Supplementary Materials

Supplemental figure legends.

figure S1. *htr1a*, *htr1b*, *htr2a*, and *htr4* mutant mice are fully sensitive to the motor deficit induced by chronic FLX. Motor behavior was evaluated using rotarod test in *htr1a* (**a**), *htr1b* (**b**), *htr2a* (**c**) and *htr4* mutant mice (**d**), treated for 3 weeks with Veh or FLX. FLX treatment reduced the latency to fall in all genotypes tested. (n = 5 - 54 mice per group) (*p < 0.05; **p < 0.01; ***p < 0.001).

Main effect of genotype: *htr1a* at 20rpm: $F_{2,57} = 0.037$, P = 0.9638, *htr1a* at 30rpm: $F_{2,57} = 0.511$, P = 0.6027, *htr1b* at 20rpm: $F_{2,66} = 0.14$, P = 0.8698, *htr1b* at 30rpm: $F_{2,66} = 0.25$, P = 0.7798, *htr2a* at 20rpm: $F_{1,48} = 0.337$, P = 0.5641, *htr2a* at 30rpm: $F_{1,48} = 0.661$, P = 0.4202, *htr4* at 20rpm: $F_{2,150} = 2.335$, P = 0.0984, *htr4* at 30rpm: $F_{2,150} = 0.047$, P = 0.9538

Main effect of treatment: *htr1a* at 20rpm: $F_{1,57} = 47.347$, P < 0.0001, *htr1a* at 30rpm: $F_{1,57} = 23.387$, P < 0.0001, *htr1b* at 20rpm: $F_{1,66} = 47.079$, P < 0.0001, *htr1b* at 30rpm: $F_{1,66} = 27.886$, P < 0.0001, *htr2a* at 20rpm: $F_{1,48} = 10.104$, P = 0.0026, *htr2a* at 30rpm: $F_{1,48} = 18.175$, P < 0.0001, *htr4* at 20rpm: $F_{1,150} = 187.455$, P < 0.0001, *htr4* at 30rpm: $F_{1,150} = 150.835$, P < 0.0001

Genotype x treatment interaction: (*htr1a* at 20rpm: $F_{2,57} = 0.118$, P = 0.889, *htr1a* at 30rpm: $F_{2,57} = 0.826$, P = 0.4432, *htr1b* at 20rpm: $F_{2,66} = 0.943$, P = 0.3948, *htr1b* at 30rpm: $F_{2,66} = 0.129$, P = 0.8794, *htr2a* at 20rpm: $F_{1,48} = 0.008$, P = 0.9274, *htr2a* at 30rpm: $F_{1,48} = 0.012$, P = 0.9118, *htr4* at 20rpm: $F_{2,150} = 0.241$, P = 0.7858, *htr4* at 30rpm: $F_{2,150} = 0.518$, P = 0.5965)

figure S2. 5-HT3 receptor antagonism does not reverse motor deficits elicited by chronic FLX treatment, and 5-HT2C agonism mimics chronic FLX induced motor deficits. Motor behavior was evaluated using the rotarod test. Acute ondansetron treatment (5-HT3 receptor antagonist, 0.1 - 1 mg/kg, i.p.) did not reverse chronic FLX-induced reductions in the latency to fall (n = 11 - 15 mice per group).

figure S3. Striatal 5-HT2C receptors do not mediate the effect of chronic 5-HTT blockade on motor behavior. Motor behavior was evaluated using rotarod test. (**a**) Cannulated mice were treated for 3 weeks with FLX (10 mg/kg/day). (**b - e**) SB242084 (selective 5-HT2C receptor antagonist, 0.1 ug) or saline acutely infused into the dorsal striatum (dStr) had no effect on the latency to fall at 20 rpm (**b, c**) or at 40 rpm (**d, e**), (n = 7 mice per group).

figure S4. **Optogenetic mapping of SNr projection to the SNc and optogenetic identification of dopamine neurons in the SNc**. (**a**) SNr projections to the SNc were mapped in VGAT-Ires-Cre mice injected with cre-dependent ChR2 virus into the SNr. (**b**) Light stimulation of SNr projections at 0.1 Hz and 10 Hz frequencies evoked IPSCs in SNc DA neurons with onset and rise times of <10 ms and low variability, indicative of monosynaptic inhibitory connections. (**c – f**) SNc DA neuron identity was verified *in vivo* using optogenetic tagging. (**c**) Extracellular single unit recordings were coupled with optogenetic stimulation to verify the identity of DA-like cells in DAT Cre::Ai32 animals. (**d**) Spontaneous spike and a light-triggered spike demonstrated similar waveforms. (**e**) Histogram of the latency to spike with respect to onset of light stimulation (dotted line) recorded from 5 identified dopamine neurons indicated <10 ms spike latency, indicating a direct effect of light on the activity of DA neurons. (**f**) Responses (black bars) of a representative neuron to 5 Hz stimulation (cyan bars) demonstrates high reliability of spiking in response to light stimulation.

figure S5. Additional firing properties of DAergic SNc neurons after chronic FLX and acute SB242084 administration. (**a**) Firing properties of DAergic SNc neurons were assessed in anesthetized mice through extracellular single unit recordings. Chronic FLX treatment (10 mg/kg/day) reduced and acute SB242084 treatment (5 mg/kg) increased the average amount of spikes per burst (**b**) (n = 20 - 45 cells per group). Neither chronic FLX nor acute SB242084 treatment altered the firing rate outside of bursts (n = 20 - 45 cells per group) (**c**) or within bursts (n = 20 - 44 cells per group) (**d**). (**p < 0.01; ***p < 0.001).

figure S6. Activation or inhibition of SNc DAergic neurons does not impact locomotor activity. Ambulatory locomotor activity was evaluated in the open field test in response to inhibition (**a**) or stimulation (**b**) of SNc DAergic cells. DAT-IRES-Cre::Ai35 (DAT::Ai35), DAT-IRES-Cre::Ai32 (DAT::Ai32), or their respective single mutant control mice were implanted bilaterally with a fiber optic cable in the SNc. DAT::Ai32, and their control mice were treated for at least 3 weeks with FLX (10 mg/kg/day). Optical inhibition (532 nm, continuous stimulation, 8 mW) did not affect ambulation (n = 7 - 8 mice per group; no significant genotype x stimulation interaction detected, **a**). Optical stimulation (473 nm, 20 Hz, 10 ms pulse duration, 8 mW) did not affect ambulation (n = 9 - 10 mice per group; no significant genotype x stimulation interaction detected, **b**).

figure S7. Chronic FLX and acute SB242084 treatments do not alter food deprivation induced weight loss, food consumption or locomotor activity. Food deprivation increases the drive to feed. To assess whether this drive is differentially affected by chronic FLX or acute SB242084 treatment, we assessed % weight loss after 24 h of food deprivation (**a, c**) and home-cage food consumption during 5 minutes after 24 h of food deprivation (**b, d**). Locomotor activity was assessed using the open field test and analyzed for two independent variables (**e**) and collapsed for acute treatment (**f**). (g) Chronic FLX and acute SB242084 enhanced time spent mobile in the forced swim test. FLX was administered at 10 mg/kg/day through the drinking water and SB242084 was administered at 1 mg/kg (**a, b, e, f**), 0.5 mg/kg (**g**) or 0.2 mg/kg (**c, d**) i.p., (n = 5 – 12 mice per group). (***$p < 0.001$).

figure S8. Interaction of chronic FLX and acute SB242084 treatment in the shuttle box learned helplessness test. Behavior was assessed in the learned helplessness test. Exposure to inescapable shock was followed by chronic FLX treatment for 21 days and acute SB24084 was administered on the day of shuttle box escape testing. Escape latency increased after chronic FLX (10 mg/kg/day) and was reduced with acute SB242084 treatment at 1 mg/kg (**a**), with no effect on intra-trial ambulatory activity (**b**) (n = 8 - 10 mice per group). (*$p < 0.05$; ***$p < 0.001$).

Figure S9. A circuit model of 5-HTergic regulation of SNr activity and its consequences on basal ganglia function and behavior. Increasing serotonergic (5-HT) tone either via 5-HTT ablation or chronic FLX administration, results in prolonged activation and increased signaling of 5-HT2C receptors located on GABAergic neurons within the SNr. These 5-HT2C-expressing GABAergic SNr neurons in turn project to and inhibit DAergic neurons in the SNc, suppressing DAergic input to the dorsal striatum. The resulting decrease in DAergic basal ganglia output leads to motor coordination deficits and behavioral consequences that are detrimental in the context of antidepressant psychopharmacology. (RN: raphe nuclei).