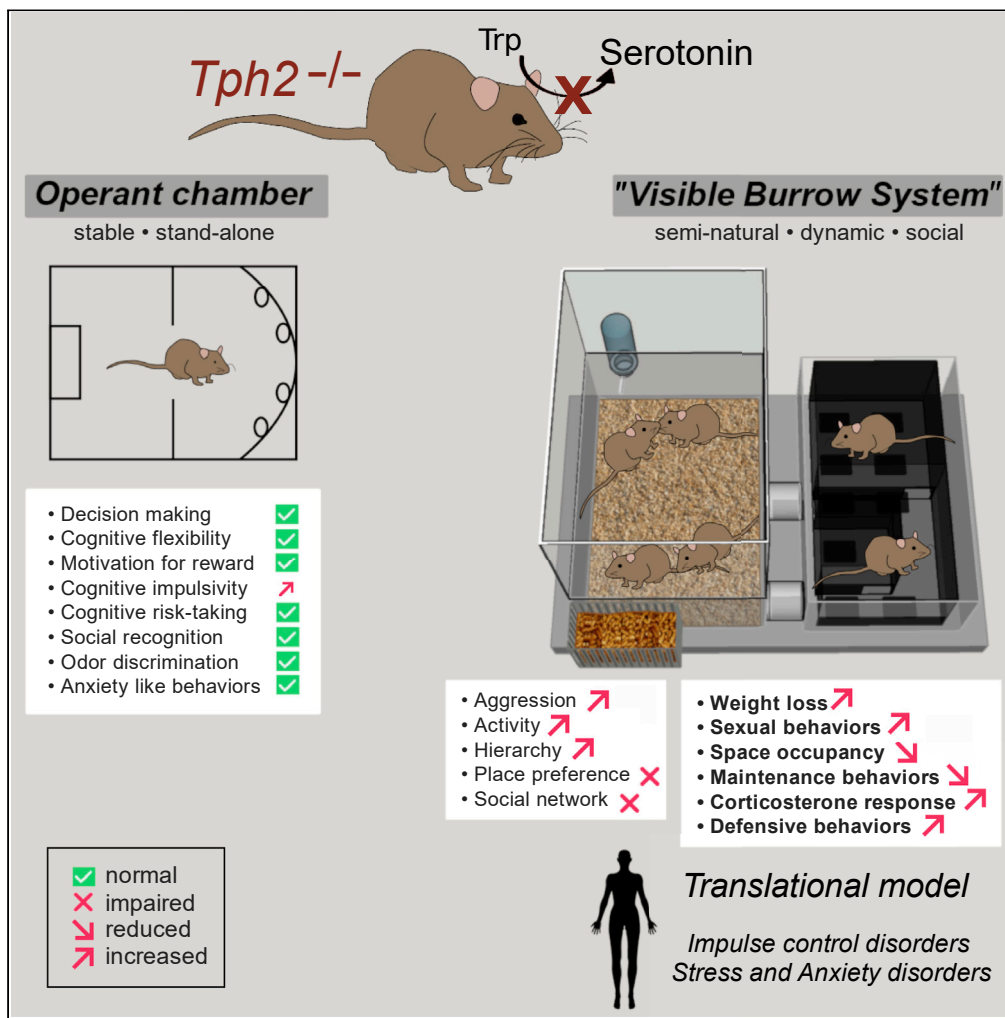


Article

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Lucille Alonso,
Polina Peeva,
Sabrina Stasko,
Michael Bader,
Natalia Alenina,
York Winter,
Marion Rivalan

alenina@mdc-berlin.de (N.A.)
marion.rivalan@gmail.com
(M.R.)

Highlights

Brain serotonin depletion did not impact cognitive abilities in classical procedures

Brain serotonin depletion compromised everyday behaviors in naturalistic home-cage

Most critical behaviors resembled symptoms of impulse control and anxiety disorders

Multidimensional testing and naturalistic conditions offered high translational value

Alonso et al., iScience 26, 105998
February 17, 2023 © 2023 The Authors.
<https://doi.org/10.1016/j.isci.2023.105998>



Article

Constitutive depletion of brain serotonin differentially affects rats' social and cognitive abilities

Lucille Alonso,^{1,2} Polina Peeva,³ Sabrina Stasko,¹ Michael Bader,^{2,3} Natalia Alenina,^{3,*} York Winter,^{1,2} and Marion Rivalan^{1,2,4,*}

SUMMARY

Central serotonin appears a promising transdiagnostic marker of psychiatric disorders and a modulator of some of their key behavioral symptoms. In adult male *Tph2*^{-/-} rats, constitutively lacking central serotonin, we tested individual's cognitive, social and non-social abilities and characterized group's social organization under classical and ethological testing conditions. Using unsupervised machine learning, we identified the functions most dependent on serotonin. Although serotonin depletion did not affect cognitive performances in classical testing, in the home-cage it induced compulsive aggression and sexual behavior, hyperactive and hypervigilant stereotyped behavior, reduced self-care and exacerbated corticosterone levels. This profile recalled symptoms of impulse control and anxiety disorders. Serotonin appeared essential for behavioral adaptation to dynamic social environments. Our animal model challenges the essential role of serotonin in decision-making, flexibility, impulsivity, and risk-taking. These findings highlight the importance of studying everyday life functions within the dynamic social living environment to model complexity in animal models.

INTRODUCTION

The complex nature of psychiatric disorders makes them some of the least understood and most incapacitating of all pathological conditions.^{1–4} A challenge for biomedical research today is to develop efficient and specific treatments that can reverse dysfunctional conditions and improve psychiatric patients' quality of life. However, the current diagnosis of mental disorders lacks biological markers specific to given pathological conditions.³ Beyond the categorical classification of psychiatric disorders, the search for combinations of behavioral symptoms associated with a specific biological profile is necessary for identifying neurocognitive markers of mental disorders.^{5–7}

The monoamine serotonin (5-hydroxytryptamine) is a neuromodulator of the central nervous system (CNS). In the CNS, its synthesis is restricted to the raphe nuclei neurons, which innervate the whole brain with a vast axonal network.^{8–11} Serotonin, through its action on numerous post- and presynaptic receptors,¹² is essential for mood regulation and treating mood disorders (anxiety, bipolar, and depressive disorders)^{10,13} and other neuropsychiatric disorders, such as addiction,^{14–16} attention deficit hyperactivity disorder,¹⁷ suicidal behavior,^{18,19} obsessive-compulsive disorder,^{20,21} psychopathy,²² and other aggression-related disorders.^{23,24} At the behavioral level, serotonin is known to be critical in modulating several executive functions and aspects of social behavior. Disadvantageous decisions,^{25,26} impulsive choices and actions,^{27–29} inflexibility,^{27,28,30} aggression, and socially inappropriate behavior^{31,32} are characteristic impairments of affective, impulse control, or substance-related disorders.^{33–40} Similarly, such cognitive and social deficits are induced in non-clinical humans and rodents after experimental reduction of serotonin levels.^{41–48}

Overall, the serotonergic system appears a promising transdiagnostic marker of apparently distinct psychiatric disorders and a common modulator of some of their key behavioral symptoms. Despite the appeal to reduce mental disorders to impairments studied in isolation, the reality is that the complexity of human mental disorders cannot be explained only in terms of their components, as their interaction plays a critical role in the emergence of the pathology.^{49–52} Using a multidimensional profiling approach,⁵³ we studied the effect of brain serotonin depletion on the expression of several cognitive, social, and affective functions in

¹Humboldt-Universität zu Berlin, Berlin, Germany

²Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany

³Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

⁴Lead contact

*Correspondence: alenina@mdc-berlin.de (N.A.), marion.rivalan@gmail.com (M.R.)

<https://doi.org/10.1016/j.isci.2023.105998>



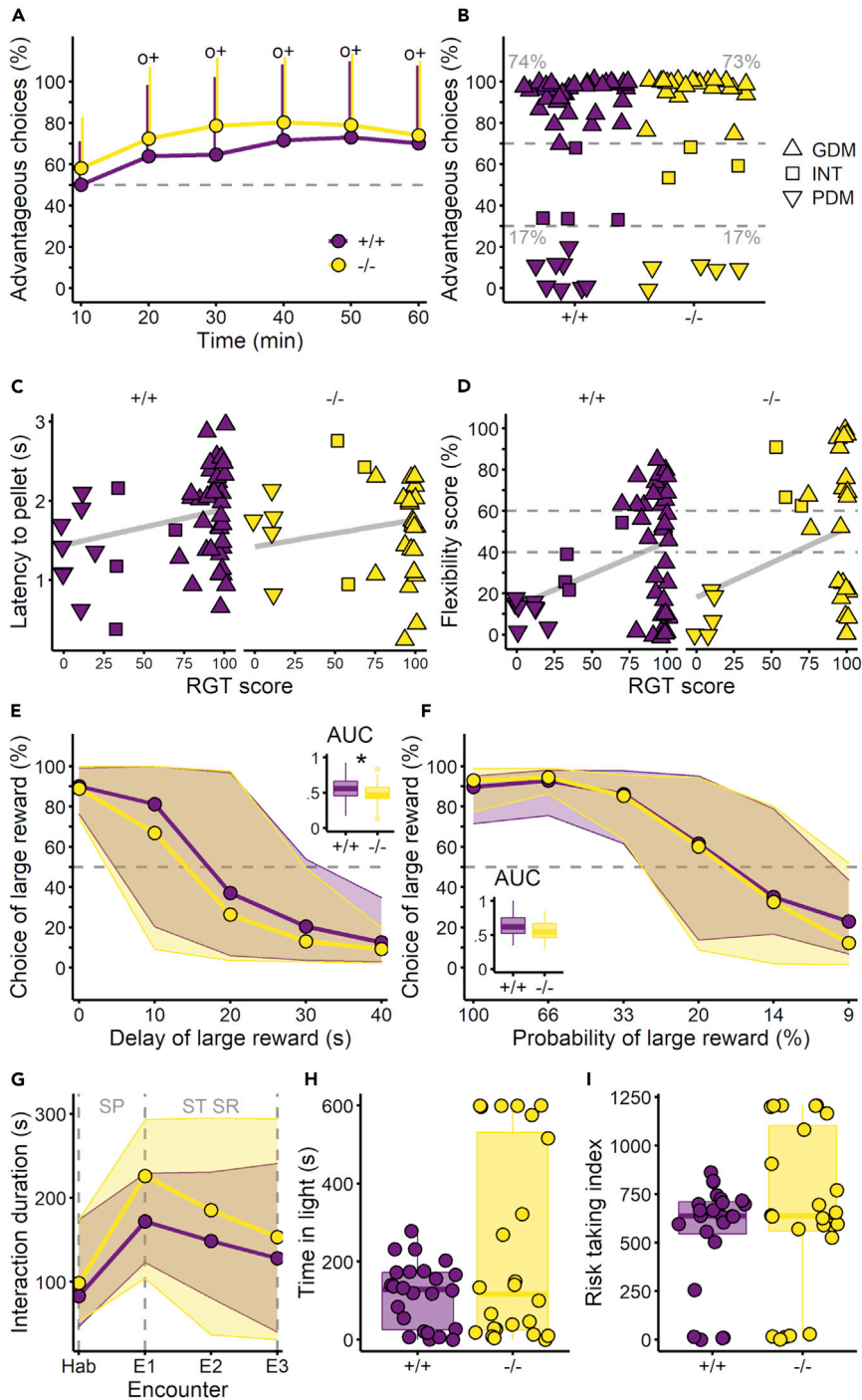


Figure 1. Cognitive abilities of the *Tph2*^{+/+} and *Tph2*^{-/-} rats

(A) Advantageous choices in the rat gambling task (RGT). Lines indicate mean +SD, one-sample t-test compared to 50% with ^o p value <0.05 for +/+ and + p value <0.05 for -/-.

(B) Individual (mean) scores during the last 20 min of the RGT. The dashed lines at 70% and 30% of advantageous choices visually separate good decision-makers (GDMs, above 70% of advantageous choices in the last 20 min, upward triangle), intermediates (INTs, between 30% and 70% of advantageous choices in the last 20 min, square), and poor decision-makers (PDMs, below 30% of advantageous choices in the last 20 min, downward triangle).

(C) Latency to collect the reward in the RGT after a choice for GDMs (upward triangle), INTs (square), and PDMs (downward triangle). Linear regression (gray line) representing the positive correlation.

Figure 1. Continued

(D) Flexibility scores in the reversed-RGT corresponding to the preference for the new location of the preferred option in the RGT for GDMs (upward triangle), INTs (square), and PDMs (downward triangle). Linear regression (gray line) representing the positive correlation. The dashed lines at 60% and 40% visually separate flexible individuals (above 60%) from inflexible individuals (below 40%). The flexibility score is the preference for the location of the non-preferred option during the RGT.

(E) Choice of the large reward option as a function of the delay in reward delivery in the delay discounting task (DDT). Lines show medians, and shaded areas show 5th to 95th percentiles. The dashed line indicates the 50% chance level. Inset showing the area under the curve (AUC) for the preference for the large reward, Wilcoxon rank-sum test between +/+ and -/-, * p value <0.05.

(F) Choice of the large reward option as a function of the probability of reward delivery in the probability discounting task (PDT). Lines show medians, and shaded areas show 5th to 95th percentiles. Dashed line shows 50% chance level. Inset showing the AUC for the preference for the large reward.

(G) Duration of interaction in the social recognition task (SRt). Lines show the medians, and shaded areas show the 5th to 95th percentiles, social preference (SP), short-term social recognition (ST SR), habituation with empty cage (Hab), successive encounters with same conspecific placed in the small cage (E1–3).

(H) Time in the open part of the dark-light box (DL-box). Individual data over the boxplot.

(I) Risk-taking index for the DL-box test. Individual data over the boxplot. Boxplots classically represent the median, 25th and 75th percentiles, 1.5IQR and “outlying” points when individual data are not shown Panels A–D: +/+ n = 47, -/- n = 30, E: +/+ n = 48, -/- n = 30, F: +/+ n = 24, -/- n = 24, G: +/+ n = 30, -/- n = 30, H–I: +/+ n = 24, -/- n = 24. *Tph2*^{+/+} in purple and *Tph2*^{-/-} in yellow.

the same individual. The aim of the study was to identify which of these functions were most affected by the absence of central serotonin and discuss how those key symptoms compare to mental conditions observed in humans.

Genetic modifications are among the most specific methods to target central serotonin in animals. In our study we took advantage of the recently created rats with genetic deletion of tryptophan hydroxylase 2 (TPH2),⁵⁴ the rate-limiting enzyme for serotonin synthesis in the brain.⁵⁵ Constitutive lack of brain serotonin in these animals^{54,56} results in delayed growth and impaired autonomic responses, which normalize at adult age. At the behavioral level, TPH2-deficient (*Tph2*^{-/-}) rats showed increased aggression in the resident intruder paradigm.⁵⁷ However, more subtle social and cognitive deficits remain to be characterized.

Based on previous studies where executive and social functions were individually tested after pharmacological, genetic, or dietary alteration of central serotonin, we hypothesized that the absence of serotonin would simultaneously alter the rats' cognitive and executive functions, social abilities, activity level, and affective responses in both classical testing contexts and more dynamic home-cage environments. We used a version of a visible burrow system (VBS)⁵³ to create an ethologically relevant environment and identify novel real-life markers of serotonergic function.⁵⁸ The *Tph2*^{-/-} phenotype was characterized by multiple behavioral changes only detected in the dynamic social context. With unsupervised machine learning we uncovered that the most critical impairments in these animals resembled transdiagnostic symptoms of impulse control disorders.

RESULTS

Central serotonin deficiency does not affect decision-making, cognitive flexibility, sensitivity to reward, motor impulsivity, social memory, and anxiety

All the animals started the rat gambling task (RGT) without preference for either option (first 10 min, Figure 1A) and preferentially chose the advantageous options over the disadvantageous ones after 10 min of the test (Figure 1A, one-sample t-test, 20 min: +/+ : 0.95CI [53.7, 73.9], p value = 0.008; -/- : 0.95CI [59.5, 85.2], p value <0.001 and Table S1). In both *Tph2*^{+/+} and *Tph2*^{-/-} groups, this dynamic was driven by a majority of good decision-makers (GDMs; Figures 1B and S1). Unexpectedly, both groups presented the same proportion of good (+/+ : 74%; -/- : 73%), intermediate (+/+ : 9%; -/- : 10%), and poor decision-makers (PDMs, +/+ : 17%; -/- : 17%; Figure 1B). Regardless of their genotype but consistent with their typical decision-makers' profile,⁵⁹ PDMs were faster to collect rewards after a choice compared to GDMs (Figure 1C, Wilcoxon rank-sum test, PDMs vs. GDMs: +/+ : W = 203, p value = 0.049; -/- : W = 89, p value = 0.033). PDMs were incapable of flexibility in the reversed-RGT test (Figure 1D; Wilcoxon rank-sum test, PDMs versus GDMs: +/+ : W = 217, p value = 0.016; -/- : W = 90.5, p value = 0.028). *Tph2*^{+/+} and *Tph2*^{-/-} GDMs made either flexible choices (40% and 45%, respectively), inflexible choices (40% and 45%), or were undecided (20% and 10%, Figure 1D). GDMs and PDMs did not differ in any other tests

or between genotypes (Table S2). For the remainder of the study, only genotype comparisons are presented. In the delay discounting task (DDT, Figure 1E) and probability discounting task (PDT, Figure 1F), rats' preference for the large reward progressively decreased as the associated discounting factor (delay or uncertainty) increased. Rats of both genotypes switched preference for the (immediate) smaller reward at delay 20 s [Figure 1E, linear mixed model (lmer), *delay*: $F(4, 289) = 1$, p value <0.001] and at probability 20% [Figure 1F, lmer, *probability*: $F(5, 202) = 173$, p value <0.001]. In the DDT, $Tph2^{-/-}$ rats presented a smaller total area under the curve (AUC) than $Tph2^{+/+}$ animals (Figure 1E inset, Wilcoxon rank-sum test, $W = 916$, p value = 0.044). In the PDT, both genotypes presented similar AUC (Figure 1F inset, Wilcoxon rank-sum test, $W = 373$, p value = 0.081). Animals of both genotypes presented similar anticipatory and perseverative responses during the fixed-interval and extinction phases of the fixed-interval and extinction schedule of reinforcement test (FIEXT, Figure S2). Despite a similar social preference for an unfamiliar partner (E1, Figures 1G and S3) and recognition abilities (E2, E3, Figures 1G, and S3) in both groups, $Tph2^{-/-}$ rats presented a higher interest in the social partner than the $Tph2^{+/+}$ rats [Figure 1G, lmer, *genotype*: $F(1, 40) = 8$, p value = 0.006]. $Tph2^{-/-}$ and $Tph2^{+/+}$ rats showed similar abilities in the odor discrimination test (Figure S4). Anxiety and risk-taking levels in the dark-light box (DL-box) test were similar between genotypes, although $Tph2^{-/-}$ rats showed high variability in responses (Figures 1H and 1I).

Central serotonin deficiency disrupts daily activity, place preference, body weight, and corticosterone levels of group-housed rats within the VSB

In the VBS, $Tph2^{-/-}$ rats were more active than $Tph2^{+/+}$ rats in reaction to novelty (Figure 2A, post-hoc test after lmer, day 1 – dark phase: $SE = 20$, z -value = 7, p value <0.001) and over days (Figure 2A, glmmMCMC, *genotype*: post mean = 8.32, credible interval [5.97, 11.03], p value <0.001). Circadian fluctuation of day/night activity was preserved in both groups (Figure 2A, glmmMCMC, *phase*: post mean = -9.14, credible interval [-9.95, -8.42], p value <0.001) although it was less pronounced for $Tph2^{-/-}$ during light phases (glmmMCMC, *genotype x phase*: post mean = 4.13, credible interval [2.83, 5.81], pMCMC <0.001). $Tph2^{-/-}$ rats had a lower roaming entropy (RE) index, than the $Tph2^{+/+}$ rats overall (Figure 2B, Wilcoxon rank-sum test, $W = 1183$, p value <0.001) and over days (Figure 2C, lmer, *genotype*: $F(1, 19) = 27$, p value <0.001) indicating a more restricted use of the whole cage space than the $Tph2^{+/+}$ rats. About place preference within the cage, $Tph2^{-/-}$ rats were detected less often at the feeding and drinking areas and in the large chamber than the $Tph2^{+/+}$ rats (Figure 2D, on heatmaps, the more purple the more $Tph2^{+/+}$ rats were detected compared to $Tph2^{-/-}$ rats). They stayed more in the covered tunnels close to the open area (burrow area) and in the center of the open area than $Tph2^{+/+}$ rats (Figure 2D, on heatmaps, the more yellow the less $Tph2^{+/+}$ rats were detected compared to $Tph2^{-/-}$ rats). $Tph2^{-/-}$ rats lost more weight during the VBS stay than $Tph2^{+/+}$ rats (Figure 2E, Wilcoxon rank-sum test, $W = 1397$, p value <0.001). Only in $Tph2^{-/-}$ rats, VBS housing largely increased the corticosterone metabolite level [Figure 2F, lmer, *genotype x time*: $F(1, 94) = 69$, p value <0.001].

Central serotonin deficiency disrupts social behaviors, social networks, group organization, and hierarchy in the VBS

Overall, $Tph2^{-/-}$ animals showed less huddling, eating, struggling at the feeder, and grooming behaviors than $Tph2^{+/+}$ animals and more general aggression, exploratory (sniffing), and sexual behaviors (Figure 3A, Wilcoxon rank-sum test, huddling: $W = 1240.5$, p value <0.001 ; eating: $W = 1267$, p value <0.001 ; struggling at feeder: $W = 1227.5$, p value <0.001 ; grooming: $W = 914.5$, p value = 0.0459; general aggression: $W = 29$, p value <0.001 ; sniffing: $W = 429$, p value = 0.0028; sexual behaviors: $W = 67$, p value <0.001 , and all behaviors are presented in Figure S5 and defined in Table 1). On day 1, for aggression and sexual behavior, $Tph2^{-/-}$ networks were more dense, with most pairs of rats displaying these behaviors, whereas fewer pairs connected for huddling and struggling at the feeder compared to $Tph2^{+/+}$ networks [Figure 3B, lmer, *genotype*, general aggression: $F(1, 43) = 40.9$, p value <0.001 ; sexual behavior: $F(1, 44) = 167$, p value <0.001 ; huddling: $F(1, 43) = 32.5$, p value <0.001 ; struggling at feeder: $F(1, 43) = 15.2$, p value <0.001 ; and Table S3]. On the following days and by day 4, the $Tph2^{-/-}$ network densities for huddling (Figure 3C-left representative network), sniffing, and general aggression (Figure 3C-right representative network) normalized to the level of the $Tph2^{+/+}$ networks (Figure 3B); network densities for sexual behaviors always remained higher for $Tph2^{-/-}$ [Figure 3B; lmer, *genotype x day*: $F(3,38) = 11$, p value <0.001] and for struggling at the feeder remained stable for both genotypes [Figure 3B; lmer, *day, +/-*: $F(3,23) = 2$, p value = 0.13; $-/-$: $F(3,14) = 0.2$, p value = 0.89]. The average path length (mean number of steps between any pair of the network) indicated similar results to density, and the out-degree centralization (distribution of out-interactions) was low for all networks (median at 0.20, Figure S6). In both genotypes, individual hierarchical ranks emerged progressively (Figure 3D). The rats' final Glicko ratings were broadly distributed

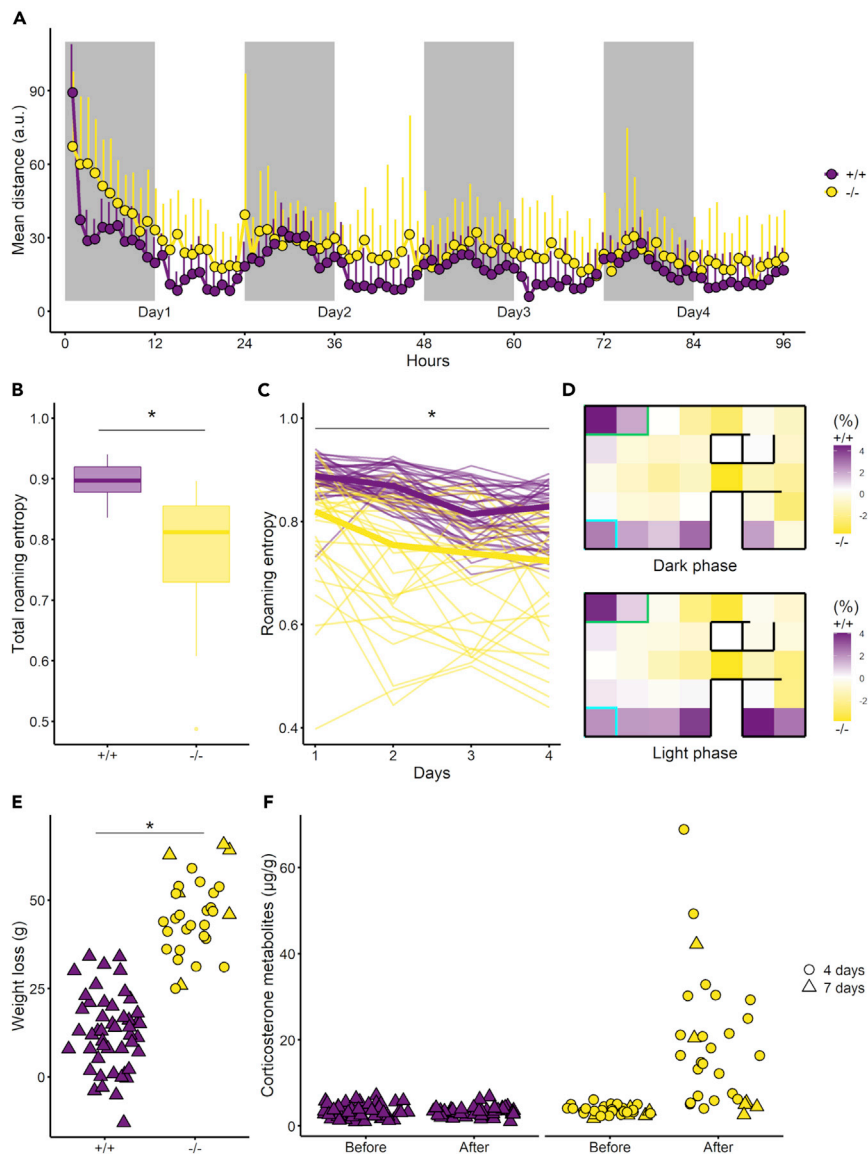


Figure 2. Activity, roaming entropy, and place preference of the $Tph2^{+/+}$ and $Tph2^{-/-}$ rats in the automated visible burrow system (VBS)

(A) Activity as mean index of distance traveled in arbitrary unit per hour. Lines indicate mean +SD

(B) Total roaming entropy, boxplots classically represent the median, 25th and 75th percentiles, 1.5IQR and “outlying” points, Wilcoxon rank-sum test between +/+ and -/-, * p value <0.05.

(C) Roaming entropy per day, thick lines indicate the median values, and thin lines indicate the individual values, lmer between +/+ and -/-, * p value <0.05.

(D) Difference in place preference (frequency of detections) in percent between +/+ and -/- over 4 days of VBS housing for dark (above) and light (below) phases. A top view of the VBS is represented and each zone corresponds to one of the 32 radio-frequency identification [RFID] detectors located beneath the VBS cage. Rectangles indicate the locations of the feeder (green) and water bottle (cyan). Positive difference (purple shade) indicates a higher place preference of the +/+ and negative difference (yellow shade) indicates a higher place preference of the -/- at each zone.

(E) Weight loss in grams after the stay in the automated VBS. A 4-day stay is indicated with circles, and a 7-day stay is indicated with triangles, Wilcoxon rank-sum test between +/+ and -/-, * p value <0.05.

(F) Corticosterone metabolites in μg/g of feces before and after VBS housing for both genotypes. A 4-day stay is indicated with circles, and a 7-day stay is indicated with triangles; post-hoc test after lmer between -/- and after -/- (SE = 1.4, z-value = 10.5, p value <0.001), * p value <0.05. Panels A and D–F: +/+ n = 48, -/- n = 30 and B–C: +/+ n = 42, -/- n = 30. $Tph2^{+/+}$ in purple and $Tph2^{-/-}$ in yellow.

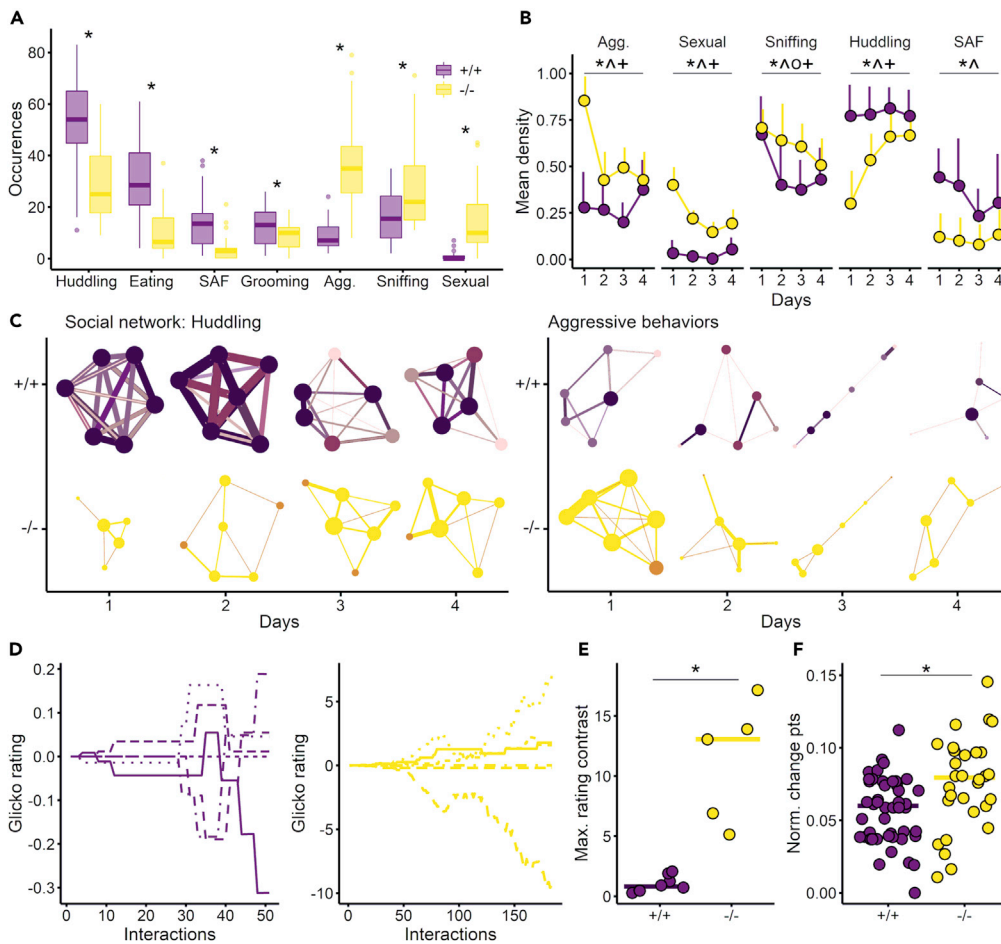


Figure 3. Social abilities and dominance of $Tph2^{+/+}$ and $Tph2^{-/-}$ in the automated VBS

(A) Number of occurrences of behaviors in 4 days in the VBS for the most expressed behaviors, struggling at the feeder (SAF), general aggression including all aggressive behaviors except struggling at the feeder (Agg.), all sniffing behaviors (Sniffing), sexual behaviors including embracing and mounting behaviors (Sexual; Table 1). Boxplots classically represent the median, 25th and 75th percentiles, 1.5IQR and “outlying” points. Wilcoxon rank-sum test between $Tph2^{+/+}$ and $Tph2^{-/-}$, * p value < 0.05 .

(B) Social network density along days. Lines indicate mean \pm SD, lmer genotype: * p value < 0.001 , lmer genotype \times day: \wedge p value < 0.01 , lmer day: \circ p value < 0.001 for $+/+$ and \circ p value < 0.01 for $-/-$. The network density is the proportion of potential connections in the network that are existing connections between rats; the development of the social network density over days can be visualized by the number of edges in the networks in the panel C.

(C) Representative social networks of two $Tph2^{+/+}$ and $Tph2^{-/-}$ groups from days 1–4 for huddling (left) and aggression (right) behaviors and for illustration of data of panel B. The color intensity and thickness of the edges represent the number of behaviors exchanged (weight), and the color intensity and size of the nodes represent the number of edges received and sent out (node-degree). As in B, in this representative network of huddling, in $Tph2^{+/+}$, the density was the highest at day 1 and remained high over days as shown by the number of edges and large node size; in $Tph2^{-/-}$, the density was the lowest at day 1 and increased over days. In the aggression networks, in $Tph2^{+/+}$, the density was stable and low over days; in the $Tph2^{-/-}$ group, the density of connection strongly decreased after day 1.

(D) Glicko rating representation for the six individuals of one representative $Tph2^{+/+}$ group (left) and for the six individuals of one representative $Tph2^{-/-}$ group (right).

(E) Maximum difference in the final Glicko rating between the lowest and highest individuals (Max. rating contrast) for each group, Wilcoxon rank-sum test between $+/+$ and $-/-$, * p value < 0.05 .

(F) Individual proportion of Glicko rating change points, normalized number of change points to the total number of interaction (Norm. change pts); a change point indicates an increase or decrease in the individual rating, Wilcoxon rank-sum test between $+/+$ and $-/-$, * p value < 0.05 . Panels A, B, and F: $+/+$ $n = 48$, $-/-$ $n = 30$, panel E: $+/+$ $n = 8$ groups, $-/-$ $n = 5$ groups and panels C and D representative groups of each genotype. $Tph2^{+/+}$ in purple and $Tph2^{-/-}$ in yellow.

Table 1. Ethogram of the behaviors scored in the VBS

Category	Behavior	Definition	Grouped category
Affiliative	Allogrooming	Gentle grooming of another rat that is not pinned on its back	
Affiliative	Attending	Orienting the head, ears, and possibly the whole body toward another rat	
Affiliative	Huddle	Lying in contact with another rat	
Affiliative	Sniffing – anogenital	Nose contact to the anogenital zone or base of tail of another rat	Sniffing
Affiliative	Sniffing – nose	Nose contact to the nose of another rat for longer than 1 s	Sniffing
Affiliative	Sniffing – body	Nose contact to the fur of another rat, sniffing it and exploring the other animal	Sniffing
Aggressive	Struggling at feeder	Rats pushing each other to obtain the place at the feeder	
Aggressive	Aggressive grooming	Vigorous grooming of another rat while pinning it	General aggression
Aggressive	Attack: Attack bite, jump, and lateral attack	Sudden bite toward neck and back of another rat. Sudden jump toward another rat. Arched-back posture oriented toward another rat, often including shoving and piloerection	General aggression
Aggressive	Following	Rat runs after another one	General aggression
Aggressive	Fight	Rough-and-tumble of two animals	General aggression
Aggressive	Mutual upright posture	Both rats standing in front of each other with vertical movements of the forepaw	General aggression
Aggressive	Pinning	Being above another rat usually lying on its back and holding it with the forepaw	General aggression
Aggressive	Struggle in tunnels	Rats pushing each other to pass in the tunnel, struggling with the paws	General aggression
Sexual	Mounting	Rat encircles the back, hips, or waist of another rat with its forelimb and shakes its hips	Sexual
Sexual	Embracing	Rat encircles the back, hips, or waist of another rat with its forelimb without shaking its own hips	Sexual
Defensive	Flight	Rapid movement away from another rat	
Defensive	Freezing	Being immobile or maintaining a specific posture (crouching)	
Defensive	Lateral defense	Exposing the flank to another rat	
Defensive	Supine posture	Lying on the back (exposure of the belly) because of another rat	
Defensive	Upright defense	Exposing the belly to another rat in a half-erect posture	
Maintenance	Drinking	Drinking water	
Maintenance	Eating	Eating food	
Maintenance	Grooming	Self-grooming: a rat is cleaning itself with rapid little nibbles	

below and above the initial rating score (Figure 3D), with one dominant individual identified in each group (except for one *Tph2^{-/-}* group with two dominant individuals, Figure S7). The two hierarchical scales, the non-aggression Blanchard dominance and the Glicko rating scores, correlated positively in *Tph2^{+/+}* ($r = 0.30$, p value = 0.0405) and negatively in *Tph2^{-/-}* ($r = -0.45$, p value = 0.0132). Compared to *Tph2^{+/+}*, *Tph2^{-/-}* dominant animals were more aggressive toward subordinates (higher rank divergence; Figure 3E, Wilcoxon rank-sum test, $W = 0$, p value = 0.0015) and the *Tph2^{-/-}* group's hierarchy was more unstable (higher number of change points; Figure 3F, Wilcoxon rank-sum test, $W = 453$, p value = 0.0061). Finally, in *Tph2^{+/+}* rats, the higher the Glicko rating, the higher the hub centrality in the general aggression network ($r = 0.40$, p value = 0.0051). This correlation was not found in *Tph2^{-/-}* rats ($r = 0.04$, p value = 0.8543), indicating that the dominant's aggression did not influence this network.

Central serotonin deficiency differentially impacts cognitive abilities and group-housed behaviors

Among all measured behaviors, those most impacted by the lack of brain serotonin were identified using a random forest (RF) classifier (with an average accuracy of 98.5%, $SD = 0.54$, Table S4) and confirmed by a Principal component analysis (PCA, Table S5). The PCA revealed a clear separation of the genotypes along its first dimension (Figure 4A-left). The variables contributing the most to dimension 1 were also those discriminating the best between genotypes using the RF classifier (Figures 4B, Tables S6 and S7). Dimension 1 was mainly loaded by weight loss, maintenance (drinking, eating, grooming) behavior, RE, corticosterone variation, and defensive and sexual behaviors (Figure 4A-right). From the RF, the other relevant variables comprised total distance traveled, Glicko rating score, affiliative (allogrooming, attending, huddling, sniffing) and aggressive (struggling at the feeder and general aggression; Table 1) behaviors, and the presence in the VBS open area (Figure 4B). None of the cognitive variables predicted the animals' genotypes (Figure 4A-right and B).

DISCUSSION

In this multidimensional study, we used classical and ethological approaches of testing to evaluate the effects of brain serotonin deficiency on the expression of cognitive, social, and affective functions in different contexts and in the same animals. With unsupervised statistics, we identified which functions were primarily affected by the absence of brain serotonin. Surprisingly, no function evaluated in the classical testing appeared altered by its absence. However, in the day-to-day context of the home-cage, the absence of brain serotonin most strikingly affected the animals' sexual, maintenance (eating, drinking, grooming), and defensive behaviors, levels of home-cage RE, weight, and corticosterone. These discriminative markers of serotonin function, consistent with the constellation of other behavioral impairments observed in *Tph2^{-/-}* rats, are reminiscent of common symptoms found in human impulse control disorders (ICD; e.g. disruptive, impulse control, and conduct disorders, compulsive sexual behavior disorder, and behavioral addictions) and stress and anxiety disorders (e.g. obsessive-compulsive, post-traumatic stress, and generalized anxiety disorders), which also share a high comorbidity level with ICDs (Table S8).^{60–65}

Under the complex and experimenter-free conditions of their home-cage, *Tph2^{-/-}* rats showed increased corticosterone levels, exacerbated repetitive aggression, and exploratory (sniffing) and sexual behaviors while neglecting affiliative (huddling), self-caring (grooming), and self-sustaining (feeding, poor maintenance of body weight) essential behaviors. Although the dynamics of interactions eventually normalized for aggressive, exploratory, and affiliative behaviors, it did not for sexual behaviors. In clinical settings, cortisol disturbances, uncontrolled repetitive violent or sexual outbursts with poor consequences for others (harm) and self (neglect of health and personal care) are characteristic of disruptive, impulse control, and conduct disorders^{66–70} and compulsive sexual behavior disorder.⁷¹ At the group level, *Tph2^{-/-}* dominance was emphasized by increased aggression toward subordinate. This is in line with a despotic style of hierarchy which could compare, to a certain extent, to macaques' social organizations, and in particular the expression of escalated aggression that is found inversely dependent on serotonin turnover and controlled by serotonergic gene polymorphism.^{72,73} Nonetheless, *Tph2^{-/-}* hierarchical ranks appeared less stable and did not reflect in the structure of aggression networks (i.e. hub centrality) as was the case in the *Tph2^{+/+}* groups. *Tph2^{-/-}* groups were disorganized overall. In line with the work of Kiser et al. (2012)³¹ *Tph2^{-/-}* rats might present a more reactive type of aggression with persistent sexual activity and outbursts of aggression, appearing devoid of long-term goals (e.g. reproduction, secure food resource, hierarchical structure) and of specificity (e.g. occurred between random conspecifics). In addition, *Tph2^{-/-}* rats expressed a hypervigilant defensive profile with higher day/night activity and smaller territories, ignoring food sources

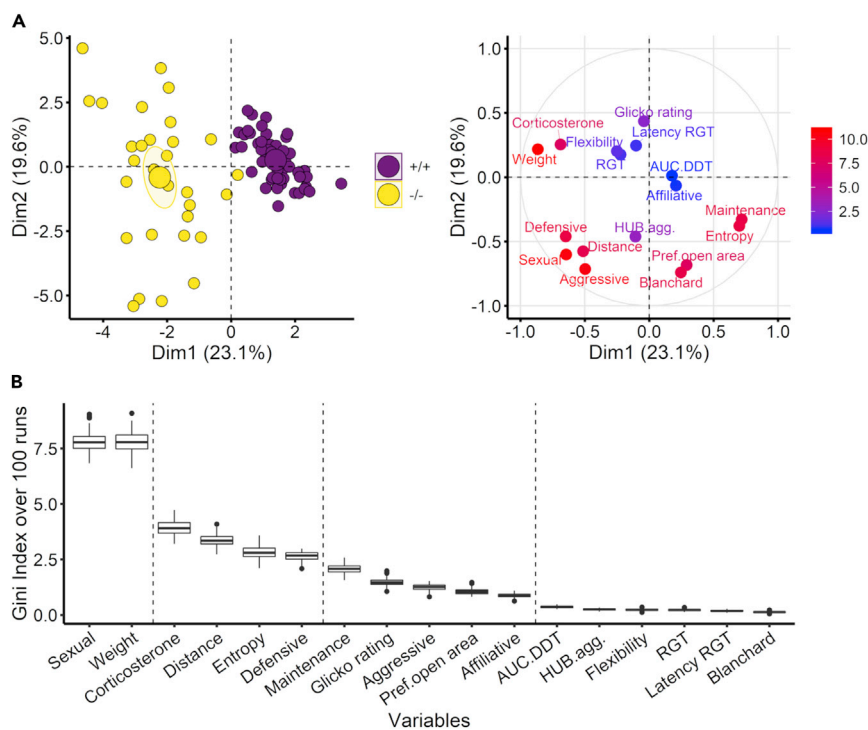


Figure 4. Principal component analysis (PCA) and random forest (RF) classification

(A-left) Separation of the genotypes along dimension 1 but not along dimension 2 of the principal component analysis, *Tph2*^{+/+} in purple and *Tph2*^{-/-} in yellow; large symbols show group centroids and ellipses show the 0.95 confidence interval. (A-right) Contribution of the variables to dimensions 1 and 2 of the principal component analysis; higher contribution with warmer color (red, points with higher coordinates values closer to the circle) and lower contribution with colder color (blue, points with lower coordinates values closer to the center).

(B) Gini index of the RF classification over 100 runs indicating the importance of the variable for the genotype dissimilarity. Boxplots classically represent the median, 25th and 75th percentiles, 1.5IQR and “outlying” points. The dashed line indicates the groups of variables resulting from the k-means clustering of the Gini indexes over 100 runs. Total occurrences of sexual behaviors (Sexual), percentage of weight variation (Weight), percentage of corticosterone metabolite variation (Corticosterone), total distance traveled (Distance), total roaming entropy (Entropy), total occurrences of defensive behaviors (Defensive), total occurrences of maintenance behaviors (Maintenance; drinking, eating, grooming), total occurrences of aggressive behaviors (Aggressive), total preference for the open area (Pref.open area), total occurrences of affiliative behaviors (Affiliative), area under the curve in the delay discounting task (AUC.DDT), hub centrality in aggression network (HUB.agg), flexibility score in reversed-RGT (Flexibility), preference in last 20 min of rat gambling task (RGT), latency to collect pellet in RGT (Latency RGT), Blanchard dominance score (Blanchard). Panels A–B: +/+ n = 48, -/- n = 30.

but favoring hiding and escaping options. Concerning the physiological changes, possible explanations could be that the downstream glucocorticoid receptor pathway’s disruption by serotonin depletion may have maintained elevated corticosterone levels in *Tph2*^{-/-} rats,^{74,75} and weight loss may have resulted from social stress-inducing feeding pattern modifications.^{76,77} Finally, the rich phenotype of the *Tph2*^{-/-} rats within the VBS confirmed the potential of this line to model transdiagnostic features of human disorders and revealed behavioral dysfunctions at the group level and the essential role of serotonin in modulating social and non-social daily life behaviors.

However, outside the home cage, the same animals had normal scores under the controlled conditions of cognitive testing. *Tph2*^{-/-} rats solved complex and risky decision-making tasks. They showed normal cognitive flexibility, typical sensitivity to reward, satisfactory motor control, good social recognition and odor discrimination abilities, and normal levels of anxiety and risk-taking. Only in the DDT, they appeared more sensitive to the discounting effect of the delay on their preference for the larger reward. Such preserved cognitive performance in the absence of brain serotonin was highly unexpected, as it contrasted with the dominant literature indicating an essential role of serotonin in modulating these higher-order

functions using the same classical tests,^{30,42,78–87} although see^{88–96} However, before these results might indicate a more limited role for serotonin in modulating executive functions (decision-making, impulsivity, flexibility, social recognition), it is necessary to consider other potential explanations.

The lack of cognitive impairments could be because of the specific animal model we used. Knockout models specifically target one gene.⁹⁷ Compared to pharmacological models, they prevent potential off-target effects associated with compound specificity, dosage, and application route. In a previous study, we confirmed normal cognitive and social abilities in *Tph2*^{+/+} Dark Agouti rats,⁵³ excluding the risk of a flooring effect in *Tph2*^{-/-} rats. However, a limitation of constitutive knockout models is their propensity to develop unexpected compensatory mechanisms, which might neutralize the genetic perturbation and result in a lack of phenotype.⁹⁸ Following this hypothesis, TPH2-deficiency in mice and rats led to an increase in brain-derived neurotrophic factor (BDNF) levels in the hippocampus and prefrontal cortex^{99–101} and serotonergic hyperinnervation.¹⁰¹

Studies in *Tph2*^{-/-} mice showed that “serotonergic neurons” were morphologically conserved in these animals, despite their inability to produce serotonin.^{102–104} Considering the physiological co-transmissions of glutamate, dopamine, or GABA neurotransmitters by serotonergic neurons,^{105–112} activity of the “serotonergic circuitry” could have occurred in the absence of serotonin in *Tph2*^{-/-} rats. The hypothesis of such a compensatory scheme, counteracting the absence of brain serotonin in classical stand-alone cognitive tests, would suggest the existence of powerful biological targets for cognitive remediation, which remain to be studied.

Although it is unclear which compensatory mechanisms could have counterbalanced the absence of serotonin in classical tests, these mechanisms showed their limits under the less controlled, experimenter-free conditions of the social home-cage. In this more cognitively challenging and dynamic environment, *Tph2*^{-/-} rats presented altered daily life, social, and group behaviors compared to control rats. In classical tests, the cognitive demand is minimized to evaluating a few given functions, unlike natural environments where complex cognition is encouraged.⁵⁸ Behavioral adaptation in social environments is known to be facilitated by serotonin through its influence on neural plasticity.^{31,113,114} Despite normal performances in classical cognitive tests, in the VBS, the highly dysfunctional social profile of *Tph2*^{-/-} rats indicates poor impulse control (e.g. sustained aggression), limited ability to adjust choices over time (e.g. sexual activity), and lack of goal-directed behavior (e.g. reduced eating and struggling at the feeder). Consistent with the context-specific role of central serotonin in modulating cognition,^{113,114} serotonin proved essential in supporting daily cognitive life in complex and social contexts.

Finally, an intriguing result concerns their social exploratory dynamic. Sniffing one another is a critical behavior in acquiring information,¹¹⁵ communicating dominance status,¹¹⁶ and pacifying interactions.¹¹⁷ *Tph2*^{-/-} rats showed slower reduction of sniffing network density in the VBS and a higher interest in the social partner in the social recognition test. They might be slower at integrating and transmitting social cues and thus at adjusting their behavior. The lack of structure of the aggression network may indicate disrupted transmission of hierarchical information in *Tph2*^{-/-} groups. Thus, communication deficits may have played a significant role in maintaining aggression, hierarchical disorganization, social stress, and the uncertainty level of the VBS, potentiating the serotonin depletion effects. A deeper investigation of the communication strategy of *Tph2*^{-/-} rats would help understand which functions affected by serotonin depletion are responsible for these deficits.

To conclude, in this study, using adult *Tph2*^{-/-} rats, we showed that central serotonin was not essential for expressing cognitive abilities when tested in classical tests. However, central serotonin was a key modulator of essential naturalistic home-cage behaviors when living in undisturbed social groups. Context complexity must be integrated into experimental designs to investigate the role of the serotonergic system in the subtle modulation of different aspects of social and non-social behaviors. Only when facing the dynamic complexity and uncertainty of naturalistic conditions of choices were *Tph2*^{-/-} rats unable to adjust their behavior and were revealed as a promising model for studying transdiagnostic markers of ICDs and anxiety. The decision-making, flexibility, and impulsivity of the *Tph2*^{-/-} rats should be further studied under complex naturalistic conditions.^{118–120} In the complex social contexts, the unsupervised analysis of multidimensional results and analysis of network dynamics and hierarchy are essential additions to classical methods. They are necessary to expose the complexity of animals’ phenotypes and demonstrate the translational value of results.

Limitations of the study

The primary purpose of this study was not to compare the different test environments but rather use a variety of tests to establish an extended behavioral profile of the *Tph2*^{-/-} rat model. Two limitations in the design of this study can be notified, as we were unable to apply blinding and randomization and those limitations should be addressed in future studies. There is also a strong need to include female subjects in future studies to examine or rule out potential differences with the present profile exclusively established in male rats.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.105998>.

ACKNOWLEDGMENTS

This work was funded by a DFG grant (RI 2474/2-1 to Marion Rivalan and AL 1197/5-1 to Natalia Alenina). Natalia Alenina and Michael Bader were supported by the EU H2020 MSCA ITN projects “Serotonin and Beyond” (N 953327). Support was also received through DFG funding to the Center of Excellence NeuroCure DFGEXC 257. We want to thank Nina Soto, Vladislav Nachev, Patrik Bey, Miléna Brunet, Tania Fernández del Valle Alquicira, Franca Eltner, Arnau Ramos-Prats, Diane Delgado, Melissa Long, Alexej Schatz, Martin Dehnhard, Mareen Albrecht and Katrin Paschmionka for their help with the experiments, data extraction, and analysis. We thank Annegret Dahlke, Monique Bergemann, Laura Rosenzweig, Susanne da Costa Goncalves, Bettina Müller, Reimunde Hellwig-Träger, and the FEM team for their technical assistance with the animals. We thank our colleagues of the Winter Laboratory who made insightful comments on a previous version of the manuscript. Special thanks to Chloé Alonso who designed the graphical abstract. The first version of this manuscript was posted on the preprint server bioRxiv.¹²¹

AUTHOR CONTRIBUTIONS

Conceptualization: M.R., L.A., N.A., and Y.W. Methodology: M.R., L.A., and Y.W. Software: M.R., L.A., and Y.W. Validation: M.R. and L.A. Formal analysis: M.R., L.A., P.P., N.A., and Y.W. Investigation: L.A., P.P., and

S.S. Resources: M.R., N.A., M.B., and Y.W. Data curation: M.R. and L.A. Writing – original draft: M.R., L.A., N.A., and Y.W. Writing – review and editing: M.R., L.A., N.A., Y.W., M.B., and P.P. Visualization: M.R. and L.A. Supervision: M.R., L.A., and Y.W. Project administration: M.R., L.A., and Y.W. Funding acquisition: M.R., N.A., M.B., and Y.W.

DECLARATION OF INTERESTS

Y.W. owns PhenoSys equity. All other authors declare no conflict of interest.

Received: December 6, 2021

Revised: September 30, 2022

Accepted: January 12, 2023

Published: February 17, 2023

REFERENCES

- Collins, P.Y., Patel, V., Joestl, S.S., March, D., Insel, T.R., Daar, A.S., Scientific Advisory Board and the Executive Committee of the Grand Challenges on Global Mental Health, Anderson, W., Dhansay, M.A., Phillips, A., Shurin, S., et al. (2011). Grand challenges in global mental health. *Nature* 475, 27–30. <https://doi.org/10.1038/475027a>.
- Fried, E.I., and Robinaugh, D.J. (2020). Systems all the way down: embracing complexity in mental health research. *BMC Med.* 18, 205. <https://doi.org/10.1186/s12916-020-01668-w>.
- Hyman, S.E. (2008). A glimmer of light for neuropsychiatric disorders. *Nature* 455, 890–893. <https://doi.org/10.1038/nature07454>.
- WHO, 66th World Health Assembly (2013). *Comprehensive Mental Health Action Plan 2013–2020*.
- Gottesman, I.I., and Gould, T.D. (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *Am. J. Psychiatry* 160, 636–645. <https://doi.org/10.1176/appi.ajp.160.4.636>.
- Gould, T.D., and Gottesman, I.I. (2006). Psychiatric endophenotypes and the development of valid animal models. *Genes Brain Behav.* 5, 113–119. <https://doi.org/10.1111/j.1601-183X.2005.00186.x>.
- Robbins, T.W., Gillan, C.M., Smith, D.G., de Wit, S., and Ersche, K.D. (2012). Neurocognitive endophenotypes of impulsivity and compulsivity: towards dimensional psychiatry. *Trends Cogn. Sci.* 16, 81–91. <https://doi.org/10.1016/j.tics.2011.11.009>.
- Baumgarten, H.G., and Grozdanovic, Z. (1995). Psychopharmacology of central serotonergic systems. *Pharmacopsychiatry* 28, 73–79. <https://doi.org/10.1055/s-2007-979623>.
- Gaspar, P., Cases, O., and Maroteaux, L. (2003). The developmental role of serotonin: news from mouse molecular genetics. *Nat. Rev. Neurosci.* 4, 1002–1012. <https://doi.org/10.1038/nrn1256>.
- Heinz, A., Mann, K., Weinberger, D.R., and Goldman, D. (2001). Serotonergic dysfunction, negative mood states, and response to alcohol. *Alcohol Clin. Exp. Res.* 25, 487–495.
- Maddaloni, G., Bertero, A., Pratelli, M., Barsotti, N., Boonstra, A., Giorgi, A., Migliarini, S., and Pasqualetti, M. (2017). Development of serotonergic fibers in the post-natal mouse brain. *Front. Cell. Neurosci.* 11, 202. <https://doi.org/10.3389/fncel.2017.00202>.
- Filip, M., and Bader, M. (2009). Overview on 5-HT receptors and their role in physiology and pathology of the central nervous system. *Pharmacol. Rep.* 61, 761–777.
- Grieb, Z.A., and Ragan, C.M. (2019). The effects of perinatal SSRI exposure on anxious behavior and neurobiology in rodent and human offspring. *Eur. Neuropsychopharmacol.* 29, 1169–1184. <https://doi.org/10.1016/j.euroneuro.2019.07.239>.
- Hodgins, D.C., Stea, J.N., and Grant, J.E. (2011). Gambling disorders. *Lancet* 378, 1874–1884. [https://doi.org/10.1016/S0140-6736\(10\)62185-X](https://doi.org/10.1016/S0140-6736(10)62185-X).
- Kirby, L.G., Zeeb, F.D., and Winstanley, C.A. (2011). Contributions of serotonin in addiction vulnerability. *Neuropharmacology* 61, 421–432. <https://doi.org/10.1016/j.neuropharm.2011.03.022>.
- Müller, C.P., and Homberg, J.R. (2015). The role of serotonin in drug use and addiction. *Behav. Brain Res.* 277, 146–192. <https://doi.org/10.1016/j.bbr.2014.04.007>.
- King, J.A., Tenney, J., Rossi, V., Colamussi, L., and Burdick, S. (2003). Neural substrates underlying impulsivity. *Ann. N. Y. Acad. Sci.* 1008, 160–169.
- Underwood, M.D., Kassir, S.A., Bakalian, M.J., Galfalvy, H., Dwork, A.J., Mann, J.J., and Arango, V. (2018). Serotonin receptors and suicide, major depression, alcohol use disorder and reported early life adversity. *Transl. Psychiatry* 8, 279. <https://doi.org/10.1038/s41398-018-0309-1>.
- Waider, J., Araragi, N., Gutknecht, L., and Lesch, K.-P. (2011). Tryptophan hydroxylase-2 (TPH2) in disorders of cognitive control and emotion regulation: a perspective. *Psychoneuroendocrinology* 36, 393–405. <https://doi.org/10.1016/j.psyneuen.2010.12.012>.
- Lissemore, J.I., Sookman, D., Gravel, P., Berney, A., Barsoum, A., Diksic, M., Nordahl, T.E., Pinard, G., Sibon, I., Cottraux, J., et al. (2018). Brain serotonin synthesis capacity in obsessive-compulsive disorder: effects of cognitive behavioral therapy and sertraline. *Transl. Psychiatry* 8, 82. <https://doi.org/10.1038/s41398-018-0128-4>.
- Stein, D.J., Costa, D.L.C., Lochner, C., Miguel, E.C., Reddy, Y.C.J., Shavitt, R.G., van den Heuvel, O.A., and Simpson, H.B. (2019). Obsessive-compulsive disorder. *Nat. Rev. Dis. Primers* 5, 52. <https://doi.org/10.1038/s41572-019-0102-3>.
- Baou, M., Boumba, V.A., Petrikis, P., Rallis, G., Vougiouklakis, T., and Mavreas, V. (2016). A review of genetic alterations in the serotonin pathway and their correlation with psychotic diseases and response to atypical antipsychotics. *Schizophr. Res.* 170, 18–29. <https://doi.org/10.1016/j.schres.2015.11.003>.
- Romero-Martínez, Á., Murciano-Martí, S., and Moya-Albiol, L. (2019). Is sertraline a good pharmacological strategy to control anger? Results of a systematic review. *Behav. Sci.* 9, 57. <https://doi.org/10.3390/bs9050057>.
- Tanke, M.A.C., Kema, I.P., Dijk-Brouwer, J., Doornbos, B., De Vries, E.G.E., and Korf, J. (2008). Low plasma tryptophan in carcinoid patients is associated with increased urinary cortisol excretion. *Psychoneuroendocrinology* 33, 1297–1301. <https://doi.org/10.1016/j.psyneuen.2008.07.005>.
- Cavedini, P., Zorzi, C., Piccinni, M., Cavallini, M.C., and Bellodi, L. (2010). Executive dysfunctions in obsessive-compulsive patients and unaffected relatives: searching for a new intermediate phenotype. *Biol. Psychiatry* 67, 1178–1184. <https://doi.org/10.1016/j.biopsych.2010.02.012>.
- O'Brien, J.W., Lichenstein, S.D., and Hill, S.Y. (2014). Maladaptive decision making and substance use outcomes in high-risk individuals: preliminary evidence for the role of 5-HTTLPR variation. *J. Stud. Alcohol*

- Drugs 75, 643–652. <https://doi.org/10.15288/jsad.2014.75.643>.
27. Kim, K.L., Christensen, R.E., Ruggieri, A., Schettini, E., Freeman, J.B., Garcia, A.M., Flessner, C., Stewart, E., Conelea, C., and Dickstein, D.P. (2019). Cognitive performance of youth with primary generalized anxiety disorder versus primary obsessive-compulsive disorder. *Depress. Anxiety* 36, 130–140. <https://doi.org/10.1002/da.22848>.
 28. Logue, S.F., and Gould, T.J. (2014). The neural and genetic basis of executive function: attention, cognitive flexibility, and response inhibition. *Pharmacol.Biochem.Behav.* 123, 45–54. <https://doi.org/10.1016/j.pbb.2013.08.007>.
 29. Robbins, T.W., and Arnsten, A.F.T. (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annu. Rev. Neurosci.* 32, 267–287. <https://doi.org/10.1146/annurev.neuro.051508.135535>.
 30. Izquierdo, A. (2017). Functional heterogeneity within rat orbitofrontal cortex in reward learning and decision making. *J. Neurosci.* 37, 10529–10540. <https://doi.org/10.1523/JNEUROSCI.1678-17.2017>.
 31. Kiser, D., Steemers, B., Branchi, I., and Homberg, J.R. (2012). The reciprocal interaction between serotonin and social behaviour. *Neurosci.Biobehav. Rev.* 36, 786–798. <https://doi.org/10.1016/j.neubiorev.2011.12.009>.
 32. Seo, D., Patrick, C.J., and Kennealy, P.J. (2008). Role of serotonin and dopamine system interactions in the neurobiology of impulsive aggression and its comorbidity with other clinical disorders. *Aggress. Violent Behav.* 13, 383–395. <https://doi.org/10.1016/j.avb.2008.06.003>.
 33. Ajilchi, B., and Nejati, V. (2017). Executive functions in students with depression, anxiety, and stress symptoms. *Basic Clin.Neurosci.* 8, 223–232. <https://doi.org/10.18869/nirp.bcn.8.3.223>.
 34. Bernhardt, N., Nebe, S., Poese, S., Sebold, M., Sommer, C., Birkenstock, J., Zimmermann, U.S., Heinz, A., and Smolka, M.N. (2017). Impulsive decision making in young adult social drinkers and detoxified alcohol-dependent patients: a cross-sectional and longitudinal study. *Alcohol Clin. Exp. Res.* 41, 1794–1807. <https://doi.org/10.1111/acer.13481>.
 35. Fond, G., Bayard, S., Capdevielle, D., Del-Monte, J., Mimoun, N., Macgregor, A., Boulenger, J.-P., Gely-Nargeot, M.-C., and Raffard, S. (2013). A further evaluation of decision-making under risk and under ambiguity in schizophrenia. *Eur. Arch. Psychiatry Clin. Neurosci.* 263, 249–257. <https://doi.org/10.1007/s00406-012-0330-y>.
 36. Gottwald, J., de Wit, S., Apergis-Schoute, A.M., Morein-Zamir, S., Kaser, M., Cormack, F., Sule, A., Limmer, W., Morris, A.C., Robbins, T.W., et al. (2018). Impaired cognitive plasticity and goal-directed control in adolescent obsessive-compulsive disorder. *Psychol. Med.* 48, 1900–1908. <https://doi.org/10.1017/S0033291717003464>.
 37. Higgs, S., Spetter, M.S., Thomas, J.M., Rotshtein, P., Lee, M., Hallschmid, M., and Dourish, C.T. (2017). Interactions between metabolic, reward and cognitive processes in appetite control: implications for novel weight management therapies. *J. Psychopharmacol.* 31, 1460–1474. <https://doi.org/10.1177/0269881117736917>.
 38. Kim, H.W., Kang, J.I., Namkoong, K., Jhung, K., Ha, R.Y., and Kim, S.J. (2015). Further evidence of a dissociation between decision-making under ambiguity and decision-making under risk in obsessive-compulsive disorder. *J. Affect. Disord.* 176, 118–124. <https://doi.org/10.1016/j.jad.2015.01.060>.
 39. de Wit, H. (2009). Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addict. Biol.* 14, 22–31. <https://doi.org/10.1111/j.1369-1600.2008.00129.x>.
 40. Zhang, L., Tang, J., Dong, Y., Ji, Y., Tao, R., Liang, Z., Chen, J., Wu, Y., and Wang, K. (2015). Similarities and differences in decision-making impairments between autism spectrum disorder and schizophrenia. *Front. Behav.Neurosci.* 9, 259. <https://doi.org/10.3389/fnbeh.2015.00259>.
 41. Asher, D.E., Craig, A.B., Zaldivar, A., Brewer, A.A., and Krichmar, J.L. (2013). A dynamic, embodied paradigm to investigate the role of serotonin in decision-making. *Front. Integr.Neurosci.* 7, 78. <https://doi.org/10.3389/fnint.2013.00078>.
 42. Koot, S., Zoratto, F., Cassano, T., Colangeli, R., Laviola, G., van den Bos, R., and Adriani, W. (2012). Compromised decision-making and increased gambling proneness following dietary serotonin depletion in rats. *Neuropharmacology* 62, 1640–1650. <https://doi.org/10.1016/j.neuropharm.2011.11.002>.
 43. Mosienko, V., Bert, B., Beis, D., Matthes, S., Fink, H., Bader, M., and Alenina, N. (2012). Exaggerated aggression and decreased anxiety in mice deficient in brain serotonin. *Transl. Psychiatry* 2, e122. <https://doi.org/10.1038/tp.2012.44>.
 44. Passamonti, L., Crockett, M.J., Apergis-Schoute, A.M., Clark, L., Rowe, J.B., Calder, A.J., and Robbins, T.W. (2012). Effects of acute tryptophan depletion on prefrontal-amygdala connectivity while viewing facial signals of aggression. *Biol. Psychiatry* 71, 36–43. <https://doi.org/10.1016/j.biopsych.2011.07.033>.
 45. Schweighofer, N., Bertin, M., Shishida, K., Okamoto, Y., Tanaka, S.C., Yamawaki, S., and Doya, K. (2008). Low-serotonin levels increase delayed reward discounting in humans. *J. Neurosci.* 28, 4528–4532. <https://doi.org/10.1523/JNEUROSCI.4982-07.2008>.
 46. Skandali, N., Rowe, J.B., Voon, V., Deakin, J.B., Cardinal, R.N., Cormack, F., Passamonti, L., Bevan-Jones, W.R., Regenthal, R., Chamberlain, S.R., et al. (2018). Dissociable effects of acute SSRI (escitalopram) on executive, learning and emotional functions in healthy humans. *Neuropsychopharmacology* 43, 2645–2651. <https://doi.org/10.1038/s41386-018-0229-z>.
 47. Worbe, Y., Savulich, G., de Wit, S., Fernandez-Egea, E., and Robbins, T.W. (2015). Tryptophan depletion promotes habitual over goal-directed control of appetitive responding in humans. *Int. J. Neuropsychopharmacol.* 18, pyv013. <https://doi.org/10.1093/ijnp/pyv013>.
 48. Young, S.N., and Leyton, M. (2002). The role of serotonin in human mood and social interaction. *Insight from altered tryptophan levels. Pharmacol.Biochem.Behav.* 71, 857–865.
 49. Assary, E., Vincent, J.P., Keers, R., and Pluess, M. (2018). Gene-environment interaction and psychiatric disorders: review and future directions. *Semin. Cell Dev. Biol.* 77, 133–143. <https://doi.org/10.1016/j.semdb.2017.10.016>.
 50. Auxéméry, Y. (2012). [Posttraumatic stress disorder (PTSD) as a consequence of the interaction between an individual genetic susceptibility, a traumatogenic event and a social context]. *Encéphale* 38, 373–380. <https://doi.org/10.1016/j.encep.2011.12.003>.
 51. Dean, J., and Keshavan, M. (2017). The neurobiology of depression: an integrated view. *Asian J. Psychiatr.* 27, 101–111. <https://doi.org/10.1016/j.ajp.2017.01.025>.
 52. Grahek, I., Shenhav, A., Musslick, S., Krebs, R.M., and Koster, E.H.W. (2019). Motivation and cognitive control in depression. *Neurosci.Biobehav. Rev.* 102, 371–381. <https://doi.org/10.1016/j.neubiorev.2019.04.011>.
 53. Alonso, L., Peeva, P., Ramos-Prats, A., Alenina, N., Winter, Y., and Rivalan, M. (2020). Inter-individual and inter-strain differences in cognitive and social abilities of Dark Agouti and Wistar Han rats. *Behav. Brain Res.* 377, 112188. <https://doi.org/10.1016/j.bbr.2019.112188>.
 54. Kaplan, K., Echert, A.E., Massat, B., Puissant, M.M., Palygin, O., Geurts, A.M., and Hodges, M.R. (2016). Chronic central serotonin depletion attenuates ventilation and body temperature in young but not adult Tph2 knockout rats. *J. Appl. Physiol.* 120, 1070–1081. <https://doi.org/10.1152/japplphysiol.01015.2015>.
 55. Walther, D.J., Peter, J.-U., Bashammakh, S., Hörtnagl, H., Voits, M., Fink, H., and Bader, M. (2003). Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* 299, 76. <https://doi.org/10.1126/science.1078197>.
 56. Mouradian, G.C., Kilby, M., Alvarez, S., Kaplan, K., and Hodges, M.R. (2021). Mortality and ventilatory effects of central serotonin deficiency during postnatal development depend on age but not sex. *Physiol. Rep.* 9, e14946. <https://doi.org/10.14814/phy2.14946>.

57. Peeters, D.G.A., de Boer, S.F., Terneusen, A., Newman-Tancredi, A., Varney, M.A., Verkes, R.-J., and Homberg, J.R. (2019). Enhanced aggressive phenotype of Tph2 knockout rats is associated with diminished 5-HT1A receptor sensitivity. *Neuropharmacology* 153, 134–141. <https://doi.org/10.1016/j.neuropharm.2019.05.004>.
58. Matusz, P.J., Dikker, S., Huth, A.G., and Perrodin, C. (2019). Are we ready for real-world neuroscience? *J. Cogn. Neurosci.* 31, 327–338. https://doi.org/10.1162/jocn_e_01276.
59. Rivalan, M., Ahmed, S.H., and Dellu-Hagedorn, F. (2009). Risk-prone individuals prefer the wrong options on a rat version of the Iowa Gambling Task. *Biol. Psychiatry* 66, 743–749. <https://doi.org/10.1016/j.biopsych.2009.04.008>.
60. Weintraub, D., David, A.S., Evans, A.H., Grant, J.E., and Stacy, M. (2015). Clinical spectrum of impulse control disorders in Parkinson's disease. *Mov. Disord.* 30, 121–127. <https://doi.org/10.1002/mds.26016>.
61. Fontenelle, L.F., Mendlowicz, M.V., and Versiani, M. (2005). Impulse control disorders in patients with obsessive-compulsive disorder. *Psychiatry Clin. Neurosci.* 59, 30–37. <https://doi.org/10.1111/j.1440-1819.2005.01328.x>.
62. Seo, D., Lacadie, C.M., and Sinha, R. (2016). Neural correlates and connectivity underlying stress-related impulse control difficulties in alcoholism. *Alcohol Clin. Exp. Res.* 40, 1884–1894. <https://doi.org/10.1111/acer.13166>.
63. Dunsmoor, J.E., and Paz, R. (2015). Fear generalization and anxiety: behavioral and neural mechanisms. *Biol. Psychiatry* 78, 336–343. <https://doi.org/10.1016/j.biopsych.2015.04.010>.
64. Silva, B., Canas-Simião, H., and Cavanna, A.E. (2020). Neuropsychiatric aspects of impulse control disorders. *Psychiatr. Clin. North Am.* 43, 249–262. <https://doi.org/10.1016/j.psc.2020.02.001>.
65. Fanning, J.R., Lee, R., and Coccaro, E.F. (2016). Comorbid intermittent explosive disorder and posttraumatic stress disorder: clinical correlates and relationship to suicidal behavior. *Compr. Psychiatry* 70, 125–133. <https://doi.org/10.1016/j.comppsy.2016.05.018>.
66. Grant, J.E., and Potenza, M.N. (2004). Impulse control disorders: clinical characteristics and pharmacological management. *Ann. Clin. Psychiatry.* 16, 27–34. <https://doi.org/10.1080/10401230490281366>.
67. Buchanan, T.W., McMullin, S.D., Mulhauser, K., Weinstock, J., and Weller, J.A. (2020). Diurnal cortisol and decision making under risk in problem gambling. *Psychol. Addict. Behav.* 34, 218–229. <https://doi.org/10.1037/adb0000474>.
68. Djamshidian, A., O'Sullivan, S.S., Papadopoulos, A., Bassett, P., Shaw, K., Auerbeck, B.B., and Lees, A. (2011). Salivary cortisol levels in Parkinson's disease and its correlation to risk behaviour. *J. Neurol. Neurosurg. Psychiatry* 82, 1107–1111. <https://doi.org/10.1136/jnnp.2011.245746>.
69. Lieb, K., Rexhausen, J.E., Kahl, K.G., Schweiger, U., Philipsen, A., Hellhammer, D.H., and Bohus, M. (2004). Increased diurnal salivary cortisol in women with borderline personality disorder. *J. Psychiatr. Res.* 38, 559–565. <https://doi.org/10.1016/j.jpsychires.2004.04.002>.
70. American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.* Washington DC. <https://www.psychiatry.org/psychiatrists/practice/dsm>
71. World Health Organization (2019). *International Classification of Diseases 11th Revision.* <https://icd.who.int/en>.
72. Westergaard, G.C., Suomi, S.J., Higley, J.D., and Mehlman, P.T. (1999). CSF 5-HIAA and aggression in female macaque monkeys: species and interindividual differences. *Psychopharmacology* 146, 440–446. <https://doi.org/10.1007/pl00005489>.
73. Wendland, J.R., Lesch, K.-P., Newman, T.K., Timme, A., Gachot-Neveu, H., Thierry, B., and Suomi, S.J. (2006). Differential functional variability of serotonin transporter and monoamine oxidase a genes in macaque species displaying contrasting levels of aggression-related behavior. *Behav. Genet.* 36, 163–172. <https://doi.org/10.1007/s10519-005-9017-8>.
74. Sbrini, G., Brivio, P., Bosch, K., Homberg, J.R., and 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* 11, 1248. <https://doi.org/10.3390/genes1111248>.
75. Zhou, J., Li, L., Tang, S., Cao, X., Li, Z., Li, W., Li, C., and Zhang, X. (2008). Effects of serotonin depletion on the hippocampal GR/MR and BDNF expression during the stress adaptation. *Behav. Brain Res.* 195, 129–138. <https://doi.org/10.1016/j.bbr.2008.06.009>.
76. Bhatnagar, S., Vining, C., Iyer, V., and Kinni, V. (2006). Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. *J. Neuroendocrinol.* 18, 13–24. <https://doi.org/10.1111/j.1365-2826.2005.01375.x>.
77. Jean, A., Laurent, L., Delaunay, S., Doly, S., Dusticier, N., Linden, D., Neve, R., Maroteaux, L., Nieoullon, A., and Compan, V. (2017). Adaptive control of dorsal raphe by 5-HT4 in the prefrontal cortex prevents persistent hypophagia following stress. *Cell Rep.* 21, 901–909. <https://doi.org/10.1016/j.celrep.2017.10.003>.
78. Baarendse, P.J.J., and Vanderschuren, L.J.M.J. (2012). Dissociable effects of monoamine reuptake inhibitors on distinct forms of impulsive behavior in rats. *Psychopharmacology* 219, 313–326. <https://doi.org/10.1007/s00213-011-2576-x>.
79. Brigman, J.L., Mathur, P., Harvey-White, J., Izquierdo, A., Saksida, L.M., Bussey, T.J., Fox, S., Deneris, E., Murphy, D.L., and Holmes, A. (2010). Pharmacological or genetic inactivation of the serotonin transporter improves reversal learning in mice. *Cereb. Cortex* 20, 1955–1963. <https://doi.org/10.1093/cercor/bhp266>.
80. Clarke, H.F., Dalley, J.W., Crofts, H.S., Robbins, T.W., and Roberts, A.C. (2004). Cognitive inflexibility after prefrontal serotonin depletion. *Science* 304, 878–880. <https://doi.org/10.1126/science.1094987>.
81. Fonseca, M.S., Murakami, M., and Mainen, Z.F. (2015). Activation of dorsal raphe serotonergic neurons promotes waiting but is not reinforcing. *Curr. Biol.* 25, 306–315. <https://doi.org/10.1016/j.cub.2014.12.002>.
82. Homberg, J.R., van den Bos, R., den Heijer, E., Suer, R., and Cuppen, E. (2008). Serotonin transporter dosage modulates long-term decision-making in rat and human. *Neuropharmacology* 55, 80–84. <https://doi.org/10.1016/j.neuropharm.2008.04.016>.
83. Ishii, H., Ohara, S., Tobler, P.N., Tsutsui, K.-I., and Iijima, T. (2015). Dopaminergic and serotonergic modulation of anterior insular and orbitofrontal cortex function in risky decision making. *Neurosci. Res.* 92, 53–61. <https://doi.org/10.1016/j.neures.2014.11.009>.
84. Kirkpatrick, K., Marshall, A.T., Smith, A.P., Koci, J., and Park, Y. (2014). Individual differences in impulsive and risky choice: effects of environmental rearing conditions. *Behav. Brain Res.* 269, 115–127. <https://doi.org/10.1016/j.bbr.2014.04.024>.
85. de Visser, L., Homberg, J.R., Mitsogiannis, M., Zeeb, F.D., Rivalan, M., Fitoussi, A., Galhardo, V., van den Bos, R., Winstanley, C.A., and Dellu-Hagedorn, F. (2011). Rodent versions of the Iowa gambling task: opportunities and challenges for the understanding of decision-making. *Front. Neurosci.* 5, 109. <https://doi.org/10.3389/fnins.2011.00109>.
86. Winstanley, C.A., Dalley, J.W., Theobald, D.E.H., and Robbins, T.W. (2003). Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. *Psychopharmacology* 170, 320–331. <https://doi.org/10.1007/s00213-003-1546-3>.
87. Winstanley, C.A., Theobald, D.E.H., Dalley, J.W., Cardinal, R.N., and Robbins, T.W. (2006). Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. *Cereb. Cortex* 16, 106–114. <https://doi.org/10.1093/cercor/bhi088>.
88. Angoa-Pérez, M., Kane, M.J., Briggs, D.I., Sykes, C.E., Shah, M.M., Francescutti, D.M., Rosenberg, D.R., Thomas, D.M., and Kuhn, D.M. (2012). Genetic depletion of brain 5HT reveals a common molecular pathway

- mediating compulsivity and impulsivity. *J. Neurochem.* 121, 974–984. <https://doi.org/10.1111/j.1471-4159.2012.07739.x>.
89. Carlson, K.S., Whitney, M.S., Gadziola, M.A., Deneris, E.S., and Wesson, D.W. (2016). Preservation of essential odor-guided behaviors and odor-based reversal learning after targeting adult brain serotonin synthesis. *eNeuro* 3. ENEURO.0257-16.2016. <https://doi.org/10.1523/ENEURO.0257-16.2016>.
 90. Cools, R., Blackwell, A., Clark, L., Menzies, L., Cox, S., and Robbins, T.W. (2005). Tryptophan depletion disrupts the motivational guidance of goal-directed behavior as a function of trait impulsivity. *Neuropsychopharmacology* 30, 1362–1373. <https://doi.org/10.1038/sj.npp.1300704>.
 91. Crean, J., Richards, J.B., and de Wit, H. (2002). Effect of tryptophan depletion on impulsive behavior in men with or without a family history of alcoholism. *Behav. Brain Res.* 136, 349–357.
 92. Faulkner, P., Mancinelli, F., Lockwood, P.L., Matarin, M., Dolan, R.J., Wood, N.W., Dayan, P., and Roiser, J.P. (2017). Peripheral serotonin 1B receptor transcription predicts the effect of acute tryptophan depletion on risky decision-making. *Int. J. Neuropsychopharmacol.* 20, 58–66. <https://doi.org/10.1093/ijnp/pyw075>.
 93. Neukam, P.T., Kroemer, N.B., Deza Araujo, Y.I., Hellrung, L., Pooeh, S., Rietschel, M., Witt, S.H., Schwarzenbolz, U., Henle, T., and Smolka, M.N. (2018). Risk-seeking for losses is associated with 5-HTTLPR, but not with transient changes in 5-HT levels. *Psychopharmacology* 235, 2151–2165. <https://doi.org/10.1007/s00213-018-4913-9>.
 94. van der Plasse, G., and Feenstra, M.G.P. (2008). Serial reversal learning and acute tryptophan depletion. *Behav. Brain Res.* 186, 23–31. <https://doi.org/10.1016/j.bbr.2007.07.017>.
 95. Tanaka, S.C., Schweighofer, N., Asahi, S., Shishida, K., Okamoto, Y., Yamawaki, S., and Doya, K. (2007). Serotonin differentially regulates short- and long-term prediction of rewards in the ventral and dorsal striatum. *PLoS One* 2, e1333. <https://doi.org/10.1371/journal.pone.0001333>.
 96. Thirkettle, M., Barker, L.-M., Gallagher, T., Nayeib, N., and Aquili, L. (2019). Dissociable effects of tryptophan supplementation on negative feedback sensitivity and reversal learning. *Front. Behav. Neurosci.* 13, 127. <https://doi.org/10.3389/fnbeh.2019.00127>.
 97. Jo, Y.-I., Kim, H., and Ramakrishna, S. (2015). Recent developments and clinical studies utilizing engineered zinc finger nuclease technology. *Cell.Mol. Life Sci.* 72, 3819–3830. <https://doi.org/10.1007/s00018-015-1956-5>.
 98. Kreiner, G. (2015). Compensatory mechanisms in genetic models of neurodegeneration: are the mice better than humans? *Front. Cell. Neurosci.* 9, 56. <https://doi.org/10.3389/fncel.2015.00056>.
 99. Brivio, P., Sbrini, G., Peeva, P., Todiras, M., Bader, M., Alenina, N., and Calabrese, F. (2018). TPH2 deficiency influences neuroplastic mechanisms and alters the response to an acute stress in a sex specific manner. *Front. Mol. Neurosci.* 11, 389. <https://doi.org/10.3389/fnmol.2018.00389>.
 100. Kronenberg, G., Mosienko, V., Gertz, K., Alenina, N., Hellweg, R., and Klempin, F. (2016). Increased brain-derived neurotrophic factor (BDNF) protein concentrations in mice lacking brain serotonin. *Eur. Arch. Psychiatry Clin. Neurosci.* 266, 281–284. <https://doi.org/10.1007/s00406-015-0611-3>.
 101. Migliarini, S., Pacini, G., Pelosi, B., Lunardi, G., and Pasqualetti, M. (2013). Lack of brain serotonin affects postnatal development and serotonergic neuronal circuitry formation. *Mol. Psychiatry* 18, 1106–1118. <https://doi.org/10.1038/mp.2012.128>.
 102. Gutknecht, L., Araragi, N., Merker, S., Waider, J., Sommerlandt, F.M.J., Mlinar, B., Baccini, G., Mayer, U., Proft, F., Hamon, M., et al. (2012). Impacts of brain serotonin deficiency following Tph2 inactivation on development and raphe neuron serotonergic specification. *PLoS One* 7, e43157. <https://doi.org/10.1371/journal.pone.0043157>.
 103. Pratelli, M., and Pasqualetti, M. (2019). Serotonergic neurotransmission manipulation for the understanding of brain development and function: learning from Tph2 genetic models. *Biochimie* 161, 3–14. <https://doi.org/10.1016/j.biochi.2018.11.016>.
 104. Alenina, N., Kikic, D., Todiras, M., Mosienko, V., Qadri, F., Plehm, R., Boyé, P., Vilianovitch, L., Sohr, R., Tenner, K., et al. (2009). Growth retardation and altered autonomic control in mice lacking brain serotonin. *Proc. Natl. Acad. Sci. USA* 106, 10332–10337. <https://doi.org/10.1073/pnas.0810793106>.
 105. Belin, M.F., Nanopoulos, D., Didier, M., Aguera, M., Steinbusch, H., Verhofstad, A., Maitre, M., and Pujol, J.F. (1983). Immunohistochemical evidence for the presence of γ -aminobutyric acid and serotonin in one nerve cell. A study on the raphe nuclei of the rat using antibodies to glutamate decarboxylase and serotonin. *Brain Res.* 275, 329–339. [https://doi.org/10.1016/0006-8993\(83\)90994-0](https://doi.org/10.1016/0006-8993(83)90994-0).
 106. Larsen, M.B., Sonders, M.S., Mortensen, O.V., Larson, G.A., Zahniser, N.R., and Amara, S.G. (2011). Dopamine transport by the serotonin transporter: a mechanistically distinct mode of substrate translocation. *J. Neurosci.* 31, 6605–6615. <https://doi.org/10.1523/JNEUROSCI.0576-11.2011>.
 107. Sengupta, A., Bocchio, M., Bannerman, D.M., Sharp, T., and Capogna, M. (2017). Control of amygdala circuits by 5-HT neurons via 5-HT and glutamate cotransmission. *J. Neurosci.* 37, 1785–1796. <https://doi.org/10.1523/JNEUROSCI.2238-16.2016>.
 108. Trudeau, L.-É. (2004). Glutamate co-transmission as an emerging concept in monoamine neuron function. *J. Psychiatry Neurosci.* 29, 296–310.
 109. Wang, H.-L., Zhang, S., Qi, J., Wang, H., Cachope, R., Mejias-Aponte, C.A., Gomez, J.A., Mateo-Semidey, G.E., Beaudoin, G.M.J., Paladini, C.A., et al. (2019). Dorsal raphe dual serotonin-glutamate neurons drive reward by establishing excitatory synapses on VTA mesoaccumbens dopamine neurons. *Cell Rep.* 26, 1128–1142.e7. <https://doi.org/10.1016/j.celrep.2019.01.014>.
 110. Zhou, F.-M., Liang, Y., Salas, R., Zhang, L., De Biasi, M., and Dani, J.A. (2005). Corelease of dopamine and serotonin from striatal dopamine terminals. *Neuron* 46, 65–74. <https://doi.org/10.1016/j.neuron.2005.02.010>.
 111. Liu, Z., Zhou, J., Li, Y., Hu, F., Lu, Y., Ma, M., Feng, Q., Zhang, J.E., Wang, D., Zeng, J., et al. (2014). Dorsal raphe neurons signal reward through 5-HT and glutamate. *Neuron* 81, 1360–1374. <https://doi.org/10.1016/j.neuron.2014.02.010>.
 112. Fischer, A.G., and Ullsperger, M. (2017). An update on the role of serotonin and its interplay with dopamine for reward. *Front. Hum. Neurosci.* 11, 484.
 113. Matias, S., Lottem, E., Dugué, G.P., and Mainen, Z.F. (2017). Activity patterns of serotonin neurons underlying cognitive flexibility. *Elife* 6, e20552. <https://doi.org/10.7554/eLife.20552>.
 114. Branchi, I. (2011). The double edged sword of neural plasticity: increasing serotonin levels leads to both greater vulnerability to depression and improved capacity to recover. *Psychoneuroendocrinology* 36, 339–351. <https://doi.org/10.1016/j.psyneuen.2010.08.011>.
 115. Deschênes, M., Moore, J., and Kleinfeld, D. (2012). Sniffing and whisking in rodents. *Curr. Opin. Neurobiol.* 22, 243–250. <https://doi.org/10.1016/j.conb.2011.11.013>.
 116. Wesson, D.W. (2013). Sniffing behavior communicates social hierarchy. *Curr. Biol.* 23, 575–580. <https://doi.org/10.1016/j.cub.2013.02.012>.
 117. Lee, W., Fu, J., Bouwman, N., Farago, P., and Curley, J.P. (2019). Temporal microstructure of dyadic social behavior during relationship formation in mice. *PLoS One* 14, e0220596. <https://doi.org/10.1371/journal.pone.0220596>.
 118. de Chaumont, F., Ey, E., Torquet, N., Lagache, T., Dallongeville, S., Imbert, A., Legou, T., Le Sourd, A.M., Faure, P., Bourgeron, T., et al. (2019). Real-time analysis of the behaviour of groups of mice via a depth-sensing camera and machine learning. *Nat. Biomed. Eng.* 3, 930–942. <https://doi.org/10.1038/s41551-019-0396-1>.
 119. Rivalan, M., Munawar, H., Fuchs, A., and Winter, Y. (2017). An automated, experimenter-free method for the standardised, operant cognitive testing of rats. *PLoS One* 12, e0169476. <https://doi.org/10.1371/journal.pone.0169476>.

120. Torquet, N., Marti, F., Campart, C., Tolu, S., Nguyen, C., Obertero, V., Benallaoua, M., Naudé, J., Didiene, S., Debray, N., et al. (2018). Social interactions impact on the dopaminergic system and drive individuality. *Nat. Commun.* 9, 3081. <https://doi.org/10.1038/s41467-018-05526-5>.
121. Alonso, L., Peeva, P., Stasko, S., Bader, M., Alenina, N., Winter, Y., and Rivalan, M. (2021). Constitutive depletion of brain serotonin differentially affects rats' social and cognitive abilities. Preprint at bioRxiv. <https://doi.org/10.1101/2021.09.23.461469>.
122. Touma, C., Sachser, N., Möstl, E., and Palme, R. (2003). Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *Gen. Comp. Endocrinol.* 130, 267–278. [https://doi.org/10.1016/S0016-6480\(02\)00620-2](https://doi.org/10.1016/S0016-6480(02)00620-2).
123. Janvier-Labs. Rat DARK AGOUTI. Janvier-Labs. https://janvier-labs.com/fiche_produit/dark-agouti-rat/
124. Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., et al. (2020). Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* 18, e3000411. <https://doi.org/10.1371/journal.pbio.3000411>.
125. Myerson, J., Green, L., and Warusawitharana, M. (2001). Area under the curve as a measure of discounting. *J. Exp. Anal. Behav.* 76, 235–243. <https://doi.org/10.1901/jeab.2001.76-235>.
126. Zoratto, F., Sinclair, E., Manciocco, A., Vitale, A., Laviola, G., and Adriani, W. (2014). Individual differences in gambling proneness among rats and common marmosets: an automated choice task. *BioMed Res. Int.* 2014, 927685. <https://doi.org/10.1155/2014/927685>.
127. Adriani, W., and Laviola, G. (2006). Delay aversion but preference for large and rare rewards in two choice tasks: implications for the measurement of self-control parameters. *BMC Neurosci.* 7, 52. <https://doi.org/10.1186/1471-2202-7-52>.
128. Zoratto, F., Laviola, G., and Adriani, W. (2012). Choice with delayed or uncertain reinforcers in rats: influence of timeout duration and session length. *Synapse* 66, 792–806. <https://doi.org/10.1002/syn.21570>.
129. Rivalan, M., Valton, V., Seriès, P., Marchand, A.R., and Dellu-Hagedorn, F. (2013). Elucidating poor decision-making in a rat gambling task. *PLoS One* 8, e82052. <https://doi.org/10.1371/journal.pone.0082052>.
130. Dellu-Hagedorn, F. (2006). Relationship between impulsivity, hyperactivity and working memory: a differential analysis in the rat. *Behav. Brain Funct.* 2, 10. <https://doi.org/10.1186/1744-9081-2-10>.
131. Dellu-Hagedorn, F., Rivalan, M., Fitoussi, A., and De Deurwaerdère, P. (2018). Inter-individual differences in the impulsive/compulsive dimension: deciphering related dopaminergic and serotonergic metabolisms at rest. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 373, 20170154. <https://doi.org/10.1098/rstb.2017.0154>.
132. Hardy, M.P., Sottas, C.M., Ge, R., McKittrick, C.R., Tamashiro, K.L., McEwen, B.S., Haider, S.G., Markham, C.M., Blanchard, R.J., Blanchard, D.C., et al. (2002). Trends of reproductive hormones in male rats during psychosocial stress: role of glucocorticoid metabolism in behavioral dominance. *Biol. Reprod.* 67, 1750–1755. <https://doi.org/10.1095/biolreprod.102.006312>.
133. Arakawa, H., Blanchard, D.C., and Blanchard, R.J. (2007). Colony formation of C57BL/6J mice in visible burrow system: identification of eusocial behaviors in a background strain for genetic animal models of autism. *Behav. Brain Res.* 176, 27–39. <https://doi.org/10.1016/j.bbr.2006.07.027>.
134. Burman, O., Owen, D., Aboulsmaïl, U., and Mendl, M. (2008). Removing individual rats affects indicators of welfare in the remaining group members. *Physiol. Behav.* 93, 89–96. <https://doi.org/10.1016/j.physbeh.2007.08.001>.
135. Rademacher, D.J., Schuyler, A.L., Kruschel, C.K., and Steinpreis, R.E. (2002). Effects of cocaine and putative atypical antipsychotics on rat social behavior: an ethopharmacological study. *Pharmacol. Biochem. Behav.* 73, 769–778. [https://doi.org/10.1016/S0091-3057\(02\)00904-8](https://doi.org/10.1016/S0091-3057(02)00904-8).
136. Whishaw, I.Q., and Bryan, K. (2004). Behavior of the Laboratory Rat: A Handbook with Tests - Oxford Scholarship. <http://www.oxfordscholarship.com/view/10.1093/acprof:oso/9780195162851.001.0001/acprof-9780195162851>.
137. Glickman, M.E. (1999). Parameter estimation in large dynamic paired comparison experiments. *J. R. Statist. Soc. C* 48, 377–394. <https://doi.org/10.1111/1467-9876.00159>.
138. So, N., Franks, B., Lim, S., and Curley, J.P. (2015). A social network approach reveals associations between mouse social dominance and brain gene expression. *PLoS One* 10, e0134509. <https://doi.org/10.1371/journal.pone.0134509>.
139. Stephenson, A., and Sonas, J. (2020). PlayerRatings: Dynamic Updating Methods for Player Ratings Estimation.
140. Adams, R.P., and MacKay, D.J.C. (2007). *Bayesian Online Change-point Detection*.
141. Blanchard, R.J., Dulloog, L., Markham, C., Nishimura, O., Nikulina Compton, J., Jun, A., Han, C., and Blanchard, D.C. (2001). Sexual and aggressive interactions in a visible burrow system with provisioned burrows. *Physiol. Behav.* 72, 245–254. [https://doi.org/10.1016/S0031-9384\(00\)00403-0](https://doi.org/10.1016/S0031-9384(00)00403-0).
142. Freund, J., Brandmaier, A.M., Lewejohann, L., Kirste, I., Kritzler, M., Krüger, A., Sachser, N., Lindenberger, U., and Kempermann, G. (2013). Emergence of individuality in genetically identical mice. *Science* 340, 756–759. <https://doi.org/10.1126/science.1235294>.
143. Csardi, G., and Nepusz, T. (2006). The igraph software package for complex network research. *Int. J. Complex Syst.* 1695.
144. Bonacich, P. (1987). Power and centrality: a family of measures. *Am. J. Sociol.* 92, 1170–1182.
145. Kleinberg, J.M. (1999). Authoritative sources in a hyperlinked environment. *J. ACM* 46, 604–632.
146. Lepschy, M., Touma, C., Hruby, R., and Palme, R. (2007). Non-invasive measurement of adrenocortical activity in male and female rats. *Lab. Anim.* 41, 372–387. <https://doi.org/10.1258/002367707781282730>.
147. R Core Team (2019). R: The R Project for Statistical Computing (Vienna, Austria: R Foundation for Statistical Computing). <https://www.R-project.org/>. <https://www.r-project.org/>.
148. Hervé, M. (2020). *RVAideMemoire: Testing and Plotting Procedures for Biostatistics*.
149. Torchiano, M. (2020). *Effsize: Efficient Effect Size Computation*.
150. Schielzeth, H., Dingemanse, N.J., Nakagawa, S., Westneat, D.F., Allogue, H., Teplitsky, C., Réale, D., Dochtermann, N.A., Garamszegi, L.Z., and Araya-Ajoy, Y.G. (2020). Robustness of linear mixed-effects models to violations of distributional assumptions. *Methods Ecol. Evol.* 11, 1141–1152. <https://doi.org/10.1111/2041-210X.13434>.
151. Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., and Jensen, S.P. (2017). lmerTest: Tests in Linear Mixed Effects Models.
152. Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.* 50, 346–363.
153. Hadfield, J.D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* 33, 1–22.
154. Harrell Frank, E., Jr., and Dupont, C. (2019). *Hmisc: Harrell Miscellaneous*.
155. Liaw, A., and Wiener, M. (2002). Classification and regression by randomForest. *R. News* 2, 18–22.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-5 α -pregnane-3 β ,11 β ,21-triol-20-one-CMO:BSA	Touma et al. ¹²²	Lab-code: 37e
Deposited data		
Data and analysis	This paper	https://doi.org/10.5281/zenodo.4912528
Experimental models: Organisms/strains		
Rats: Dark Agouti	Janvier Labs ¹²³	Cat#DA/HanRj
Rats: TPH2-ZFN	Kaplan et al. ⁵⁴	DA-Tph2em2Mcow
Software and algorithms		
R 3.6.1	R Core Team	https://www.R-project.org/
R Studio 1.1.456	Posit	https://www.rstudio.com/categories/rstudio-ide/
PhenoSoft	Phenosys, Germany	https://www.phenosys.com/
CamUniversal	CrazyPixels, Germany	http://www.crazypixels.com/products/camuniversal
Other		
Sweet pellets 45 mg	TestDiet, USA	Cat#5TUL

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Marion Rivalan (marionrivalan@gmail.com). Specific requests about TPH2-rats should be directed to corresponding author Natalia Alenina (alenina@mdc-berlin.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All original data are publicly available in the Zenodo repository: <https://doi.org/10.5281/zenodo.4912528>. All original codes are publicly available in the Zenodo repository: <https://doi.org/10.5281/zenodo.4912528>. Any additional information required to reanalyze the data reported in this paper is available from the lead contact Marion Rivalan (marionrivalan@gmail.com) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

Dark Agouti rats (originally from Janvier Labs, France¹²³) and TPH2 rats⁵⁴ on Dark Agouti background were bred at the Max Delbrück Center for Molecular Medicine (Berlin) and transferred to the experimental facility of the Charité between five and nine weeks of age. To generate experimental *Tph2*^{+/+} and *Tph2*^{-/-} animals, 13 *Tph2*^{+/+} dams were bred with *Tph2*^{+/+} males; and 3 and 12 dams of *Tph2*^{-/-} and *Tph2*^{+/-} genotype, respectively, were bred with *Tph2*^{-/-} males. One to five siblings per litter were taken from each dam. The *Tph2*^{-/-} pups showed a 10% mortality rate, whereas no preweaning loss was observed for *Tph2*^{+/-} and *Tph2*^{+/+} pups. Monoamine levels were controlled by HPLC: serotonin was undetectable in the brain of *Tph2*^{-/-} animals (data not shown), confirming previously published data.^{54,56} Genotyping of animals was performed according to the previously published protocol.⁷⁴

In total, 48 $Tph2^{+/+}$ and 30 $Tph2^{-/-}$ male rats (24 born from $Tph2^{+/+}$ and 6 born from $Tph2^{-/-}$ dams) were used in the study. The $Tph2^{+/+}$ group consisted of 10 Dark Agouti and 38 $Tph2^{+/+}$ rats originating from the TPH2-breeding. Animals were housed in pairs of the same genotype in standard rat cages (Eurostandard Type IV, 38 cm × 59 cm) in two temperature-controlled rooms (22°C–24°C and 45%–55% humidity) with inverted 12-h light-dark cycles. We used 8 $Tph2^{+/+}$ and 5 $Tph2^{-/-}$ cohorts, 6 animals each. Groups of 12 animals (6 $Tph2^{+/+}$ and 6 $Tph2^{-/-}$) were tested either in the morning or in the afternoon (i.e. 24 animals per day) depending on the light cycle of the housing room (lights on at 20:00 in room 1 or 01:00 in room 2) in order to maximize the use of our four operant cages and minimize potential circadian effect (rats were all tested in RGT within 3h and 1h after start of dark phase).

Animals had *ad libitum* access to water throughout the experiment. They were fed *ad libitum* with standard maintenance food (V1534-000, Ssniff, Germany) except during the operant training and testing, when they were maintained at 95% of their free-feeding weight. After their daily operant testing rats were fed up to 20 g per animal depending on the amount of reward (sweet pellets) they received in the operant chamber and following an unpredictable schedule (one to several hours after the end of test) to avoid their anticipation of feeding. Rats were weighed every two to three days allowing for adjustment of their portion of standard food. After the VBS and before the DDT rats were given as many days as necessary to be back at 100% \pm 2% of their pre-VBS bodyweight.

After staying a week undisturbed in the animal facility, animals were handled daily by the experimenters. Since $Tph2^{-/-}$ animals were very reactive to manual handling all animals were handled using a 6 cm diameter gray polypropylene tube that was added in the cage as enrichment and used by the animals as shelter preventing fights and mounting behavior. Two weeks before the beginning of the training phase, rats were marked individually, subcutaneously in the ventral left lower quadrant with a radio-frequency identification (RFID) chip (glass transponder 3 × 13 mm, Euro I.D.) under short isoflurane anesthesia. Rats were between 8 and 14 weeksold when first trained in the operant procedures.

Ethical statement

All procedures followed the national regulations in accordance with the European Union Directive 2010/63/EU. The protocols were approved by the local animal care and use committee (LaGeSo Berlin) and under the supervision of the animal welfare officer of our institution.

METHOD DETAILS

The study was reported in accordance with the ARRIVE Guidelines¹²⁴ (Table S9). Numbers of animals for each test are reported in the Table S10. The number of animals was decided following a *priori* power analysis ($n = 51$, G^* power 3.1.2). It was reduced because of the difficulty to obtain $Tph2^{-/-}$ animals due to their higher post-natal mortality rate. Unless stated otherwise, rats were trained and tested following established procedures described previously.⁵³ The order of the tests and inter-test pauses were chosen to minimize any interference of one test on another (Figure S8). Training and testing started 1 h after the beginning of the dark phase. Animals were habituated to the experimental room conditions for 30 min before the start of the test. The order of testing of the animals was mixed and balanced in order to minimize potential confounders such as the time of the day or experimenter-related factors. A randomly generated sequence was not used for that. Blinding of the experimenter to the genotype of the animals was not possible during the conduct of experiment due to important behavioral differences at baseline. Automatic outcome assessment was used for data collection for all tests except dark light box test, social recognition task, odor discrimination test and video scoring of the visible burrow system test.

Operant system

Four operant cages (Imetronic, France) were used with either a curved wall equipped with one to four nose-poke holes or a straight wall equipped with one central lever, depending on the test. On the opposite wall was a food magazine connected to an outside pellet dispenser filled with 45 mg sweet pellets (5TUL, TestDiet, USA). A clear partition with a central opening in the middle of the operant cage ensured an equal distance to all nose-poke holes from this central opening for an approaching rat.

Rat gambling task

We used the rat gambling task (RGT) to assess complex decision-making. The operant cages were equipped with four nose-poke holes on the operant wall. The training 1 started with the four nose-pokes lit and active, a single nose-poke generated the delivery of one pellet. The selected hole remained lit until the collection of the pellet into the magazine while all the other holes were inactive. A visit to the magazine induced the reactivation and illumination of all the nose-poke holes. The training 1 continued daily until rats obtained 100 pellets in a session (30 min cut-off), then they could start the training 2. In training 2, two consecutive nose-pokes at the same hole were required to obtain one pellet and the same criterion had to be reached (100 pellets in 30 min cut-off). In training 3, two pellets were delivered after a choice (two consecutive nose-pokes) during a short session (maximum 30 pellets and 15 min cut-off). A forced training⁵³ was applied to counter any side preference developed during the training procedure: if the choices for the two holes of one side were superior to 60% during the last session of training 2. During the first part of the forced-training, the two nose-poke holes on the non-preferred side were active and lit, two consecutive nose-pokes into the active holes induced the delivery of one pellet. The holes on the preferred side were inactive and not lit. After the collection of 15 pellets, the second part of the forced training started with the four holes active and lit. Two consecutive nose-pokes into holes of the preferred side induced the delivery of one pellet with a probability of 20% whereas choosing the non-preferred side induced the delivery of one pellet with a probability of 80%. The cut-off was 50 pellets or 30 min. The training procedure lasted six to ten days and the test was performed the next day.

During the test, each of the four holes was associated with an amount of reward and a possible penalty (time-out) which was unknown to the rat. Two holes on one side were rewarded by two pellets and associated with unpredictable long time-outs (222s and 444s with the probability of occurrence $\frac{1}{2}$ and $\frac{1}{4}$ respectively), in the long term those options were disadvantageous. The two holes on the other side were rewarded by one pellet and associated with unpredictable short time-outs (6s and 12s with the probability of occurrence $\frac{1}{2}$ and $\frac{1}{4}$ respectively), in the long term those options were advantageous. After a choice (two consecutive nose-pokes), the reward was delivered and the selected hole remained lit until a visit to the magazine or the duration of the time-out. During this time all the nose-poke holes were inactive. The test lasted 1 h (or cut-off 250 pellets). The theoretical maximum gain of the advantageous options was five times higher than the disadvantageous options at the end of the test (60 min). The percentage of advantageous choices for the last 20 min of RGT was used to identify good decision-makers (GDMs) > 70% of advantageous choices, poor decision-makers (PDMs) < 30% of advantageous choices and intermediate animals. The percentage of advantageous choices per 10 min indicated the progression of the preference over time. An index of the motivation for the reward was measured as the mean latency to visit the feeder after a choice.

Reversed rat gambling task

We used the reversed rat gambling task (reversed-RGT) to assess cognitive flexibility. The animals were tested in the reversed-RGT 48 h after the RGT. The same advantageous and disadvantageous options as in the RGT were used but they were switched from one side to the other. The test lasted 1 h (or cut-off 250 pellets). A flexibility score was calculated as the preference for the location (side) of the non-preferred options during the RGT. Flexible rats had >60% of such choices during the last 20 min, undecided rats had between 40% and 60% of choices, and inflexible rats had <40%. Inflexible animals are unable to adjust their behavior to follow the options previously preferred (RGT) but rather keep choosing at the same location (indifferent of the outcomes newly associated with the nose-pokes) as established before.

Delay discounting task

We used the delay discounting task (DDT) to assess cognitive impulsivity. The operant cages were equipped with two nose-poke holes the furthest from each other on the operant wall. One nose-poke hole (NP1) was associated with a small immediate reward (1 pellet) and a second nose-poke hole (NP5; 25 cm between the two holes) with a large (5 pellets) reward. During the training, the large reward was obtained immediately (delay 0s) after the choice (two consecutive nose-pokes). After the pellet delivery, the magazine and house lights were turned on for a 60s time-out. The session lasted 30 min (or cut-off 100 pellets). A percentage of choice of the large reward $\geq 70\%$ on two following sessions with $\leq 15\%$ variation (stability criterion) was required to start the test. Minimum three training sessions were done. During the test, choosing NP5 induced the delivery of the large reward after a designated delay, NP5 stayed lit during the duration of the delay. After the pellet delivery of the large reward the magazine and the house lights were turned on for a

time-out of 60s minus the duration of the delay. The delay was fixed for a day and increased by 10s from 0s to 40s according to a stability criterion $\leq 10\%$ variation of choice of the large reward during two consecutive sessions. The test sessions lasted 60 min (or cut-off 100 pellets). The preference for the large delayed reward was calculated as the mean percentage of NP5 choices during two stable sessions. To calculate AUC which represents the sensitivity to delay, for each individual the preference for the large delayed reward for each delays was normalized to the preference for the large delayed reward during the training and plotted against delay as a proportion of maximum delay¹²⁵; the area under this normalized curved was then calculated.

Probability discounting task

We used the probability discounting task (PDT) to assess risky decision-making. The operant cages were equipped with two nose-poke holes the furthest from each other on the operant wall. This test is an adaptation of the test of Koot et al., 2012⁴² previously described in Alonso et al., 2019⁵³ with the addition of a stability criterion. During the training, the large reward was always delivered after choosing NP5 (probability $p = 1$), which allowed the rats to develop a preference for NP5. Two consecutive nose-pokes induced the delivery of the reward after 4s, during this time the selected hole stayed lit. Then the magazine light turned on for a 15s time-out. The session lasted 25 min (or cut-off 100 pellets). A percentage of choice of the large reward $\geq 70\%$ on two following sessions with $\leq 15\%$ variation (stability criterion) was required to start the test. At least three training sessions were done. During the test, the probability we used were $p = 0.66, 0.33, 0.20, 0.14$ and 0.09 . Probabilities were generated by a constant pseudo-random sequence of reward and omission. There was a non significant variation between experienced probability and theoretical probability (Figure S9). The probability was fixed for a day and increased the next day only after reaching the stability criterion of $\leq 10\%$ variation of choice of the large reward during two consecutive sessions. This stability criterion ensured stability in the individual performance at a given probability. The session lasted 25 min (or cut-off 100 pellets). The percentage of preference for the large and uncertain reward was calculated for each probability as the percentage of NP5 choices during the two stable sessions. To calculate the AUC which represents the sensitivity to probabilistic uncertainty and risk taking, for each individual the preference for the large reward for each probability was normalized to the preference for the large reward during training and plotted against probabilities expressed as odds¹²⁶ with odds = $(1/P) - 1$; the area under this normalized curved was then calculated.

To further study impulsivity and compare the respective traits assessed in DDT and PDT, the use of an unbalanced-DDT design¹²⁷ (with unique time-out duration) is possible to be considered. Another version of the PDT offering a fully stochastic generation of reward delivery and omission is also available to mimic casino games' settings and gamblers' experience.¹²⁸

FIEXT schedule of reinforcement test

We used the fixed-interval and extinction schedule of reinforcement test (FIEXT) to assess motor impulsivity. The operant cages were equipped with a central single nose-poke hole or a single lever. The fixed-interval (FI) consists of two phases: a fixed time interval during which choices are not rewarded, followed by a phase where a choice can be rewarded.¹²⁹ The extinction (EXT) is a longer, fixed time interval during which no choices are rewarded. Both FI and EXT are conditions that cause frustration in the animal. A session consisted of the repetition of seven FI and one EXT of 5 min. The maximum number of pellets was 14 during a single session. FI lasted 30 s for the first four sessions, 1 min for the next four sessions, 2 min for the next three sessions and 1 min for the final four sessions. The final four sessions with a 1 min FI were the actual test. During the FI, the house light was on and the central nose-poke hole was inactive. At the end of the FI, the house light turned off and the central nose-poke was lit and became active; two consecutive nose-pokes induced the delivery of one pellet, the central nose-poke light was turned off and the tray light was lit. A visit to the tray induced the start of the next FI. After seven consecutive FI, the EXT period started, with all lights off and no consequences associated with nose poking.

When the operant cages were equipped with a lever, the scheme was similar. During the FI, the house light was on and any press on the lever had no consequence. At the end of the FI, a cue light above the lever turned on and the first press was rewarded by a pellet. The cue light above the lever stayed on until pellet collection. A visit to the tray induced the start of the next FI. After seven repetitions of the FI and pellet collection the EXT started. During EXT the house light was off and any press on the lever had no consequence.

As described earlier,¹³⁰ the data from the first FI of the session and the first FI after the first EXT were excluded. The total number of nose pokes and mean number of nose pokes were determined for each FI and EXT period. We summed nose pokes for 10 s intervals during FI to visualize the anticipatory activity of the rats. Likewise, we summed nose pokes for 1 min intervals during EXT to visualize the perseverative activity.

Social recognition task

We used the Social recognition task (SRT) to assess social preference and social recognition memory. The test took place in a square open field (OF, 50 × 50 cm), a small cage was placed in one corner of the OF. To improve the setup, a foam PVC partition was placed around this intruder's cage to avoid the test rat hiding behind the cage. The unfamiliar conspecifics were older male Wistar Han rats, accustomed to the procedure. A video camera on top of the OF recorded the experiment. Each rat was tested on two consecutive days. On the first day, the subject was placed in the OF containing the empty cage in a corner for a habituation of 15 min. Then, the unfamiliar conspecific was placed in the small cage and the subject was allowed to freely explore the open field for 5 min (E1). After that the small cage with the conspecific was removed from the open field, and the subject remained alone in the open field for a break of 10 min. The encounter procedure was repeated two more times with the same conspecific (E2, E3). On the second day, the first 15 min habituation phase was followed by a fourth encounter (E4) of 5 min encounter with the same conspecific as in day 1. After this encounter, a break of 30 min took place, in which the subject remained alone in the open field. Then, the last encounter took place, but a new unfamiliar conspecific was placed in the same small cage for 5 min (Enew). The time spent in close interaction with the intruder was measured for each encounter and for the first 5 min of Habituation (Hab) when the subject smelled at the grid of the empty cage. The social preference was calculated as the ratio of the interaction time in E1 and Hab. The short-term social recognition was calculated as the ratio of the interaction time in E1 and E3. The long-term social recognition was calculated as the ratio of the interaction time in E4 and Enew.

Odor discrimination test

We used the odor discrimination test to assess odor discrimination ability. The test took place in a square OF (50 × 50 cm). Two plastic petri dishes filled with either spoiled (from older Wistar Han rats) or fresh bedding were placed in two opposite corners of the OF. A video camera on top of the OF recorded the experiment. The test rat explored the OF for 5 min. The time spent in close interaction with each dish was measured and the preference for the spoiled bedding (social odor) was calculated.

Dark-light box test

We used a box of 45 cm × 22.5 cm × 35 cm with two compartments, one compartment made of transparent plastic was bright and one compartment made of black opaque plastic and with a lid of the same material was dark. A gate (9 cm × 10 cm) enabled the rats to pass from one compartment to the other. Room light was on and extra lamps were positioned above the box providing a high light intensity in the bright compartment >500 lux. Inside the dark box there was no appreciable illumination (i.e. 2 lux). The rat was brought into the bright compartment (with the home-cage tube) and allowed to explore the apparatus for 10 min. After the test, the apparatus was cleaned with 5% ethanol before the next rat was assessed. We recorded each test with a video camera placed above the bright compartment. We measured the number and duration of visits to each compartment, number of risk assessments which included head poking through the door and body stretches, the latency to leave the bright compartment the first time and the duration of the first visit to the dark compartment. Risk taking index¹³¹ was calculated as the sum of the duration of the first visit to the dark compartment, the number of risk assessment into the light compartment and the time spent in the dark compartment, for clarity this number was subtracted to the maximum score in order to get ascending values.

Automated visible burrow system

We used the automated visible burrow system (VBS) to assess spontaneous social and non-social behaviors, activity, spatial occupation (see also [roaming entropy](#)), social hierarchy (see [glicko rating](#) and [blanchard dominance score](#)), social network analysis (see [social network analysis](#)) and physiological responses (see also [corticosterone metabolite measurements](#)). The automated VBS consisted of an open area connected through two transparent tunnels to a burrow system. Food and water were available at all time in the open area. The burrow system was kept in the dark throughout the test (infrared-transparent black

plastic) and comprised a large and a small chamber connected by tunnels. A grid of 32 RFID detectors was placed underneath the VBS in order to automatically determine individual animal positions using the program PhenoSoft (PhenoSys, Germany). An infrared camera (IP-Camera NC-230WF HD 720p, Tri-Vision Tech, USA) mounted above the VBS recorded a 30 s video every 10 min (CamUniversal, CrazyPixels, Germany). The software PhenoSoft ColonyCage (PhenoSys, Germany) was used to superimpose colored dots (one color per animal) to the videos to allow visual identification of each individual of the group. Six rats of the same genotype were housed in the VBS for seven days in a humidity- and temperature-controlled room (temperature 23–24°C, humidity 45–50%) containing two VBS systems. The animals were visually checked every day. After the first cohort (6 *Tph2^{+/+}* and 6 *Tph2^{-/-}*), the duration of the VBS housing was reduced from seven to four days¹³² for the *Tph2^{-/-}* animals due to noticeable weight loss. The videos of the first 4 h of the dark and light phases were scored by trained experimenters using a scan sampling method.¹³³ For each rat at a time, and for each behavior expressed that was listed in Table 1,^{133–136} the experimenter reported in a behavioral ethogram 1) the type and the 2) duration of the behavior, 3) where it took place in the cage and 4) the ID of the receiver (*i.e.* the rat with which the focal rat was interacting with during the behavior). All six animals in a video were observed, one focal animal at a time. The videos were scored by three trained observers, trained together to specifically and similarly recognize the behaviors described in Table 1. The same observer scored all videos of a given group of rats. Consistency between observers was evaluated as such: for each group, one observer would randomly select 10 videos of experimental day 1 she did not yet annotate, score these videos and compare her results with the other observer's results. If results differed, the two observers discussed discrepancies and adjusted their scorings' strategies accordingly before further scoring. This was repeated until scorings were similar between observers. All aggressive behaviors except "struggling at feeder" were grouped under "general aggression" and sexual behaviors grouped under "sexual" (Table 1). We present the most expressed behaviors (median >5): huddling, sniffing, eating, grooming, general aggression, struggling at feeder and sexual. All scored behaviors (Table 1) are shown in the Figure S5. The body weight of the animals was measured before and after VBS housing (4 or 7 days); the difference of weight was calculated. Although wounds were rarely observed during this study, they were documented at the end of VBS housing. The activity (distance traveled) and the place preference were extracted using the software PhenoSoft analytics (PhenoSys, Germany) for the first four days of VBS housing. The time spent in the open area of the VBS was measured using the data collected from the grid of detectors.

Glicko rating

For each VBS group, the social ranking of the rats was defined using a Glicko rating system.^{137,138} The individual rank was dynamically updated for each individual following the outcome of each aggressive and sexual interaction during the dark phase (R package PlayerRating)^{138,139} within the group. The direction of the interaction defined the winning animal (initiator) and losing animal (receiver). We considered all types of aggressive and sexual behaviors for the first four days because both aggressive and sexual behaviors elicited defensive behaviors sometimes together with vocalizations from the receiver indicating perceived threat from the receiver. We detected the change points of the Glicko rating over time for each individuals (R package online CPD)¹⁴⁰ and determined the stability of the rating. Because the total number of agonistic interactions varied between VBS groups, we calculated a normalized number of change points dividing by the group total number of interactions. For each group the divergence or maximum rating contrast was the difference between the highest and the lowest individual final ratings. Dominant animals' ratings were higher than 1/3 of the maximum rating contrast of the group.

Blanchard dominance score

The Blanchard dominance score¹⁴¹ is a dominance score established in the original VBS. It originally combines three classical parameters: the number and location of wounds, the time spent in the open area and the weight loss. A wound is a visible alteration of the skin of an animal such as scratches and scabs. A wounded animal was monitored closely until complete skin healing. In our study wounds rarely occurred. Over the 78 rats tested, only nine rats presented one to 6 wounds in total (over 4 to 7 days in VBS). Because of its sporadic occurrence, the number of wounds could not be considered in the calculation of the Blanchard dominance score. For each individual within a group, time spent in open area and weight loss for the entire stay in the VBS (4 or 7 days) were ranked from 1 to 6, the average of both ranks was the Blanchard dominance score.

Roaming entropy

The Roaming Entropy (RE) within the VBS is the probability that an individual will be at a certain place at a given time. RE indicates the spatial dispersion of the rats within the automated VBS with high RE, indicating broader use of the cage space. RE calculation was based on the method described previously.¹⁴² Continuous location recordings from the RFID grid were cleaned and filtered; we selected the data from the dark phase of the first four days. We sliced the data into 1 s detections for each rat in order to weigh longer detections. We calculated the observed frequencies or probabilities, $p_{i,j,d}$ of detection of each animal i at each reader j on a day d . These frequencies were then used to compute the RE for each day, following the equation of Shannon: $RE_{i,d} = -\sum (p_{i,j,d} \log p_{i,j,d}) / \log(k)$ where k is the number of detectors in the automated VBS. In the VBS, the spatial dispersion of the rats was evaluated through the total and daily RE.

Social network analysis

We developed the method to social network analysis to understand the qualitative aspects of the social interactions between the individuals. It allows uncovering individual and group dynamics such as information transmission or power distribution. Behavioral interactions between two individuals were organized into matrices for each category of behavior (huddling, sniffing, struggling at feeder, aggression, and sexual behavior). The matrices were weighted and directed, meaning that the number of occurrences of interactions was used and that all interactions weren't always reciprocal in a pair of rats. We used the R package *igraph*¹⁴³ to calculate the parameters and visualize the networks. We measured three global network parameters: density, average path length and out-degree centralization to understand the structure of the networks.¹³⁸ Density is the proportion of possible ties that can exist in the network. Average path length is the mean number of steps between any pair of individuals in the network. Out-degree centralization indicates the differences of initiated connections between the individuals. We measured five individual network parameters: in- and out-degree, betweenness centrality, closeness centrality, Bonacich's power centrality and Hub centrality, to understand the roles of individuals within networks.¹³⁸ In- and out-degree is the number of interactions an individual receives and initiates respectively. Betweenness centrality indicates how much an individual connects two other individuals. Closeness centrality indicates how much an individual directly connects with other individuals. Bonacich's Power Centrality defines the influence of an individual based on the connections of its neighbors, powerful individuals are connected to many individuals that themselves are less connected to others.¹⁴⁴ Hub centrality also depends on the connection of an individual's neighbors, powerful individuals (authorities) are connected to many individuals highly connected to others (hubs).¹⁴⁵

Corticosterone metabolite measurements

One day before and immediately after VBS housing, both times at the same time of the day, the rats were housed in individual cages with food, water and clean bedding for 4 h maximum. Every 30 min, feces produced were collected in microtubes and stored at -20°C until extraction. Then, the samples were defrozen, 0.1 g of feces was added to 0.9 mL of 90% methanol, agitated for 30 min and then centrifuged at 3000 rpm for 15 min. A 0.5 mL aliquot of the supernatant was added to 0.5 mL of water, this extract was stored at -20°C . Measurements of corticosterone metabolites with a 5α - 3β , 11β -diol structure were performed with enzyme immunoassay (EIA) using a polyclonal antibody (rabbit) against 5α -pregnane- 3β , 11β , 21 -triol- 20 -one (linked to carboxymethyloxim) coupled with BSA¹²² following the method of Lepschy et al.¹⁴⁶ in the laboratory of Dr. Dehnhard at the Leibniz Institute of Zoo and Wildlife Research. Briefly, a double antibody technique was used in association with a peroxidase conjugate generating a signal quantitatively measurable by photometry. Corticosterone metabolite concentrations were expressed in micrograms/grams of feces.

QUANTIFICATION AND STATISTICAL ANALYSIS

R (version R – 3.6.1)¹⁴⁷ and R studio (version 1.1.456) were used for statistical analyses. Before comparing the genotypes, we compared the performance of Dark Agouti ($n = 10$) and $Tph2^{+/+}$ from the TPH2-breeding ($n = 38$) animals in all the tests using the Wilcoxon rank-sum test. The results from Dark Agouti and $Tph2^{+/+}$ from the TPH2-breeding animals were not different and these animals were grouped together to form the $Tph2^{+/+}$ group (control group, $n = 48$). During the data analysis the experimenter was not blind to the genotype of the animals. We do not expect our data to follow a normal distribution; hence we used non-parametric statistical tests. We used the Wilcoxon rank-sum test to compare the two genotypes ($Tph2^{+/+}$ vs. $Tph2^{-/-}$) against each other, the Fisher exact test to compare the number of GDMs and

PDMs in $Tph2^{+/+}$ and $Tph2^{-/-}$ groups, the one sample t-test to compare the performance of the animals to a theoretical value in RGT and the Wilcoxon sign test (R package RVAideMemoire)¹⁴⁸ to compare the performance of the animals to a theoretical value in DDT, PDT, SRt and odor discrimination test. Differences in performance between GDMs and PDMs were evaluated with the cohen's effect size (R package effsize).¹⁴⁹ Linear mixed-effect models (lmer models) can be used robustly on non-normal data.¹⁵⁰ We used lmer models (R package lmerTest)¹⁵¹ to compare genotypes (or decision maker groups) over several time points and with individual and batch information as nested random effects. Post-hoc multiples comparisons were done on the linear models (R package multcomp),¹⁵² there the pvalues were adjusted using the Holms method for multiple comparisons. Because of their ability to model over-dispersion, we used generalized linear models with Markov chains (MCMCglmm; R package MCMCglmm)¹⁵³ to compare the distance traveled of genotypes over light cycles and hours with individual and batch information as random effects. The fitting of the MCMCglmm models was assessed with the plots of the fixed effects and random effects. The lower deviance information criterion (DIC) was used to choose the best MCMCglmm model. We used Spearman's correlation (R package Hmisc)¹⁵⁴ to assess the link between hierarchy variables (Glicko and Blanchard scores), individual SNA centrality, roaming entropy and corticosterone level after VBS stay. For all tests, $p < 0.05$ was considered as statistically significant. Symbols, such as $^{*^{\wedge}+}$, represent significant pvalue and may differentiate several comparisons on the same figure.

RF and PCA were used to identify the functions most affected by brain serotonin depletion in tests. The RF (R package randomForest)¹⁵⁵ predicts the genotype of each individual based on their scores in each test and returns the importance of each variable for the classification. We used a Leave-One-Out cross-validation and ran the RF for 100 runs. A k-means clustering (R package stats)¹⁴⁷ grouped the variables by importance; the number of clusters ($n = 4$) was chosen to maximize homogeneity within a cluster and minimize homogeneity between clusters (Figure 4B). The PCA (R package stats)¹⁴⁷ summarizes the dataset in new dimensions representing which is the most variable between individuals. RF and PCA were run on the same datasets. As both methods cannot handle missing data; they were run on a selection of variables including all animals of the study, and additionally on two other sets with more variables but excluding some groups of animals (see Tables S5, S6, S7 and S8).

One $Tph2^{+/+}$ animal was excluded from the RGT because it did not sample the options (Table S10). One $Tph2^{-/-}$ animal was excluded from the odor discrimination test because it did not explore the open field. One group of six $Tph2^{+/+}$ rats was excluded from RE analysis due to a grid malfunction on days 1 and 2. One group of six $Tph2^{-/-}$ animals were born from $Tph2^{-/-}$ dams. In order to control for potential carryover effects of the mother's genetic background over the offsprings' behaviors, we compared their results with the results of the other $Tph2^{-/-}$ animals (born from $Tph2^{+/+}$ dams). Both groups of $Tph2^{-/-}$ animals behave similarly in all tests (Wilcoxon rank-sum test, data not shown). They did not form a subgroup different from other $Tph2^{-/-}$ rats.