Wallace et al.

The influence of ΔFosB in the nucleus accumbens on natural reward-related behavior

Supplemental Material:

- Supplemental Methods

- Supplemental Table S1

Supplemental Methods

Sexual behavior. Male Sprague-Dawley rats (Charles River Laboratories) arrived at 7-8 weeks of age and were housed two per cage in a 12-hr light/12-hr dark cycle room (lights on between 7 AM and 7 PM; all experiments were conducted in the dark phase of the light cycle between 8 PM and 2 AM). Male sexual behavior was conducted under red light conditions in circular arenas (60 cm diameter) containing wood chips on the floor and behavior was recorded by videotape. For experiments using viral injected animals, the observer scoring the behavior was blind to the viral condition. The first session of sexual behavior was measured approximately 3 weeks after viral surgery (to allow maximum expression of the viral transgene). Further testing for each session followed 5-7 days between each session.

For sexually experienced comparisons, males were assigned randomly to a control or sexually experienced group. Sexually experienced males were generated by allowing copulation with a receptive female 1-2 times per week, for approximately 8-10 weeks, for a total of 14 sessions. For each session, males were given a 5-min acclimation period to the testing arena. Testing started at the end of the acclimation period by the introduction of a receptive female to the arena. Behaviors recorded were as follows: mounts, intromissions, and ejaculations. Other parameters measured also included mount latency (from the start of the test to the first mount), intromission latency (from the start of the test to the first intromission), ejaculation latency (calculated from the first

intromission), the number of mounts and intromissions necessary to reach ejaculation, and postejaculatory interval (time from ejaculation to the first following intromission).

Sprague–Dawley ovariectomized female rats (Charles River Breeding Laboratories) were used in these experiments. Receptivity of the female was induced by injection of estradiol benzoate (50 μg, s.c.) and progesterone (500 μg, s.c.) 48 and 4 – 6 hr before testing, respectively. One week before the experiments, the females were trained for one copulatory series with an experienced male. Before testing on the experimental day, female receptivity was verified by exhibition of lordosis, in the presence of the experienced male, and accepted intromission.

The olfaction control group was generated by similar handling and introduction to the behavioral arena as described above. The arena consisted of the same wood chips from a previous mating session mixed with fresh wood chips. Males were allowed to explore the arena for the same amount of time spent in the arena by the experimental males. A female was never introduced to the arena with the olfaction control males.

The no-sex control group was generated by similar methods as described above. The females used with these males were treated in the same manner as those used with the sexually experienced males, but were introduced to the control no-sex males before entering their estrous cycle and therefore did not exhibit lordosis. Although the males attempted to mount/intromit with these females, the females were not receptive. The males were exposed to the non-

receptive females for the same amount of time as the sexually experienced males spent in the arena. However, if the males became too aggressive with the non-receptive females, they were removed early from the arena.

In all experimental conditions, males were never exposed to the same female more than once.

Sucrose consumption. To habituate to drinking from two bottles, all animals were given two bottles of water for one week before any testing was conducted. To ensure the animals did not have a drinking side preference, left versus right, water and sucrose bottles were weighed and refilled and the placement of the bottles was switched every 24 hours.

Two-bottle choice test. All testing and pre-testing for the two-bottle choice was conducted in separate cages. Animals were habituated to two bottles of water for one week in their home cages. One hour before dark cycle, animals were taken from home cages, separated in novel cages and allowed access to food but no liquids. Testing commenced when lights went out, and one bottle of water and one bottle of 1% sucrose were presented at the same time, with half of the animals receiving sucrose on the left side, the other half receiving sucrose on the right side in a random balanced order for all animals. Both bottles were removed and weighed 30 min after being presented and animals were returned to their home cages.

Western blotting. For the sucrose experiments, animals had access to both sucrose and water before they were decapitated to eliminate a possible withdrawal-like effect from sucrose. For the sexual behavior experiments, animals were treated as in previous sessions, and returned to their home cage and holding rooms after the last behavioral session. Decapitation took place approximately 18 hours later. Blots were visualized using ECL Plus (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions. Bands were quantified using the NIH Scion image analysis software.

Immunohistochemistry. For both sucrose and sexual behavior experiments, eighteen to 24 h after their last treatment, animals were deeply anesthetized with pentobarbital and intracardially perfused using previously published methods (Perrotti et al., 2005). Brains were removed, stored overnight in 4% paraformaldehyde at 4 degrees and then transferred to a 20% glycerol in 0.01 m PBS solution for cryoprotection. Coronal sections (40 μm) were cut on a freezing microtome (Leica, Bannockburn, IL, USA) and then processed for immunohistochemistry. Since the exposure to the natural rewarding behavior occurred 18-24 hours later, we used a pan-FosB antibody, (SC-48; Santa Cruz Biotechnology, Santa Cruz, CA, USA) considering any acute FosB protein would be degraded within the 18-24 hour time period, and the only remaining protein differences detected would be ΔFosB (Perrotti et al., 2004, 2005). (These differences were also confirmed by Western Blotting results with differences only observed in the ΔFosB band). FosB-like staining was revealed by use of the

avidin–biotin peroxidase complex method with diaminobenzidine as a substrate using the same methods as described previously (Perrotti et al., 2005).

Viral-Mediated Gene Transfer. Surgery was performed on male Sprague-Dawley rats. AAV vectors were injected bilaterally (1.5 μl per side), over 7.5 min, into the NAc (relative to bregma: rat, anterior-posterior = +1.8, lateral = ±2.4, dorsal-ventral = -6.7 mm below dura, with a 10 degree angle) as previously described (Barrot et al., 2005). At the end of the experiment, the animals used in behavior were perfused, and the injection placements were evaluated for each animal on 40 μm cresyl-violet stained coronal sections. Only animals with correct bilateral placements were used for analysis; less than 10% of all animals had to be excluded for incorrect placements.

The titers of the viral vectors used are carefully monitored and only preparations with equivalent titers that are validated for each viral batch are used. Batches are validated by comparing the levels of ΔFosB with AAV-GFP infection versus AAV-ΔFosB infection.

Supplemental Table S1

Parameters of sexual behavior are shown for naïve (Session 1) and experienced (Sessions 2 and 3) rats that received intra-NAc injections of AAV-ΔFosB or -GFP. Data represent mean ± standard error, measured in seconds. Naïve behavior, number of intromissions: *p=0.04. (T(30)=2.145, n=15-17); Post-ejaculation interval: #p=0.065 (T(30)=1.916, n=15-17). ML: mount latency; IL: intromission latency; EL: ejaculation latency; #M: number of mounts to reach ejaculation; #I: number of intromissions to reach ejaculation; IR: intromission ratio (number of intromissions/(number of intromissions + number of mounts); PEI: post-ejaculation interval.