AMPA Receptor Plasticity in Accumbens Core Contributes to Incubation of Methamphetamine Craving

Supplemental Information

Supplemental Methods

Subjects and Surgery

All experimental procedures were approved by the Rosalind Franklin University Institutional Animal Care and Use Committee in accordance with the USPHS Guide for Care and Use of Laboratory Animals. Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN), 250-275 g upon arrival, were housed 3/cage under a reverse 12-h light-dark cycle (food and water freely available). Rats were acclimated to the animal colony for at least 5 days before intravenous catheter surgery. As described previously (1,2), rats were anesthetized with ketamine-xylazine (80-10 mg/kg, i.p., respectively). A silastic catheter (Plastics One, Roanoke, VA) was inserted into the right jugular vein and passed subcutaneously to the mid-scapular region. For rats destined for the experiment using 1-naphthyl acetyl spermine (naspm), following jugular catheterization, guide cannulae (23-gauge, Plastics One) were implanted bilaterally 1.5 mm above the NAc core. Coordinates (3) were: AP -1.4 mm, ML ±2.5 mm (6° angle), DV -5.5 mm. Banamine was administered as an analgesic after surgery and the rats were allowed to recover for 6-8 days. During recovery and self-administration training, catheters were flushed every 24-48 h with 0.9% sterile saline or cefazolin (15 mg, i.v.; Webster Veterinary Supply, Devens, MA) in sterile saline and rats were singly housed. The experiments consisted of three phases: self-administration training, a withdrawal period, and either electrophysiological recordings or tests for cue-induced methamphetamine seeking.

Drug Self-Administration Training

Rats were trained to self-administer methamphetamine or saline (control condition) during a total of 10 daily sessions, each lasting 6 h, conducted over 11-12 days with 1-2 days off, under a fixed-ratio-1 reinforcement schedule. Methamphetamine was dissolved in saline and self-administered at a dose of 0.1 mg/kg/infusion (0.065 mL/infusion). Control rats were trained to self-administer saline (0.065 mL/infusion) under the same schedule. Self-administration sessions began at the onset of the dark cycle and were conducted in operant chambers equipped with two nose-poke holes. Active hole responses activated the infusion pump and led to the delivery of a 20-sec light cue (white light illuminating the active hole). Each infusion was followed by a 20-sec timeout period during which nose pokes were recorded, but did not result in drug delivery. Nose poking in the inactive hole had no consequences but was recorded as a measure of nonspecific behavioural activation. Any rats that did not learn to self-administer methamphetamine and/or had faulty catheters were euthanized. During the withdrawal phase, rats remained in home cages and were handled 2-3 times per week.

Naspm Injections Into the NAc Core

Intracranial injections of naspm (40 µg/site, injection volume 0.5 µl) were made with 10-µl Hamilton syringes (Hamilton Co., Reno, NV) that were connected via polyethylene-50 tubing to 30-gauge injectors (Plastics One, Roanoke, VA). The intracranial dose of naspm was based on our previous studies (1). Naspm or vehicle was infused over 1 min and the injector was left in place for an additional 1 min to allow for diffusion. Rats were returned to their cages for 15 min and then placed in the operant chamber for the 30-min seeking test. Each rat received only one intra-NAc infusion (naspm or vehicle). After the experiment, cannulae placements were verified in Cresyl Violet-stained sections.

Whole-Cell Patch-Clamp Recordings

Electrophysiological procedures were performed as described previously (1,2,4-7). All MSN were recorded from the NAc core subregion. Briefly, rats were anesthetized with chloral hydrate (400-600mg/kg, i.p.) and brains were rapidly removed. Coronal slices (350 μm) were cut with a vibrating microtome in ice-cold oxygenated (95% O_2-5% CO_2) artificial cerebrospinal fluid solution (aCSF) containing (in mM): 122.5 NaCl, 20 glucose, 25 $NaHCO_3$, 2.5 KCl, 0.5 $CaCl_2$, 3 $MgCl_2$, 1 NaH_2PO_4, 1 ascorbic acid. Slices were then incubated in warm aCSF (32-34°C) for at least 1 h before transferring into the recording chamber. In the recording aCSF, $CaCl_2$ was increased to 2.5 mM and $MgCl_2$ was reduced to 1 mM. Both picrotoxin (0.1 mM) and (2R)-amino-5-phosphonopentanoate (0.05 mM) were added into the recording aCSF to pharmacologically isolate AMPAR transmission. All recordings were conducted at 32-34°C using patch pipettes (6-8 MΩ) filled with a Cs-based/spermine-containing internal solution (in mM): 140 CsCl, 10 HEPES, 2 $MgCl_2$, 5 NaATP, 0.6 NaGTP, 2 QX-314, 0.1 spermine. A bipolar tungsten stimulating electrode placed ~200 μm from the recording site was used to elicit excitatory postsynaptic currents in MSN. Only neurons that exhibited a stable synaptic response (<15% variability) during 15 min of baseline recording were included.

Reagents

All chemicals were purchased from Tocris (Minneapolis, MN), except for picrotoxin (Sigma-Aldrich, St. Louis, MO). Methamphetamine was obtained from the National Institute on Drug Abuse and dissolved in 0.9% saline. The selective mGlu1 PAM SYN119 (9H-Xanthene-9-carboxylic acid (4-trifluoromethyl-oxazol-2-yl)-amide), also referred to as compound 14a (8) or Ro0711401 (9), was a generous gift from Dr. M. Foster Olive (University of Arizona) and was synthesized by EAG Labs (Tempe, AZ).

Drug Solutions

For systemic administration, SYN119 was dissolved in vehicle consisting of 10% Tween-80 (vol/vol, #P4780, Sigma-Aldrich) in 0.9% saline. For patch-clamp recordings, SYN119 was dissolved in 100% DMSO as a stock solution (stored at −20°C) and used at a final concentration of ≤0.05% DMSO (vol/vol). Naspm was dissolved in 1x phosphate buffered saline for intracranial infusions and diluted into aCSF for patch-clamp recordings.

References for Supplemental Methods

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