

Supplemental Figures

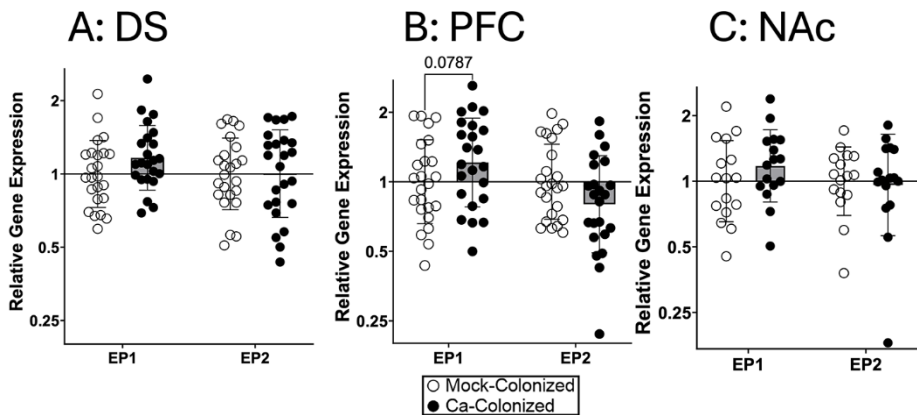


Figure S1

Figure S1: *Ep* receptor expression were not different in *C. albicans*-colonized mice vs mock-colonized mice in various brain regions. Single housed female C57BL/6 mice were orally inoculated with *C. albicans* strain CKY101 or PBS and subjected to the 2-bottle choice experiment as in Figure 1. On day 2, mice were euthanized, and brains were collected. *Ep* receptor expression was measured in various brain regions of mock-colonized or *C. albicans*-colonized mice by RT-qPCR using the ddCT method. Geometric mean and geometric standard deviation are shown. A Two-way ANOVA was performed for statistics and p-values are shown. (A) Shows expression in dorsal striatum (DS) (B) Shows expression in the prefrontal cortex (PFC) (C) Shows expression in the nucleus accumbens (NAc)

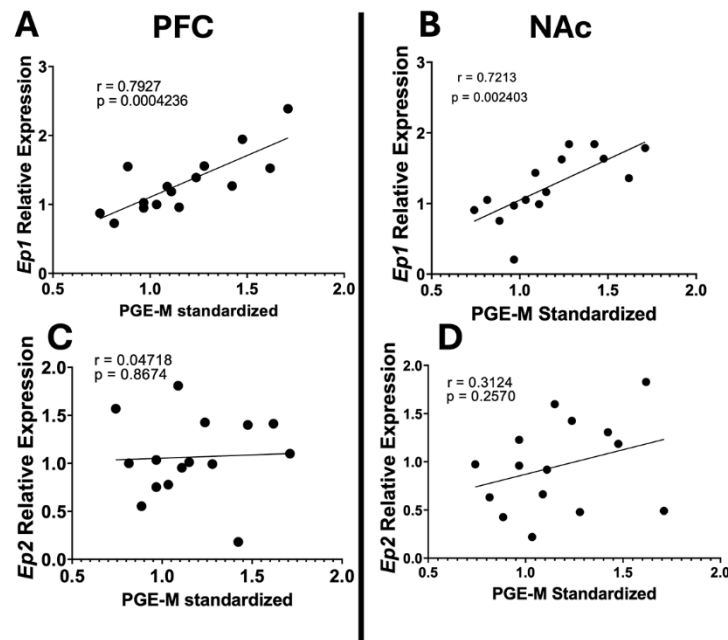


Figure S2

Figure S2: Serum PGE-metabolite correlates with EP1 Receptor expression in the PFC and NAc of *C. albicans*-colonized mice. Single housed female C57BL/6 mice were orally inoculated with *C. albicans* strain CKY101 or PBS and subjected to the 2-bottle choice experiment as in Figure 1. On day 2, mice were euthanized, and brain and serum were collected. Expression of *Ep* genes relative to average expression in mock-colonized mice from the same experimental trial, plotted as a function of concentration of PGE-metabolite standardized to the average in mock-colonized mice in the same experimental trial. Each symbol represents an individual mouse. **(A,C)** show gene expression of *Ep1* and *Ep2*, respectively, in the prefrontal cortex (PFC). **(B,D)** show gene expression of *Ep1* and *Ep2*, respectively, in the nucleus accumbens (NAc). Pearson correlations were used to test for significant correlations, r =correlation strength and p =statistical significance. Significant correlations were observed between *Ep1* expression and concentration of PGE-M in the PFC and NAc of *C. albicans*-colonized mice.

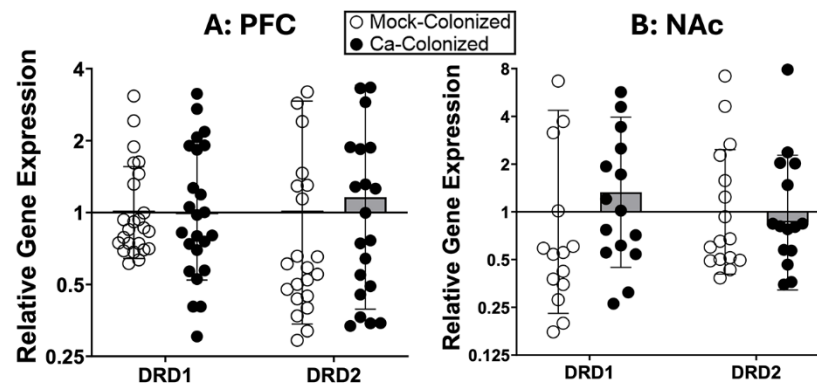


Figure S3

Figure S3: *Drd* gene expression was not different between *C. albicans*-colonized and mock-colonized mice in the PFC and NAc. Single housed female C57BL/6 mice were orally inoculated with *C. albicans* strain CKY101 or PBS and subjected to the 2-bottle choice experiment as in Figure 1. On day 2, mice were euthanized, and brains were collected. *Drd* receptor expression was measured in the prefrontal cortex (PFC) (A) or nucleus accumbens (NAc) (B) of mock-colonized or *C. albicans*-colonized mice by RT-qPCR using the ddCT method. Geometric mean and geometric standard deviation are shown. Multiple t-tests performed for statistics—no significant differences were observed.

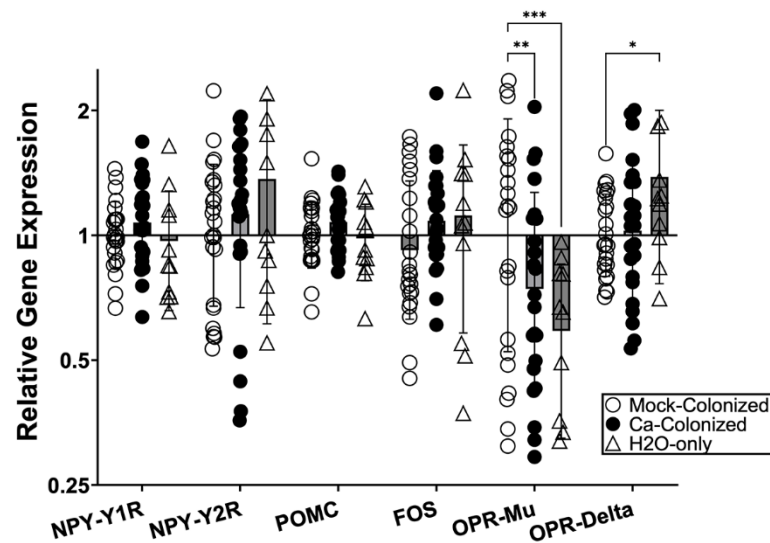


Figure S4

Figure S4: Expression of other addiction-implicated genes in the Dorsal Striatum of *C. albicans*-colonized mice. Single housed female C57BL/6 mice were orally inoculated with *C. albicans* strain CKY101 or PBS and subjected to the 2-bottle choice experiment as in Figure 1. On day 2, mice were euthanized, and brains were collected. Groups of mice that consumed ethanol (Ca-colonized or Mock-colonized) or mice that were mock-colonized and only given access to water (H2O-only) are shown. The expression of genes implicated in addiction was measured in the dorsal striatum of the different groups by RT-qPCR using the ddCT method. Geometric mean and geometric standard deviation are shown. A two-way ANOVA with Dunnett's correction was performed for statistics. * p=0.0315; ** p=0.0065; *** p=0.0001.

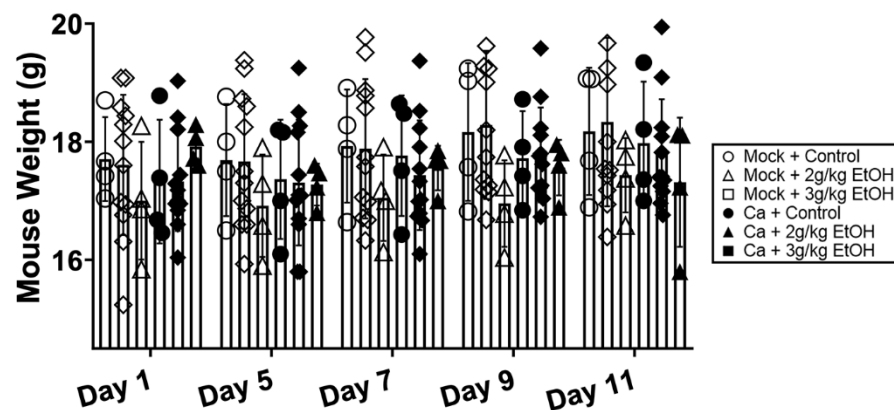


Figure S5

Figure S5: No differences in mouse weight were observed in the ethanol-induced conditioned taste aversion experiment. Single housed female C57BL/6 mice were trained to drink their daily liquid in the late morning. Mice orally inoculated with *C. albicans* strain CKY101 or PBS were given one hour of access to a novel tastant, 1.2% saline solution, and then injected intraperitoneally with ethanol (2 g/kg or 3 g/kg) or control (sterile saline). Mouse weight is shown in grams. Mock-colonized, control (open circles); mock-colonized + 2g/kg EtOH (open triangles); mock-colonized + 3 g/kg EtOH (open squares); *C. albicans*-colonized, control (closed circles); *C. albicans*-colonized +2 g/kg EtOH (black triangles); *C. albicans*-colonized + 3 g/kg EtOH (closed squares). Bars show means, error bars show standard deviation, and each symbol represents one mouse. A two-way ANOVA corrected for repeated measures was performed for statistical significance—there were no significant comparisons.

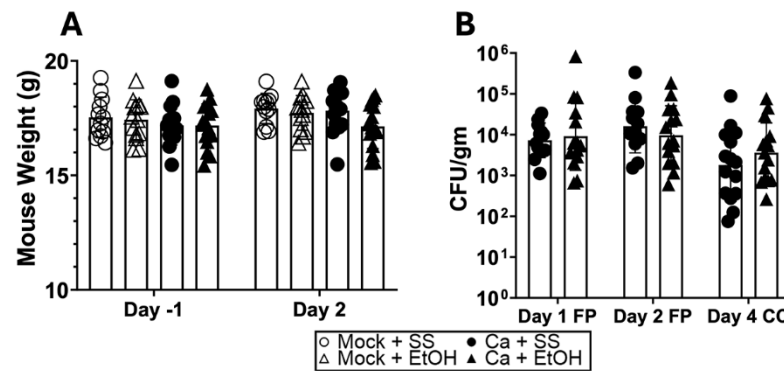


Figure S6

Figure S6: No differences in mouse weight or colonization were observed in the LORR, OFT, and balance beam experiments. Single housed female C57BL/6 mice were orally inoculated with *C. albicans* strain CKY101 or PBS on days 0 and 2, and subjected to the behavioral tests illustrated in **Fig. 8A**. **(A)** Mouse weights on days -1 and 2 are shown. **(B)** CFU/gm fecal pellets (FP) or cecum contents (CC) collected on days indicated. Bars show means with the standard deviation (A) or geometric mean with geometric standard deviation (B). Symbols represent individual mice. Two-way ANOVAs corrected for multiple repeated measures were performed for statistics and there were no significant differences.

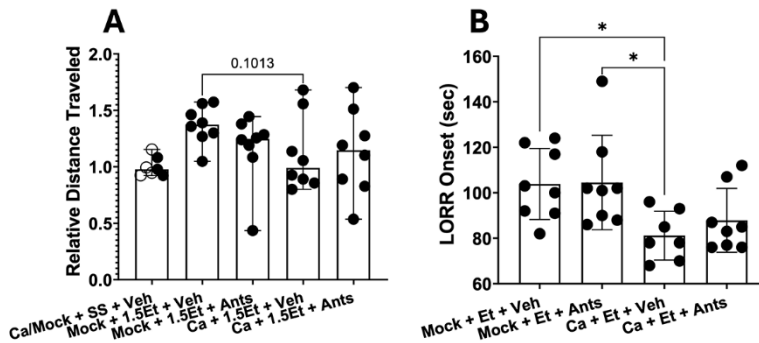


Figure S7

Figure S7: Antagonism of Ep receptors did not change the behavior of mice in the OFT or LORR tests. Single housed female C57BL/6 mice were orally inoculated with *C. albicans* strain CKY101 or PBS on days 0 and 2, and subjected to the behavioral tests illustrated in **Figure 8A**, except that mice were injected intraperitoneally with EP1 and EP2 antagonists or vehicle daily on days 0-4 approximately one hour before each behavioral test. **(A)** Groups are defined as: Ca/Mock + SS + Veh: mock-colonized or *C. albicans*-colonized, received sterile saline ip injections on days 1 and 3 and vehicle ip injections daily; Mock + EtOH + Veh: mock-colonized, received 1.5g/kg ethanol ip injected on days 1 and 3 and vehicle ip injections daily; Mock + EtOH + Ants: mock-colonized, received 1.5g/kg ethanol ip injected on days 1 and 3 and antagonist ip injections daily; Ca + EtOH + Veh: *C. albicans*-colonized, received 1.5g/kg ethanol ip injected on days 1 and 3 and vehicle ip injections daily; Ca + EtOH + Ants: *C. albicans*-colonized, received 1.5g/kg ethanol ip injected on days 1 and 3 and antagonist ip injections daily. **(B)** Groups are defined as: Mock + Et + Veh: mock-colonized, received 1.5g/kg ethanol ip injected on days 1 and 3 and 3.5g/kg EtOH on day 4 and vehicle daily; Mock + Et + Ants: mock-colonized, received 1.5g/kg ethanol ip injected on days 1 and 3 and 3.5g/kg EtOH on day 4 and antagonists daily; Ca + Et + Veh: *C. albicans*-colonized, received 1.5g/kg ethanol ip injected on days 1 and 3 and 3.5g/kg EtOH on day 4 and vehicle daily; Ca + Et + Ants: *C. albicans*-colonized, received 1.5g/kg ethanol ip injected on days 1 and 3 and 3.5g/kg EtOH on day 4 and antagonists daily. **(A)** distance traveled in the open field test in a ten-minute trial relative to the average for sterile saline-injected (SS) mice within the same group (mock-colonized or *C. albicans*-colonized). A Brown-Forsythe and Welch ANOVA test was run for statistical significance. **(B)** time after injection (seconds) for the mouse to lose its righting reflex (LORR onset). An ordinary One-way ANOVA was completed for statistical significance. * $p < 0.0465$.

967 **Table S1: Primers used in this study**

Primer Name for RT-qPCR	Sequence	Source
GAPDH-FW	TGTAGACCATGTAGTTGAGGTCA	(1)
GAPDH-RV	AGGTCGGTGTGAACGGATTTG	
EP1-FW	TGCTTGCCATCGACCTAGC	(2)
EP1-RV	CACCCAGGAAATGACACGC	
EP2-FW	CAGCTCGGTGATGTTCTCGG	(2)
EP2-RV	GAGCACCAATTCCGTTACCAG	
NPY-Y1R-FW	GACTCTCACAGGCTGTCTT	(3)
NPY-Y1R-RV	TTGGTCTCACTGGACCTGT	
NPY-Y2R-FW	TTTTCGGAGGCTACCAATGT	(3)
NPY-Y2R-RV	AATACAATGGGAGGTCTGCA	
POMC-FW	GAGGCCTTTCCCCTAGAGTT	(4)
POMC-RV	CACCGTAACGCTTGTCCTT	
FOS-FW	GGGACAGCCTTTCCCTACTAC	(5)
FOS-RV	GGGATAAAGTTGGCACTAGAG	
OPR-MU-FW	GAGCCACAGCCTGTGCCCT	(6)
OPR-MU-RV	CGTGCTAGTGGCTAAGGCATC	
OPR-DELTA-FW	GCTCGTCATGTTTGGCATC	(6)
OPR-DELTA-RV	AAGTACTTGGCGCTCTGGAA	
DRD1-FW	GAACCCAGAAGACAGGTGGA	(7)
DRD1-RV	GCTTAGCCCTCACGTTCTTG	
DRD2-FW	TATGCCCTGGGTCGTCTATC	(7)
DRD2-RV	AGGACAGGACCCAGACAATG	

968

969 **Supplemental Bibliography:**

- 970 1. Markey L, Hooper A, Melon LC, Baglot S, Hill MN, Maguire J, et al. Colonization with the
971 commensal fungus *Candida albicans* perturbs the gut-brain axis through dysregulation of
972 endocannabinoid signaling. *Psychoneuroendocrinology*. 2020 Nov;121:104808.
- 973 2. Chen L, Ji X, Wang M, Liao X, Liang C, Tang J, et al. Involvement of TLR4 signaling
974 regulated-COX2/PGE2 axis in liver fibrosis induced by *Schistosoma japonicum* infection.
975 *Parasites Vectors*. 2021 Dec;14(1):279.
- 976 3. Shi Y -C., Ip CK, Reed F, Sarruf DA, Wulff BS, Herzog H. Y5 receptor signalling counteracts
977 the anorectic effects of PYY 3-36 in diet-induced obese mice. *J Neuroendocrinology*. 2017
978 Oct;29(10):e12483.
- 979 4. Luque RM, Gahete MD, Hochgeschwender U, Kineman RD. Evidence that endogenous
980 SST inhibits ACTH and ghrelin expression by independent pathways. *American Journal of*
981 *Physiology-Endocrinology and Metabolism*. 2006 Aug;291(2):E395–403.
- 982 5. Almeida A, Paul Thierry J, Magdelénat H, Radvanyi F. Gene expression analysis by real-time
983 reverse transcription polymerase chain reaction: influence of tissue handling. *Analytical*
984 *Biochemistry*. 2004 May;328(2):101–8.

6. Reiss D, Ceredig RA, Secher T, Boué J, Barreau F, Dietrich G, et al. Mu and delta opioid receptor knockout mice show increased colonic sensitivity. *European Journal of Pain*. 2017 Apr;21(4):623–34.
7. Braunstein KE, Eschbach J, Róna-Vörös K, Soylu R, Mikrouli E, Larmet Y, et al. A point mutation in the dynein heavy chain gene leads to striatal atrophy and compromises neurite outgrowth of striatal neurons. *Human Molecular Genetics*. 2010 Nov 15;19(22):4385–98.