Altered behaviour, dopamine and norepinephrine regulation in stressed mice heterozygous in TPH2 gene

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1. Introduction

Pathological aggression is a common feature of many neuropsychiatric diseases (Van Voorhees et al., 2014; Yu et al., 2019; Kunik et al., 2020; Slaughter et al., 2020). Aggression is also associated with a higher incidence of suicide attempts (Dumais et al., 2005;
Conjero et al., 2019; Reich et al., 2019). Moreover, as a consequence of the ongoing COVID-19 pandemic, the incidence of mental disorders is expected to rise, as levels of stress, anxiety, aggression and depression increase, both through direct cases of viral illness and through negative social and economic consequences (Chaturvedi, 2020; Wind et al., 2020). Yet, excessive aggression is currently treated in conjunction with other psychiatric disorders using non-specific drugs with serious side effects, such general sedation, dizziness and rebound effect (Lane et al., 2011; Zaman et al., 2018).

The unmet medical need for therapy for aggression requires the development of improved animal models of excessive aggression. Recent genome-wide association studies suggest that there is greater variability in the genetic mechanisms thought to underpin neuropsychiatric disorders in comparison to neurological diseases (Lee et al., 2019), which further support the need to better understand the role of gene x environment interaction in psychiatric pathologies (Lesch, 2005; Lesch and Mössner, 2006). The majority of the translational studies performed to date have employed either conventional laboratory animals that have been exposed to a stressor, e.g., predation (Costa-Nunes et al., 2014; Strekalova et al., 2015), social defeat (Pari et al., 2013), maternal separation (Auth et al., 2018; Köser et al., 2018; Veenema et al., 2007; Weidner et al., 2019), ultrasound (Gorlova et al., 2019; Pavlov et al., 2019) and others (Mineur et al., 2003; Malki et al., 2016), or they have used naïve genetically manipulated mice and rats (Nelson and Chiavegatto, 2001; Manchia et al., 2017).

Recently, we proposed a new aggression paradigm in which the contribution of gene x environment interaction in the mechanisms of pathological aggression is modelled in heterozygous (Tph2+/−) mice by a five-day rat exposure (Gorlova et al., 2020). The interaction of the Tph2+/− genotype and stress had also been explored in earlier studies of Gutknecht et al. (2012) and Weidner et al. (2019). Behavioural trends seemed to be present, but they were not significant, which was felt likely to be a consequence of the mild chronic stress paradigm and maternal separation that were employed. Conceptually, the stress studies with the Tph2+/− mutants were based on the reported relationships between single nucleotide polymorphisms (SNPs) in the Tph2 gene e.g., rs1843809, rs4570625, and rs6582071 and psychiatric symptoms of excessive aggression (Oades et al., 2008; Perez-Rodriguez et al., 2010; Plemenitas et al., 2015; Laas et al., 2017), personality disorder (Perez-Rodriguez et al., 2010), negative emotionality (Lesch et al., 2012), depression (Wigner et al., 2018), mood disorder and suicidality (Ottenhof et al., 2018). Importantly, the incidence of these associations can be increased by a history of life stressors (Forssman et al., 2014; Xu et al., 2016).

While naïve mice with a complete lack of Tph2 gene (Tph2−/−) recapitulate many symptoms related to SNPs of the Tph2 gene, showing increased aggression, decreased sociability (Angoa-Perez et al., 2012; Weidner et al., 2019; Lieb et al., 2019), hyperactivity, impulsive and compulsive behaviours (Jia et al., 2014; Angoa-Perez et al., 2012; Waider et al., 2017) and enhanced fear learning (Lesch et al., 2012; Gutknecht et al., 2015; Weidner et al., 2019), but also signs of stress resilience, altered depressive-like behaviour, decreased anxiety and increased neurogenesis (Savelieva et al., 2008; Jia et al., 2014; Gutknecht et al., 2012, 2015; Zhang et al., 2016; Weidner et al., 2019), these changes were not found in unchallenged Tph2−/− mice. However, in Tph2−/− mice, predation stress elicits excessive aggressiveness with pathological behavioural patterns of impulsive aggression, as well as changes in the brain concentrations of serotonin, its precursor hydroxytryptophan, and its metabolite 5-hydroxyindoleacetic acid, which were studied in different regions of the brain (Gorlova et al., 2020). Remarkably, behavioural changes in mutants were found to be the opposite to those found in Tph2−/− mice exposed to the predation, i.e., males and females on a C57BL/6 J background display a decrease in aggressive and dominant behaviours in response to predation (Gorlova et al., 2020). As such, the use of predation stress model in Tph2−/− mice offers a promising model to explore the neurobiology of pathological aggression. In this paradigm, the stressed mutants have been shown to have altered measures of plasticity compared to the controls (Gorlova et al., 2020). These results are in keeping with other molecular abnormalities found in the Tph2−/− mice subjected to a maternal separation, where the stressed mutants exhibited altered brain expression of the sodium-dependent serotonin transporter (Lieb et al., 2019) and changes in the DNA methylation of cholecystokinin receptor (Weidner et al., 2019).

Here, we sought to extend our studies with the Tph2−/− mice exposed to stress in order to examine how other endpoints are altered following environmental challenges. In particular, we were keen to understand how the gene x environment interactions affect the levels of the monoamines dopamine (DA) and norepinephrine (NE), whose role in aggression is well established in a clinical context (Comai et al., 2012) and in animal studies (Hutchins and Pearson, 1975; Lewis et al., 1994; Adamczyk et al., 2012). We also sought to discover what other behaviours would be affected by the Tph2−/− genotype in response to predation stress or to other environmental challenges including exposure to a novel environment and to a modified forced swim test (modFST), which is known to induce helplessness in mice (Strekalova et al., 2016).

Here, we found increased aggression and dominancy, and suppressed sociability in the Tph2−/− mice that were exposed to a predation stress procedure. In response to stress, anxiety-like behaviours were only observed in the WT mice, but not in the Tph2+/−. These behaviours were associated with significant genotype-dependent differences in the concentrations of DA and NE and of their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylglycol (MHPG), respectively, as well as DA and NE turnover rates. Tph2−/− mice challenged with a novel environment exhibited increased vertical exploratory activity, and, in the modFST, they exhibited no floating behaviour potentional, suggesting the presence of a behavioural resilience to stress. Thus, stressed Tph2−/− mutants display a behavioural profile that resembles that of unstressed Tph2−/− animals, and the combination of predation stress and the Tph2−/− genotype presents itself as a promising model of excessive human aggression induced by changing environmental conditions.

2. Materials and methods
2.1. Animals

We used 10-12-week-old male Tph2−/− mice, and their wild-type littermates (Tph2+/+) as controls, which were bred and genotyped in the facilities of the University of Würzburg (Gutknecht et al., 2015). 12-week-old male CD1 mice were used as intruders for a resident-intruder test and 2-to-5-month-old male Wistar rats (Charles River, Janvier, France) were used for the predator stress. Tph2+/− and Tph2−/− littermates were housed individually, while CD1 mice and rats were housed in groups of five. Animals were kept under controlled laboratory conditions (22 ± 1 °C, 55% humidity, food and water ad libitum, lights on: 21:00 h). Studies were carried out in accordance with the European Communities Council Directive for the care and use of laboratory animals (permission issued by Ethical Committee of C. Bernard University of Lyon).

2.2. Study outline

Two cohorts of animals (Fig. 1A&B) were exposed predation stress in which Tph2−/− mutants, and littermate controls, were subjected to a daily rat-exposure stress for 5 days (Costa-Nunes et al., 2014; Vignisse et al., 2017; see below). The groups of mice were balanced according to their body weight, and for latency to attack in a baseline resident-intruder test (Couch et al., 2016; Strekalova et al., 2018). Beginning 24 h after the end of the predation stress procedure, the first set of animals were tested in the elevated O-maze (day 6), dark/light box (day 6), and, on days 6–10, in a resident-intruder test, with 3 h inter-test
intervals (see below, Fig. 1A). In the second cohort, 24 h after the end of the last predator stress session, the mice were killed and their brains were isolated and dissected (see below) for subsequent HPLC tissue assay (see below, Fig. 1 B). An additional cohort of Tph2<sup>+/−</sup> mutants and their littermate Tph2<sup>+/+</sup> control mice were subjected to a novel cage test and to the modified swim test (Fig. 1C). In each part of the experiment, an average of seven to ten mice per group were used (group sizes are indicated in Figure legends).

2.3. Predation stress

Mice were introduced into a spacious transparent glass cylinder (15 cm high x 8 cm diameter) and placed into the rat cage as described elsewhere (Costa-Nunes et al., 2014; Vignisse et al., 2017; Gorlova et al., 2020). A 15-h long exposure was performed between 18:00 and 9:00 for five consecutive nights. Mice had free access to food and water in their home cages between the stress sessions. The timing of the rat exposure model was designed to minimize the impact of food and water deprivation, as the predation period overlaps with the light (inactive) phase of activity of the mice when food and water consumption is minimal. While there is no doubt that food and water deprivation during this period might add to the stress, our earlier studies, with another version of this stress model, revealed that if food and water is made available during the predation stress the C57/Bl6 mice do not consume it (Strekalova et al., 2004; Strekalova and Steinbusch, 2009, 2010). Thus, while the environment undoubtedly results in a mixture of stresses, the principal stress is based on the fear of predation.

2.4. O-maze test

The O-maze apparatus (Technoplast, Rome, Italy) consisted of a circular path (runway width 5.5 cm, diameter 46 cm) placed 50 cm above the floor. Two opposing arms were protected by walls (height 10 cm), and the illumination strength was 25 Lux. Anxiety-like behaviour was assessed using previously validated parameters as described elsewhere (Couch et al., 2016; Costa-Nunes et al., 2020). Mice were placed in one of the closed arm compartments of the maze. Total duration of time spent in the open arms of the maze and the number of visits to the open arms were scored as established measures of anxiety-like behaviour during the first 5 min period.

2.5. Dark/light box test

At the start, mice were placed into the dark compartment (15 cm x 20 cm x 25 cm) of the box from where they could visit the lit box (30 cm x 20 cm x 25 cm, Technoplast, Rome, Italy). The time spent by the mice in the lit part of the box, which was set to 25 Lux on the floor of the apparatus, was recorded over a 5-min period, as described elsewhere (Costa-Nunes et al., 2014; Strekalova et al., 2018).

2.6. Resident-intruder test

The resident-intruder test procedure was carried out on five consecutive days (Strekalova et al., 2004, 2018; Costa-Nunes et al., 2014; Gorlova et al., 2020). Mice were placed individually in an observation cage (30 cm x 60 cm x 30 cm) for 30 min, after which a CD1 mouse was introduced. During the next 4 min, mice were separated by a transparent wall with holes it is, which was removed for the following 4-min-long period. Latency to attack and the duration of attacks were recorded. In addition, measures of neutral social exploration were evaluated; the latency to contact and the number of nose/anal and nose/nose contacts were recorded as described elsewhere (Couch et al., 2016; Veniaminova et al., 2017).

Fig. 1. Experiment design. Tph2<sup>+/+</sup> and Tph2<sup>−/−</sup> mice were subjected to rat exposure stress for 5 consecutive nights, and 24 h after last stress session both groups were (A) studied in a battery of behavioural tests or (B) killed and brains were dissected for HPLC study. (C) Naïve Tph2<sup>+/+</sup> and Tph2<sup>−/−</sup> mice were studied for 5 consecutive days in novel cage test and modified swim test.
2.7. Novel cage test

A 5-min long novel cage test was carried out to assess exploration in a new environment as described elsewhere (Strekalova et al., 2004; Couch et al., 2016; Veniaminova et al., 2020). Mice were introduced into a standard plastic cage (21 cm × 21 cm × 15 cm) filled with fresh sawdust. The number of exploratory rears was counted in white light for 5 min; the strength of illumination was set to 25 Lux.

2.8. The modified swim test to assess helplessness

Mice were subjected to two swimming sessions with an interval of 24 h. After the first two swim session a third swim session was carried out on day 5 as previously described (Strekalova et al., 2016; Pavlov et al., 2017, 2020). All sessions were 6-min long and were performed by placing a mouse in a transparent cylinder (Ø 17 cm) filled with water (+23 °C, water height 13 cm, height of cylinder 20 cm). The duration of floating behaviour that was defined as the absence of any directed movements of the head or body, which was scored by an observer unaware of the identity of the animal with Noldus EthoVision XT 8.5 (Noldus Information Technology, Wageningen, The Netherlands) as described elsewhere (Malatynska et al., 2012). It is of note that the increase in floating behaviour, which is observed on day 5 compared to day 2, is reversible by pre-treatment common antidepressant compounds (Markova et al., 2017). For this reason, the increase in day 5 floating is regarded as a measure of learning in an adverse context and helplessness (Pavlov et al., 2017, 2020).

2.9. Tissue collection

Mice were terminally anaesthetized with an intraperitoneal injection of sodium pentobarbitone. The left ventricle was perfused with 10 ml of ice-cold saline and the brains were removed and dissected (Couch et al., 2013). The prefrontal cortex, striatum, amygdala, hippocampus and dorsal raphe were isolated and stored at −80 °C as described elsewhere (Gorlova et al., 2020). Selected brain structures were microdissected using Paxinos atlas as a guide (Paxinos and Franklin, 2001). As the HPLC method requires a substantial amount of tissue, all brain structures were analysed as a whole.

2.10. High-performance liquid chromatography (HPLC) tissue assay

The concentrations of dopamine, norepinephrine, and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylglycol (MHPG), respectively, were measured in the parts of the brain mentioned above using HPLC with electrochemical detection using the method of Waider et al. (2017). Dopamine turnover was calculated as a ratio of DOPAC/DA, and norepinephrine turnover was calculated as MHPG/NE.

2.11. Statistical analysis

Data were analysed using GraphPad Prism v.8.3.0 for Windows (San Diego, CA). Three-way, or two-way, or repeated measures ANOVA were employed followed by post-hoc testing were appropriate. For the two-group comparisons in the novel cage test and modFST, unpaired t-test were used. The level of confidence was set at 95% (p < 0.05). For non-parametric data (O-maze exits) a Kruskal-Wallis rank sum test was performed with post-hoc pairwise comparisons using Wilcoxon rank sum test.

3. Results

3.1. Increased aggressive behaviour in stressed Tph2+/− mice

A three-way ANOVA revealed that there was no interaction between predator stress, genotype, and the day of testing for the latency to attack in the resident-intruder test (F1,21 = 0.095; p = 0.76). There was also no significant interaction between stress and the day was present independent of genotype (F1,46 < 0.01, p = 0.89, two-way ANOVA). However, a significant interaction, independent of day, was found between predator stress and genotype (F1,46 = 17.44, p < 0.01, two-way ANOVA). Post hoc analysis revealed that there was a significantly shorter latency to attack in the stressed Tph2+/− group than in either the non-stressed Tph2+/− or the stressed Tph2+/+ mice (both p < 0.01, Sidak’s test). A significant day × genotype interaction, independent of predator stress, was also present (F1,23 = 6.215, p = 0.02, two-way ANOVA). Sidak’s post-hoc testing revealed that significant decrease in the latency to attack on the day 5 in Tph2+/− group compared to day 1 (p < 0.01; Fig. 2A).

Total duration of attacks was found to be dependent on an interaction between predator stress, genotype, and the day of testing as revealed by a three-way ANOVA (F1,21 = 5.046, p = 0.04). Post hoc analysis showed that stress increased the duration of attacks in the Tph2+/− animals on days 1 and 5 compared to non-stressed Tph+/− animals or the stressed Tph+/− animals (all p < 0.01, Bonferroni test). In the mutant mice the duration of attacks was higher on day 5 than on day 1 (p = 0.01; Fig. 2B). Thus, predator stress appears to have the opposite effect on the duration of attacks in the Tph+/− animals subjected to.

3.2. Stressed Tph2+/− mice display a decrease of neutral social exploration

Both latency and number of nose/anal contacts were dependent on a stress, genotype and the day of testing (F1,21 = 7.06, p = 0.015 and F1,20 = 12.13, p < 0.01, respectively, three-way ANOVA). Post hoc testing revealed a significant increase in the latency to nose/anal contact on day 5 in stressed Tph2+/− animals in comparison to stressed Tph2+/+ animals and to the non-stressed Tph2+/− animals on the same day (p = 0.03 and p = 0.04, respectively, Bonferroni test) and in comparison to stressed Tph2+/− on day 1 (p = 0.02; Fig. 2C). On day 5, stress significantly reduced the number of nose/anal contacts in the stressed Tph2+/− compared to the stressed Tph2+/+ animals (p < 0.01), while in Tph2+/− mice stress increase the number of contacts (p = 0.04).

Three-way ANOVA also showed the presence of an interaction between stress, genotype, and the day for both latency to nose/nose contact and the total number of nose/nose contacts (F1,20 = 6.23, p = 0.02 and F1,21 = 13.46, p < 0.01, respectively). On day 5, the latency to a nose/nose contact was significantly longer in the predator stressed Tph2+− mice as compared to the stressed Tph2+/+ animals (p = 0.04). Number of nose/nose contacts was significantly elevated in the stressed Tph2+− animals from day 1 to day 5 (p < 0.01), which was not observed in the Tph2+− animals.

3.3. Anxiety-like behaviour after stress exposure does not alter in Tph2+/− mice

In the elevated O-maze, using Kruskal-Wallis rank sum test it was found that there was an interaction between the genotype and stress (chi-squared = 11.932, df = 3, p-value =0.0076). Subsequent pairwise comparisons using Wilcoxon rank sum test revealed that there were significant differences between the stressed Tph2+/− group and both the Tph2+/− stressed (p = 0.030) and non-stressed animals (p = 0.026). Stressed Tph2+/− mice displayed shorter latency to exit to an open arm than non-stressed Tph2+/− (p = 0.03, Tukey’s test, data not shown). In stressed Tph2+/− animals the number of exits was significantly lower in comparison to non-stressed controls (p = 0.03; Fig. 3A). The main effect of genotype, but not main stress effect or stress × genotype interaction was significant for the time spent the open arms of the O-maze (F1,20 = 6.12, p = 0.02; F1,20 = 1.55, p = 0.23 and F1,20 = 4.087, p = 0.06, respectively; Fig. 3B). Thus, in essence, Tph2+/− mice do not exhibit the anxiety-like behaviours that are observed in Tph2+/+ group in the
elevated O-maze.

In the dark-light box test, no significant stress x genotype interaction was found for latency to entry into the lit box, number of entries and time spent in the lit box (F_{1,20} = 0.027, p = 0.87; F_{1,20} = 1.903, p = 0.18 and F_{1,20} = 0.95, p = 0.34, respectively; Fig. 3C,D), as well as no significant stress effects (F_{1,20} = 1.56, p = 0.23; F_{1,20} = 0.95, p = 0.34 and F_{1,20} = 1.9, p = 0.18, respectively; Fig. 3C,D) and no genotype effects (F_{1,20} = 0.48, p = 0.49; F_{1,20} = 2.13, p = 0.16 and F_{1,20} < 0.01, p > 0.99, respectively; Fig. 3C,D).

3.4. Stress-induced changes in dopamine metabolism in the brain of Tph2^{+/−} mice

The was a significant interaction between stress and genotype for the concentration of dopamine in the amygdala and prefrontal cortex, but not in hippocampus, dorsal raphe or striatum (F_{1,27} = 5.75, p = 0.02; F_{1,28} = 8.41, p < 0.01; F_{1,28} < 0.01, p = 0.98; F_{1,27} = 0.04, p = 0.84 and F_{1,26} = 0.05, p = 0.82, respectively, two-way ANOVA). In the hippocampus, dorsal raphe, and striatum the concentration of dopamine was significantly affected by stress (F_{1,28} = 28.58, p < 0.01; F_{1,27} = 5.08, p = 0.03 and F_{1,26} = 47.73, p < 0.01, respectively). A post-hoc analysis revealed significantly lower concentrations of dopamine in the amygdala of the stressed Tph2^{+/−} mice in comparison to both the non-stressed Tph2^{+/−} and the stressed Tph2^{+/+} animals (p < 0.01 and p = 0.02, respectively Tukey’s test. Fig. 4A). The concentration of dopamine in the prefrontal cortex was decreased in stressed Tph2^{+/−} mice compared to the non-stressed mutants (p < 0.01; Fig. 4D). Finally, the concentration of dopamine was lower in the stressed controls and mutants in comparison with non-stressed groups in the hippocampus (both p < 0.01, Tukey’s test; Fig. 4G) and striatum (both p < 0.01; Fig. 4M).

In contrast to the levels of dopamine, there was found to be an interaction between stress and genotype for DOPAC in the amygdala and in the dorsal raphe, but not in prefrontal cortex, hippocampus, or striatum (F_{1,28} = 12.86, p < 0.01; F_{1,27} = 4.25, p < 0.05; F_{1,28} = 1.09, p = 0.31; F_{1,28} = 0.76, p = 0.39 and F_{1,26} = 3.87, p = 0.06, respectively). Stress alone significantly affected DOPAC concentrations in the prefrontal cortex and striatum (F_{1,28} = 4.42, p = 0.04 and F_{1,26} = 21.49, p < 0.01, respectively) and post hoc analysis revealed that in the amygdala of stressed Tph2^{+/+} mice the DOPAC concentration was elevated compared to non-stressed Tph2^{+/+} animals or predator stressed Tph2^{+/−} mice (both p < 0.01, Tukey’s test; Fig. 4B). The concentration of DOPAC was increased in stressed Tph2^{+/+} mice in comparison with non-stressed controls in dorsal raphe (p = 0.06; Fig. 4K). It was also elevated in striatum of stressed Tph2^{+/+} compared to the non-stressed mutants (p < 0.01; Fig. 4N).

A significant interaction between stress and genotype on the dopamine turnover rate was found in the prefrontal cortex (F_{1,28} = 11.30, p < 0.01, two-way ANOVA), but not in amygdala, hippocampus, dorsal...
raphe, or striatum ($F_{1,27} = 1.13, p = 0.30; F_{1,28} < 0.05, p = 0.86; F_{1,27} = 1.77, p = 0.19$ and $F_{1,26} = 0.46, p = 0.5$, respectively). However, stress had significant main effect on dopamine turnover in amygdala, hippocampus, and dorsal raphe ($F_{1,27} = 30.07, p < 0.01; F_{1,28} = 51.13, p < 0.01$ and $F_{1,27} = 5.03, p = 0.03$, respectively). Post hoc analysis revealed an increase in turnover rate in the stressed controls and mutants in comparison with non-stressed groups in the amygdala ($p = 0.02$ and $p < 0.01$, Tukey’s test, respectively; Fig. 4C) and hippocampus (both $p < 0.01$; Fig. 4I), while in the prefrontal cortex of stressed Tph2$^{+/−}$ mice it was increased compared to both non-stressed Tph2$^{+/−}$ or Tph2$^{+/+}$ animals (both $p < 0.01$; Fig. 4F).

3.5. Altered brain metabolism of norepinephrine in stressed Tph2$^{+/−}$ mice

There was no significant interaction between stress and genotype for norepinephrine concentrations in amygdala, prefrontal cortex, hippocampus, dorsal raphe and striatum ($F_{1,28} < 0.01, p = 0.89; F_{1,28} = 0.11, p = 0.75; F_{1,28} = 1.77, p = 0.19; F_{1,27} = 1.21, p = 0.28$ and $F_{1,28} = 0.78, p = 0.39$, respectively, two-way ANOVA). Stress significantly affected concentrations of norepinephrine in prefrontal cortex, hippocampus, and striatum ($F_{1,28} = 7.96, p < 0.01; F_{1,28} = 6.00, p = 0.02$ and $F_{1,28} = 50.82, p < 0.01$, respectively). Post hoc analysis revealed that there was a significant increase in norepinephrine levels in the stressed Tph2$^{+/−}$ and Tph2$^{+/−}$ mice compared to non-stressed animals of respective genotype (both $p < 0.01$, Tukey’s test; Fig. 5M).

MHPG concentrations exhibited a stress x genotype interaction in the amygdala, but not in the prefrontal cortex, hippocampus, dorsal raphe, or striatum ($F_{1,28} = 13.45, p < 0.01; F_{1,28} = 0.86, p = 0.36; F_{1,28} = 1.7, p = 0.2; F_{1,27} = 0.42, p = 0.52$ and $F_{1,28} < 0.01, p = 0.78$, respectively). Main effects for stress and genotype were observed in the hippocampus ($F_{1,28} = 28.75, p < 0.01$ and $F_{1,28} = 6.34, p = 0.02$, respectively; Fig. 5H). In the stressed Tph2$^{+/−}$ group, the MHPG level in the amygdala was significantly lower than in both the non-stressed Tph2$^{+/−}$ mutants and stressed Tph2$^{+/−}$ mice (both $p = 0.01$, Tukey’s test; Fig. 5B).

There was a significant interaction between genotype and stress for norepinephrine turnover rate in the amygdala, but not in prefrontal cortex, hippocampus, dorsal raphe, or striatum ($F_{1,28} = 11.31, p < 0.01; F_{1,28} = 0.25, p = 0.62; F_{1,28} < 0.01, p = 0.95; F_{1,27} = 5.9, p = 0.45$ and $F_{1,28} = 2.95, p = 0.14$, respectively). Norepinephrine turnover rate was significantly lower in the stressed Tph2$^{+/−}$ compared to the non-stressed Tph2$^{+/−}$ in the amygdala ($p < 0.01$, Tukey’s test; Fig. 5C). Stress alone significantly changed norepinephrine turnover rate in prefrontal cortex, hippocampus, and striatum ($F_{1,28} = 18.32, p < 0.01; F_{1,28} = 6.17, p = 0.02$ and $F_{1,28} = 27.84, p < 0.01$, respectively). Norepinephrine turnover rate was decreased in the prefrontal cortex of stressed Tph2$^{+/−}$ in comparison to controls ($p = 0.01$; Fig. 5F). It also was decreased in the striatum of stressed Tph2$^{+/−}$ and Tph2$^{+/−}$ mice compared to the non-stressed groups of same genotypes (both $p < 0.01$ and $p < 0.05$, respectively; Fig. 5O).

3.6. Tph2$^{+/−}$ mice display increased novelty exploration and reduced helplessness in the modFST test

In the modFST test, there was an interaction between day and genotype in the duration of floating, but not in the latency to float or the number of floating episodes ($F_{2,22} = 3.73, p = 0.04; F_{2,22} = 3.56, p = 0.07$ and $F_{2,22} = 0.69, p = 0.51$, respectively, two-way ANOVA). A main effect of the day was observed for the latency to float and number of floating episodes ($F_{1,124,1,42} = 9.80, p < 0.01$ and $F_{1,901,20,91} = 6.44, p < 0.01$, respectively). No significant group differences in latency to float were found on day 1 ($p = 0.14$, Bonferroni test; Fig. 6A). Latency to float was shortened in Tph2$^{+/−}$ mice on day 5 compared to day 1 ($p = 0.02$, Bonferroni test; Fig. 6A), the number of floating episodes was increased in Tph2$^{+/−}$ on day 5 compared to day 1 ($p = 0.04$; Fig. 6B). On day 5, the duration of floating was significantly shorter in Tph2$^{+/−}$ than in the Tph2$^{+/−}$ animals ($p < 0.01$; Fig. 6C).

In the novel cage test, Tph2$^{+/−}$ mice showed significantly higher number of exploratory rears than their Tph2$^{+/−}$ littermates ($p = 0.04,$
unpaired t-test; Fig. 6D). Together, observations in these two behavioural tests suggest that there is a lower propensity to float and increased exploratory activity in the naïve Tph2+/− mice when they are exposed to environmental challenges, which may reflect increased stress-resilience in these animals.

4. Discussion

Tph2+/− mice subjected to the predation stress paradigm displayed a marked increase of aggression, dominance and a suppression of neutral sociability, whereas the stressed Tph2+/+ group showed opposing changes in social behaviour. Rat exposure paradigm increased anxiety-like behaviour in Tph2+/− control mice, but did not alter it in the Tph2+/+ mutants. The separate cohort of Tph2+/− mice exhibited increased exploration in a novel environment and a lack of potentiating of floating behaviour over the course of repeated sessions of the ModFST. The HPLC tissue assays revealed profound changes in the content and metabolism of brain DA and NE in the stressed and in the non-stressed Tph2+/− mice, suggesting that this monoamine dysregulation might contribute to the altered behaviours that are observed in the mutants.

In accordance with previous results, we found that Tph2+/− mice subjected to a five-day predation stress paradigm displayed excessive aggression, which was the opposite of the changes found in the littermate controls (Gorlova et al., 2020) and reminiscent to aggressive behaviour typical of non-stressed Tph2+/− mice (Gutknecht et al., 2012, 2015). Additional analysis of the social behaviour showed that the stressed Tph2+/− mice also displayed increased measures of dominancy and decreased scores of neutral social exploration, which are known to correlate with the appearance of aggressive traits in small laboratory rodents, including the naïve null Tph2+/− mice (Gutknecht et al., 2012, 2015; Jia et al., 2014; Weidner et al., 2019), and the present findings further supports the view of phenotypical resemblance between naïve Tph2+/− and stressed...
Tph2+/− mice (Gorlova et al., 2020).

Previous work with stress paradigm that was employed here demonstrated altered brain concentrations and metabolism of serotonin, a major regulator of aggression and anxiety (Nelson and Chiavegatto, 2001; Abela et al., 2020; Quah et al., 2020), in the stressed Tph2+/− mice (Gorlova et al., 2020). The serotonergic system closely interacts with brain dopaminergic system of the brain, reciprocally modulating dopaminergic activity (Millan et al., 1998; De Deurwaerdere et al., 2005; Seo et al., 2008; Rosell and Siever, 2015). Lower levels of dopamine were previously measured in the brains of naive Tph2+/− mice in the hippocampus and in the frontal cortex (Gutknecht et al., 2012). Similarly, in the present study, stress resulted in diminished DA concentrations in the amygdala and in the prefrontal cortex of Tph2+/− mice that was not found in Tph2+/− animals. In the stressed Tph2+/− mice, DA concentrations were also lower in the hippocampus and striatum in comparison with naive mutants. Thus, changes in the dopaminergic system of the stressed Tph2+/− mice are strikingly different from those found in the Tph2+/− animals and overly resemble those observed in Tph2−/− mice; they can underpin stress-induced excessive aggression and other behavioural changes reported in this study.

Changes to the serotonergic system are often associated with changes to NE neurotransmission, particularly, in amygdala (Pucilowski et al., 1987; Millan et al., 1998). Here, stress exposure resulted in similar genotype-related increases of NE concentration and NE turnover in the striatum, elevated concentrations of NE metabolite MHPG in the hippocampus and decreased NE turnover in the prefrontal cortex. However, genotype differences between stressed groups were shown for MHPG concentrations in the amygdala, whose values in the Tph2+/− group were lower than in stressed Tph2+/− mice. Stress significantly decreased this measure, as well as NE turnover in the amygdala of Tph2+/− mice, but not in the littermate Tph2−/− controls. These data further show the contribution of amygdala and suggest the role of NE in described here stress-induced behavioural abnormalities of Tph2+/− mice.

Together, the HPLC data presented here provides evidence for genotype-governed differences in the stress-induced changes of DA and NE regulation in the amygdala and prefrontal cortex. These are brain areas in which genotype changes in the TPH2-deficient mice were also shown for serotonin precursor 5-hydroxytryptophan (5-HTP). This led to the suggestion that these inter-related changes in the three monoamines in the amygdala might underlie excessive aggression and altered social behaviour in general of stressed Tph2+/− mutants. This suggestion

Fig. 5. Stress-induced alterations in concentrations of norepinephrine, its metabolite MHPG, and norepinephrine turnover rate in Tph2+/− and Tph2−/− mice. (A) In the amygdala, no significant group differences were observed in the norepinephrine concentration. (B) MHPG concentration was significantly lower in the stressed Tph2+/− compared to both stressed Tph2+/− and non-stressed Tph2+/− mice. (C) Norepinephrine turnover rate was significantly lower in stressed Tph2+/− in comparison to non-stressed Tph2+/− mice. No significant changes between the groups were found in (D) norepinephrine concentration and (E) MHPG concentration in the prefrontal cortex. (F) However, norepinephrine turnover rate in the prefrontal cortex was significantly lower in stressed Tph2+/− compared to non-stressed Tph2+/− mice. (G) No significant group changes in the norepinephrine concentration were observed in the hippocampus. (H) In the hippocampus, MHPG concentration was elevated significantly in both genotypes in stressed groups compared to non-stressed animals, but (I) no significant changes between the groups were found in the norepinephrine turnover rate. (J - L) No significant group changes in the norepinephrine metabolism were found in the dorsal raphe. (M) In the striatum, in both genotypes, there were significant increases in the norepinephrine concentrations in stressed groups compared to non-stressed animals of the same genotype. (N) MHPG concentration in the striatum did not show any significant changes between the groups. (O) Norepinephrine turnover ratio was significantly lowered in both genotypes in stressed groups in comparison to non-stressed animals. *p < 0.05, two-way ANOVA and post hoc Tukey’s test. 7-9 animals per group were used. Bars represent Mean ± SEM. NE = norepinephrine, MHPG = 3-Methoxy-4-hydroxyphenylglycol.
is keeping with previously reported monoamine changes in the amygdala of stressed Tph2\(^{-/-}\) mice in relation to GABA metabolism (Waider et al., 2017), as well as the altered methylation profile of CCK in this brain structure (Weidner et al., 2019). These findings are in line with the well-documented primary role of the amygdala in the regulation of aggression (Haller et al., 2017) and stress response (Tottenham and Galván, 2016). Moreover, they are also in agreement with clinical data showing that the amygdala has a key function in the response to emotional stimuli of valence in carriers of SNPs of the Tph2 genes G-844 T and G-703 T (Brown et al., 2005; Canli et al., 2005).

Regarding other brain structures that could be potentially involved in behavioural changes reported here in the Tph2\(^{-/-}\) mice, it should be noted that prefrontal cortex was shown to regulate amygdala-dependent processing of social cues in fMRI studies in humans with acute tryptophan depletion (Passamonti et al., 2012). Reduced inhibitory control of the amygdala by the prefrontal cortex is considered to result in impulsive aggression (Jackson and Moghaddam, 2001; Haller, 2017). Here, we found diminished DA levels in the prefrontal cortex in stressed Tph2\(^{-/-}\) group. These changes might lead to a decrease in the top-down control of the amygdala (Rosenkranz and Grace, 2001) resulting in excessive aggression that is in keeping with earlier studies. For example, the hypoactivation of the prefrontal cortex in rats was associated with elevated activation of the amygdala in a single-prolonged stress paradigm (Piggott et al., 2019).

Chronic restraint stress in C57BL/6 mice was shown to enhance amygdala-prefrontal cortex interactions, and optogenetic activation of these circuits elicited anxiety-like and hyperactivity in naïve mice (Lowery-Gionta et al., 2018). The changes of NE regulation observed here after stress in the prefrontal cortex may alter the function of the amygdala that is keeping with previous findings (Van Bockstaele et al., 1998).

Our previous study showed a decrease of 5-HTP and the serotonin metabolite 5-Hydroxyindoleacetic acid (5-HIAA) in the striatum of stressed Tph2\(^{-/-}\) mice, but not in other experimental groups (Gorlova et al., 2020). Together with the findings of a decreased DOPAC concentration in the striatum in stressed Tph2\(^{-/-}\) mice, this suggests a role for this brain area in differential effects of stress in these mutants compared to the Tph2\(^{+/+}\) controls. The striatum, and, particularly, the nucleus accumbens, is essential in the function of ‘limbic-motor’ interface, which is functionally connected to the amygdala (Cardinal et al., 2003; Ambroggi et al., 2008) and to the prefrontal cortex (Del Arco and Mora, 2008; Janak and Tye, 2015). In rats, lesions of the nucleus accumbens provoked excessive aggression and muricide behaviour (Lee and Ueki, 1986; Pucilowski and Valzelli, 1986). Whereas social defeat stimulated dopamine signalling by increasing both DA release and uptake (Anstrom et al., 2009; Deal et al., 2018), isolation stress supressed baseline DA levels, but increased the DA-mediated response to various stimuli (Yorgason et al., 2016; Karkhanis et al., 2016). While nucleus accumbens is distinct from dopaminergic region of striatum functionally, many data suggest unidirectional stress-induced changes in DA metabolism in these areas (Abercrombie et al., 1989; Porcelli et al., 2012). A decrease of DA concentrations in the striatum of stressed mice in our study may resemble previously reported effects of chronic isolation stress on basal DA levels in rats (Yorgason et al., 2016; Karkhanis et al., 2016). Thus, in addition to the changes reported here in the whole striatum, the studies highlighted above argue for the importance of the striatal DA system in the mechanisms of stress associated with social determinants.

The stress model employed here is based largely on social stress. The model was designed to minimize the impact of physical stressors such as food and water deprivation, and the loss of consummatory behaviour in mice during the rat exposure was shown to be secondary to the fear of predation (Strekalova and Steinbusch, 2009, 2010). Yet, the role of these stressors, as well as other potential factors cannot be completely ruled out (Nakamura et al., 1996; Bekkevold et al., 2013), thus, the nature of the applied stress model should be considered in the broader context.

Separate experiments on Tph2\(^{-/-}\) mice revealed elevated vertical exploratory activity in a situation of novelty and a lack of increase in floating behaviour between days 2 and 5, which is a marker of enhanced acquisition of despair behaviour (Strekalova et al., 2016; Pavlov et al., 2020). Both features can be interpreted in a light of increased resilience to environmental challenges and stressors, as diminished rearing activity in the novel cage and increased floating behaviour were associated with a susceptibility of mice to chronic stress (Strekalova et al., 2004; Cline et al., 2012, 2015). Elevated locomotor activity in home cage conditions was reported in naïve Tph2\(^{-/-}\) mice (Zhang et al., 2018), which is reminiscent of the finding reported here. Notably, increased aggression scores were found to inversely correlate with the stress response and the development of depressive behaviour in mice (Strekalova et al., 2004; Comai et al., 2012). Together, these findings suggest the presence of similar behavioural patterns in Tph2\(^{-/-}\) mice and the null mutants, which were also described to show decreased signs of stress resilience (Savelieva et al., 2008; Jia et al., 2014; Weidner et al., 2019).

Fig. 6. Reduced propensity to float and increased vertical exploratory activity in naïve Tph2\(^{-/-}\) mice. In the modified swim test, (A) naïve Tph2\(^{-/-}\) showed significant decrease in the latency to the first immobility episode between days 1 and 5 of the test. (B) Total number of immobility episodes significantly increased from day 1 to day 5 in naïve Tph2\(^{-/-}\) mice. (C) Total duration of floating was significantly lower in naïve Tph2\(^{-/-}\) mice compared to naïve Tph2\(^{+/+}\) mice on day 5 of the test. Both in Tph2\(^{-/-}\) and Tph2\(^{+/+}\) mice total immobility time was significantly lower on day 1 in comparison with day 2 and day 5. (D) In the novel cage test, naïve Tph2\(^{-/-}\) mice showed significantly higher number of rearing in comparison to naïve Tph2\(^{+/+}\) mice. *p < 0.05; in A-C repeated measures two-way ANOVA and post hoc Tukey’s test; in D unpaired t-test. 5–8 animals per group were used. Bars represent Mean ± SEM.
5. Conclusions

Thus, the present findings suggest that Tph2-altered mutants exposed to the predation stress model display changes in social behaviour that are the opposite of those observed in control mice. In summary, these results, along with data on anxiety-like behaviour and the outcome from studies on mutants challenged with novelty and repeated swimming, suggest that under stress conditions, Tph2-altered mice demonstrate a less anxious profile that is reminiscent of naïve Tph2-altered animals. This behavioural repertoire is associated with increased aggressive behaviour, decreased stress-induced anxiety and other signs of stress resilience, such as reduced floating and elevated exploratory activity. In addition to previously demonstrated changes in serotonin metabolism in the amygdala, altered dopamine concentration and turnover in this brain structure are likely to mediate excessive aggression and reduced sociability of stressed Tph2-altered mice. These neurochemical changes, along with genotype differences in monoamine regulation in the prefrontal cortex and striatum and a lack of changes in DOPAC levels in the dorsal raphe in stressed mutants are likely to contribute to the behavioural phenotype of these mice. In conclusion, present paradigm can be considered as a promising model of gene x environment interactions underlying excessive human aggression.

Author statement

All authors confirm that they have contributed to the manuscript “Altered behavior, dopamine and norepinephrine regulation in stressed mice heterozygous in TPH2 gene”, have read and approved its final form and Response to reviewers.

Ethical statement

All experiments described in the manuscript were performed in accordance with the European Communities Council Directive for the care and use of laboratory animals upon approval by the local governmental body of animal care and welfare.

Declaration of Competing Interest

All authors express a lack of any conflict of interests in connection with the manuscript “Altered behavior, dopamine and norepinephrine regulation in stressed mice heterozygous in TPH2 gene”.

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References

Abercrombie, E.D., Keefe, K.A., DifRischia, D.S., Zigmond, M.J., 1989. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial prefrontal cortex. J. Neurochem. 52, 1655–1658. https://doi.org/10.1111/j.1471-5204.1989.tb09224.x.

Afanas’ev, A.V., Meijas, R., Takamaki, K., Yocum, J., Kranova, L.N., Calderon, J., Cadet, J., L., Huganir, R.L., Pietrasik, M.V., Wang, T., 2012. GluA3-deficiency in mice is associated with increased social and aggressive behavior and elevated dopamine in striatum. Behav. Brain Res. 229, 265–272. https://doi.org/10.1016/j.bbr.2012.01.007.

Abercrombie, E.D., Keefe, K.A., DifRischia, D.S., Zigmond, M.J., 1989. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial prefrontal cortex. J. Neurochem. 52, 1655–1658. https://doi.org/10.1111/j.1471-5204.1989.tb09224.x.

Abercrombie, E.D., Keefe, K.A., DifRischia, D.S., Zigmond, M.J., 1989. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial prefrontal cortex. J. Neurochem. 52, 1655–1658. https://doi.org/10.1111/j.1471-5204.1989.tb09224.x.

Abercrombie, E.D., Keefe, K.A., DifRischia, D.S., Zigmond, M.J., 1989. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial prefrontal cortex. J. Neurochem. 52, 1655–1658. https://doi.org/10.1111/j.1471-5204.1989.tb09224.x.
Del Arco, A., Mora, F. 2008. Prefrontal cortex-nucleus accumbens interaction: in vivo modulation by dopamine and glutamate in the prefrontal cortical circuit. Pharmacol. Biochem. Behav. 91, 84–94. https://doi.org/10.1016/j.pbb.2008.04.001.

Dumais, A. S., Ber, L., Seage, A.D., Phil, M., Alaa, M., Rouleau, G., Ph, D., Dumont, M., Chawkly, N., Ph, M., Roy, M., Mann, J.J., Benkefat, C., Turecki, G., Ph, D. 2005. Risk Factors for Suicide Completion in Major Depression: A Case-Control Study of Impulsive and Aggressive Behaviors in Men. p. 2116–2124.

Forssman, L., Ytrehus, S., Puura, K., Mononen, N., Lhtimákki, T., Leppanen, J.M. 2014. Regulatory variants of the TPH2 gene and early life stress are associated with heightened attention to social signals of fear in infants. J. Child Psychol. Psychiatry 55, 793–801. https://doi.org/10.1111/jcpp.12181.

Gorlava, A., Pavlov, D., Anthony, D.C., Ponmorev, E.D., Sambon, P., Proshin, A., Shafarevich, I., Babavyskyana, D., Lesch, K.P., Bettendorf, L., Strekalova, T. 2019. The role of behavioral and functional ultrasonic vocalizations of cage-mate aggression, normalize AMPA receptor expression and plasticity markers, and reduce oxidative stress in mice. Neuropharmacology 156, 105743. https://doi.org/10.1016/j.neuropharm.2019.02.029.

Gorlava, A. Orthoped, E., Babavyskyana, N., Veniaminovna, E., Proshin, A., Kaluev, A., Anthony, D.C., Lesch, K.P., Strekalova, T. 2020. Stress-induced aggression in heterogeneous TPH2 mutant mice is associated with alterations in serotonin turnover and expression of 5-HT6 and AMPA subunit 2A receptors. J. Affec. Disord. 272, 440–451. https://doi.org/10.1016/j.jad.2020.04.034.

Gutknecht, L., Aranagi, M., Merker, S., Waider, J., Sommerlandt, F.M.J., Minari, B., Bacci, G., Mayer, U., Proff, F., Hamon, M., Schmitt, A.G., Corradetti, R., Lanfumey, L., Lesch, K.P. 2012. Impacts of brain serotonin deficiency following Typhoid development and rape neuropathic sensitization. PLoS One 7. https://doi.org/10.1371/journal.pone.0043515.

Gutknecht, L., Pop, S., Waider, J., Sommerlandt, F.M.J., Gipperp, C., Pest, A., Reif, A., Van Den Hove, D., Strekalova, T., Schmitt, A., Colacino, M.B.N., Sommer, C., Pehl, E., Lesch, K.P. Interaction of behavior and synaptogenesis with stress and sex differentially impact emotional behavior in Tph2 knockout mice. Psychopharmacology 232, 2429–2441. https://doi.org/10.1007/s00213-015-3879-9.

Haller, J. 2017. The role of central and medial amygdala in normal and abnormal aggression: a review of classical approaches. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2017.10.029.

Hutchins, D.A., Pearson, Z.D.M. 1975. Striatal metabolism of dopamine in mice, p. 241. Elsevier.

Lesch, K.P., M. 2002. Effects of food-restriction stress on immune response in mice. J. Neuroimmunol. 30, 235. https://doi.org/10.1016/j.jneuroim.2002.04.011.

Malki, K., Tosto, M.G., Pain, O., Shyter, F., Mineur, Y.S., Cruise, W.E., de Boer, S., Lysko, A., Kalueff, A.V., Ksenzenova, E., Schvolyvka, L.C., Asherson, P., 2016. Comparative mRNA analysis of behavioral and genetic mouse models of aggression. Am. J. Med. Genet. Part B Neurogenet. 171, 427–436. https://doi.org/10.1002/ajmg.b.32271.

Manchon, M., Carpinello, B., Valtorta, V., Comai, S. 2017. Serotonin dysfunction, aggressive behavior, and mental illness: exploring the link using a dimensional approach. ACS Chem. Neurosci. 8, 961–972. https://doi.org/10.1021/acschemneuro.6b00427.

Markova, K., Babenovna, N., Anthony, D.C., Vinignovn, J., Sivatanon, A., Lesch, K.P. Bettendorf, L., Strekalova, T. 2017. Thiamine and benfotiamine improve cognition and ameliorate GSK3β-assisted stress-induced behaviours in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 75, 148–156. https://doi.org/10.1016/j.pnpbp.2017.01.001.

Millan, M.J., Dekeyne, A., Gobert, A. 1998. Serotonin (5-HT1C) receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. Neuropharmacology 37, 953–955. https://doi.org/10.1016/S0028-3908(98)00078-1.

Mineur, Y.S., Prasolos, D.J., Belzung, C., Cruise, W.E. 2003. Agonistic behavior and unpredictable chronic mild stress in mice. Behav. Genet. 33, 513–519. https://doi.org/10.1021/acschemneuro.6b00427.

Mosienko, V., Bert, B., Davis, M., Y., Chen, C.H., Tsai, M.H., Lu, T.P., Huang, M.C., Kuo, P.H., 2019. Transcriptome analysis of the brain in mice with high levels of aggression. Neuropsychopharmacology 10, 115–122. https://doi.org/10.1016/j.npp.2018.08.010.

Ottenhof, K.W., Sild, M., Lévesque, M.L., Ruhe, G.H., Booij, L. 2018. TPH2 polymorphisms across the spectrum of psychiatric morbidity: a systematic review and meta-analysis. Neurosci. Biobehav. Rev. 92, 29–42. https://doi.org/10.1016/j.neubiorev.2018.05.018.

Passamonti, L., Crockett, M.J., Apergis-Schoute, A.M., Clark, L., Rowe, J.B., Calder, A.J., Robbins, T.W. 2012. Effects of acute tryptophan depletion on prefrontal-amygdalectomy connectivity while viewing facial signals of aggression. Biol. Psychiatry 71, 713–719. https://doi.org/10.1016/j.biopsych.2011.07.033.

Patki, G., Solanki, N., Atroz, F., Allam, F., Salim, S. 2013. Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. Brain Res. 1539, 73–86. https://doi.org/10.1016/j.brainres.2013.07.035.

Pavlov, D., Markova, N., Bettendorf, L., Chekhonin, P., Pomytkin, I., Lioudyno, V., Sivatanon, A., Ponmorev, E., Lesch, K.P., Strekalova, T. 2017. Elucidating the neurobiological basis of aberrant hippocampal plasticity in a mouse model of social stress and lack of affective deficits in 18-month-old C57Bl/6 mice: implications for modeling elderly depression. Exp. Gerontol. 47, 552–564. https://doi.org/10.1016/j.exger.2012.04.010.

Pavlov, D., Gorlava, A., Loupy, K.M., Yeritsyan, N.B., Vignisse, J., Bachurin, S., Strekalova, T. 2012. Anhedonic-like traits are associated with affective deficits in 18-month-old C57Bl/6 mice: implications for modeling elderly depression. Exp. Gerontol. 47, 552–564. https://doi.org/10.1016/j.exger.2012.04.010.

Pavlov, D., Markova, N., Bettendorf, L., Kaluev, A.A., Umrathkin, A., Proshin, A., Lysko, A., Landgraf, R., Anthony, D.C., Strekalova, T. 2020. Enhanced conditioning of adverse memories in the mouse modified swim test is associated with pronounced neuroinflammatory changes - effects that are susceptible to antidepressants. Neurobiol. Learn. Mem. 172, 107227. https://doi.org/10.1016/j.nlm.2020.107227.

Paxinos, G., Franklin, K.B.J., 2001. Paxinos and Franklin’s The Mouse Brain in Stereotaxic Coordinates, 2nd edition. Academic Press, San Diego. p. 246.

Pavlova, N., M., Weidner, N., Weidner, N., Aviles, A., Boey, L., Yuan, Q., Zhou, Z., Hodgkinson, C., Goodman, M., Koeningberg, H.W., Goldman, D., Stever, L.J., 2010. Tryptophan-hydroxylase 2 haplotypes association with borderline personality disorder.
disorder and aggression in a sample of patients with personality disorders and healthy controls. J. Psychiatr. Res. 44, 1075–1081. https://doi.org/10.1016/j.jpsych.res.2009.11.016.
Piggott, V.M., Bosse, K.E., Lisienk, M.J., Strader, J.A., Stanley, J.A., Conti, A.C., Ghodousian, F., Perrine, S.A., 2019. Single-prolonged stress impairs prefrontal cortical control of amygdala and striatum in rats. Front. Behav. Neurosci. 13, 1–9. https://doi.org/10.3389/fnbeh.2019.00018.
Flemenitas, A., Kores Plesniar, B., Kastelic, M., Porcelli, S., Serretti, A., Dolzan, V., 2015. Genetic variability in tryptophan hydroxylase 2 gene in alcohol dependent and alcohol-related psychopathological symptoms. Neurosci. Lett. 604, 86–90. https://doi.org/10.1016/j.neulet.2015.07.027.
Porcelli, A.J., Lewis, A.H., Delgado, M.R., 2012. Acute stress influences neural circuits of alcohol-related psychopathological symptoms. Neurosci. Lett. 604, 86–90. https://doi.org/10.1016/j.neulet.2015.07.027.

Strekalova, T., Markova, N., Shevtsova, E., Zubareva, O., Bakhmet, A., Steinbusch, H.M., Strekalova, T., Steinbusch, H.W.M., 2010. Measuring behavior in mice with chronic stress. Behav. Brain Res. 217, 227–231. https://doi.org/10.1016/j.bbr.2010.09.029.
Van Bockstaele, E.J., Colago, E.E.O., Valentino, R.J., 1987. Amygdaloid corticotropin-releasing factor targets locus coeruleus dendrites: substrate for the co-ordination of emotional and cognitive limbs of the stress response. J. Neuroendocrinol. 10, 743–758. https://doi.org/10.1111/j.1365-2826.1987.tb00524.x.
Van Voorhees, E.E., Dennis, P.A., Elbogen, E.B., Clancy, C.P., Hertzberg, M.A., Beckham, J.C., Calhoun, P.S., 2014. Personality assessment inventory internalizing and externalizing structure in veterans with posttraumatic stress disorder: associations with aggression. Agress. Behav. 40, 562–592. https://doi.org/10.1002/ab.21554.
Veena, A.H., Bredewold, R., Neumann, I.D., 2007. Opposite effects of maternal separation on intermale and maternal aggression in C57BL/6 mice: link to hypothalamic vasopressin and oxytocin immunoreactivity. Psychoneuroendocrinology 32, 437–450. https://doi.org/10.1016/j.psyneuen.2007.02.008.
Veniaminova, E., Cespuglio, R., Cheung, C.W., Umriukhin, A., Markova, N., Shvetsova, E., Lesch, K.P., Anthony, D.C., Strekalova, T., 2017. Autism-like Behaviours and memory deficits result from a Western diet in mice. Neural Plast. 2017 https://doi.org/10.1155/2017/9489247.
Veniaminova, E., Oplatychikova, M., Bettendorf, L., Kotenkov, E., Lyuko, A., Vasilevskaya, E., Kaufel, A.V., Fedulova, L., Umriukhin, A., Lesch, K.P., Anthony, D.C., Strekalova, T., 2020. Prefrontal cortex inflammation and liver pathologies accompany cognitive and motor deficits following Western diet consumption in non-obese female mice. Life Sci. 241, 117163. https://doi.org/10.1016/j.lfs.2019.117163.
Vigneux, J., Samson, M., Gorkova, A., Pavlov, D., Caron, N., Malgrange, B., Shvetsova, E., Svitinov, A., Anthony, D.C., Markova, N., Bazhenova, N., Coumous, B., Lakaye, W., Pons, P.J., Strekalova, T., 2017. Thiamine and benfotiamine prevent stress-induced suppression of hippocampal neurogenesis in mice exposed to predation without affecting brain thiamine diphosphate levels. Mol. Cell. Neurosci. 82, 126–136. https://doi.org/10.1016/j.mcn.2017.05.005.
Waidner, J., Popp, S., Lange, M.D., Kern, R., Kohler, J.F., Kohler, J., Donner, N.C., Lowe, K.R., Malzender, J.H., Brazel, C.J., Arnold, M.R., Abagay, B., Schmitt-Böhrer, A., Lowry, C.A., Pape, H.C., Lesch, K.P., 2017. Genetically driven brain serotonin deficiency facilitates panic-like escape behavior in mice. Transl. Psychiatry 7, e1242. https://doi.org/10.1038/tp.2017.209.
Waidner, J., Popp, S., Minlar, B., Montalban, A., Bonfiglio, F., Abagay, B., Thuy, E., Kern, R., Thiel, C., Aragagi, N., Svinir, E., Schmitt-Böhrer, A.G., Corradetti, R., Lowry, C.A., Lesch, K.P., 2019. Serotonin deficiency increases context-dependent fear learning through modulation of hippocampal activity. Front. Neurosci. 13, 245 https://doi.org/10.3389/fnins.2019.00245.
Weidner, M.T., Lardonejo, R., Ejisen, L., Mogavero, F., Popp, S., Palme, R., Forstner, K.U., Reese, B.E., Kipin, T.E., 2019. Identification of Cholecystokinin by Genome-Wide Profiling as Potential Mediator of Serotonin-Dependent Behavioral Effects of Maternal Separation in the Amygdala, p. 13. https://doi.org/10.3389/fnins.2019.00460.
Wigser, P., Zarny, P., Synowiec, E., Bijk, M., Biatk, K., Talarsowna, M., Galecki, P., Szerma, J., Sliwinski, T., 2018. Association between single nucleotide polymorphisms of TPH1 and TPH2 genes, and depressive disorders. J. Cell. Mol. Med. 22, 1778–1791. https://doi.org/10.1111/jcmm.13459.
Wing, T.S., Rijkheem, M., Anderson, G., Ripper, H., 2020. The COVID-19 pandemic: the ‘black swan’ for mental health care and a turning point for e-health. Internet Interv. 20, 100317. https://doi.org/10.1016/j.invent.2020.100317.
Xu, Z., Reynolds, G.P., Yuan, Y., Shi, Y., Pu, M., Zhang, Z., 2016. TPH-2 polymorphisms interact with early life stress to influence response to treatment with antidepressant drugs. Int. J. Neuropsychopharmacol. 19, pyw070. https://doi.org/10.1093/ijnp/pyw070.
Yorgason, J.T., Calipari, E.S., Ferris, M.J., Karkhanis, A.N., Fordahl, S.C., Weiner, J.L., Jones, S.R., 2016. Social isolation rearing increases dopamine uptake and psychostimulant potency in the striatum. Neuropharmacology 101, 479–489. https://doi.org/10.1016/j.neuropharm.2015.10.025.
Yu, R., Topiwala, A., Jacoby, R., Fazel, S., 2019. Aggressive behaviors in Alzheimer disease and mild cognitive impairment: systematic review and meta-analysis. Am. J. Geriatr. Psychiatry 27, 290–300. https://doi.org/10.1016/j.jagp.2018.10.008.
Zaman, H., Sampson, S., Beck, A., Sharma, T., Clay, D., Spyridi, S., Zhao, S., Gilles, D., 2018. Benzodiazepines for psychosis-induced aggression or agitation. Schizophrenia Bull. 44, 966–969. https://doi.org/10.1093/schbul/bby056.
Zhang, X., Yan, H., Luo, Y., Huang, Z., Rao, Y., 2018. Thermoregulation-independent regulation of sleep, by serotonin revealed in mice defective in serotonin synthesis S. Mol. Pharmacol. 93, 657–664. https://doi.org/10.1124/mii.111229.