Olympus BX50WI). Neurons were visualized using infrared differential interference contrast (IR-DIC) optics and an EXi Aqua camera (QImaging, Surrey, BC, Canada). Whole-cell voltage-clamp recordings were made with patch pipettes (4-6 MΩ; Warner Instruments) coupled to a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA), low-pass filtered at 2-5 kHz, digitized (Digidata 1440A; Axon Instruments), and stored on a computer using pClamp 10 software (Axon Instruments). Series resistance was typically <10 MΩ and was continuously monitored with a hyperpolarizing 10 mV pulse. The intracellular solution used for all recordings was composed of (in mM): KCl, 145; EGTA, 5; $MgCl_2$, 5; HEPES, 10; Na-ATP, 2; and Na-GTP, 0.2. Drugs were dissolved in aCSF and applied by bath superfusion. To isolate inhibitory currents mediated by $GABA_A$ receptors, recordings (V_{hold} = -60mV) were performed in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 µM), DL-2-amino-5-phosphonovalerate (AP-5, 30 µM) and CGP55845A (1 µM). As demonstrated previously

(10), the inhibitory postsynaptic currents recorded under these conditions can be blocked by gabazine, a $GABA_A$ receptor antagonist. All voltage-clamp recordings were performed in a gap-free acquisition mode with a sampling rate per signal of 10 kHz or a total data throughput equal of 20 kHz (2.29 MB/min) as defined by pClamp 10 Clampex software.

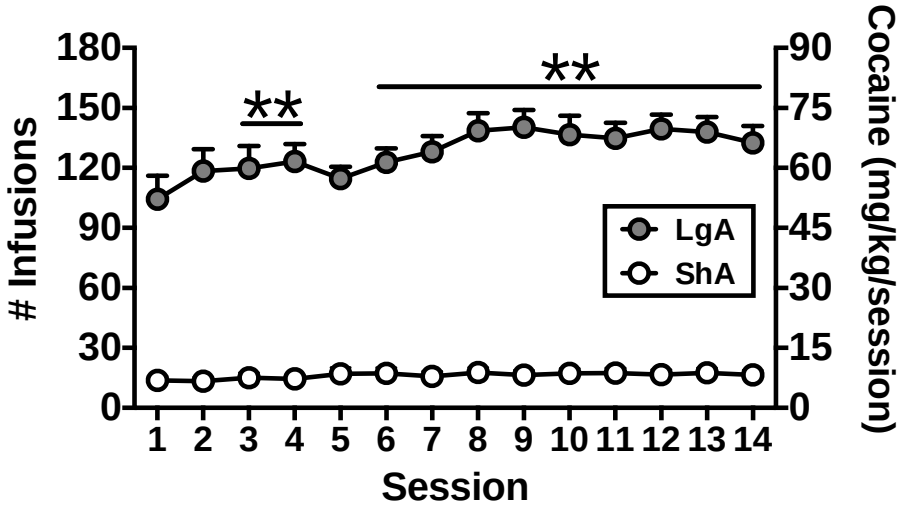

Figure S1. Cocaine self-administration under a fixed-ratio schedule of reinforcement (FR1) during the escalation period. Data are expressed as mean number of infusions (+ SEM) during each daily session for short access (1 h; ShA; *n* = 10) and long access (6 h; LgA, *n* = 9) groups. Right axis indicates total amount of cocaine (mg/kg) per session. Rats allowed LgA to cocaine self-administration significantly increased number of cocaine infusions over progressing sessions, whereas number of infusions for ShA rats remained stable over time (Group: $F_{(1,17)}= 210.8$, $p < 0.001$; Session: $F_{(13, 221)}= 8.03$, $p < 0.001$; Group x session: $F_{(13, 221)}= 5.64$, $p < 0.001$). **p < 0.01 versus Session 1 by repeated measures two-way ANOVA, Dunnett's multiple-comparison test.

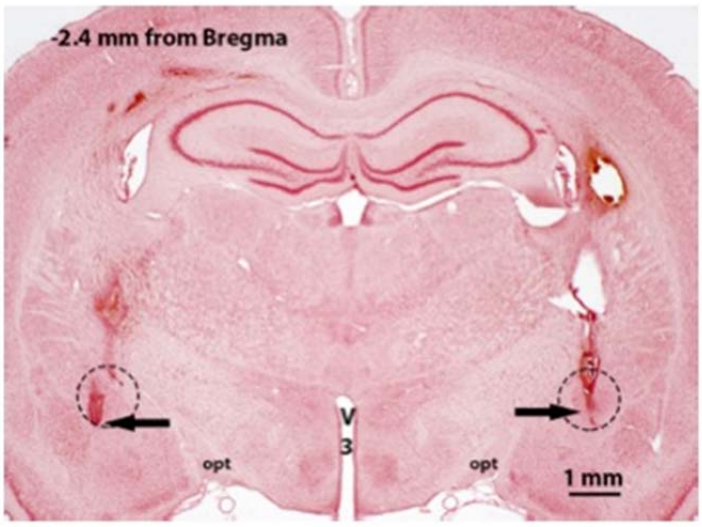

Figure S2. Representative photomicrograph of central amygdala (CeA) infusion sites in a neutral red stained coronal section (-2.4 mm from Bregma). The most ventral extent of the infusion needle tracks (arrow) is shown within the CeA region (demarcated by dashed circle). Bilateral infusions of the HCRT-R1 antagonist SB-334867 (0, 10, and 20 nmol) were made over a 2 min period. opt, optic tract; V3, third ventricle.

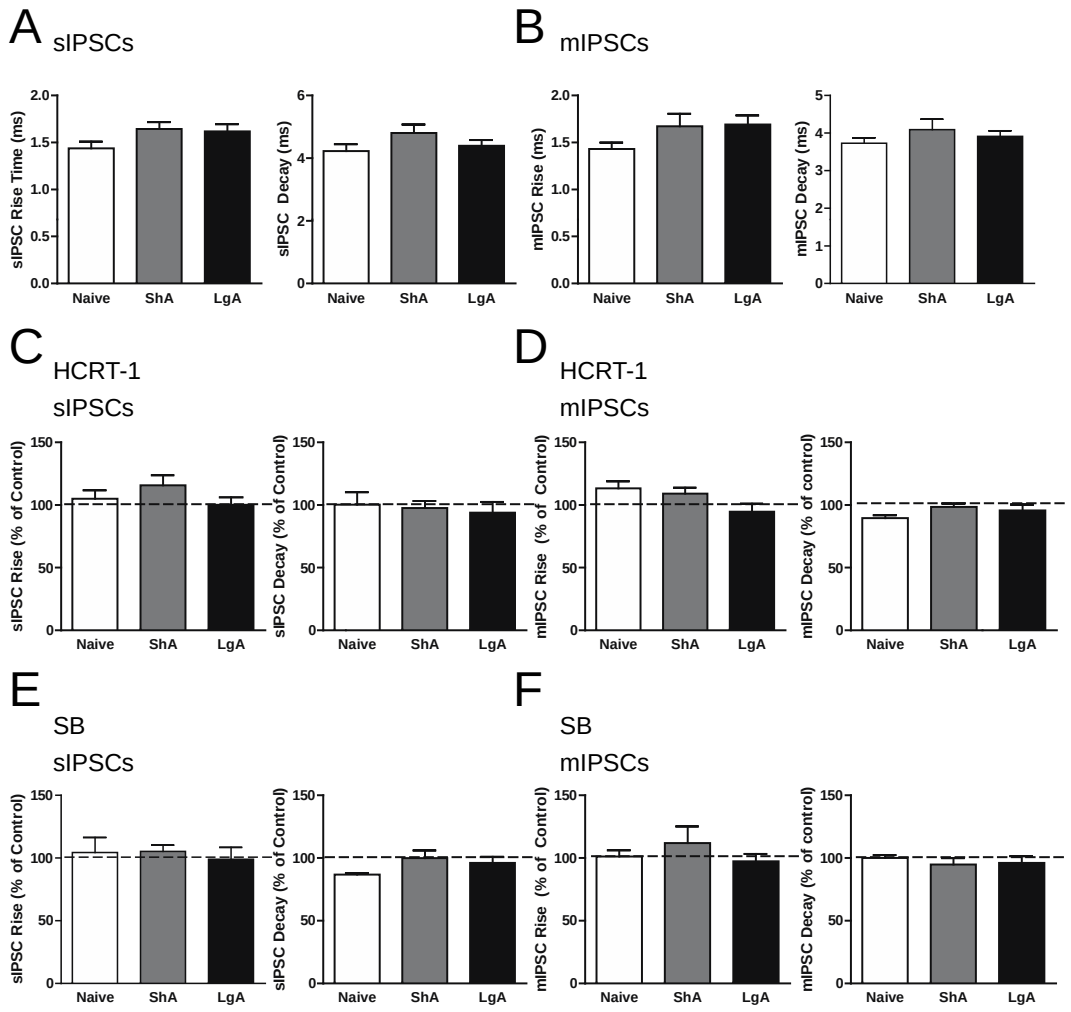

Figure S3. A. Average baseline sIPSC rise ($F_{(2,81)}$= 2.22, n.s.; left panel) and sIPSC decay ($F_{(2,81)}$= 1.66, n.s.; right panel) in CeA neurons from naïve, ShA, and LgA (*n* = 27, 27, and 30, respectively) rats. **B**. Average miniature IPSC (mIPSC) rise ($F_{(2,35)}$= 1.75, n.s.; left panel) and mIPSC decay ($F_{(2,35)}$= 0.87, n.s.; right panel) in CeA neurons from naïve, ShA, and LgA (*n* = 15, 13, and 13, respectively) rats. **C**. Average change in sIPSC rise ($F_{(2,15)}$= 1.31, n.s.; left panel) and sIPSC decay ($F_{(2,15)}$= 0.15, n.s.; right panel) in CeA neurons from naïve, ShA, and LgA (*n* = 6 each group) rats following superfusion of HCRT-1. **D**. Average change in mIPSC rise ($F_{(2,16)}$= 2.91, n.s.; left panel) and mIPSC decay ($F_{(2,16)}$= 2.00, n.s.; right panel) in CeA neurons from naïve, ShA, and LgA (*n* = 8, 6, and 6, respectively) rats following superfusion of HCRT-1. **E**. Average change in sIPSC rise ($F_{(2,18)}$= 0.17, n.s.; left panel) and sIPSC decay ($F_{(2,18)}$= 1.41, n.s.; right panel) in CeA neurons from naïve, ShA, and LgA (*n* = 5, 8, and 8, respectively) rats following superfusion of SB-334867 (SB). **F**. Average change in mIPSC rise ($F_{(2,15)}$= 0.70, n.s.; left panel) and sIPSC decay ($F_{(2,15)}$= 0.37, n.s.; right panel) in CeA neurons from naïve, ShA, and LgA (*n* = 6 each group) rats following superfusion of SB.

Supplemental References

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