Increasing Histone Acetylation in the Hippocampus-Infralimbic Network Enhances Fear Extinction

Supplemental Information

Subjects

Mice were housed four to a cage with free access to lab chow and water. Subjects were maintained on a 12-hour light/dark cycle (lights on at 0600 h). The laboratory temperature remained at 21 ± 1°C. All experiments were started at 1200 ± 1 h.

Cannulation

Briefly, mice were placed under isoflurane anesthesia with bilateral holes drilled in the skull.

For intrahippocampal infusions, cannulae guides (Plastics One, Inc., Roanoke, VA) were then inserted into the holes and glued to the skull (AP -1.7 mm, ML ± 1.5 mm). The injectors (28 ga) extended 0.5 mm below the cannula guide into the brain (2.0 mm total length). For the angled medial prefrontal cortex (mPFC) cannulations, mice were mounted on a stereotax, skulls leveled and rotated 30°. A hole was then drilled (1.7 AP, 1.67 ML) with a 7.0 mm, 26 ga guide cannula lowered –1.43 DV and glued to the skull with ketac dental cement (3M). Injectors into the mPFC extended 1.0 mm below the cannulae.

All mice were given at least 3 d to recover from surgeries. Of the 169 mice cannulated, 134 were used in the experiment. The loss of mice was due to various factors such as incorrect guide cannulae placement, guide cannulae coming loose, and health issues following surgery.

Fear Conditioning

A sound attenuating chamber, kept in a dark room, contained a circular Plexiglas arena placed on a grid floor through which a .35 mA scrambled shock was delivered by a shock

generator with an infrared activity monitor fixed to the top of each chamber to record freezing (Coulbourn Instruments; Allentown, PA). This chamber served as the context (CTX) in all experiments.

Habituation

All animals used in the systemic experiments were brought into the procedure room for one hour and handled for ~1 min/day combined with a phosphate buffered saline (PBS) injection for 3 d prior to the first CTX exposure. In the microinjection experiments, animals were habituated to microinfusion procedures over two days. On each experimental day, animals were brought into the experimental procedure room 1 h prior to each experimental session.

Analysis of Sodium Butyrate (NaB) Effects on Locomotor Behavior and Shock Response

Locomotor Activity. Experimentally naive mice received either a 1.2 g/kg NaB (n = 8) or equivalent volume of 1X PBS subcutaneously (n = 8) 15 min prior to being placed in the same circular chambers used during fear conditioning (21.5 cm diameter). Behavior was recorded for 15 min with a pinhole camera (Polaris USA Video, Inc. product EM100/E-3; Norcross, GA) mounted in the ceiling of the chamber. Velocity and distance travelled was analyzed using the EthoVision XT video tracking system (Noldus, Wageningen, Netherlands).

No difference was seen between mice pre-injected with NaB or vehicle on either average velocity or distance travelled (Figure S1A&B; all ps > 0.7).

Shock Response. This was investigated by analyzing the number of activity counts the Conditioning Group (Experiment 1; pre-session injections) made during each 2 s shock received on the CTX+ day. The activity monitors sample behavior every 100 ms, thus if movement occurred during a 100 ms block this was labeled as one activity count.

No difference between groups pre-injected with either NaB or vehicle was observed during either of the 2-shocks (Figure S1C; all *p*s > 0.2).

Pre-Exposure vs No Pre-Exposure to Conditioning Context

We investigated whether context pre-exposure influences test performance in the Conditioning groups by comparing a group that did not receive pre-exposure (handled for ~10 s on Day 1) to a group that did receive pre-exposure (3 min CTX- exposure Day 1). On Day 2, both groups received a 2 shock CTX pairing as described in Experiment 1. Mice were tested 1 d later as described above.

There was no significant behavioral difference between mice that were pre-exposed to context 1 d prior to conditioning and those that received no pre-exposure (Figure S2; $p = 0.84$).

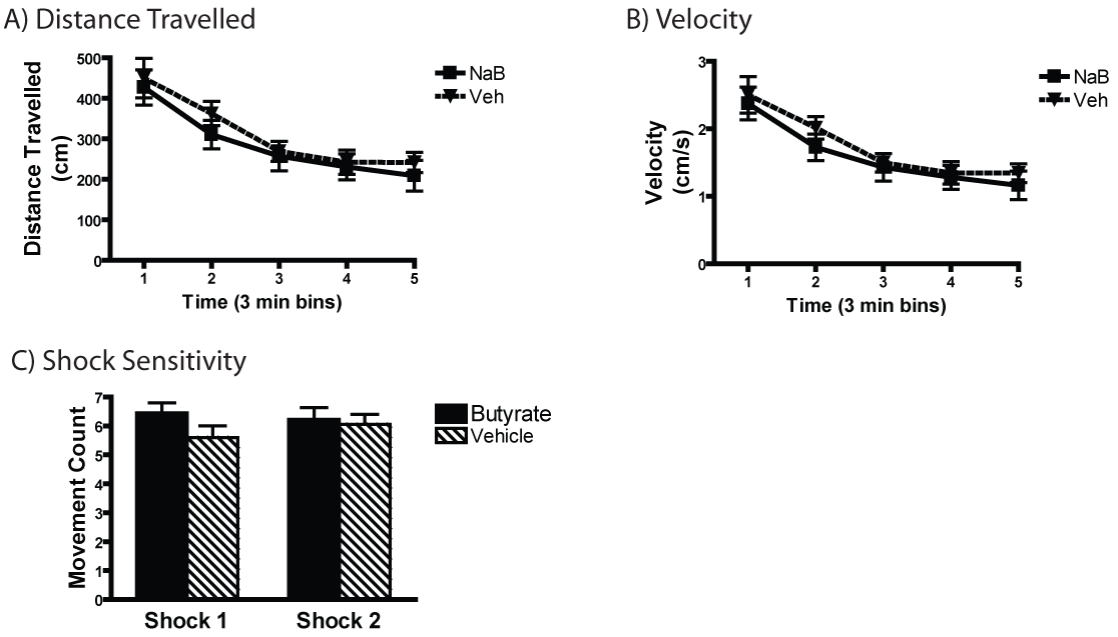

Figure S1. Sodium butyrate (NaB) effects on locomotor behavior and shock response. **(A, B)** Injection of NaB (*n* = 8) 15 min prior to a 15 min locomotor test does not alter distance travelled or velocity relative to vehicle treated mice (*n* = 8). **(C)** NaB (*n* = 11) injected 15 min prior to two 2 s footshocks does not alter response to shock during the shock relative to vehicle treated mice (*n* = 11).

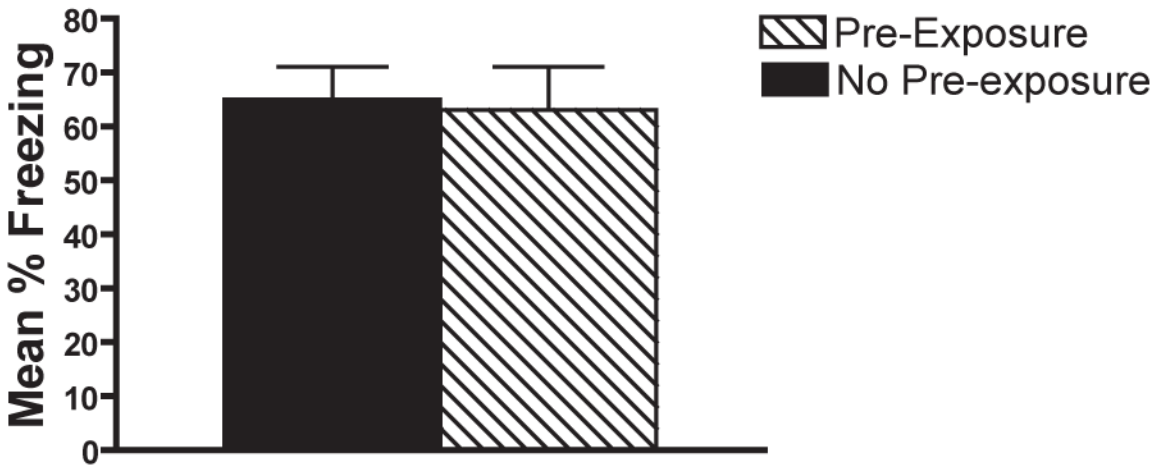

Figure S2. Context pre-exposure controls. When tested 24 hours after fear conditioning, mice that received context exposure the day prior to fear conditioning (n = 8) did not differ from those that did not (n = 7).