Supplementary Materials

This section details the procedures and results of an independent study to assess the reliability of the observation of reduced basal PFC dopamine in ethanol experienced rats relative to control rats. The overall procedures were similar to those described in the manuscript so only key differences are highlighted below.

Materials and Methods

Materials

Drinking solutions were prepared as described in the manuscript, with the exception that tap water was used instead of deionized water.

Animals

Sixteen male, young adult Long Evans rats from Charles River Laboratories (Raleigh, NC, USA; 220-240 g upon arrival). All animal procedures complied with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin. Stereotaxic surgeries were performed as described in the manuscript to implant a cannula directly above the mPFC (+3.0 AP; +0.5 ML; -1.1 DV).

Self-Administration Training and Protocols

For the replication project, operant self-administration training was conducted as described in the manuscript. However, a handling group was not necessary for this project and therefore animals were assigned to either the ethanol (10S10E) or sucrose group (10S).

Microdialysis

The microdialysis experiment followed the same procedures as described in the manuscript, with a few exceptions discussed below. During the final microdialysis session, only basal samples were collected.

Following the seventh operant session, rats were briefly anesthetized with isoflurane and a lab-constructed microdialysis probe (Pettit and Justice, 1991) was implanted through the guide cannula and into the mPFC. As described above, probes were perfused with ACSF overnight at a flow rate of 0.2 µl/min. Immediately following probe implantation and for the duration of microdialysis sampling, animals remained in their home cage next to their respective operant chamber. The next morning, the flow rate was increased to 1.0 µl/min at least 2 hours prior to the start of microdialysis. Microdialysis samples were manually collected every six minutes and were immediately frozen on dry ice. After a minimum of 4 samples was collected, the ACSF was changed to calcium-free ACSF. Calcium-free ACSF was perfused through the probe for at least 2 hours and three additional calcium-free samples were collected.

Dopamine Analysis

Dopamine content in each sample was quantified via reverse-phase high performance liquid chromatography (HPLC) with electrochemical detection. All samples were run with accompanying external standards (0.015 to 1.25 nM dopamine). Samples and standards were run using an 8125 manual injector (Rheodyne, Cotati, CA), a Luna 50 x 1.0 mm (C18, 3-µm particle size; Phenomenex, Torrance CA), and a 2 mm glassy carbon working electrode (SenCell, Antec Leyden, Netherlands) at potential + 450 mV relative to a Ag/AgCl reference electrode. Mobile phase was pumped through the HPLC system at a flow rate of 0.2 mL/min using an ISCO 65D syringe pump. The mobile phase consisted of 0.500 g octanesulfonic acid, 0.050 g decanesulfonic acid, 0.128 g ethylenediaminetetraacetic acid, and 11.08 g NaH_2PO_4 dissolved in 1 liter of deionized water, adjusted to 5.60 pH with 1 M sodium hydroxide. Methanol (6-8% v/v) was added to the mobile phase solution as the organic solvent and the solution was

sparged with helium. EZChrome Elite software (Agilent Technologies, Wilmington, DE) was used for chromatogram acquisition and peak integration. For three animals, the chromatography software was unable to determine peak heights of the dopamine signal for one or more of the samples. Therefore, peak area was used to determine the dopamine concentrations of all samples collected for these animals. The dopamine signal was required to be at least 3 times greater than the background noise.

Histological Analysis

Within three days of dialysis, animals were overdosed with sodium pentobarbital (150 mg/kg, intraperitoneal) prior to brain extraction. Brains were post-fixed with 10% formalin in saline, coronally sectioned (120 µm thick), and stained with cresyl violet for verification of the microdialysis probe placement (Paxinos et al., 1999).

Exclusion Criteria

For inclusion of rats in data analysis, dopamine concentrations in home cage baseline samples were required to have a relative standard deviation < 0.25. We also required a 40% decrease in dopamine concentration in calcium-free ACSF samples compared to basal ACSF samples to verify that dopamine release was exocytotic. Rats were required to acquire the lever-press behavior within two training sessions and the 10S10E group was required to consume at least 0.24 g/kg in session 5, 0.43 in session 6, and 0.58 g/kg in session 7. These minimum values for consumption were determined from the ethanol consumption data for the animals included in the original experiment.

Statistical Analysis

Raw dopamine concentrations (nM) were analyzed via a one-sided student's t-test to determine differences in basal PFC dopamine content between the 10S10E and 10S groups. Data were analyzed using SPSS software (IBM). Significance was assigned if $p < 0.05$; ns= not significant.

Results

Consumption during operant self-administration sessions

Ethanol and sucrose consumption data for the seven operant sessions are represented in Table S1. In the group consuming sweetened ethanol, rats increased their ethanol intake over the eight sessions and consumed at least 1 g/kg during the four sessions prior to microdialysis. The consumption data for the 10S10E and 10S groups resembled those of the original experiment.

Table S1: Ethanol and sucrose consumption per session

Training Day	Ethanol in 10% Sucrose Drinking Solution	Ethanol Intake (g/kg)*	Sucrose Intake (g/kg)
1	0%	n/a	2.80 ± 0.65
2	2%	0.27 ± 0.06	2.52 ± 0.31
3	2%	0.47 ± 0.06	3.03 ± 0.43
4	5%	1.06 ± 0.12	3.17 ± 0.45
5	5%	1.05 ± 0.18	2.89 ± 0.47
6	10%	1.43 ± 0.33	3.13 ± 0.60
7 (Tethered)	10%	1.18 ± 0.25	3.00 ± 0.50

* indicates mean ± SEM.

Analysis of raw basal PFC dopamine concentrations

As observed in the original experiment, animals with about a week of sweetened ethanol experience demonstrated significantly lower basal dopamine concentrations in the PFC relative to sucrose-only controls (t_{14}= 1.76, $p<0.05$; Fig S1).

Histologies and calcium-dependent dopamine concentration

Histologies indicated that at least 50% of the probe active area for each animal was in the infralimbic and prelimbic regions (Figure S2). Calcium-dependent dopamine release was confirmed by a minimum 40% dialysate dopamine concentration decrease when calcium-free ACSF was perfused through the probe. 10S10E and 10S groups showed an average of 63 ± 5% and 57 ± 4% decrease in dopamine in calcium-free

ACSF samples compared to concentrations at the conclusion of the operant session, respectively.

Figure S1:

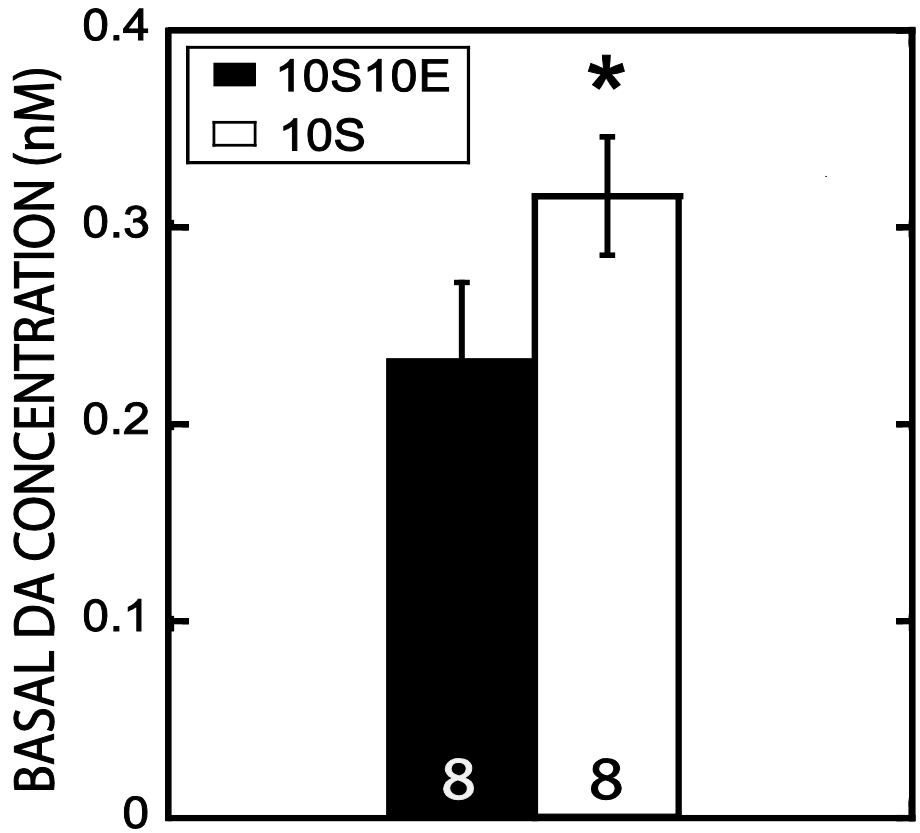

Figure S1. Basal dopamine concentrations in the medial PFC of rats trained to consume 10S or 10S10E. Animals with prior ethanol self-administration experience (10S10E) demonstrate significantly lower basal dopamine concentrations than control rats with only 10S experience. Means ± SEM are represented. *T(14)=1.76, p(10S10E<10S) < 0.05.

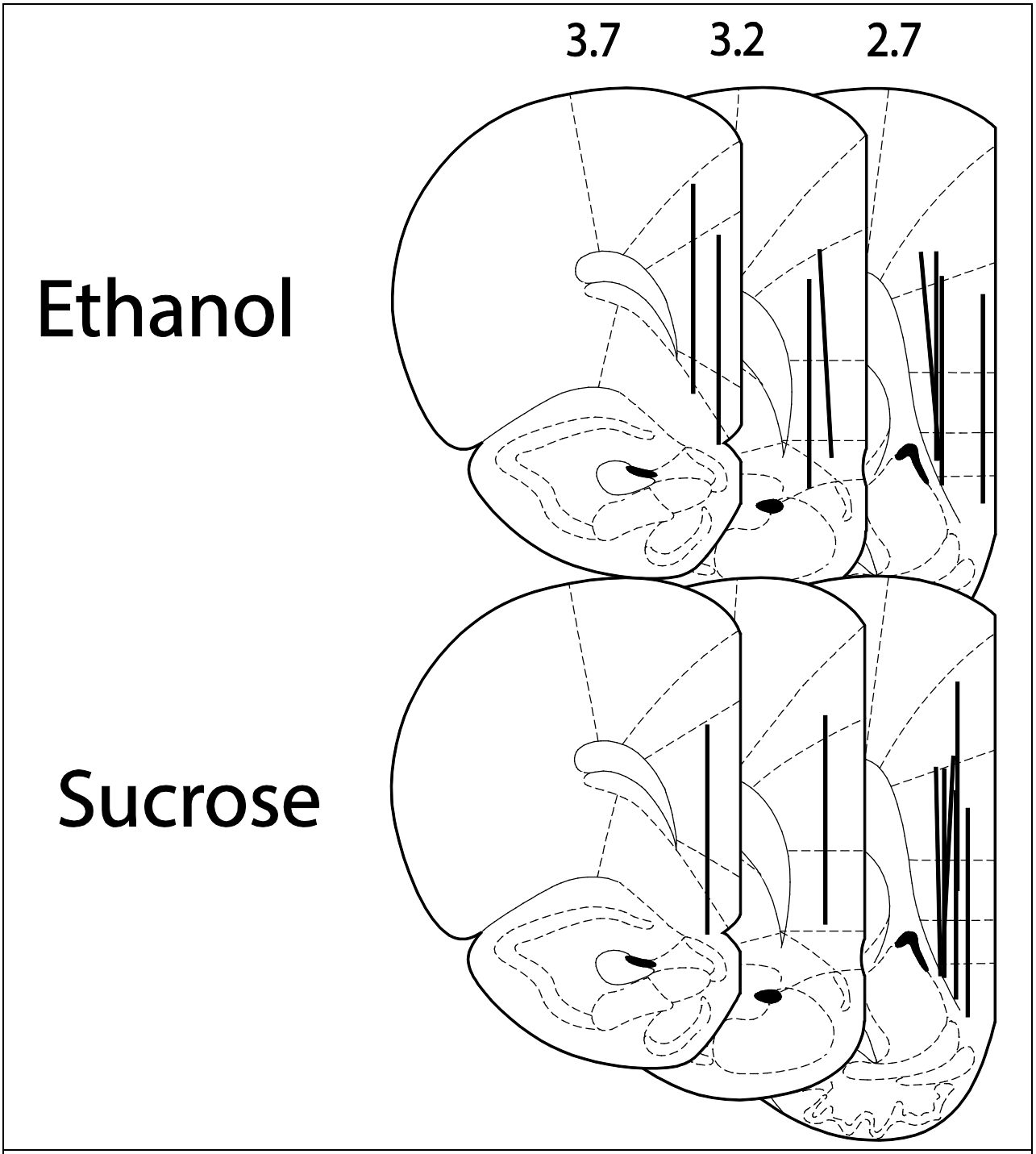

Figure S2: Microdialysis probe placements within the medial prefrontal cortex. Coronal slices 2.7, 3.2, and 3.7 mm from bregma showing placements of the active area (3.0 mm) of the microdialysis probes for each experimental group. Ethanol indicates the group that consumed 10% sucrose + 10% ethanol. Sucrose indicates the group that consumed sucrose only. It should be noted that for one animal in the 10S group, the brain extraction occurred about 72 hours following probe removal. To ensure that a probe track would be visible, the probe was reinserted immediately prior to euthanasia. The animal ended up with 2 separate probe tracks, but based on the extent of the tissue damage, we made an assumption as to which one was the original tract. Histology figure adapted from Paxinos & Watson (1998).