Supporting Information

Ohtsuka *et al.* 10.1073/pnas.0806180105

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X-Gal Staining. The Dlx 5/6-Cre line was also crossed with a reporter line that has *LacZ* between the *β-actin* promoter and the stop cassette flanked by two *loxP* sequences (1) to monitor the recombination pattern. The double transgenic mice were perfused transcardially with 4% PFA in 0.1M phosphate buffer (PB, ph7.4) and subjected to cryoprotection procedure. Sagittal and coronal serial sections were prepared by cryostat at 35 μm for X-gal staining. Sections were incubated in 1 mg/ ml X-gal, 4 mM $K_4Fe(CN)_6H_2O$, 4 mM $K_3Fe(CN)_6$ and 2 mM $MgCl_2$ at 37°C for 18 h. Since the P16 conditional knockout pups had a smaller body size, their brain size was also slightly smaller.

Western Blotting. For NR1 immunoblotting, the crude synaptosome fraction was used. P16 mice pups were decapitated and brains were placed on an ice-cold glass plate then the striatum, hippocampus and cortex were dissected. Tissue was homogenized in 0.32 M sucrose, 5 mM Tris·HCl, pH 7.4 and centrifuged at 800×*g* for 10 min. The resulting supernatant was further centrifuged at either 10,000×*g* for 20 min. The pellet was resuspended in 10 volume of the same buffer and centrifuged again. Protein samples were separated on 4% SDS/PAGE gel. Anti-NR1 C-terminal region rabbit polyclonal antibody was used as a primary antibody (provided by M. Watanabe). Next the blot was subjected to a secondary antibody reaction with anti-rabbit IgG-HRP from Upstate at a dilution of 1:50,000. After detection using ECL plus from Amersham, all of the antibodies were stripped in Reblot Plus Mild Antibody Stripping Solution from Chemicon, then incubated with anti-*β*-actin-HRP monoclonal antibody from Sigma and developed as described above.

***In Vitro* Brain Slice Recording.** Sagittal slices of the ventral striatum (240 μm) were prepared as described (2). Slices were recovered in a holding chamber at least 1 h before use. During recording, they were superfused with artificial cerebrospinal fluid (22–23°C) saturated with 95% O_2/5% CO_2 and containing 119 mM NaCl, 2.5 mM KCl, 1.0 mM NaH_2PO_4, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$ and 11 mM glucose. Picrotoxin (100 μM) was added to block $GABA_A$ receptor-mediated IPSCs. Cells were visualized using IR-DIC optics and MSNs were identified by their morphology and high resting membrane potential (−75 to −85 mV). To assess excitatory synaptic transmission, neurons were voltage-clamped at −80 mV using a Multiclamp 700A amplifier (Axon Inst.). Electrodes (3–5 MΩ) contained 117 mM cesium gluconate, 2.8 mM NaCl, 20 mM Hepes, 0.4 mM EGTA, 5 mM TEA-Cl, 2 mM MgATP and 0.3 mM MgGTP, pH 7.2–7.4 (285–295 mOsm). Series resistance (10–40 MΩ) and input resistance were monitored on-line with a 4 mV depolarizing step (100 ms) given with each afferent stimulus. Afferents were stimulated at 0.1 Hz by glass monopolar microelectrodes placed at the prelimbic cortex–ventral striatal border. Data were filtered at 2 kHz, digitized at 5 kHz, collected and analyzed using custom software (Igor Pro, Wavemetrics). NMDAR/AMPAR ratios were computed from EPSCs at +40 mV with or without 50 μM D-AP5 as described (2). Traces in figures have had stimulus artifacts removed and are averages of consecutive responses collected during 2–5 min of recording.

***In Vivo* Local Field Potential Recording.** The surgery and construction of multielectrodes in stereotrode format are similar to methods previously described by our group (3). In these experiments, the headstages, which had a total weight of 0.3 g, were modified to minimize the weight. P16 pups were anesthetized with an i.p. injection of 37.5 mg/kg of ketamine (Bedford Laboratories) and 0.5 mg/kg of dormitor (Pfizer Animal Health). The mouse's head was immobilized in a stereotaxic frame, and its eyes were coated with sterile ocular lubricant (Puralube Vet Ointment, Pharmaderm). After the hair above the skull had been removed, Betadine solution was applied to the skin surface, and an incision was made along the midline of the skull. The edges of the cut skin were held to the sides with small clips, and the membranous layer was removed to expose the skull. Hydrogen peroxide was applied to the skull surface to permit visualization of the bregma and the lambda positions along the midline. The positions for the two bundles were 1.8 mm lateral to bregma and 0.6 mm rostral to the bregma on both the right and left sides based on the adult animal (4) and scaled accordingly. Holes for the two stereotrode bundles were then drilled, and the dura was removed carefully. The stereotaxic apparatus was then used to lower the stereotrode bundles into these holes and gradually inserted at a depth of 3 mm below the surface of the brain. The gaps surrounding the stereotrodes were filled with softened paraffin and the headstage was stabilized with dental cement. One end of the reference wire was attached to the dura through a small hole and the other end was soldered to the reference wire affixed to the connector pin arrays of the headstage, and copper mesh was wrapped around the entire headstage to protect the wires from potential damage. The connector pin arrays on the headstage were first attached to preamplifiers with extended cables to allow for the monitoring of neuronal signals using the Plexon system. A helium-filled mylar balloon was tied to the cables for alleviating the weight of the apparatus and cables, thereby enabling the mouse to move freely. The mouse was then aroused with an injection of 1.25 mg/kg Antisedan and returned to its home cage. The mouse was allowed to recover completely at least for 2 h before recordings. The recovery was validated by its ambulatory activity, and the survival rate for the surgery was 100%. Local field potentials were recorded as previously described (3, 5). At the end of the experiments, the mouse was anesthetized and a small amount of current was applied to four channels in the headstage to mark the positioning of the electrodes. The actual electrode positions were confirmed by histological staining using 1% cresyl echt violet.

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2. Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001) Long-term depression in the nucleus accumbens: A neural correlate of behavioral sensitization to cocaine. *Nat Neurosci* 4:1217–1223.
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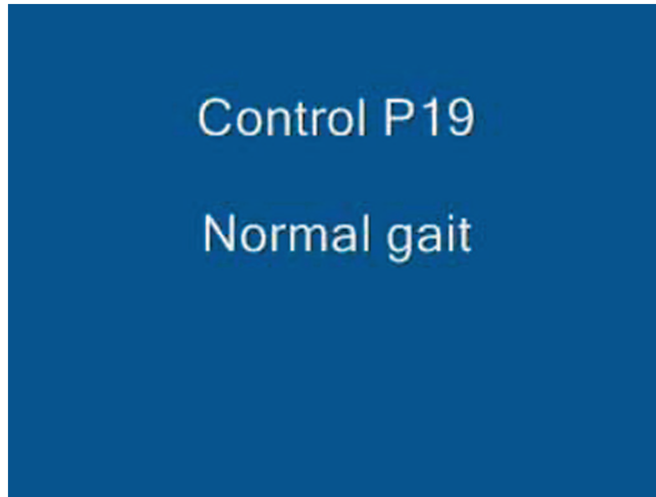
5. Lin L, *et al.* (2005) Identification of network-level coding units for real-time representation of episodic experiences in the hippocampus. *Proc Natl Acad Sci USA* 102:6125–6130.



Movie S1. P19 mutants started to show abnormal gait with their hind legs that are often sprawled while any obvious abnormal voluntary movements such as axial and limbic abnormality was not observed until P18.

Movie S1 (MP4)

Movie S2. STR-KO mutants are impaired to terminate nipple-suckling. This movie shows an example of a P16 mutant. When pups were suckling from their mother's nipples and the mother was gently lifted, mutant pups hung onto the mother by biting nipples for more than 10 seconds while all control litter mates immediately released. This phenotype becomes severe at P16 ($P<0.0001$, Fisher's exact probability test). This indicates dysfunction of the oral motor control in mutants.

Movie S2 (MP4)