Supplemental Information

Supplementary Methods

Assessment of vaginal estrus condition

Every morning between 8:00-9:00am, the vaginal estrus condition of each female donor was assessed. Vaginal secretions were collected with a plastic pipette filled with 10 µl of saline (NaCl 0.9%) by inserting the tip into the mouse vagina. Vaginal fluid was transferred on a glass slide and mounted under a cover-slip with a trace of methylene blue to add contrast and bring out the nuclei. The four stages (proestrus, estrus, metaestrus and diestrus) of the estrus cycle were determined by examining the proportion and morphology of leukocytes and epithelial cells present in the smear under 400× magnification of a light microscope (1). To obtain urine from the female mice in estrus, we placed female donor mice on a flat surface covered with clean aluminum foil while holding them by skin on the nape. Handling was usually sufficient to induce urination, but if not the ventral area was stroked in an anterior-to-posterior direction, which was usually effective (2). The urine on the foil was collected immediately, and 60 µl were pipetted directly from the aluminum surface onto a clean cotton swab (3). The experimenter wore gloves at all times, and the aluminum foil was replaced after every urine collection.

Novel odors and the FUST paradigm

We assessed the duration of sniffing for two novel odors—banana and almond—and found that time spent sniffing both odors was very short compared to time spent sniffing estrus female urine. In addition, no USVs were detected when mice were exposed to the novel banana and almond odors. We extended the experiment using a novel odor (almond) to a larger group of animals (n=10 in each group) to contrast the sniffing preference of rodents for female estrus urine vs. almond extract, and obtained similar results to those seen in the preliminary

experiments that used water as a control (Figure S1). Given the lack of effect of these other odors, we chose to use water as a control in the FUST paradigm in order to avoid mixing the odors presented to the animals (almond versus urine).

Surgery and microdialysis

Surgery and microdialysis were conducted as previously described (4, 5). Male 129S1/SVImJ mice (n=5) were anesthetized by inhalation of 3% isoflurane in oxygen and placed in the stereotaxic apparatus. A unilateral 6 mm long guide shaft (cannula) made of 21-gauge stainless-steel tubing (Plastics One, Roanoke, VA) was stereotaxically implanted, aimed at the NAc (coordinates: A 10.0 mm, L 1.2 mm, V 4.0) with respect to bregma and the midsagittal sinus, and fixed to the skull using cranioplast and two jeweler screws (6). All mice recovered for at least one week prior to the experiments.

A microdialysis probe (4) was inserted through the guide cannula. The probe (active membrane, MW cutoff 13 kDA) protruded 2 mm from the base of the guide cannula shaft so that it reached the NAc. Inlet tubing was attached to a Hamilton syringe mounted on a microinfusion pump (Stoelting, Wood Dale, IL). In vivo microdialysis probes were perfused continuously (1 µl/minute) with artificial CSF (136 mM NaCl, 3.7 mM KCl, 1.2 mM CaCl2, 1.0 mM MgCl2, 10.0 mM NaHCO3 at pH=7.4). Probes were inserted and fixed in place at least four hours before each experiment to allow stabilization of the neurotransmitter recovery. Samples were collected for two minutes in polyethylene tubes containing 1 µl of 0.1 M HCl and 100 mM EDTA. Three microdialysate samples per animal per time point (a total of 15 samples) were collected at the following time points: 1) baseline, 2) during water exposure, 3) between water and urine exposure, 4) during urine exposure, and 5) after removing the cotton applicator.

Dopamine levels were determined in dialysates immediately after collection using high pressure liquid chromatography with electrochemical detection (HPLC-EC) with a microbore column (100 x 1 mm, 3 μm particle, ODS column from Bioanalytical Systems, West Lafayette, IN). To elute analytes, a mobile phase consisting of 75 mM sodium phosphate, 1.55 mM octane sulfonic acid, 1 mM EDTA, and 9% acetonitrile (vol/vol) was conducted and analyzed in a radial flow carbon working electrode; a Ag/AgCl reference electrode was set at an oxidation voltage of 700 mV in a digital detector (Epsilon, Bioanalytical Systems). The average retention time for dopamine was seven minutes, and the detection range was 2 nA. Animals with probe misplacement were excluded before measurement. Figure S2 provides descriptive data for levels of dopamine release in the NAc after exposure to the urine-dipped applicator for the four different mouse groups (WT vs GluR6KO, and saline vs chronic lithium-treated (21 days)).

The LH paradigm

The LH paradigm comprises three parts: deficit induction using uncontrollable, inescapable foot-shocks of varying intervals; deficit screening; and deficit re-testing (after a period of spontaneous recovery or treatment) (7, 8). The key deficit measure is helplessness, defined as 20 failures to escape over 30 foot-shock escaping trials in the screening and re-testing sections. Roughly 50-80% of mice display helplessness behavior after induction (7, 8).

The LH paradigm was conducted as previously described (8). Briefly, a Gemini Avoidance System (San Diego Instruments, San Diego, Ca) was used to assess this behavior in manipulation-naïve mice (C57BL/6J; n=15). Induction profile: 180 inescapable shocks (0.3 mA, 15-second duration, at random intervals with an average of 30 seconds); screening profile: 30 trials (0.3 mA; three-second duration for conditioned stimulus and three-second duration for unconditioned stimulus, conducted at random intervals with a mean of 30 seconds); testing

profile: 30 trials (0.3 mA; three-second duration for conditioned stimulus and 15-second for unconditioned stimulus, conducted at random intervals with a mean of 30 seconds). The number of escape failures and latency to escape were recorded for each mouse. Mice were defined as having developed helplessness when they showed at least 20 escape failures. FUST behavior was compared between LH and NLH mice.

Saccharin preference test

In order to compare the reward-seeking behavior of GluR6KO mice in the FUST to a widely used non-operant appetitive paradigm, we conducted the saccharin preference test in GluR6KO mice and their WT controls (n=13 per group). Animals were given one bottle of tap water and one of saccharin (Sigma, St. Louis, MO) diluted in tap water to a final concentration ranging from 0.005 to 0.35% overnight (7pm-10am). Bottle position was switched daily to control for any side preference. Total consumption of water and saccharin was measured in grams, and the percentage of saccharin preference per day was calculated as follows: (consumed saccharin*100)/(consumed water + consumed saccharin).

Figure S1. Time spent sniffing female urinary pheromone odors vs. almond scent by wild-type (WT) and GluR6 knockout (GluR6KO) mice.

In an attempt to explore whether estrus female urine had a different effect on reward-seeking behavior compared with other novel stimuli, we conducted an experiment using a clear almond extract (McCormick, MD) rather than water versus estrus female urine. One hour before the test, rodents were habituated to a sterile cotton-tipped applicator inserted into their home cage. For the test, rodents were transferred to a dimly-lit room (~3 lux lighting). The test had three phases: (1) one exposure (three minutes) to the cotton tip dipped in almond scent, during which sniffing duration was measured; (2) an interval of 45 minutes during which no cotton tip was presented to the animal; and (3) one exposure (three minutes) to a cotton tip applicator infused with fresh urine collected from females of the same strain in estrus, during which time spent sniffing the cotton was measured. Sniffing duration was measured in GluR6KO mice and their WT littermates (n=10). Two-way repeated measures ANOVA was used to assess differences between these groups.

Both GluR6KO and WT males spent more time sniffing urine than almond scent in the FUST paradigm [water vs. almond scent: $F(1,18)=91.89$; $p<0.01$]. Overall, GluR6KO mice spent

more time sniffing the cotton tipped applicator than did the WT mice [genotype: $F(1,18)=4.46$, $p<0.05$]; however, a significant interaction revealed that GluR6KO mice sniffed the female urine more, and spent less time sniffing the almond scent than their WT controls [$F(1,18)=6.95$; $p<0.01$; Bonferroni post-hoc; almond scent $t=0.3$; N.S.; Urine $t=3.29$; $p<0.001$].

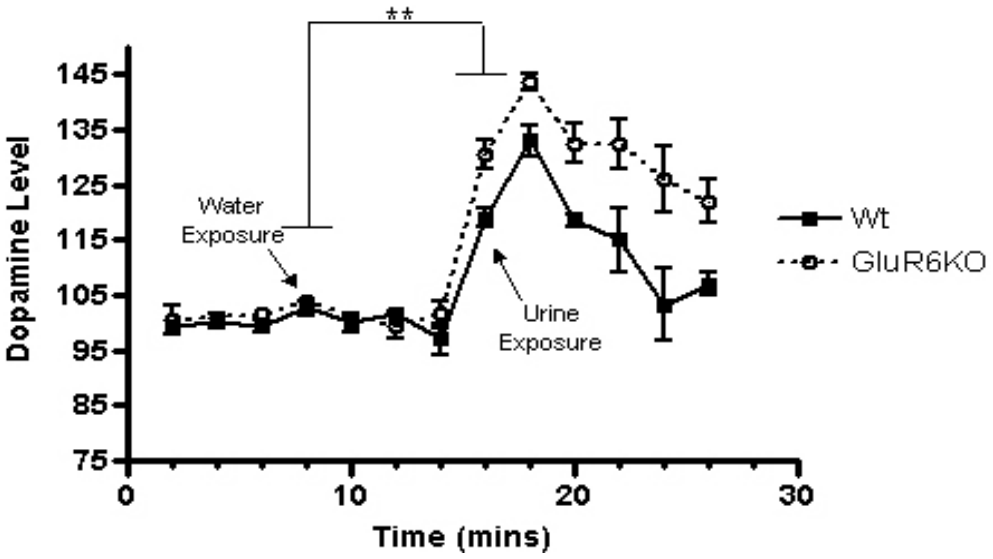

Figure S2. Changes in NAc extracellular dopamine levels during the FUST.

NAc extracellular dopamine levels during the FUST were determined for two different groups of mice: 129S1/SVImJ wild-type (WT) vs. GluR6 knockout (KO) mice. Differences in dopamine levels were analyzed by two-way repeated measures ANOVA. Overall mice displayed significantly elevated levels of dopamine released in the NAc after exposure to the urine-dipped applicator vs. after exposure to the water-dipped applicator [$F(5,15)=66.72$; $p<0.01$]. In addition, GluR6KO mice had higher levels of NAc extracellular dopamine than WT mice [$F(1,3)=15.14$; $p<0.05$]. The interaction between the strains and the FUST periods were significant [$F(5,15)=5.78$; $p<0.01$]. These interactions show that the differences between the WT and the GluR6KO mice occurred during periods of urine exposure as well as the two periods after them. This suggests that as a result of urine exposure, GluR6KO mice exhibited higher extracellular dopamine elevations, and that these levels remained higher longer than in the WT mice.

These results correlate with the behavioral results showing that GluR6KO mice overall show more reward-seeking behaviors (both in the FUST and saccharin preference paradigm) and higher levels of dopamine during the FUST.

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