Brain serotonin deficiency leads to social communication deficits in mice

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A deficit in brain serotonin is thought to be associated with deteriorated stress coping behaviour, affective disorders and exaggerated violence. We challenged this hypothesis in mice with a brain-specific serotonin depletion caused by a tryptophan hydroxylase 2 (TPH2) deficiency. We tested TPH2-deficient (Tph2−/−) animals in two social situations. As juveniles, Tph2−/− mice displayed reduced social contacts, whereas ultrasonic vocalizations (USVs) were unchanged within same-sex same-genotype pairings. Interestingly, juvenile females vocalized more than males across genotypes. Sexually naive adult males were exposed to fresh male or female urine, followed by an interaction with a conspecific, and re-exposed to urine. Although Tph2−/− mice showed normal sexual preference, they were hyper-aggressive towards their interaction partners and did not vocalize in response to sexual cues. These results highlight that central serotonin is essential for prosocial behaviour, especially USV production in adulthood, but not for sexual preference.

1. Introduction

A deficit in central serotonin (5-HT) is thought to be associated with deteriorated stress coping behaviour, affective disorders and exaggerated violence [1]. In rodents, genetically or pharmacologically induced 5-HT reduction in the central nervous system (CNS) is related to a loss of avoidance behaviour and increased aggression [2,3], and is suggested to cause the loss of sexual preference [4,5]. However, a detailed analysis of social communication deficits associated with the observed behavioural alterations [6] across development is still missing.

We recently generated mice that are constitutively deficient for the rate-limiting enzyme of 5-HT synthesis, tryptophan hydroxylase 2 (TPH2), which is expressed solely in 5-HT-producing cells within the CNS. Mice lacking TPH2 are almost completely devoid of brain 5-HT (less than 4% of wild-type levels), exhibit growth retardation during the first weeks of life but are vital and do not show obvious malfunctions in adulthood, probably owing to compensatory mechanisms evoked by the lifelong absence of 5-HT [7,8]. Here, we used these mice to investigate if central 5-HT is essential for social behaviour and communication in non-violent conditions, i.e. juvenile interaction and sexual behaviour at adult age. Our data reveal an important role of central 5-HT for the expression of (a)social, but not sexual behaviours.

2. Material and methods

Behavioural tests were conducted in wild-type (Tph2+/+), heterozygous (Tph2+/−) and homozygous (Tph2−/−) mutant mice [7] on a highly social C57BL/6N background [2] (see details in the electronic supplementary material). Offspring from Tph2−/− breeding couples were weaned around postnatal day (PND) 21, group
housed and maintained on a 12:12 light/dark cycle (lights off at 18.00 for juvenile or at 06.00 for adult mice) with standard chow and water ad libitum.

Juvenile social interaction was investigated after 1 day of single housing around PND25 in a new cage. An unfamiliar naive animal of the same sex, age and genotype was introduced after 1 min. Behaviour and ultrasonic vocalizations (USVs) were measured for 5 min under red light to reduce the stress level.

Sexually naive males at 15 weeks of age were singly housed for three weeks in a female-free husbandry room. Behavioural tests were conducted under red light during the dark phase following a modified protocol [9]. On day 1 and day 2, Tph2+/+ and Tph2−/− mice were cross-balanced exposed for 5 min (figure 2a) to a new cage containing 50 µl of either fresh male or female urine, collected from FVB/N mice a maximum of 4 h earlier. On day 3 and day 4, animals had 1 min of habituation to a new cage followed by 15 min of cross-balanced interaction with either a male or oestrus-synchronized female FVB/N mouse (group housed, three months of age). Finally, on day 5 and day 6, sexually experienced mice were re-exposed to urine of each sex. Avisoft Bioacoustics (Germany) and ViewSonic software (Biobserve, Germany) were used to analyse USV emission and behaviour (see details in the electronic supplementary material).

For comparing social behaviour and USV production between genotypes two-way ANOVAs for repeated measurements with the between-subject factors 'genotype' and 'sex' were calculated, followed by least significant difference (LSD) post hoc tests when appropriate. To analyse urine preference and social behaviour during dyadic interactions, paired and unpaired t-tests, or non-parametric tests (Mann–Whitney U-test and Wilcoxon paired test) were performed. A p-value of less than 0.05 was considered statistically significant. The Kolmogorov–Smirnov test was used to evaluate if groups meet the Gaussian distribution. Accordingly, parametric (for Gaussian distribution) and non-parametric (for non-Gaussian distribution) tests were used for the analysis.

3. Results

(a) Juvenile social interaction

To evaluate the impact of central 5-HT deficiency on juvenile mice, we first investigated social behaviour and USV production at PND25 in Tph2+/+, Tph2−/− and Tph2−/− mice during interaction with an unfamiliar conspecific of the same genotype, sex and age. Genotypes differed in juvenile social interaction behaviour, with significant genotype differences being detectable in all three parameters determined, namely number of social interactions (ANOVA, \( F_{2,48} = 5.660; \ p = 0.006; \) figure 1a), total contact duration (ANOVA, \( F_{2,48} = 5.657; \ p = 0.006; \) figure 1b) and average contact duration (ANOVA, \( F_{2,48} = 8.715; \ p = 0.001; \) figure 1c).

Specifically, juvenile Tph2−/− displayed fewer social contacts than Tph2+/− (LSD post hoc, \( p = 0.002 \)) and Tph2+/− (LSD post hoc, \( p = 0.018 \)) littersmates. However, total contact duration was higher in juvenile Tph2+/− than in Tph2−/− (LSD post hoc, \( p = 0.002 \)) and Tph2+/− (LSD post hoc, \( p = 0.038 \)) pairs. This is due to the fact that the average contact duration was longer in juvenile Tph2−/− than Tph2+/− (LSD post hoc, \( p < 0.001 \)) and Tph2+/− (LSD post hoc, \( p = 0.002 \)) littersmates.

In all three parameters determined, Tph2−/− and Tph2+/− did not differ from each other. The genotype-dependent alterations in juvenile social interaction behaviour were also seen in a more detailed temporal analysis (figure 1a′–c′). Sex had no effect on juvenile social interaction.

In contrast to social contacts, genotype affected total duration of USV emission (ANOVA, \( F_{2,43} = 4.414; \ p = 0.019; \) figure 1d), but not total number of USVs (ANOVA, \( F_{2,43} = 2.390; \ p = 0.106; \) figure 1d). Furthermore, during juvenile social interactions sex had a strong impact on USV emission, with females producing more USVs (ANOVA, \( F_{1,43} = 11.648; \ p = 0.002; \) figure 1d) and calling for longer (ANOVA, \( F_{1,43} = 16.287; \ p < 0.001; \) figure 1e) than males. In females, differences in total calling time were also genotype-dependent (ANOVA, \( F_{1,43} = 4.411; \ p = 0.038; \) Tph2−/− females spent more time vocalizing than Tph2+/− (LSD post hoc, \( p = 0.009 \)) and Tph2−/− (LSD post hoc, \( p = 0.044 \)), whereas in males, no differences were observed. The sex-dependent difference in USV pattern was confirmed in a more detailed temporal analysis (figure 1d′,e′): while males exhibited a fast drop in USV number and total calling time after the first minute of interaction, females of all genotypes showed a blunted decrement.

3. Results

(b) Adult social interaction

To evaluate the impact of central 5-HT deficiency on adult social behaviour, we analysed 15-week-old Tph2+/+ and Tph2−/− male mice before (naive), during and after their first social (male–male) or sexual (male–female) interaction in a cross-balanced manner (figure 2a).

(i) Urine exposure

When being exposed to a drop of female urine, naive Tph2+/+, but not Tph2−/− male mice spent more time in the corner with the female urine spot than in the opposite corner (paired t-test, \( t_{13} = 2.394; \ p = 0.032 \) and \( t_{13} = 1.736; \ p = 0.106, \) respectively), yet genotypes did not differ in the time spent in proximity to the female urine spot (t-test, \( t_{26} = 1.001; \ p = 0.326; \) figure 2b).

After the first sexual experience Tph2−/− male mice still did not display a preference for the side with the female urine spot, in contrast to Tph2+/+ animals (paired t-test, \( t_{13} = 1.972; \ p = 0.070 \) and \( t_{13} = 4.751; \ p < 0.001; \) respectively). Furthermore, after sexual experience Tph2−/− mice spent less time in proximity to the female urine spot than Tph2+/+ mice (t-test, \( t_{26} = 2.099; \ p = 0.046; \) figure 2d).

Irrespective of the genotype, no side preference was evoked by male urine in socially naive males (figure 2b).

However, after social interaction both genotypes displayed a preference for the side containing male urine (paired t-test, \( t_{13} = 3.490; \ p = 0.004 \) and \( t_{13} = 2.205; \ p = 0.046, \) respectively), with similar time spent in proximity to male urine (t-test, \( t_{26} = 0.371; \ p = 0.714; \) figure 2d).

Before social interaction, some male Tph2−/−, but no Tph2−/− mice emitted USVs when exposed to female urine, with both genotypes emitting no USV in response to male urine (U-test; n.s.; figure 2f).

After social interaction, however, Tph2+/+ male mice emitted USVs to both male and female urine, whereas male Tph2−/− mice were almost silent in response to both stimuli (U-test, \( U = 41.5; \ p = 0.008 \) and \( U = 44.5; \ p = 0.012, \) respectively; figure 2f).

(ii) Social interaction

Compared with Tph2+/+ male mice, Tph2−/− male mice displayed more aggressive attacks on both partners during male–male and male–female social interaction (t-test, \( t_{21,153} = -2.672; \ p = 0.014 \) and \( t_{25} = -2.401; \ p = 0.024, \) respectively), with attacks on males occurring more often in both Tph2+/+ and Tph2−/− mice (Wilcoxon paired test, \( t_{13} = 3.363; \ p = 0.005 \) and \( t_{13} = 4.130; \ p = 0.001 \)).
Figure 1. Juvenile interaction of Tph2+/+, Tph2+/− and Tph2−/− mouse pairs. (a) Total number of physical contacts, (b) total contact duration, (c) mean contact duration and (d–f′) the respective time-dependent change of all parameters of Tph2+/+ (n = 19, open blue bars or solid line), Tph2+/− (n = 19, hatched green bars or dashed line) and Tph2−/− (n = 10, filled red bars or dotted line) male–male and female–female pairs (mean ± s.e.m.). (d) Total number, (e) total duration and (f′–e′) the respective time-dependent change of ultrasonic vocalizations (USVs) of Tph2+/+ (n = 9/8), Tph2+/− (n = 9/8) and Tph2−/− (n = 4/5) male–male/female–female pairs (mean ± s.e.m.). Asterisk and section symbols indicate statistically significant differences between genotypes (*Tph2−/− versus Tph2+/+; $Tph2+/− versus Tph2+/+; $Tph2+/− versus Tph2−/−); hash symbols indicate statistically significant differences between sexes. (Online version in colour.)
Sexual stimuli exposition (male or female urine) are shown for behaviour (mean and bars (left), p = 0.006, respectively). Finally, both Tph2+/+ and Tph2–/– mice showed a preference for female urine in contrast to Tph2+/+ animals, whereas the behaviour towards male urine did not differ between the genotypes, with no preference in naive conditions and clear preference after first social experience. Importantly, both male and female urine did not evoke USV in Tph2–/– mice, whereas experienced Tph2+/+ mice produced USVs to male and female urine (courtship syllables).

While environmental exploration during dyadic interactions was identical in both genotypes irrespective of the partner, social behaviour was highly influenced by the partner’s sex in both genotypes. Similar to Tph2+/+ mice, Tph2–/– males showed less aggression towards and more mountings and sniffing of female than male partners. However, Tph2–/– had fewer contacts with other males in comparison with Tph2+/+ mice and with their own response to females. Thus, a loss of sexual preference, as was suggested in recent publications [4,5], could not be verified in our cross-balanced study.

4. Discussion

Here, we investigated how lifelong depletion in brain 5-HT affects social and sexual behaviour in juvenile and adult Tph2-deficient mice. During social interaction, juvenile Tph2–/– mouse pairs displayed a reduced number but longer duration of physical contacts in comparison with Tph2+/+ and Tph2–/– mice, indicating alterations in juvenile social behaviour. Analysis of ultrasonic communication revealed a sexual dimorphism in USV production during development, but did not reveal an overall genotype effect, highlighting that 5-HT depletion does not affect the ability of either male or female juvenile mice to vocalize.

In adulthood, neither naive nor experienced Tph2–/– male mice showed a preference for female urine in contrast to Tph2+/+ animals, whereas the behaviour towards male urine did not differ between the genotypes, with no preference in naive conditions and clear preference after first social experience. Importantly, both male and female urine did not evoke USV in Tph2–/– mice, whereas experienced Tph2+/+ mice produced USVs to male and female urine (courtship syllables).
consequence of non-balanced experimental protocols (in reference [4]) that can enhance the experience-biased reactions during tests [11,12].

In summary, we conclude that central 5-HT activity is essential for control of aggression and fine-tuning of prosocial behaviour, but does not affect sexual preference.

Ethics statement. Procedures were approved by the ethical committee of the local government (LAGeSo, Berlin and Regierungspräsidium Gießen, Germany).

Data availability. The data is available via the supplementary files submitted along with the manuscript.

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Author contributions. D.B. and M.W. conceived and designed the experiments; D.B. performed the experiments; D.B., M.F. and K.H. analyzed the data; M.W. contributed reagents/materials/analysis tools; D.B., N.A. and M.W. wrote the paper; M.W., N.A. and M.B. gave final approval of the manuscript.

Competing interests. The authors have no competing interests.

References


Correction to ‘Brain serotonin deficiency leads to social communication deficits in mice’


Following publication of our article, we found that Dr Valentina Mosienko was omitted from the list of authors.

Therefore, the author list should appear as follows:

D. Beis, K. Holzwarth, M. Flinders, V. Mosienko, M. Bader, M. Wöhr† and N. Alenina†

†These authors contributed equally to this study.

The authors’ contributions section should read as follows:

Conceived and designed the experiments: D.B., M.W., V.M., N.A. and M.B.; performed the experiments: D.B.; analysed the data: D.B., M.F. and K.H.; contributed reagents/materials/analysis tools: M.W. and V.M.; wrote the paper: D.B., M.W. and N.A.; critically revised the paper: K.H., M.F., V.M. and M.B. and final approval of the manuscript: D.B., K.H., M.F., V.M., M.B., M.W. and N.A.

The competing interests statement remains the same:

Authors have no competing interests.