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The cortico-habenular axis reactivity and anxiety behavior in male and female rats with dopamine or serotonin transporter knockout

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Abstract

Based on available knowledge, the present study aimed to test the hypothesis that serotonin transporter knockout (SERT-KO) and dopamine transporter knockout (DAT-KO) affect exploratory and anxiety-like behaviour and the excitability of habenular neurons in response to prefrontal cortical stimulation, but in distinct patterns. Because of limited information on potential sex differences, the experiments were conducted in both male and female rats. DAT-KO and SERT-KO rats aged 6–7 weeks were compared with age-matched wild-type (WT) controls. Exploratory and anxiety-like behaviors were assessed using the open-field test. Using extracellular single-cell in vivo electrophysiology, the firing activity of glutamatergic lateral habenula (LHb) neurons was recorded under basal conditions and following medial prefrontal cortex (mPFC) stimulation. DAT-KO rats exhibited hyperlocomotion accompanied by sex-specific alterations in anxiety-like behavior with lower number of entries and time spent in the central zone in females. In SERT-KO rats only mild sex differences in anxiety-like behavior were observed. Furthermore, the reactivity of the cortico-habenular pathway to mPFC stimulation differed between male and female DAT-KO and SERT KO rats. In WT animals, basal firing activity of LHb neurons was associated with anxiety-like behavior in a sex-dependent manner; however, this relationship was absent in both DAT-KO and SERT-KO rats. These findings identify sex-dependent effects of DAT and SERT knockout on behavior and cortico-habenular circuit function and reveal a disruption of the association between lateral habenula neuronal activity and anxiety-like behavior.

Introduction

Dopamine and serotonin are major modulators of locomotor activity, emotional processing and adaptive behaviour. Their extracellular availability is tightly regulated by the dopamine transporter (DAT) and serotonin transporter (SERT), which remove dopamine and serotonin, respectively, from the extracellular space. Lifelong deletion of these transporters produces marked but partly contrasting behavioural phenotypes.

The lateral habenula (LHb) represents a potential neural substrate through which altered dopamine and serotonin signalling may influence behavioural adaptation. It integrates forebrain information related to aversive outcomes, reward omission and other behaviourally relevant events and influences midbrain dopaminergic and serotonergic systems through direct and indirect pathways (Matsumoto & Hikosaka, 2007; Stamatakis & Stuber, 2012; Hu et al., 2020). The medial prefrontal cortex (mPFC), which contributes to contextual processing, action–outcome evaluation and behavioural control, is embedded within cortico-striato-pallidal networks that influence the LHb (Howland et al., 2022; Tooley et al., 2018). Measuring LHb neuronal responses to mPFC stimulation may therefore provide a functional assessment of how cortical activation is processed within circuits involved in motivation and aversion.

Previous studies have consistently demonstrated that dopamine transporter knockout (DAT-KO) animals exhibit markedly elevated locomotor activity compared to heterozygous (HET) and wild-type (WT) controls (Giros et al., 1996). Hyperlocomotion has long been considered the primary phenotypic hallmark

of DAT gene deletion (Apryatin et al., 2023). Across studies, DAT-KO rodents show significantly greater total distance travelled, higher locomotor velocity, and increased time spent in motion relative to HET and WT individuals. These effects were observed both during 24-hour home-cage monitoring (Leo et al., 2018) and in short-duration open-field tests (Apryatin et al., 2019; Reinwald et al., 2022; Mallien et al., 2022; Lloyd et al., 2022; Hlavacova et al., 2023; Apryatin et al., 2023; Sadighi et al., 2025). Hyperlocomotion is therefore a robust feature of the DAT-KO phenotype, whereas information on potential sex differences remains limited.

In addition to hyperactivity, DAT-KO animals frequently display anxiety-like behaviors. These include a reduced number of entries into the central zone of the open-field arena (Apryatin et al., 2019; Reinwald et al., 2022; Lloyd et al., 2022) and repetitive stereotypic behaviors, such as wall-following or circling, accompanied by central-zone avoidance (Reinwald et al., 2022; Apryatin et al., 2023; Sadighi et al., 2025).

Structural neuroimaging analyses indicate that both hyperlocomotor activity and anxiety-like behavior correlate with reduced striatal volume and increased cerebellar volume in DAT-KO rats, suggesting that these neuroanatomical alterations may underlie the observed behavioral phenotype (Reinwald et al., 2022). Dopaminergic signaling in the dorsal striatum plays a central role in regulating motor function. Prior studies have demonstrated that elevated dopamine concentrations modulate striatal circuitry and influence motor output (Kreitzer & Malenka, 2008). However, chronic or excessive dopamine exposure has been shown to exert neurotoxic effects, leading to disruptions in synaptic architecture and contributing to postsynaptic neurodegeneration (Cadet et al., 2010).

In DAT-KO mice, structural alterations in the striatum, including reduced striatal volume, have been attributed primarily to the loss of GABAergic interneurons (Cyr et al., 2003). The degeneration of these inhibitory interneurons may lead to disinhibition within striatal and cortical networks. Such disinhibition has been proposed to contribute to hyperactivity and the cortical hyperconnectivity observed in related neuropathological conditions (Berlanga et al., 2011).

Serotonin transporter knockout (SERT-KO) rodents have been reported to display anxiety-like behavior as measured using the elevated plus maze test (Olivier et al., 2008; Verheij et al. 2018; Sbrini et al. 2020; Sun et al. 2024; Guo et al., 2025). In the open field test anxiety-like behavior in SERT-KO rats was demonstrated by decreased time spent in the central zone of the open-field arena (Olivier et al., 2008; Golebiowska et al., 2019; Diniz et al., 2021). Johnson et al. (2019) found that male SERT-KO rats spent significantly less time in the central zone compared to HET animals, although this difference did not reach significance in comparison with WT control animals.

Reduced locomotor activity in SERT-KO rats compared with WT controls was also observed; however, these latter differences were mostly not statistically significant (Olivier et al., 2008; Normann et al., 2018; Sbrini et al., 2020; Willadsen et al., 2021; Diniz et al., 2021). In contrast, Golebiowska et al. (2019) demonstrated significantly increased locomotor activity and exploratory behavior in SERT-KO rats relative to WT animals. Overall, the available findings on locomotor phenotypes in SERT-KO rats remain inconsistent, and no sex-dependent differences have been reported.

It has been reported that SERT-KO rats may have altered glutamatergic neurotransmission in the LHb. Thus, SERT removal led to reduced baseline LHb mRNA levels of critical determinants of glutamate transmission, such as SLC1A2, the main glutamate transporter, and N-methyl-D-aspartate (Grin1, Grin2A, and Grin2B) as well as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (Gria1 and Gria2) receptor subunits (Caffino et al., 2019). However, the effects of both SERT and DAT knockouts on the excitability of habenular neurons and on the reactivity of the cortico-habenular axis have not yet been directly examined.

Based on available knowledge, the present studies aimed to test the hypothesis that SERT and DAT knockouts affect open-field behavior and the excitability of habenular neurons in response to cortical stimulation, but in distinct patterns. Because of limited information on potential sex differences, the experiments were conducted in both male and female rats.

Methods

Animals

Male and female serotonin (SERT) and dopamine (DAT) transporter knockout (SERT-KO and DAT-KO, respectively) rats were bred at the animal facility of Radboud University (Nijmegen, Netherlands) and transported to the animal facility at the Biomedical Research Center of the Slovak Academy of Sciences at the age of 3–4 weeks. The number of rats used is stated in the Results and Figure legends. The SERT and DAT KO rats were generated on a Wistar Unilever and Wistar-Han genetic background, respectively, as previously described (Homberg et al. 2007, Leo et al. 2018). Each knockout group of animals, for each sex, was accompanied by a corresponding control group of wild-type (WT) animals. Upon arrival at the animal facility, animals were allowed to habituate to the housing conditions for at least three weeks. Rats were housed 2–3 per cage under standard conditions with a constant 12:12 h light/dark cycle (lights on at 6 a.m.), temperature (22°C ± 2°C), and humidity (55 ± 10%). Food and water were available ad libitum. All experimental procedures were approved by the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic (Approval No.3566/19–221) and conformed to the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Open field (OF) test

Locomotor activity and anxiety-like behavior were evaluated in an open-field test. A square-shaped OF apparatus (Ekoplast, Telc, Czech Republic) with dimensions of 100 x 100 cm, consisting of a rubber floor and plastic walls 52 cm in height, was used. The illumination intensity in the central zone was 40–45 lux, and that in the peripheral area of the apparatus was 15–25 lux. At the beginning of the test, each rat was placed in a corner of the OF apparatus and allowed to explore freely for 15 min. The entire test was continuously recorded on a video camera. All recordings were analyzed using the EthoVision XT 10

software. The anxiety-like behaviour was assessed by the time spent and the number of entries into the central zone. Locomotor activity and velocity were also measured.

Electrophysiology

Animals were anesthetized with urethane (1.25 g/kg, intraperitoneally: i.p.) and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Their body temperature was maintained using a water-heating pad (Gaymor Instruments, Orchard Park, NY, USA). Two holes were drilled in their skull, using David Kopf Stereotaxic NH-180 High-Speed Drill (38,000 RPM): one above the medial prefrontal cortex (mPFC), and one above the LHb. A custom-made, steel fork-shaped stimulation electrode was inserted into the mPFC. Single-barrel recording glass electrodes were prepared using DMZ-Universal Puller (Zeitz-Instruments GmbH, Martinsried, Germany), filled with 2M NaCl solution, and inserted into the LHb. Glutamate-secreting LHb neurons were recognized according to their firing pattern, described in previous papers (Li *et al.*, 2019a), and their basal activity was recorded for at least two minutes. Afterward, the mPFC was stimulated with square-wave current pulses of 0.2 ms duration and 1–2 mA amplitude, delivered at a frequency of 1 Hz for 60 s, as previously described (Varga *et al.*, 2003), using a Medicor Pulse Generator (Budapest, Hungary). The firing activity of LHb neurons was recorded for at least five minutes after mPFC stimulation. To avoid the residual effect of mPFC stimulation, only one neuron per animal was recorded.

Statistical analysis

Data distribution was assessed using the Shapiro–Wilk test for normality. As the data were normally distributed, parametric statistical tests were used. Two-way analysis of variance (ANOVA) was performed to assess the effects of sex (male vs. female) and genotype (DAT-KO vs WT or SERT-KO vs. WT) on behavioral parameters, including total distance travelled, velocity, number of entries into the central zone, and time spent in the central zone. Basal firing rates of habenular neurons were also analyzed by two-way ANOVA with sex and genotype as factors, with DAT-KO and SERT-KO rats evaluated separately against their corresponding WT controls. In order to assess the effect of mPFC stimulation on the firing rate of habenular neurons, the neuronal firing activity before mPFC stimulation (the baseline) was set to 100%; the firing rate of each neuron after mPFC stimulation was calculated as a percentage of the basal firing rate of the same neuron. Two-way ANOVA for repeated measures (RM ANOVA) was used to compare the firing rates of habenular neurons before and after mPFC stimulation (factor one: time) and between DAT-KO or SERT-KO rats (factor two: genotype) and their corresponding WT controls. Pairwise comparisons between groups were performed with Bonferroni correction for multiple comparisons. Spearman's correlation was used to examine associations between behavioral measures (number of entries and time spent in the central zone) and electrophysiological parameters (basal firing rate of habenular neurons).

Results

Knockout of the DAT led to an increase in locomotor activity and a decrease in the number of entries and time spent in the central zone during the OF test

DAT-KO rats showed increased locomotor activity in the OF test compared to WT rats (Fig. 1A). Two-way ANOVA revealed a significant main effect of sex ($F_{1,38}$ =15.022, p < 0.001) and genotype ($F_{1,38}$=47.926, p < 0.001) on the total distance travelled. No significant interaction between sex and genotype was observed. Bonferroni-adjusted pairwise comparisons revealed that female DAT-KO rats (n = 9) covered a greater distance (p = 0.001) than male DAT-KO rats (n = 12). Furthermore, there was a significant difference (p < 0.001) in the total distance travelled between female DAT-KO rats (n = 9) and female WT rats (n = 11). A significant difference was observed between male DAT-KO rats (n = 12) and male WT rats (n = 10) (p = 0.005) according to the Bonferroni post hoc test. For velocity (Fig. 1B), significant main effects of sex ($F_{1,38}$ = 15.004, p < 0.001) and genotype ($F_{1,38}$ = 47.917, p < 0.001) were observed. No significant interaction between sex and genotype was revealed. Regarding entries into the central zone (Fig. 1C), significant main effects were observed for sex ($F_{1, 38}$=4.742, p = 0.036) and genotype ($F_{1, 38}$=7.19, p = 0.011). Bonferroni comparisons revealed a significant difference between female DAT-KO rats and female WT rats (p = 0.03). No significant interaction between sex and genotype was found. The two-way ANOVA of data on time spent in the central zone (Fig. 1D) showed a significant main effect of sex ($F_{1, 38}$ = 5.531, p = 0.024) and genotype ($F_{1,38}$=6.157, p = 0.018). No significant interaction between sex and genotype was observed. Bonferroni comparisons revealed that female DAT-KO rats spent significantly less time in the central zone compared to female WT rats (p = 0.039). Additionally, female WT rats spent significantly more time in the central zone than male WT rats (p = 0.040).

Knockout of the SERT resulted in a decrease in the number of central zone entries during the OF test

SERT-KO rats (n = 10 group/sex) exhibited more pronounced anxiety behavior in the OF test compared to WT (n = 10 group/sex) controls (Fig. 2). Two-way ANOVA revealed significant main effects of sex ($F_{1, 36}$ =8.81, p = 0.005) and genotype ($F_{1, 36}$=5.074, p = 0.03) on the total distance traveled (Fig. 2A). Similarly, velocity analysis (Fig. 2B) showed significant effects of sex ($F_{1, 36}$=8.88, p = 0.005) and genotype ($F_{1, 36}$=5.125, p = 0.03). Female rats showed higher locomotor activity than males, and within both WT and SERT-KO groups, this activity was greater in WT controls. No significant interaction between sex and genotype on total distance traveled and velocity was observed. Regarding the number of entries into the central zone, significant main effects were observed for sex ($F_{1, 36}$=5.488, p = 0.025) and genotype ($F_{1, 36}$=7.412, p = 0.01), as shown in Fig. 2C. Bonferroni comparisons revealed a significant difference between female SERT-KO and female WT (p = 0.041). There was a significant effect on time spent in the central zone (Fig. 2D) between SERT-KO and WT rats ($F_{1,35}$=4.337, p = 0.045), whereas no such effect was observed between male and female rats. No significant interaction between sex and genotype was found.

Knockout of the DAT led to attenuated reactivity of the cortico-habenular axis

Electrical stimulation of the mPFC inhibited 100% of the habenular neurons detected in female DAT-KO rats; a representative recording from a habenular neuron showing an inhibitory response to mPFC stimulation is shown in Fig. 3A.

In male DAT-KO and male and female WT rats, approximately two-thirds of the habenular neurons were inhibited by mPFC stimulation as well. Circa one-third of the neurons, however, showed a stimulatory response to mPFC stimulation; a representative recording from a habenular neuron with a stimulatory response to PFC stimulation is shown in Fig. 3B. The exact numbers of neurons with inhibitory and stimulatory responses to mPFC stimulation in each group of animals are shown in Table 1A.

The basal firing rate of habenular neurons, however, did not differ statistically between the sexes or between DAT-KO rats and their corresponding WT controls (Fig. 4A). Two-way RM ANOVA of the summary firing rates of habenular neurons before and after mPFC stimulation and between female DAT-KO rats and corresponding female WT controls revealed a significant effect of time ($F_{1,31}$=11.86, p = 0.004) and genotype, and a significant time × genotype interaction ($F_{1,31}$=4.58 and p = 0.05 in both cases). Bonferroni post hoc tests confirmed a significant reduction in habenular neuronal firing activity after mPFC stimulation ($p < 0.001$ compared with baseline) and a significant decrease in post-stimulation habenular neuronal excitability in female DAT-KO rats ($p < 0.01$ compared with control WT females). Two-way RM ANOVA comparisons of the neuronal firing activity before and after mPFC stimulation and between male DAT-KO rats and corresponding male WT controls revealed no statistically significant differences (Fig. 4B).

Knockout of SERT does not alter the reactivity of the cortico-habenular axis

Habenular neurons in male and female SERT-KO rats, as well as in corresponding male and female WT controls, showed either inhibitory (circa 70%) or excitatory (circa 30%) responses to the mPFC stimulation. The exact numbers of neurons with inhibitory and stimulatory responses to mPFC stimulation in male and female SERT-KO and WT rats are shown in Table 1B. There were no effects of sex or genotype on the basal activity of the habenular neurons (Fig. 5A), nor on post-stimulatory excitability (Fig. 5B).

Association of habenular neuronal firing activity and anxiety behavior

The relationship between basal habenular neuronal activity and anxiety behavior, namely entries and time spent in the central zone of the OF test, was examined using Spearman´s correlation analyses. In female WT rats, basal habenular activity exhibited a positive correlation with the number of entries into the central zone (r = 0.49, p = 0.061), with a marginal statistical significance. Moreover, basal habenular activity was significantly positively correlated with the time spent in the central zone (r = 0.58, p = 0.024).

Interestingly, no significant correlations were revealed in DAT-KO or SERT-KO rats, irrespective of sex or genotype. Similarly, no significant associations were detected in male WT rats (data not shown).

Discussion

The results of the present study show that increased locomotion in DAT-KO rats is accompanied by sex specific changes in parameters used to reflect anxiety. Sex differences were also observed in the anxiety-like behavior of SERT-KO rats. Moreover, the reactivity of the cortico-habenular axis was differently modified in male and female DAT-KO rats. Importantly, habenular neuronal firing activity was found to be linked with rats' anxiety behavior in a sex-specific manner in the WT controls but not in DAT and SERT transporter knockout animals.

DAT-KO rats exhibited significant hyperlocomotor activity, consistent with results from several previous studies (for review, see Kim et al. 2024). In agreement with existing knowledge (Dubovicky et al. 1999), female rats were more active than male rats, and this difference was significantly greater in DAT-KO animals than in WT controls.

Parameters of anxiety-like behavior measured in the open field test in DAT-KO rats indicate their higher anxiety. However, DAT-KO rats showed a stereotypic pattern of running along the borders, which could lead to avoidance of the central zone. There are several other potentially modifying factors, and the previously published findings on anxiety behavior in DAT-KO rats are inconsistent. One of the factors that modulates anxiety-like behavior appears to be illumination. In the present study, illumination intensity ranged from 40–45 lux in the central zone and 15–25 lux in the peripheral area of the apparatus. Mallien et al. (2022) used a relatively low illumination level (25 lux) and reported that DAT-KO animals spent more time in the central zone than HET and WT controls. Conversely, Sadighi et al. (2025) observed reduced time spent in the center under an even lower illumination level (12 lux) in DAT-KO rats. This may partially explain the reduced tendency of animals to enter the central zone; however, DAT knockout itself certainly contributes to this behavioral phenotype. Thus, the available findings concerning center-zone behavior under low-light conditions remain contradictory.

In this study, reduced locomotor activity was observed in SERT-KO rats compared with WT controls. Previous findings in SERT-KO rats are inconsistent. The present results are consistent with findings reported in most previous studies (for a review, see Holmes et al., 2003). Some authors reported increased locomotor activity (Golebiowska et al. 2019), despite SERT-KO rodents being widely used as a model of depression-like behavior.

SERT-KO rats exhibited anxiety-like behavior, as evidenced by increased anxiety-like parameters (including reduced time spent in the central zone and decreased frequency of entries into the central zone). These findings are consistent with previous reports (Olivier et al., 2008; Golebiowska et al., 2019; Diniz et al., 2021). In addition, increased anxiety-like behavior was particularly strong in SERT-KO female rats, a finding that has not been reported previously.

Present electrophysiological measurements under urethane anesthesia showed that the LHb neurons exhibit spontaneous firing of 10–15 action potentials per second. The basal firing activity of the habenular neurons did not differ between the sexes or genotypes. These results are consistent with the observation of no sex differences in the spontaneous basal activity of LHb neurons reported in a previous study (Bell et al., 2023).

The present experiments showed that ~ 70% of LHb neurons in male and female WT animals exhibited an inhibitory response to electrical stimulation of the mPFC. The remaining ~ 30% of the neurons were excited by the cortical stimulation. It might be speculated that the activation of distinct neuronal circuits accounts for the different responses of habenular neurons to cortical stimulation. It is indeed known that LHb receives monosynaptic excitatory input from the mPFC. In addition, this structure is innervated by GABA–releasing neurons of the ventral pallidum (Hu et al., 2020). The neuronal activity of the ventral pallidum is regulated by GABAergic projections from the ventral striatum, whereas the striatal circuits themselves are controlled by inputs from the mPFC (Babiczky & Matyas, 2022; Howland et al., 2022). Therefore, the mPFC can influence LHb activity through two distinct routes: (1) directly, via its monosynaptic excitatory projection to the LHb, and (2) indirectly, via a polysynaptic mPFC → ventral striatum → ventral pallidum pathway, which can drive either excitatory or inhibitory pallidal inputs to the LHb. It may be suggested that the habenular neurons showing inhibitory response to the cortical stimulation are predominantly controlled by the GABAergic input from the ventral pallidum, while the LHb neurons that were excited by the cortical stimulation are primarily controlled by the glutamatergic input from the ventral pallidum or mPFC.

The lack of SERT in SERT-KO rats did not alter the habenular response to cortical stimulation. DAT-KO rats, however, exhibited an increase in the proportion of neurons showing an inhibitory response to mPFC stimulation; this effect was observed in female but not in male rats. Dopamine is known to modulate functional connectivity within the striatal, pallidal, and accumbal circuits, enhancing activity in some pathways while suppressing others (Dremencov et al., 2006; Hu et al., 2020). It is thus possible that dopamine modifies mPFC–LHb interactions by facilitating the indirect inhibitory mPFC → ventral striatum → ventral pallidum → LHb pathway and/or by attenuating the direct and/or indirect excitatory corticohabenular projections.

As mentioned above, the DAT-KO led to an altered reactivity of the cortico-habenular axis in female, but not in male rats. It was previously reported that female rats have a higher density of estrogen receptor alpha-positive cells in the LHb (Bell et al., 2023). Thus, estrogens may enhance the effect of dopamine on cortico-habenular neuronal circuits. Further studies should be performed to test this hypothesis.

The results of the present experiments revealed that WT female rats with a higher mean habenular neuron firing rate showed a lower degree of anxiety. No correlation between the habenular neuronal firing rate and anxiety behavior was observed in WT male rats or in both male and female SERT-KO or DAT-KO rats. The present study appears to be the first, to the best of our knowledge, to examine *in vivo* excitability of the individual habenular neurons and to correlate these data with the behavioral

characteristics within the same experimental subjects. A previous study (Li et al., 2019b) investigated the link between alcohol abstinence-induced anxiety and neural excitability within certain brain areas, including LHb. Surprisingly, the authors detected a link between habenular excitability and anxiety in male, but not in female rats. The differences between our findings and those of Li and colleagues might be explained by differences in methodological approaches. We directly measured the neuronal excitability using the single-unit *in vivo* electrophysiology. In the Li et al. study, neural excitability was indirectly assessed using ex vivo assessment of the proto-oncogene c-Fos expression.

In conclusion, the higher motor activity in female rats compared to that in males was significantly greater in DAT-KO animals than in WT controls. Increased anxiety-like behavior in SERT-KO rats was particularly strong in females, a finding that has not been reported previously. The basal firing activity of the habenular neurons did not differ between the sexes or genotypes. It is suggested that the habenular neurons showing an inhibitory response to cortical stimulation are predominantly controlled by the GABAergic, while the LHb neurons that were excited are primarily controlled by the glutamatergic input. Female DAT-KO rats exhibited an increase in the proportion of neurons showing an inhibitory response to mPFC stimulation compared to WT rats?. An intriguing finding of the present study is that WT female rats with a higher mean habenular neuron firing rate showed lower anxiety. These findings identify sex-dependent effects of DAT and SERT knockout on behavior and cortico-habenular circuit function and reveal a disruption of the association between lateral habenula neuronal activity and anxiety behavior. The present results motivate further studies on possible monoaminergic control over the mPFC-habenula circuit in anxiety.

Declarations

Author Contribution

M.R. analyzed the behavioral data, statistical analyses, writing – original draft, prepared figuresE.D. in vivo electrophysiology, statistical analyses, writing – original draftD.G. in vivo electrophysiologyN.H. behavioral experimentsC.G. J. G. reviewed and edited the manuscriptJ.R.H. project administration,experimental animals, writing – review & editingD.J. supervision, funding acquisition, conceived and designed the study, wrote the main manuscript text

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Data Availability

The data are available upon request .

References

1. Apryatin SA, Shipelin VA, Trusov NV, Mzhelskaya KV, Evstratova VS, Kirbaeva NV, Soto JS, Fesenko ZS, Gainetdinov RR, Gmoshinski IV (2019) Comparative analysis of the influence of a high-fat/high-carbohydrate diet on the level of anxiety and neuromotor and cognitive functions in Wistar and DAT-KO rats. Physiol Rep 7. https://doi.org/10.14814/phy2.13987
2. Apryatin SA, Traktirov DS, Karpenko MN, Ivleva IS, Pestereva NS, Bolshakova MV, Trofimov AN, Fesenko ZS, Klimenko VM (2023) Antioxidant system alterations and physiological characteristics of neonatal and juvenile DAT-KO rats. J Neurosci Res 101:1651–1661. https://doi.org/10.1002/jnr.25228
3. Babiczky Á, Matyas F (2022) Molecular characteristics and laminar distribution of prefrontal neurons projecting to the mesolimbic system. *eLife* 11. https://doi.org/10.7554/eLife.78813
4. Bell D, Waldron VJ, Brown PL (2023) Quantitative and qualitative sex difference in habenula-induced inhibition of midbrain dopamine neurons in the rat. Front Behav Neurosci 17:1289407. https://doi.org/10.3389/fnbeh.2023.1289407
5. Berlanga ML, Price DL, Phung BS, Giuly R, Terada M, Yamada N, Cyr M, Caron MG, Laakso A, Martone ME, Ellisman MH (2011) Multiscale imaging characterization of dopamine transporter knockout mice reveals regional alterations in spine density of medium spiny neurons. Brain Res 1390:41–49. https://doi.org/10.1016/j.brainres.2011.03.044
6. Cadet JL, Jayanthi S, McCoy MT, Beauvais G, Cai NS (2010) Dopamine D1 receptors, regulation of gene expression in the brain, and neurodegeneration. CNS Neurol Disord Drug Targets 9:526–538. https://doi.org/10.2174/187152710793361496
7. Caffino L, Verheij MMM, Que L, Guo C, Homberg JR, Fumagalli F (2019) Increased cocaine self-administration in rats lacking the serotonin transporter: a role for glutamatergic signaling in the habenula. Addict Biol 24:1167–1178. https://doi.org/10.1111/adb.12673
8. Cyr M, Beaulieu JM, Laakso A, Sotnikova TD, Yao WD, Bohn LM, Gainetdinov RR, Caron MG (2003) Sustained elevation of extracellular dopamine causes motor dysfunction and selective degeneration of striatal GABAergic neurons. Proc Natl Acad Sci U S A 100:11035–11040. https://doi.org/10.1073/pnas.1831768100
9. Diniz DM, Calabrese F, Brivio P, Riva MA, Grandjean J, Homberg JR (2021) BDNF overexpression in the ventral hippocampus promotes antidepressant- and anxiolytic-like activity in serotonin transporter knockout rats. Int J Mol Sci 22:5040. https://doi.org/10.3390/ijms22095040
10. Dremencov E, Weizmann Y, Kinor N, Gispan-Herman I, Yadid G (2006) Modulation of dopamine transmission by 5HT2C and 5HT3 receptors: a role in the antidepressant response. Curr Drug Targets 7:165–175. https://doi.org/10.2174/138945006775515491
11. Dubovický M, Skultétyová I, Jezová D (1999) Neonatal stress alters habituation of exploratory behavior in adult male but not female rats. Pharmacol Biochem Behav 64:681–686. https://doi.org/10.1016/S0091-3057(99)00166-5

12. Giros B, Jaber M, Jones S, Wightman RM, Caron MG (1996) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379:606–612. https://doi.org/10.1038/379606a0
13. Golebiowska J, Hołuj M, Potasiewicz A, Piotrowska D, Kuziak A, Popik P, Homberg JR, Nikiforuk A (2019) Serotonin transporter deficiency alters socioemotional ultrasonic communication in rats. Sci Rep 9:20283. https://doi.org/10.1038/s41598-019-56629-y
14. Guo CC, Xu Y, Shan L, Foka K, Memoli S, Mulveen C, Gijsbrechts B, Verheij MM, Homberg JR (2025) Quantifying multilabeled brain cells in the whole prefrontal cortex reveals reduced inhibitory and a subtype of excitatory neuronal marker expression in serotonin transporter knockout rats. Cereb Cortex 35:2. https://doi.org/10.1093/cercor/bhae486
15. Hlavacova N, Hrivikova K, Karailievova L, Karailiev P, Homberg JR, Jezova D (2023) Altered responsiveness to glutamatergic modulation by MK-801 and to repeated stress of immune challenge in female dopamine transporter knockout rats. Prog Neuropsychopharmacol Biol Psychiatry 126:110804. https://doi.org/10.1016/j.pnpbp.2023.110804
16. Holmes A, Murphy DL, Crawley JN (2003) Abnormal behavioral phenotypes of serotonin transporter knockout mice: parallels with human anxiety and depression. Biol Psychiatry 54:953–959. https://doi.org/10.1016/j.biopsych.2003.09.003
17. Homberg JR, Olivier JDA, Smits BMG, Mul JD, Mudde J, Verheul M, Nieuwenhuizen OFM, Ronken E, Cremers T, Schoffelmeer ANM, Ellenbroek BA, Cuppen E (2007) Characterization of the serotonin transporter knockout rat: a selective change in the functioning of the serotonergic system. Neuroscience 146:1662–1676. https://doi.org/10.1016/j.neuroscience.2007.03.030
18. Howland JG, Ito R, Lapish CC, Villaruel FR (2022) The rodent medial prefrontal cortex and associated circuits in orchestrating adaptive behavior under variable demands. Neurosci Biobehav Rev 135:104569. https://doi.org/10.1016/j.neubiorev.2022.104569
19. Hu H, Cui Y, Yang Y (2020) Circuits and functions of the lateral habenula in health and in disease. Nat Rev Neurosci 21:277–295. https://doi.org/10.1038/s41583-020-0292-4
20. Johnson PL, Molosh AI, Federici LM, Bernabe C, Haggerty D, Fitz SD, Nalivaiko E, Truitt W, Shekhar A (2019) Assessment of fear and anxiety associated behaviors, physiology and neural circuits in rats with reduced serotonin transporter (SERT) levels. Transl Psychiatry 9:33. https://doi.org/10.1038/s41398-019-0368-y
21. Kim D, Yadav D, Song M (2024) An updated review on animal models to study attention-deficit hyperactivity disorder. Transl Psychiatry 14:187. https://doi.org/10.1038/s41398-024-02893-0
22. Kreitzer AC, Malenka RC (2008) Striatal plasticity and basal ganglia circuit function. Neuron 60:543–554. https://doi.org/10.1016/j.neuron.2008.11.005
23. Leo D, Sukhanov I, Zoratto F, Illiano P, Caffino L, Sanna F, Messa G, Emanuele M, Esposito A, Dorofeikova M, Budygin EA, Mus L, Efimova EV, Niello M, Espinoza S, Sotnikova TD, Hoener MC, Laviola G, Fumagalli F, Adriani W, Gainetdinov RR (2018) Pronounced hyperactivity, cognitive

dysfunctions, and BDNF dysregulation in dopamine transporter knock-out rats. J Neurosci 38:1959–1972. https://doi.org/10.1523/JNEUROSCI.1931-17.2018

24. Li H, Pullmann D, Jhou TC (2019a) Valence-encoding in the lateral habenula arises from the entopeduncular region. *eLife* 8. https://doi.org/10.7554/eLife.41223
25. Li J, Chen P, Han X, Zuo W, Mei Q, Bian EY, Umeugo J, Ye J (2019b) Differences between male and female rats in alcohol drinking, negative affects and neuronal activity after acute and prolonged abstinence. Int J Physiol Pathophysiol Pharmacol 11:163–176
26. Lloyd JT, Yee AG, Kalligappa PK, Jabed A, Cheung PY, Todd KL, Karunasinghe RN, Vlajkovic SM, Freestone PS, Lipski J (2022) Dopamine dysregulation and altered responses to drugs affecting dopaminergic transmission in a new dopamine transporter knockout (DAT-KO) rat model. Neuroscience 491:43–64. https://doi.org/10.1016/j.neuroscience.2022.03.019
27. Mallien AS, Becker L, Pfeiffer N, Terraneo F, Hahn M, Middelman A, Palme R, Creutzberg KC, Begni V, Riva MA, Leo D, Potschka H, Fumagalli F, Homberg JR, Gass P (2022) Dopamine transporter knockout rats show impaired wellbeing in a multimodal severity assessment approach. Front Behav Neurosci 16:924603. https://doi.org/10.3389/fnbeh.2022.924603
28. Matsumoto M, Hikosaka O (2007) Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 447:1111–1115. https://doi.org/10.1038/nature05860
29. Normann C, Frase S, Haug V, von Wolff G, Clark K, Münzer P, Dorner A, Scholliers J, Horn M, Vo Van T, Seifert G, Serchov T, Biber K, Nissen C, Klugbauer N, Bischofberger J (2018) Antidepressants rescue stress-induced disruption of synaptic plasticity via serotonin transporter-independent inhibition of L-type calcium channels. Biol Psychiatry 84:55–64. https://doi.org/10.1016/j.biopsych.2017.10.008
30. Olivier JD, Van Der Hart MG, Van Swelm RP, Dederen PJ, Homberg JR, Cremers T, Deen PM, Cuppen E, Cools AR, Ellenbroek BA (2008) A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders. Neuroscience 152:573–584. https://doi.org/10.1016/j.neuroscience.2007.12.032
31. Reinwald JR, Gass N, Mallien AS, Sartorius A, Becker R, Sack M, Falfan-Melgoza C, von Clemm C, Leo D, Pfeiffer N, Middelman A, Meyer-Lindenberg A, Homberg JR, Weber-Fahr W, Gass P (2022) Dopamine transporter silencing in the rat: systems-level alterations in striato-cerebellar and prefrontal-midbrain circuits. Mol Psychiatry 27:2329–2339. https://doi.org/10.1038/s41380-022-01471-4
32. Sadighi M, Foka K, Mottarlini F, Burg E, Taddini S, Leo D, Sanna F, Caffino L, Fumagalli F, Homberg JR (2025) Subchronic amphetamine decreases hyperactivity, anti-social behaviour and anhedonia in dopamine transporter knockout rats: role of prefrontal glutamate. Neuropharmacology 279:110597. https://doi.org/10.1016/j.neuropharm.2025.110597
33. Sbrini G, Brivio P, Bosch K, Homberg JR, Calabrese F (2020) Enrichment environment positively influences depression- and anxiety-like behavior in serotonin transporter knockout rats through the modulation of neuroplasticity, spine, and GABAergic markers. Genes (Basel) 11:1248. https://doi.org/10.3390/genes11111248

34. Stamatakis AM, Stuber GD (2012) Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance. Nat Neurosci 15:1105–1107. https://doi.org/10.1038/nn.3145
35. Sun M, Brivio P, Shan L, Docq S, Heltzel LCMW, Smits CAJ, Middelman A, Vrooman R, Spoelder M, Verheij MMM, Buitelaar JK, Boillot M, Calabrese F, Homberg JR, Hanswijk SI (2024) Offspring's own serotonin transporter genotype, independently from the maternal one, increases anxiety- and depression-like behavior and alters neuroplasticity markers in rats. J Affect Disord 350:89–101. https://doi.org/10.1016/j.jad.2024.01.114
36. Tooley J, Marconi L, Alipio JB, Matikainen-Ankney B, Georgiou P, Kravitz AV, Creed MC (2018) Glutamatergic ventral pallidal neurons modulate activity of the habenula-tegmental circuitry and constrain reward seeking. Biol Psychiatry 83:1012–1023. https://doi.org/10.1016/j.biopsych.2018.01.003
37. Varga V, Kocsis B, Sharp T (2003) Electrophysiological evidence for convergence of inputs from the medial prefrontal cortex and lateral habenula on single neurons in the dorsal raphe nucleus. Eur J Neurosci 17:280–286. https://doi.org/10.1046/j.1460-9568.2003.02408.x
38. Verheij MMM, Contet C, Karel P, Latour J, van der Doelen RHA, Geenen B, van Hulten JA, Meyer F, Kozicz T, George O, Koob GF, Homberg JR (2018) Median and dorsal raphe serotonergic neurons control moderate versus compulsive cocaine intake. Biol Psychiatry 83:1024–1035. https://doi.org/10.1016/j.biopsych.2017.10.032
39. Willadsen M, Üngör M, Sługocka A, Schwarting RKW, Homberg JR, Wöhr M (2021) Fear extinction and predictive trait-like inter-individual differences in rats lacking the serotonin transporter. Int J Mol Sci 22:7088. https://doi.org/10.3390/ijms22137088

Tables

Table 1. Distribution of inhibitory and excitatory lateral habenula neuronal responses to medial prefrontal cortex stimulation in DAT-KO and SERT-KO rats and their corresponding WT controls.
Numbers indicate neurons showing excitatory or inhibitory responses to mPFC stimulation relative to the total number of recorded neurons in each sex and genotype group; corresponding percentages are given in parentheses. Only one LHb neuron was recorded per animal.

DAT-KO rats and corresponding WT controls		
Females	**DAT-KO**	**WT**
Excitatory response	0/9 neurons (0%)	2/7 neurons (30%)
Inhibitory response	9/9 neurons (100%)	5/7 neurons (70%)
Males	**DAT-KO**	**WT**
Excitatory response	3/10 neurons (30%)	1/8 neurons (12%)
Inhibitory response	7/10 neurons (70%)	7/8 neurons (88%)
SERT-KO rats and corresponding WT controls		
Females	**SERT-KO**	**WT**
Excitatory response	4/9 neurons (44%)	2/8 neurons (25%)
Inhibitory response	5/9 neurons (56%)	6/8 neurons (75%)
Males	**SERT-KO**	**WT**
Excitatory response	3/10 neurons (30%)	4/10 neurons (40%)
Inhibitory response	7/10 neurons (70%)	6/10 neurons (60%)

Figures

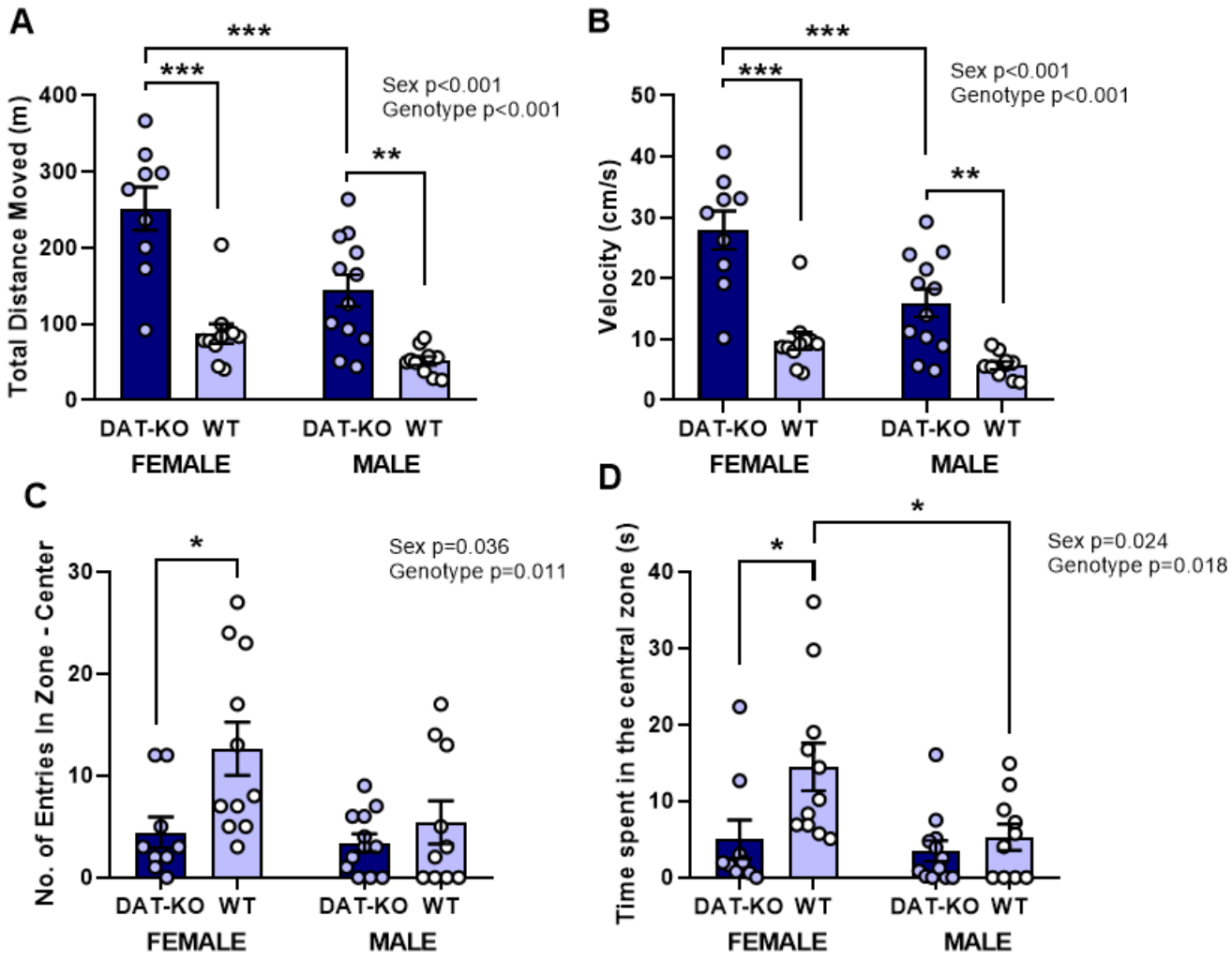


Figure 1

Locomotor activity and anxiety-like behavior in male and female DAT-KO rats and their corresponding WT controls in the open-field test.

(A) Total distance travelled, (B) mean velocity, (C) number of entries into the central zone and (D) time spent in the central zone. Group sizes were n = 9 for female DAT-KO, n = 11 for female WT, n = 12 for male DAT-KO, and n = 10 for male WT rats. Data were analyzed by two-way ANOVA with sex and genotype as factors, followed by Bonferroni pairwise comparisons. *p < 0.05, **p < 0.01, ***p < 0.001.

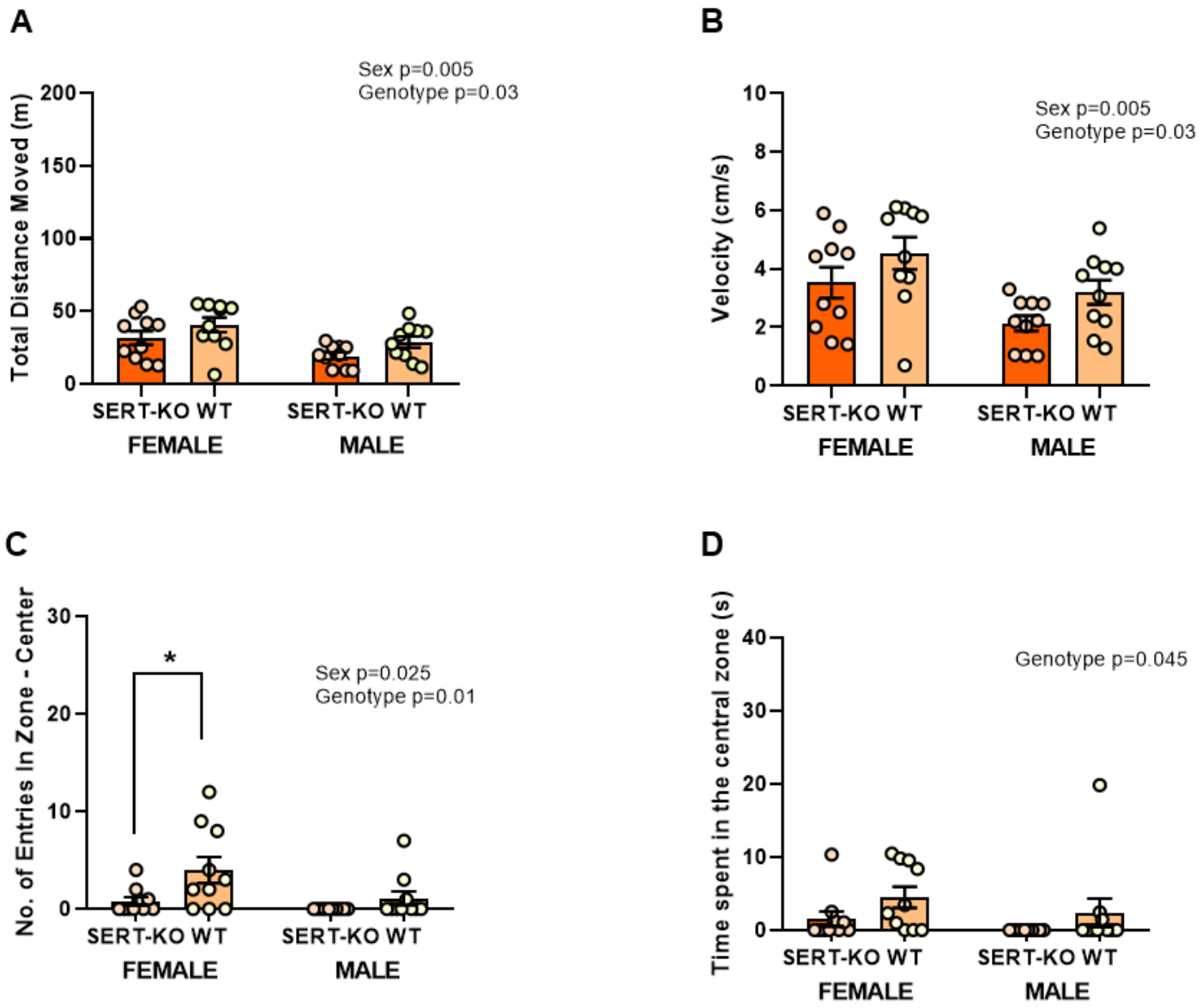


Figure 2

Locomotor activity and anxiety-like behavior in male and female SERT-KO rats and their corresponding WT controls in the open-field test.(A) Total distance travelled, (B) mean velocity, (C) number of entries into the central zone and (D) time spent in the central zone. Each sex and genotype group consisted of n = 10 animals. Data were analyzed by two-way ANOVA with sex and genotype as factors, followed by Bonferroni pairwise comparisons. *p < 0.05.

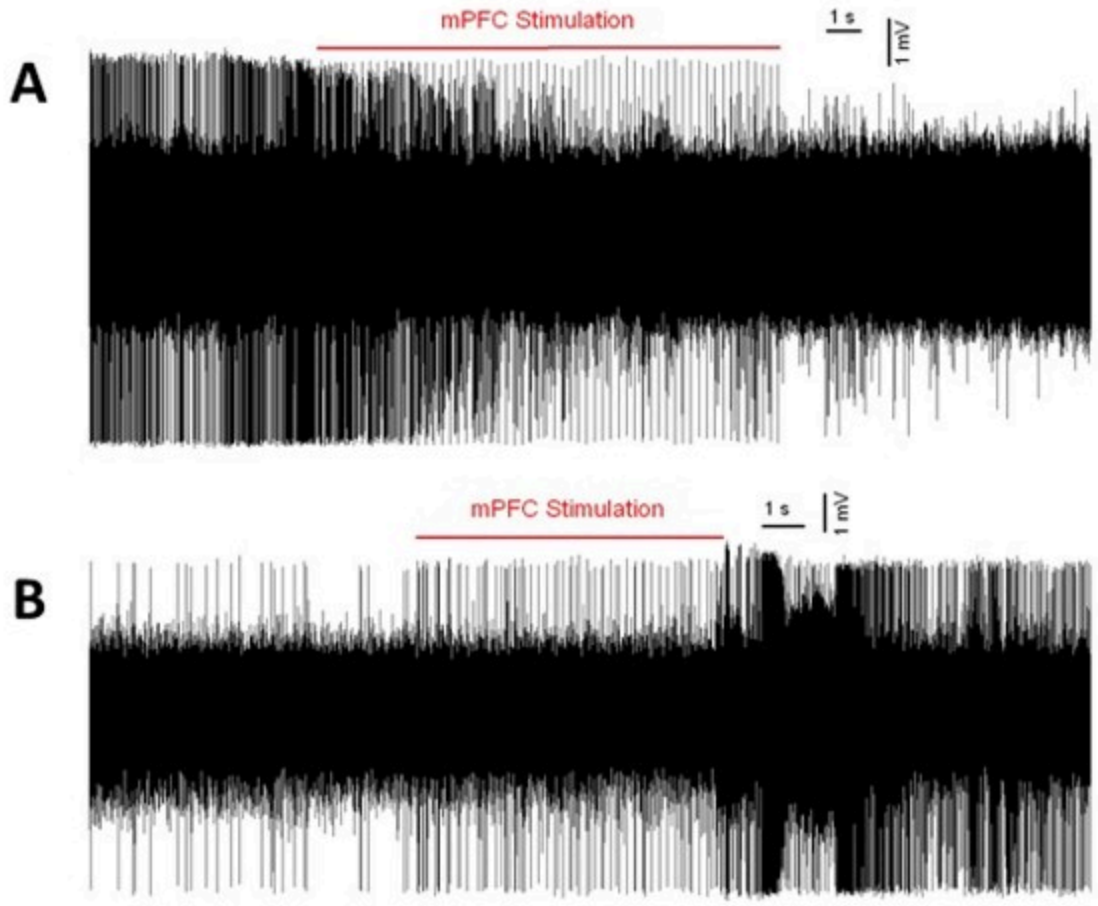


Figure 3

Representative recordings of lateral habenula neuronal responses to electrical stimulation of the medial prefrontal cortex. Representative extracellular single-unit recordings showing an inhibitory (A) and an excitatory (B) response of LHb neurons to mPFC stimulation. The horizontal bar indicates the period of mPFC stimulation.

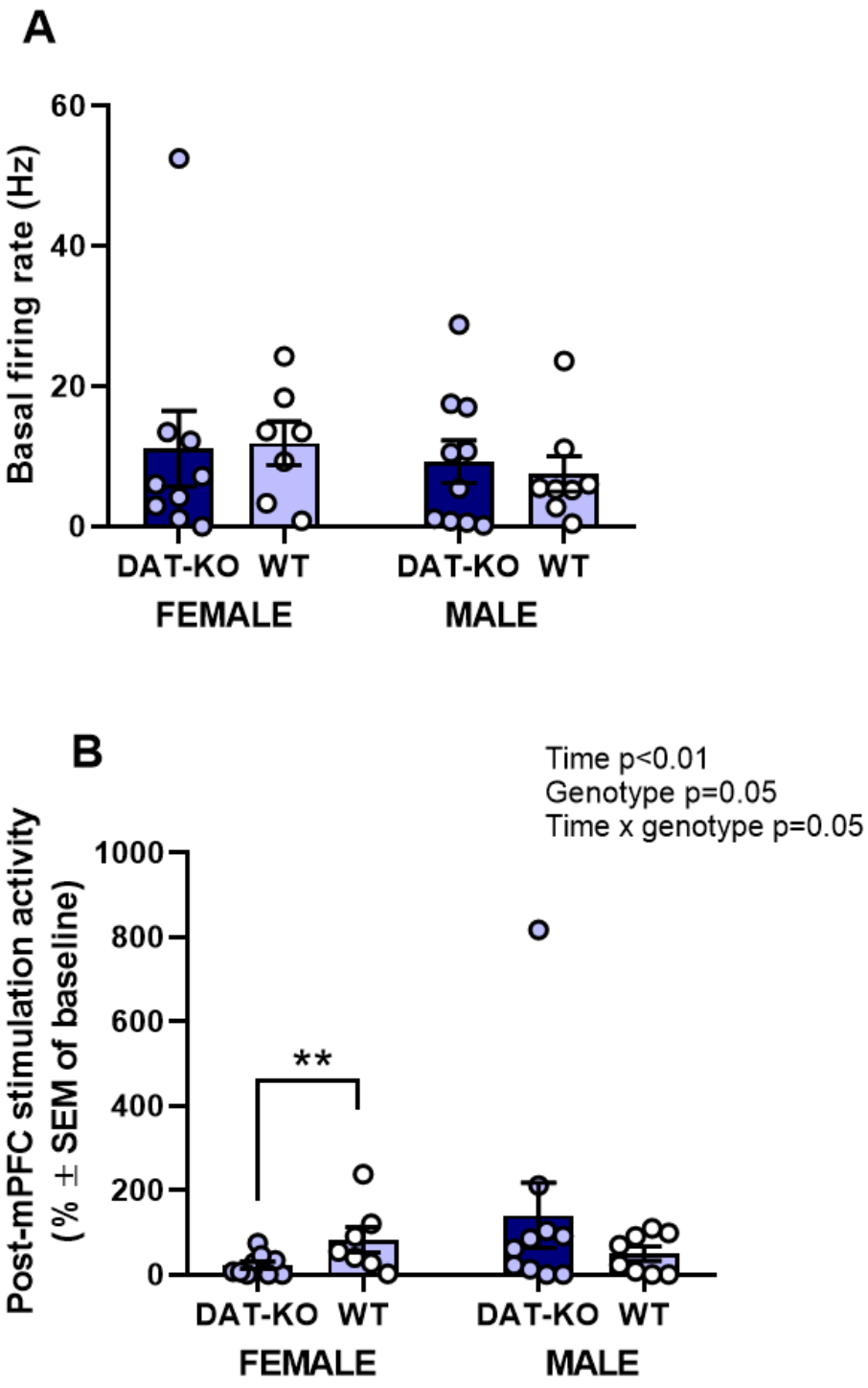


Figure 4

Basal firing activity and reactivity of lateral habenula neurons to medial prefrontal cortex stimulation in male and female DAT-KO rats and their corresponding WT controls. (A) Basal firing rate of LHb neurons. (B) Firing activity following mPFC stimulation expressed as a percentage of the respective baseline activity. Group sizes were n = 9 for female DAT-KO, n = 7 for female WT, n = 16 for male DAT-KO, and n = 10 for male WT rats. Basal firing rates were analyzed by two-way ANOVA with sex and genotype as

factors. Responses to mPFC stimulation were analyzed separately in males and females by two-way repeated-measures ANOVA with time and genotype as factors, followed by Bonferroni pairwise comparisons. **p < 0.01.

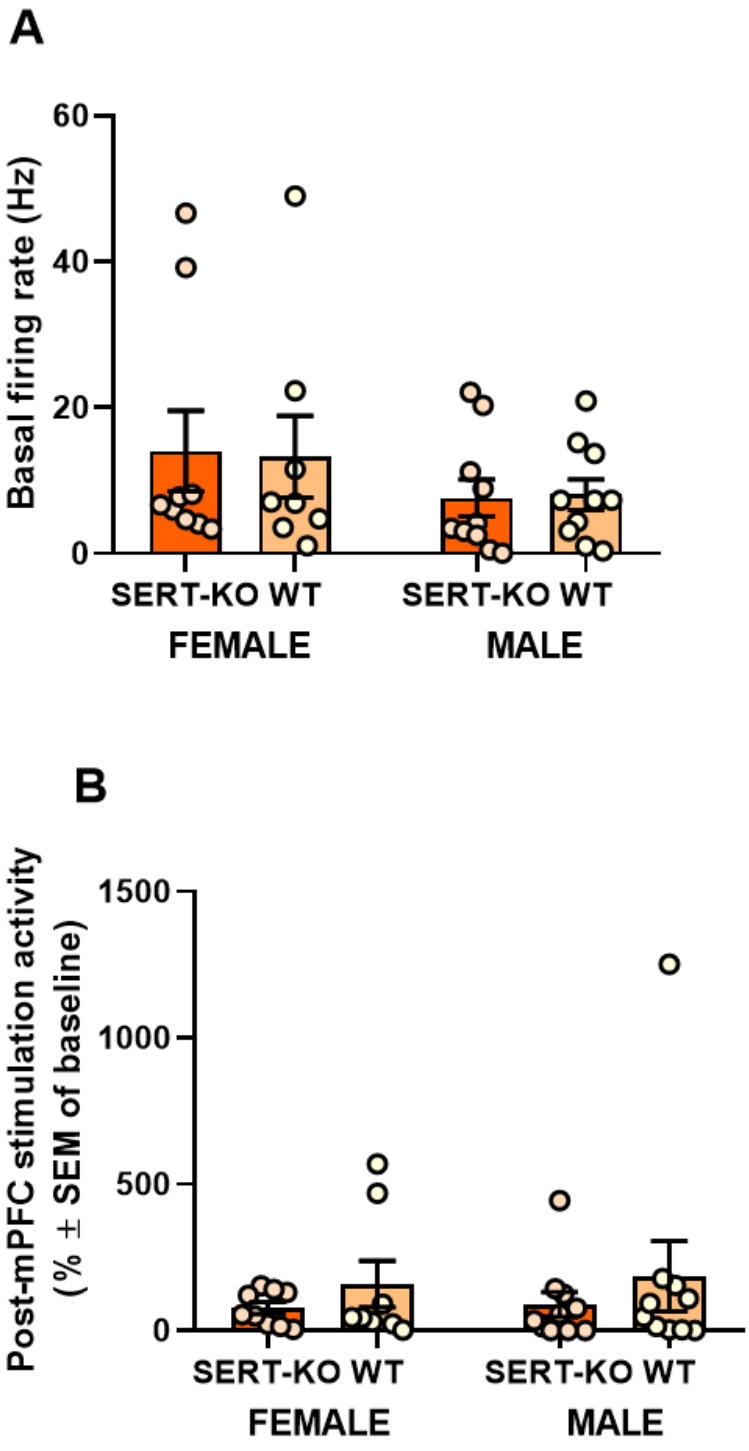


Figure 5

Basal firing activity and reactivity of lateral habenula neurons to medial prefrontal cortex stimulation in male and female SERT-KO rats and their corresponding WT controls. (A) Basal firing rate of LHb neurons. (B) Firing activity following mPFC stimulation expressed as a percentage of the respective baseline activity. Group sizes were n = 9 for female SERT-KO, n = 8 for female WT, n = 10 for male SERT-KO, and n = 10 for male WT rats. Basal firing rates were analyzed by two-way ANOVA with sex and genotype as factors. Responses to mPFC stimulation were analyzed separately in males and females by two-way repeated-measures ANOVA with time and genotype as factors.

Supplementary Files

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