DISSERTATION

Multidimensional description of behavioral phenotypes – Role of serotonin in individual profiles of behavior

Multidimensionale Beschreibung von Verhaltensphänotypen – Die Rolle von Serotonin für individuelle Verhaltensprofile

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DA: Dark Agouti

GDM: good decision maker

PDM: poor decision maker

SD: Sprague-Dawley

TPH2: Tryptophan hydroxylase 2

WH: Wistar Han

Abstract

Despite the considerable global burden of mental disorders, the development of new therapeutic options still does not meet the need. New approaches to study and understand the complexity of mental disorders are essential. The dimensional approach of mental health brings normal and pathological states on the same continuum. The worsening of a symptom along this continuum is then conceptualized as the consequence of an underlying biological change. In preclinical research, behavioral animal studies aim at identifying specific neurobiological markers of different behavioral dimensions. The study of the expression of natural evolutionary shaped behaviors and their alterations is now encouraged to bridge the translational gap between clinical and preclinical studies and to foster the development of new therapies. Studying inter-individual differences is another strategy to model spontaneous maladapted behavioral dimensions in animals and scrutinize their neurobiology. Using the Rat Gambling task, a decision-making task that mimics real-life complexity of choice, three types of decision makers, spontaneously existing in a population of rats, can be identified. Among them, individuals called “poor decision makers” make less favorable decisions on the long term in the task. These individuals also present a set of maladaptive behaviors and biological specificities including a dysregulation of the serotonergic metabolism. Serotonin is a neurotransmitter associated with the expression of several mental disorders and is the main biological target of many available clinical treatments. However, whether serotonin would modulate several behavioral dimensions simultaneously and in the same individual is not yet known. In a translational effort, I first aimed to establish a new multidimensional and differential profiling approach to characterize individual-based complex bio-behavioral profiles in healthy rats. The second objective was to evaluate how changes in brain serotonin would influence the identified bio-behavioral profiles. The role of serotonin was investigated with two genetical rat models targeting the synthesis of central serotonin. Rats with a constitutive lack of brain serotonin expressed compromised everyday life behaviors in their semi-naturalistic home-cage. Rats with an induced and moderate decrease of central serotonin presented a higher proportion of poor decision makers which presented an aggravated cognitive profile. Overall, this extensive work established the use of machine learning and multivariate analysis as essential to apprehend the complexity of animals’ phenotype. Multidimensional testing, especially in semi-naturalistic home-cages, was key in the identification of real-life markers of serotonergic function with

high translational value. This work confirmed the specific role of serotonin in the worsening of the behavioral profile of the most vulnerable individuals specifically and through their social cognition abilities.

Zusammenfassung

Die Entwicklung therapeutischer Optionen wird dem Bedarf trotz der enormen globalen Belastung durch psychische Störungen nicht gerecht. Neue Ansätze zum Verständnis der Komplexität psychischer Störungen sind unerlässlich. Im dimensionalen Ansatz zur psychischen Gesundheit werden normale und pathologische Zustände in einem Kontinuum zusammengefasst. Hier wird das Verschlechtern eines Symptoms als Folge zugrundeliegender biologischen Veränderung interpretiert. Verhaltensstudien an Tieren zielen auf die Identifikation spezifischer neurobiologischer Marker der verschiedenen Verhaltensdimensionen. Die Untersuchung natürlicher, evolutionär geprägter Verhaltensweisen und ihrer Veränderungen ist ein neuer Ansatz, um die Kluft zwischen klinischen und präklinischen Studien zu schießen und die Entwicklung neuer Therapien zu stärken. Spontane, fehlangepasste Verhaltensdimensionen bei Tieren und ihre Neurobiologie können durch interindividuelle Unterschiede untersucht werden. Mit dem “Rat Gambling task“, einer Entscheidungsaufgabe, mit einer Komplexität wie im wirklichen Leben, können drei Typen von Entscheidungsträgern in einer Rattenpopulation identifiziert werden. Hierbei treffen "schlechte Entscheider" genannte Individuen weniger günstige langfristige Entscheidungen. Diese Tiere weisen zusätzlich eine Reihe von Verhaltensstörungen und biologischen Besonderheiten auf, darunter die Dysregulation des serotonergen Stoffwechsels. Serotonin ist ein Neurotransmitter, der mit verschiedenen psychischen Störungen im Zusammenhang steht und das biologische Ziel vieler klinischer Behandlungen ist. Es ist nicht bekannt, ob Serotonin gleichzeitig mehrere Verhaltensdimensionen innerhalb eines Individuums moduliert. Im Rahmen eines translationalen Konzepts wollte ich zunächst einen neuen multidimensionalen und differenzierenden Profiling-Ansatz für individuelle komplexe biologische Verhaltensprofile bei gesunden Ratten entwickeln. Das zweite Ziel war zu untersuchen, wie Serotonin-Veränderungen im Gehirn die ermittelten Verhaltensprofile beeinflussen. Dieser Einfluss wurde durch zwei genetische Rattenmodelle mit adaptierter Synthese des zentralen Serotonins untersucht. Ratten mit einem starken Serotonin-Mangel im Gehirn zeigten in ihrem halbnatürlichen Heimkäfig beeinträchtigte Verhaltensweisen im Alltag. Ratten mit

einem moderaten Mangel wiesen einen hohen Anteil schlechter Entscheidungsträger mit verschlechtertem kognitivem Profil auf. Insgesamt hat diese umfangreiche Arbeit gezeigt, dass der Einsatz multivariater Analysen für die Erfassung der Phänotyp-Komplexität von Tieren unerlässlich ist. Multidimensionale Tests, insbesondere in semi-naturalistischen Heimkäfigen, ermöglichten die Identifizierung realer translationaler Marker der serotonergen Funktion. Diese Arbeit stärkte unser Verständnis der spezifischen Rolle von Serotonin in der Verschlechterung des Verhaltensprofils, insbesondere der sozialen kognitiven Fähigkeiten der verwundbarsten Individuen.

1 Introduction

1.1 Mental disorders and their preclinical study

The World Health Organization defines mental health as a human right (World Health Organization, 2022a). Mental disorders, are defined as “a clinically significant disturbance in an individual’s cognition, emotional regulation, or behavior” (World Health Organization, 2022b). In the world one in eight people suffer from mental disorders with anxiety disorders, depression, and bipolar disorder being the first three most prevalent disorders (Institute for Health Metrics and Evaluation (IHME), 2019). However, despite their considerable global burden, the development of new therapeutic options for the improved treatment of all mental disorders is slow (Insel and Scolnick, 2006; Menke, 2018). One key element to explain this paradox is the overall complexity of mental disorders resulting from intricated interactions of the sociocultural environment, genetic factors, development, and experience (Collins et al., 2011; Insel, 2009; Menke, 2018).

In clinical setting, mental disorders are diagnosed using the *Diagnostic and Statistical Manual of Mental Disorders* (DSM, American Psychiatric Association, 2013) or the *International Classification of Diseases* (ICD, World Health Organization, 2019). Using these manuals, each mental disorder is delineated on the basis of its symptoms and disorders are defined as separated diseases. However in practice, the diagnosis for one disease is based on the number of symptoms expressed out of a list of criteria which leads to a strong comorbidity level within groups of patients (Park et al., 2017; Zimmerman et al., 2015). In contrast to other non-mental disorders, so far, the diagnosis of mental disorders is not based on objective biological descriptions of the diseases. Moreover, the continuous distribution in the population of symptoms count, severity and genetic risk factors opposes to this purely categorical classification of ill and health (Hyman, 2021).

For the last decades, there has been a growing consensus that beyond such categorical classifications of mental disorders, normal and pathological states are on the same continuum of each existing behavioral dimension (Adam, 2013; Caspi and Moffitt, 2018; Markon et al., 2005). The dimensional approach of mental disorders also defines a disorder by measurable entities i.e. the dimensions, or symptoms, which severity is rated from normal to severely ill (Avasthi et al., 2014). One of the end goals of the dimensional

approach applied to psychiatry is to understand the biology of mental disorders by uncovering the physiopathology of extreme behaviors recognized as psychiatric symptoms (Budde et al., 2018; Waszczuk et al., 2023).

In preclinical research, animal models are used to investigate the measurable neurobiological markers underlying behavioral dimensions (Insel et al., 2010; Morris and Cuthbert, 2012; Rivalan et al., 2009b). The complexity of mental disorders is never entirely modeled in animals even if recent models try to recapitulate more than one dimension (or symptom) at a time (Rodríguez de Los Santos et al., 2021). The translational validity of an animal model is however fundamental to further foster the development of new therapeutic ways (Bolker, 2017). Recently, the use of semi-natural and home-cage testing is more largely encouraged for longitudinal studies and to allow the evaluation of the spontaneous expression of natural evolutionary shaped behaviors and their alterations (Dennis et al., 2021; Kahnau et al., 2023; Kondrakiewicz et al., 2019; Krakauer et al., 2017; Shemesh and Chen, 2023). This powerful approach holds the promise, through well validated and realistic animal models and the study of complex behaviors, to discover objective and measurable markers leading to the development of effective and curative therapies (Gould and Gottesman, 2006; Nestler and Hyman, 2010; Oikonomidis et al., 2017; Robbins et al., 2012; Robinson et al., 2019).

1.2 Inter-individual differences in animals' behavior as a model of the diversity of humans' behavior and symptoms

Behavioral inter-individual differences represent the variation in the expression of a behavioral response. The characterization of inter-individual differences allows the definition of specific subpopulations which express specific behavioral responses. Several methods can be used to define subpopulations of interest, although the grouping of the individual is always based on the variance of the data. It is possible to use the distribution of the behavioral response directly, for example with the use of the median as a threshold (subgrouping above and below) or the selection of extreme quartiles to separate the individuals. Classical methods to consider several behavioral dimensions together are the correlations and multivariate analysis techniques such as principal component analysis (PCA), linear discrimination analysis (LDA) or cluster analysis (de Boer et al., 2017; Forkosh et al., 2019; Laloyaux et al., 2018; van der Staay et al., 2009). In the field of ethology, social network analysis has been used to identify personality traits

in groups of individuals (Krause et al., 2010). Classical methods are used in combination with methods of the newest techniques such as machine learning and network analysis (Lekkas et al., 2022; Sorella et al., 2022).

Spontaneous differences in behavioral response may reflect evolutionarily conserved value and so better translate to human clinical symptoms than differences caused by an experimental intervention (Rivalan et al., 2009b; Shemesh and Chen, 2023). The study of inter-individual differences may allow to model maladapted behavioral dimensions in animals and to scrutinize their neurobiology in order to pinpoint translational biological markers, underlying cause or vulnerability associated with human psychiatric symptoms (Robbins et al., 2012).

Decision making is a fundamental and complex adaptive process. It involves a large number of cognitive functions and brains structures (cortico-subcortical circuits). It is a common symptom altered in many mental disorders (Cáceda et al., 2014; Griffiths et al., 2014; Lee, 2013). Indeed, poor decision making ranges from keeping washing hands when it is not necessary (obsessive-compulsive disorders), choosing short *vs*. long-term gratification (addiction) or avoiding social contact when it is important for well-being (anxiety disorders, depression). Importantly and in line with a continuum between health and pathology, poor decision making abilities are also observed in non-pathological individuals and in population at risk especially (Bechara and Damasio, 2002; Denburg et al., 2006; Glicksohn et al., 2007; Oswald et al., 2015; Suhr and Hammers, 2010; van den Bos et al., 2009). Similarly, in healthy populations of rats, different types of decision makers spontaneously exist. These different types of decision makers can be identified using a one session test of the Rat Gambling task (Rivalan et al., 2009a), which is a rodent version of the human Iowa Gambling task (Bechara et al., 1994). During this test, rats are confronted with an uncertain and conflicting situation of choice recapitulated in four options which contingencies are unknown to the subjects at the start. Choice after choice, rats must understand the immediate and long-term gains associated with the four options in order to earn more food at the end of the test.

In this task, similarly as in the human task, “good” decision making individuals present the most efficient strategy. They are able to figure out trial after trial which options are the most advantageous ones on the long term (although these options are the least advantageous on the short term/immediately after a choice) allowing them to collect a

maximized amount of food reward. The so-called “intermediate” individuals are those that do not develop a preference for any options during the task. And finally, the “poor” decision makers represent the most ineffective strategy. Trial after trial, “poor” decision makers develop a preference for the options that are the least rewarding on the long term. Their attraction to these options being driven by the immediate value of the options and the long-term loss being largely ignored by these individuals.

Very interestingly the poor decision makers represent a healthy sub-population of rats with an extreme phenotype on the decision making dimension. Further studies on these animals, revealed that they also expressed an ensemble of behavioral traits namely inflexibility, motor impulsivity, reward seeking (Rivalan et al., 2013). These behavioral traits are typically observed in hypo-serotonergic conditions. The behavioral profile of poor decision makers was indeed associated to a cortical-subcortical monoaminergic imbalance (Fitoussi et al., 2015). This behavioral profile gathers a set of maladaptive behaviors nevertheless they are healthy and functioning individuals which make them a good model to study the shift from vulnerability to pathology (Rivalan et al., 2009a). Our hypothesis is that manipulation of the serotonergic system will affect an individual's decision-making strategy, ability to flexibly adapt, ability to inhibit responses, sensitivity to reward, and, ultimately, the entire behavioral profile. We also hypothesize that poor decision makers constitute a vulnerability model, they are most at risk of developing a worsened profile in changing conditions such as a modification of brain functioning.

1.3 Serotonin a promising marker of mental disorder

Serotonin (5-hydroxytryptamine) is an ancient molecule that has evolved in plants, invertebrates and vertebrates. In animals, serotonin is a peripheral hormone and a neurotransmitter of the central nervous system. The central serotonergic system regulates vital functions such as the circadian rhythm, food intake, memory, cognition, pain, mood and social behavior (Bacqué-Cazenave et al., 2020). The central serotonergic system is a complex system. Serotonin synthesis happens in the raphe nuclei neurons, and is distributed throughout the whole brain through a vast axonal network (Jacobs and Azmitia, 1992). Serotonin synthesis is a two-step process. The essential amino acid tryptophan is first hydroxylated into 5-hydroxytryptophan by the enzyme tryptophan hydroxylase. Secondly hydroxytryptophan is decarboxylated into serotonin by the enzyme aromatic L-amino acid decarboxylase. Tryptophan hydroxylase is the rate-

limiting enzyme for serotonin synthesis. Tryptophan hydroxylase 2 (TPH2) is the brain specific isoform separated by the blood brain barrier from TPH1 which synthesizes the separated bodily serotonin pool (Walther et al., 2003). Alteration of the TPH2 function, directly and specifically alters the synthesis of brain serotonin without impacting peripheral serotonin.

Serotonin has been associated with several mental disorders: depression, anxiety disorder, bipolar disorder, substance-related disorder, attention-deficit/hyperactivity disorder, autism and schizophrenia (Baumgarten and Grozdanovic, 1995; Gaspar et al., 2003; Heinz et al., 2001; Maddaloni et al., 2017). Available treatments for these psychiatric conditions also mainly target the serotonergic system. The most prescribed medicine are the selective serotonin reuptake inhibitors, selective serotonin reuptake enhancers and atypical antipsychotics (Grünze and Möller, 2003; Li et al., 2012; Maher and Theodore, 2012; Spielmans et al., 2013). Serotonin receptor agonists and antagonists are also prescribed (Filip and Bader, 2009).

Animal studies have investigated the role of serotonin in modulating different socio-cognitive abilities, one at a time, which are also altered in most mental disorders. Lower serotonin concentration in the lateral orbitofrontal cortex were described in animals with lower cognitive flexibility (Barlow et al., 2015). Serotonin depletion was found to increase different types of behavioral dis-inhibition although not always cognitive impulsivity (Mobini et al., 2000; Winstanley et al., 2003). Serotonin decrease through tryptophan deficiency induced increased risk-taking impulsivity and impaired decision-making ability (Koot et al., 2012). Decision-making ability was also improved in serotonin transporter knock-out animals which supposedly extends the presence of serotonin in the synaptic cleft (Homberg et al., 2008). Social recognition memory deficit was linked to the activation of serotonergic receptors in the frontal cortex (Bert et al., 2008; Loiseau et al., 2008). Finally in mice, aggressive behavior was increased in serotonin depleted condition via tryptophan hydroxylase 2 knock-out while anxiety was decreased (Mosienko et al., 2012). It is however currently unknown how serotonin would modulate these multiple abilities simultaneously in the same individual. In humans a mental disorder is defined by the simultaneous expression of several impairments. Personalized solutions are needed for an optimal and adapted care. Understanding the impact of serotonin modulation on complex behavioral profiles at the level of the individual is indispensable.

1.4 Research questions and objectives

Serotonin is an important modulator of cognitive and social abilities and a promising biomarker of human psychiatric disorders. However, at a preclinical level it is not known how serotonin modulates several behavioral dimensions at the same time in the same individual. In a translational goal, we aimed to establish the importance of using a multidimensional profiling method to identify and characterize complex behavioral profiles in rats, at the group and individual levels. At the level of the individual, the objective was to measure a large variety of behavioral dimensions, including social and cognitive abilities, in and out of their social home-cage environment, and physiological parameters using non-invasive methods whenever possible. Furthermore, we evaluated with multivariate analysis and machine learning the interactions between all behavioral and physiological measures of the profiles. With this method, our main goal was to understand how brain serotonin modulates the identified rat's multidimensional bio-behavioral profiles. Our hypothesis was that brain serotonin deficiency would result in a bio-behavioral profile that is impaired on multiple dimensions recalling aspects of human mental disorders such as decision making, behavioral flexibility, impulsivity, social recognition, or aggression. We expected the level of brain serotonin deficiency, from complete depletion to partial and temporary decrease, to impact directly the level of phenotypic alterations. We expected to see a total absence of brain serotonin to have the strongest effect on the individual profiles and affecting all bio-behavioral dimensions. While a more moderate decrease of brain serotonin would induce a more moderate impairment affecting the most sensitive dimensions. Regarding the inter-individual differences in decision making based profiles, we expected the probability of developing a poor decision making strategy to (inversely) increase with the decrease of the levels of brain serotonin. Moreover, we expected a worsening of the socio-cognitive profile of the most vulnerable individuals specifically the low-serotonin poor decision makers.

1.4.1 Part I. Validation of the multidimensional profiling approach with a case of strain comparison in rats

Our first goal was to develop and test a multidimensional profiling method that allows to discriminate between groups and individuals based on the expression of a specific combination of physiological and behavioral factors. To this end, we used a case of rat strain comparison. We chose a group of healthy individuals of the reference strain the

Wistar Han (WH) strain and another group of healthy individuals of the inbred Dark Agouti (DA) strain. DA rats were chosen as they are the background strain of the transgenic rats used in Part II of the project. Behavioral data of this strain were largely lacking at the time of the project. This original study established the bio-behavioral profile of DA rats, testing the same behavioral traits in WH rats. The behavioral traits were selected for their documented dependence on the serotonergic system. With this study we extended the characterization of the behavioral profile of the poor decision makers to yet untested serotonin-sensitive functions such as probability-based decision making, sociability and spontaneous behaviors in undisturbed group-housed semi-natural environment.

These results were published as a preprint publication in bioRxiv in 2019 (Alonso et al., 2019a) and in the peer-reviewed journal Behavioral Brain Research in 2019 (Alonso et al., 2019b)

1.4.2 Part II. Effect of constitutive brain serotonin depletion on individual bio-behavioral profile

We investigated the effect of a complete brain serotonin depletion on the bio-behavioral profiles, established in Part I of the project, in full knock-out rats (*Tph2*$^{-/-}$ rats) constitutively devoid of brain serotonin. We characterized the expression of several cognitive, social, affective and biological functions at the group and individual level in this line of rats. We found that the most impacted dimensions were everyday life functions expressed within the undisturbed group-housed home-cage and that these extreme manifestations compared to symptoms of serotonin related mental conditions observed in humans. This important study also revealed that only the use of the semi-natural home-cage made it possible to detect the complex alterations of the phenotype of the *Tph2*$^{-/-}$ rats demonstrating the urgent need to study natural-based behavior.

These results were published as a preprint publication in bioRxiv in 2021 (Alonso et al., 2021) and in the peer-reviewed journal iScience in 2023 (Alonso et al., 2023)

1.4.3 Part III. Effect of acute and moderate brain serotonin depletion on the vulnerable behavioral profile

We investigated the effect of an acute and moderate brain serotonin decrease on the bio-behavioral profile of inducible knock-down rats (tetO-shTPh2 rats). TetO-shTPh2 rats were created on a Sprague Dawley (SD) background which behavioral characteristics are

well known, contrary to DA rats, confirming the validity to use this strain without the need to pre-screen their spontaneous profiles. We focused our study on the cognitive dimensions (in stand-alone tests) which were not impacted in Part II but were most susceptible to be worsened by serotonin decrease in the vulnerable (poor decision maker) subpopulation of rats. The behavioral profile of the poor decision makers under low-serotonin condition was indeed the one presenting most deficits compared to other individuals.

These results were published as a preprint publication in bioRxiv in 2024 (Alonso et al., 2024a) and in the peer-reviewed journal International Journal of Molecular Sciences in 2024 (Alonso et al., 2024b)

2 Methods

2.1 Animals

Animal experiments were designed and performed in accordance with the European Union Directive 2010/63/EU relating to animal welfare and ethics of animal experimentation and with the regulations of the animal care and use committee of the state of Berlin and under the supervision of the animal welfare officer of our institution.

In total, we used 42 Wistar Han rats, 48 Dark Agouti rats, 30 *Tph2*$^{-/-}$ rats (Kaplan et al., 2016), 96 male TetO-shTPH2 rats (Reichhart et al., 2017) and 24 Sprague Dawley rats. All rats were males. Unless in the Visible burrow (see below), they were housed in pairs of the same genotype (or strain) in standardly enriched rat cages (Eurostandard Type IV, 38 cm x 59 cm) in two temperature-controlled rooms (22°C–24°C and 45%–55% humidity) with inverted 12-hour light-dark cycles. Rats receive ad libitum access to water and to standard maintenance food (V1534-000, Ssniff, Germany). During operant training and testing, animals were maintained at 95% of their free-feeding weight. The *Tph2*-kd rats were 60 TetO-shTPH2 rats treated with doxycycline at a dosage of 40 mg/kg of body weight in the drinking water of the cage. The treatment lasted over the duration of the behavioral protocol. Radio-frequency identification (RFID) was done two weeks before the beginning of the behavioral training for the animals participating in the visible burrow system experiment. They received a RFID chip (glass transponder 3 × 13 mm, Euro I.D.) subcutaneously in the ventral left lower quadrant under short isoflurane anesthesia.

Animals were tested in batches of 12 animals including six animals of each experimental group. For the three experiments, the experimental groups were the strains: WH vs. DA, the genotypes: control vs. *Tph2*$^{-/-}$, the treatment: control vs. *Tph2*-kd. Two batches were usually run during the same period of time. They started the behavioral training between 9 and 13 weeks of age. Over the whole protocol, behavioral tests and biological measurements were done one after the other following the same order for all experiments. The order of tests was chosen to minimize the influence of one test over the other ones.

2.2 Behavioral tests

In this section I will describe for each test the principle of the test and the main outcome measure (variable) that was later used in the multidimensional analysis, technical details about the methods can be found in the publications of this thesis (Alonso et al., 2019b, 2023, 2024a). The individual represents the experimental unit of analysis.

2.2.1 Tests in the operant system

We used four rat operant cages (Imetronic, France). They were equipped depending on the test with one to four nose-poke holes or one lever on one side. On the opposite side was a food magazine connected to a pellet dispenser filled with 45 mg sweet pellets (5TUL Cat#1811155, TestDiet, USA).

Rat Gambling Task (RGT) and reversal (rev-RGT)

The RGT assesses complex decision making which is the ability to choose in complex and conflicting situations. It was used to identify the different types of decision makers (Rivalan et al., 2009a). For the animal it consists in identifying the most advantageous options among four unfamiliar options with different reward magnitudes and probability of penalty (waiting time before the next choice) in one hour.

We computed the percentage of advantageous choices per 10 min over time and for the last 20 minutes (RGT score). The later was used to identify the subtypes of decision makers: good decision makers with more than 70% of advantageous choices, poor decision makers with less than 30% of advantageous choices and intermediate animals in between. The mean latency to visit the feeder after a choice was used as an index for the motivation for the reward.

Animals were tested in the rev-RGT (reversal RGT) 48 h after the RGT in order to assess their behavioral flexibility. The positions of the advantageous and disadvantageous options were spatially switched. The flexibility score depended on the preference developed during RGT and was calculated as the preference for the new location of the option that was preferred during RGT.

Delay Discounting Task (DDT)

The DDT assesses cognitive impulsivity which is the ability to wait in order to get a reward of higher magnitude. The protocol was adapted from Rivalan et al. 2013. For the animal it consists in pondering the magnitude of rewards and the duration of the delay to obtain the reward among two options. The preference for the large delayed reward was calculated for each applied delay. From the resulting overall preference curve, the area under the curve (AUC) representing an index of cognitive impulsivity was calculated. The lower the preference for the delayed option the more impulsive was the animal.

Probability Discounting Task (PDT)

The PDT assesses cognitive risk-taking (or risky decision making) which is the ability to choose in a probabilistic context. The protocol was adapted from Koot et al. 2012. For the animal it consists in pondering the magnitude of rewards to the probability to obtain the reward among two options. The preference for the large and uncertain reward was calculated for each applied probability. From the resulting overall preference curve, the AUC representing an index of sensitivity to probabilistic uncertainty and risk-taking was calculated. The lower the preference for the probabilistic option the less risk-taker was the animal.

Fixed interval and extinction schedule of reinforcement (FI-EXT)

The FIEXT assesses motor impulsivity in two ways: anticipatory activity and perseveration (Rivalan et al., 2013). For the animal it consists in responding during an active period associated with reward delivery and during an inactive period. The test was run with a nose-poke hole or a lever as response manipulandum. The mean number of responses were determined for each FI and EXT period. A higher number of responses was associated with higher motor impulsivity.

2.2.2 Other tests

Social Recognition test (SRt)

The SRt assesses social preference which is the tendency to interact with a social partner over an object motor and social recognition memory which is the ability to remember a social partner. The protocol was adapted from Shahar-Gold et al., 2013 (Shahar-Gold et

al., 2013). For the animal it consists in meeting through a grid an unfamiliar social partner in a familiar context (open field) for 5 minutes. The encounter is then repeated multiple times with the same social partner or a new one. The time of interaction was measured for each encounter and during habituation to the open field without the social partner in order to compute ratio of interaction time as indexes of social preference and social recognition memory.

Odor discrimination test (Odor test)

I developed the odor discrimination test that assesses the ability to detect the smell of a social partner. For the animal it consists exploring used and fresh bedding in two petri dishes for 5 minutes in an open field. The time of interaction was measured for each bedding and a preference index was calculated.

Dark-Light box (DL-box)

DL-box test assesses risk-taking behavior which is the exploration of a bright environment over the exploration of a covered environment both unfamiliar. The risk-taking index 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.

Elevated Plus Maze (EPM)

EPM test assesses risk-taking behavior which is the exploration of an open environment over more closed environment both unfamiliar. The number of visits to open arms was measured.

2.2.3 Semi-automated Visible Burrow System (VBS)

We used the VBS to assess spontaneous social and non-social behaviors, activity, spatial occupation, social hierarchy, social network analysis and physiological responses. Six animals (three pairs) were introduced at the same time in the semi-natural enclosure. For the animal the VBS stay consisted in exploring with its cage-mate a new complex arena for up to seven days and meeting two new pairs of individuals.

The individual animal positions were recorded 24/7 by a grid of 32 RFID detectors (PhenoSys, Germany) placed underneath the VBS. 30-second videos were recorded

every 10 min by an infrared camera (IP-Camera NC-230WF HD 720p, Tri-Vision Tech, USA) mounted above the VBS.

Behavioral analyses: social network analysis, glicko rating and Blanchard dominance score

The videos of the first 4 hours of the dark and light phases were scored by trained experimenters who recorded the occurrence, duration and location of behaviors from a list of behaviors of several types: affiliative, maintenance, aggressive and defensive behaviors. The experimenter reported the ID of the initiator, the duration of the behavior, where it took place and the ID of the receiver if applicable in an ethogram. From this ethogram the number of occurrences of each type of behavior was calculated.

Social network analysis was done for the behaviors implicating an initiator and a receiver. Matrices of interaction were done for each behavior (for example: huddling, sniffing, struggling at feeder, aggression, and sexual behavior). From those matrices social networks were drawn and several global and individual parameters were computed with the package iGraph (Csardi and Nepusz, 2006). The hub centrality represented a measure of the power of individuals within networks, especially aggression networks (Kleinberg, 1999; So et al., 2015).

The glicko rating system was used to define the social rank of the individual. It was calculated from the aggressive and sexual (when applicable) interactions between individuals with the package PayerRating (Stephenson and Sonas, 2020)

The Blanchard dominance score (Blanchard et al., 2001) is another non-behavioral measure of dominance. It originally combines three parameters: the number and location of wounds, the time spent in the open area of the VBS and the weight loss. Wounds were rarely observed during the studies, they were documented at the end of VBS housing but not used to calculate the Blanchard dominance score. The time spent in the open area (see in section Location parameter analyses) and weight loss (see measurement in section Biological measures) for the entire stay in the VBS were ranked from 1 to 6, and the average of both ranks was the Blanchard dominance score.

Location data analyses

The time spent in the open area of the VBS was computed from the 24/7 location data. The activity (distance traveled) and the place preference were extracted from the 24/7 location data using the software PhenoSoft analytics (PhenoSys, Germany).

The roaming entropy is the probability to be at a certain place at a given time. In the VBS it is an index of spatial dispersion and indicates how the animals use the space i.e. the relative size of their territory. We used the classical equation of Shannon:

$$RE_{i,d} = - \Sigma (p_{i,j,d} \log p_{i,j,d})/\log(k)$$

where $p_{i,j,d}$ is the probabilities of detection of each animal *i* at each reader *j* on a day *d* and k is the number of detectors in the automated VBS.

Biological measures of weight and corticosterone metabolites

The body weight of the animals was measured before and after VBS housing. The difference of weight was calculated.

Non-invasive measurements of corticosterone metabolites were done in fecal samples. Feces collection happened one day before and immediately after VBS housing, both times at the same time of the day. Rats were housed in individual cages with food, water and clean bedding for up to 4 hours. Fecal samples were stored at −20°C until extraction and enzyme immunoassay (Lepschy et al., 2007). The variation of corticosterone metabolite concentrations was calculated and was an index of the stress response to VBS housing.

2.3 Serotonin measurements

Two days after the last test, whatever the length of the protocol, rats were anesthetized via an intraperitoneal injection of Ketamine (100mg/kg) and Xylazine (10mg/kg) under isoflurane anesthesia. Animals were transcardially perfused with phosphate-buffered saline. Brain parts were immediately collected, snap-frozen on dry ice, and stored at −80°C until measurements. High-performance liquid chromatography was used to measure brain tissue levels of tryptophan, serotonin and its metabolite 5-HIAA (Alonso et al., 2024a, 2024b).

2.4 Statistical analysis

In this doctoral work non-parametric tests were favored whenever possible as I did not expect our data to follow a normal distribution, which they did not. The analyses were done with R and R studio (R Core Team, 2019). In this report I present descriptive statistics (median and inter-quartile range). I present also the results of the Wilcoxon rank sum test that was used to compare groups of animals (strains or genotypes or treatment or decision making subgroups); a continuity correction was automatically applied to the data whenever appropriated.

2.5 Multidimensional analysis

2.3.1 One variable per function

The performance of the animals was recapitulated into variables representative of the behavioral dimensions (ex: RGT score representative of complex decision making ability in the RGT). Variables that represented the same dimension would not be included together in the analysis (ex: activity indexes from two operant tests). Combinations of variables into indexes could be made to create a new variable that better represented a dimension (ex: risk-taking index in the DL-box or weight variation in the VBS).

Our datasets consisted of a suite of variables for each individual. We used Random Forest (RF) and Principal component analysis (PCA) to identify the functions most affected by the experimental condition (strain, genotype or treatment). RF and PCA were run on the same datasets. Missing values were not allowed by the models therefore animals that had a missing value for one variable were removed from the whole analysis.

2.3.2 Random Forest (RF)

The RF (R package randomForest) can predict experimental condition (strain, genotype or treatment) of an individual based on its scores in each test. Then the RF returns the importance of each variable for the classification that was done (Gini index). Therefore, it allows to rank the variables by order of importance in the prediction of the experimental condition. We used a Leave-One-Out cross-validation which allows to use the dataset minus the individual to be classified as a training dataset to make the classification. This

supervised method of machine learning allowed us to do the analysis without a dedicated training dataset.

2.3.3 Principal component analysis (PCA)

The PCA (R package stats) is an unsupervised method of machine learning that summarizes the dataset in new non-correlated dimensions that recapitulate the variance observed between individuals. It allows to represent the contribution of each variable to the observed variance which enables to extract the variables the most influenced by the experimental condition.

2.3.2 Network analysis of behavioral profiles

We used a network analysis method to visually represent behavioral networks of subgroups of decision makers. The network is a representation of the strength of connections between the variables. I used the Package qgraph (Epskamp et al., 2012) to create association graphs with Spearman's correlations to draw the edge weights between two nodes. Partial correlations are usually used because they enable to account for the relationships of the network for each pair. However, it was not possible in our analysis to apply Spearman's partial correlation due to a small number of animals in the *Tph2*-kd poor decision maker group.

3. Results

3.1 Validation of the multidimensional profiling approach and comparison of DA and WH strains of rats, published in Behavioral Brain Research (Alonso et al., 2019b)

In order to enrich current approaches to identify multidimensional and objective strain-specific characteristics in different animal models of mental disorder, the first goal of my doctoral work was to study and compare the unknown multidimensional profile of normal males of one common (the WH) and one uncommon (DA) strain of rats. In this aim we used a combination of classical and new behavioral and statistical approaches to accommodate the complexity of their natural profiles. We used several fine-tuned classical tests to assess cognitive abilities. We developed and validated ethological-like and physiological non-invasive methods to access new social and naturalistic features within the animals' home cage. **This study is one of the few that thoroughly analyzed several dimensions of animals' spontaneous cognitive abilities, natural behaviors and physiological responses in order to delineate complex and integrated profiles.** Finally, we selected and used supervised machine learning and other statistical approaches to extract the multidimensional profiles. Thanks to this innovative approach we could identify objective differences between the two rat strains in 8 out of the 19 traits we evaluated (Table 1) and reveal the key discriminative traits of DA and WH rats (Fig. 1).

The random forest classification with a leave-one-out cross-validation was able to predict the strain of the individuals based on their behavioral traits with an accuracy of 84% (±0.72 SD over 10 runs). The Gini index provided a ranking of the differences in dimensions between the strains (Fig. 1). The most important traits to differentiate WH from DA rats were the cognitive impulsivity (AUC of the DDT) and the activity in the VBS (total distance) with the highest Gini indexes. WH rats appeared more impulsive and more active in the VBS than DA rats (Table 1). The motivation for reward (latency to collect a reward in the RGT) and the time spent in the open area in the VBS were the next more important behaviors to differentiate WH and DA rats. WH rats appeared more sensitive to reward than DA rats and more present in the open area of the VBS. Of lesser significance were the social preference, the aggressive behavior in the VBS, and the cognitive risk-taking (AUC of the PDT). WH were more aggressive in the VBS whereas DA rats expressed

more affiliative behaviors but had a weaker social preference in the SRt. Finally, WH rats were more sensitive to the absence of reward in the PDT than DA rats.

Interestingly, poor decision makers from the two strains behaved very similarly (Table 2). As expected from previous studies, WH poor decision makers which had opposite response than WH good decision makers in complex decision making task, also presented higher motivation for reward, and increased cognitive inflexibility. This was also the case of DA poor decision makers compared to DA good decision makers. Interestingly, among the new traits investigated in the semi-natural conditions of the home-cage, we showed new differences between the decision maker subtypes. Poor decision makers occupied more the open area, they were more active and tended to lose less weight than good decision makers during the VBS stay (Table 2), which confer them a more dominant profile than other decision makers.

To conclude we observed large phenotypic variations between WH and DA rats while the poor decision maker profiles kept stable between the two genetically different strains. The use of the VBS, through the variety of parameters it can measure, enriched the output of classical testing methods to draw more complete behavioral profiles.

Table 1: Comparison of the WH and DA rats' performances with the Wilcoxon rank sum test, own representation for this doctoral work.

Trait	Test	Parameter	WH Median (IQR)	DA Median (IQR)	Wilcoxon rank sum test W (p-value)
Complex decision making	RGT	RGT score	81.7 (80)	93.8 (20)	1133 (0.01)
Sensitivity to reward	RGT	Latency to collect reward	1.1 (0.3)	1.8 (0.4)	1613 (**<0.001**)
Cognitive flexibility	Rev-RGT	Flexibility score	23.5 (47)	47.1 (52)	909 (0.5)
Cognitive impulsivity	DDT	AUC	0.4 (0.3)	0.7 (0.3)	923 (**<0.001**)
Cognitive risk-taking	PDT	AUC	0.6 (0.3)	0.7 (0.2)	516 (0.006)
Anticipatory activity	FI	Mean number of responses	3.4 (4)	5.1 (5)	589 (0.03)
Perseverative activity	EXT	Mean number of responses	9.1 (6)	17.1 (15)	690 (**<0.001**)
Social preference	SRt	Ratio of interaction time	2.7 (2)	1.9 (1)	121 (0.01)
Short-term social recognition memory	SRt	Ratio of interaction time	2 (2)	1.6 (1)	186 (0.5)
Exploration EPM	EPM	Number of visits to open arms	9.5 (5)	1 (0.5)	5.5 (**<0.001**)
Affiliative behavior	VBS	Occurrences	25 (12)	37 (15)	384 (0.008)
Aggressive behavior	VBS	Occurrences	21 (16)	9 (11)	120 (**0.002**)
Maintenance behavior	VBS	Occurrences	33.5 (11)	23 (15)	172 (0.04)
Defensive behavior	VBS	Occurrences	2 (1)	1 (1)	175 (0.04)
Place preference	VBS	Preference for open area	60 (16)	36 (7)	23 (**<0.001**)
Open area occupation	VBS	Time spent	73 (29)	44 (9)	72 (**<0.001**)
Activity	VBS	Total distance	3878 (735)	3015 (404)	41 (**<0.001**)
Weight loss	VBS	Weight loss	-4.6 (8)	-6.2 (5)	543 (0.24)
Stress response	VBS	Corticosterone variation	34 (100)	-15 (87)	221 (0.35)

IQR: interquartile range; Following a Bonferroni correction for multiple testing, the significance level in Table 1 is 0.003, significant p-values are shown in bold.

Table 2: Behaviors of the good and poor decision makers in DA and WH strains. In bold the dimensions with differences between good and poor decision makers for either strains of rats, adapted from Alonso et al., 2019b.

Trait	Test	Parameter	GDM-WH Median (IQR)	PDM-WH Median (IQR)	GDM-DA Median (IQR)	PDM-DA Median (IQR)
Sensitivity to reward	RGT	**Latency to collect reward**	**1.4 (0.4)**	**1.1 (0.1)**	**1.9 (0.4)**	**1.6 (0.5)**
Cognitive flexibility	Rev-RGT	**Flexibility score**	**48.3 (52)**	**7.4 (16)**	**52.3 (53)**	**15 (8)**
Cognitive impulsivity	DDT	AUC	0.4 (0.3)	0.4 (0.3)	0.6 (0.2)	0.9 (0.1)
Cognitive risk-taking	PDT	AUC	0.6 (0.2)	0.6 (0.3)	0.8 (0.3)	0.6 (0.2)
Anticipatory activity	FI	Mean number of responses	3 (4)	2.6 (2)	4.9 (5)	6.7 (4)
Perseverative activity	EXT	**Mean number of responses**	10 (12)	9 (3)	**15 (13)**	**19 (19)**
Social preference	SRt	Ratio of interaction time	2.8 (1.5)	2.6 (1.1)	2 (1.5)	1.8 (0.6)
Short-term social recognition memory	SRt	Ratio of interaction time	2.2 (1.9)	1.5 (1)	1.5 (0.7)	2.1 (1.4)
Exploration EPM	EPM	Number of visits to open arms	10 (4)	11 (4)	1 (1)	1 (1)
Affiliative behavior	VBS	Occurrences	25 (11)	28 (12)	39 (14)	35 (18)
Aggressive behavior	VBS	Occurrences	19 (13)	22 (13)	9 (14)	8 (9)
Maintenance behavior	VBS	Occurrences	33 (9)	37 (15)	24 (13)	22 (5)
Defensive behavior	VBS	Occurrences	2 (1)	2 (1)	1 (1)	1 (0)
Place preference	VBS	Preference for open area	58 (19)	60 (14)	36 (7)	40 (4)
Open area occupation	VBS	**Time spent**	**68 (33)**	**74 (30)**	43 (9)	47 (10)
Activity	VBS	**Total distance**	**3809 (401)**	**4449 (1096)**	3051 (410)	2952 (789)
Weight loss	VBS	**Weight loss**	-6.4 (7)	-3.7 (7)	**-6.9 (5)**	**-1.7 (5)**
Stress response	VBS	Corticosterone variation	9.1 (100)	43.4 (62)	-12.3 (85)	-19 (38)

GDM: good decision makers, PDM: poor decision makers, IQR: interquartile range, AUC: area under the curve

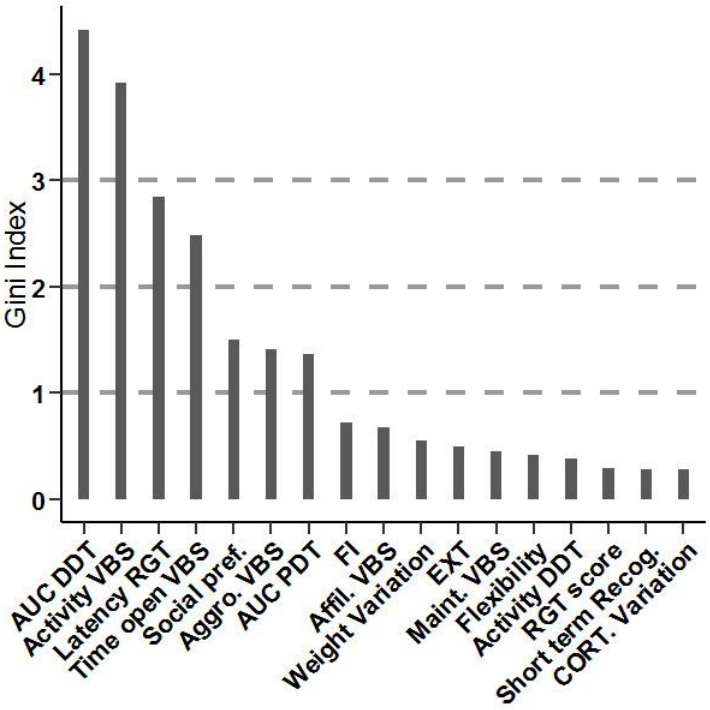

Figure 1. Random Forest classification of the DA and WH rats. Gini index indicates for each trait its importance to discriminate DA and WH strains. Area under the curve in the delay discounting task (AUC DDT), total distance traveled in VBS (Activity VBS), latency to collect pellet in RGT (Latency RGT), time spent in open area (Time open VBS), social preference ratio (Social pref.), total occurrences of aggressive behaviors (Aggro. VBS), area under the curve in the probability discounting task (AUC PDT), mean number of responses in FI (FI), total occurrences of affiliative behaviors (Affil. VBS), percentage of weight variation (Weight Variation), mean number of responses in EXT (EXT), total occurrences of maintenance behaviors (Maint. VBS), flexibility score in reversed-RGT (Flexibility), activity in the delay discounting task (Activity DDT), preference in last 20 minutes of rat gambling task (RGT score), short-term social recognition ratio (Short term Recog), percentage of corticosterone metabolite variation (CORT. Variation). WH n = 22 and DA n = 24. Figure modified from Alonso et al., 2019a.

3.2 Effect of constitutive brain serotonin depletion on the bio-behavioral profile of DA rats, published in iScience (Alonso et al., 2023)

In order to investigate the effect of the constitutive brain serotonin depletion on the socio-cognitive profile of the *Tph2*$^{-/-}$ rats (background strain DA), we used the successful multidimensional profiling and statistical approach developed in our first study. We deepened the analysis of the behaviors expressed in the semi-natural home cage (VBS) with an analysis of the social dynamics using a social network analysis to evaluate group structure evolution and Blanchard's dominance score and Glicko rating to establish

hierarchical specificities of the groups. We complemented the uses of the random forest analysis (RF) with an unsupervised approach the principal component analysis (PCA) to further confirm the key discriminant characteristics of the *Tph2*$^{-/-}$ rats.

Among all measured behaviors, the random forest classifier identified those most impacted by the lack of brain serotonin (with an average accuracy of 98.5%, ± SD = 0.54) and this was confirmed by the PCA. PCA's first dimension clearly separated both genotypes (Fig. 2A-left). The behavioral dimensions (variables) contributing the most to the PCA's first dimension (Fig. 2A-right) were also the most important to discriminate between genotypes using the RF classifier (Fig. 2B). Dimension 1 was primarily loaded by weight loss, maintenance behavior, roaming entropy, corticosterone variation, defensive behaviors and sexual behaviors (Fig. 2A-right). The RF indicated other relevant variables comprising total distance traveled in VBS, Glicko rating score, affiliative behaviors, aggressive behaviors, and occupation of VBS open area (Fig. 2B). Indeed, under the complex and experimenter-free conditions of the experimental home-cage, *Tph2*$^{-/-}$ rats presented drastic changes in their daily life (Table 3). In the VBS, brain serotonin depletion induced increased aggression and sexual behavior, increased activity but reduced territory, reduced self-care and body weight, and exacerbated corticosterone levels. Group-housed *Tph2*$^{-/-}$ rats showed strong social disorganization with disrupted social networks and hierarchical structure. None of the cognitive variables, evaluated in classical conditions of test such as operant chamber and open field, could predict the animals' genotypes (Fig. 2B) or contribute to explain the variance of dimension 1 (Fig. 2A-right and B). *Tph2*$^{-/-}$ rats presented unexpected normal cognitive abilities (Table 3) such as decision making performance in the RGT, same levels of flexibility, very similar levels of cognitive impulsivity and risk-taking and same levels of social recognition memory and odor discrimination.

Overall central serotonin appeared as a strong modulator of the expression of undisturbed group-housed behaviors, social structure and hierarchy. However, in the same animals the constitutive absence of central serotonin had little to no effect on the expression of several distinct cognitive abilities assessed in controlled conditions.

Table 3: *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ rats' performances and comparison between genotypes with the Wilcoxon rank sum test, own representation for this doctoral work.

Trait	Test	Parameter	*Tph2*$^{+/+}$ Median (IQR)	*Tph2*$^{-/-}$ Median (IQR)	Wilcoxon rank sum test W (p-value)
Complex decision making	RGT	RGT score	92 (29)	97 (30)	560 (0.13)
Sensitivity to reward	RGT	Latency to collect reward	1.8 (0.4)	1.7 (0.5)	832 (0.19)
Flexibility	Rev-RGT	Flexibility score	33 (51)	40 (58)	614 (0.34)
Cognitive impulsivity	DDT	AUC	0.6 (0.2)	0.5 (0.2)	916 (0.04)
Cognitive risk-taking	PDT	AUC	0.6 (0.2)	0.5 (0.2)	373 (0.08)
Anticipatory activity	FIEXT	Mean number of responses NP, lever	247 (254), 254 (144)	151 (99), 248 (85)	269 (0.01), 72 (1)
Perseverative activity	FIEXT	Mean number of responses NP, lever	137 (121), 87 (67)	78 (55), 84 (46)	275 (**0.009**), 84 (0.5)
Social preference	SRt	Ratio of interaction time	2.1 (1)	2.2 (1)	495 (0.6)
Short-term social recognition memory	SRt	Ratio of interaction time	1.3 (0.9)	1.2 (1.2)	498 (0.6)
Odor discrimination	Odor test	Ratio of interaction time	65 (18)	60 (31)	306 (0.5)
Risk-taking	DL-box	Risk-taking index	568 (166)	569 (544)	324 (0.47)
Affiliative behavior	VBS	Occurrences	74 (23)	56 (24)	1037 (**0.001**)
Aggressive behavior	VBS	Occurrences	23 (19)	39 (26)	227 (**<0.001**)
Sexual behavior	VBS	Occurrences	0 (1)	10 (15)	67 (**<0.001**)
Maintenance behavior	VBS	Occurrences	48 (24)	21 (16)	1242 (**<0.001**)
Defensive behavior	VBS	Occurrences	1 (2)	7 (7)	247 (**<0.001**)
Roaming entropy	VBS	Roaming entropy	0.9 (0.1)	0.8 (0.1)	1279 (**<0.001**)
Open area occupation	VBS	Number of detections	250046 (96955)	175916 (278687)	870 (0.1)
Activity	VBS	Total distance	1734 (323)	2493 (1220)	288 (**<0.001**)
Weight loss	VBS	Weight loss	-13 (13)	-46 (13)	1421 (**<0.001**)
Stress response	VBS	Corticosterone variation	-5 (53)	286 (520)	143 (**<0.001**)

IQR: interquartile range, AUC: area under the curve, NP: protocol with nose-poke. Following a Bonferroni correction for multiple testing, the significance level in table 3 is 0.003, significant p-values are shown in bold.

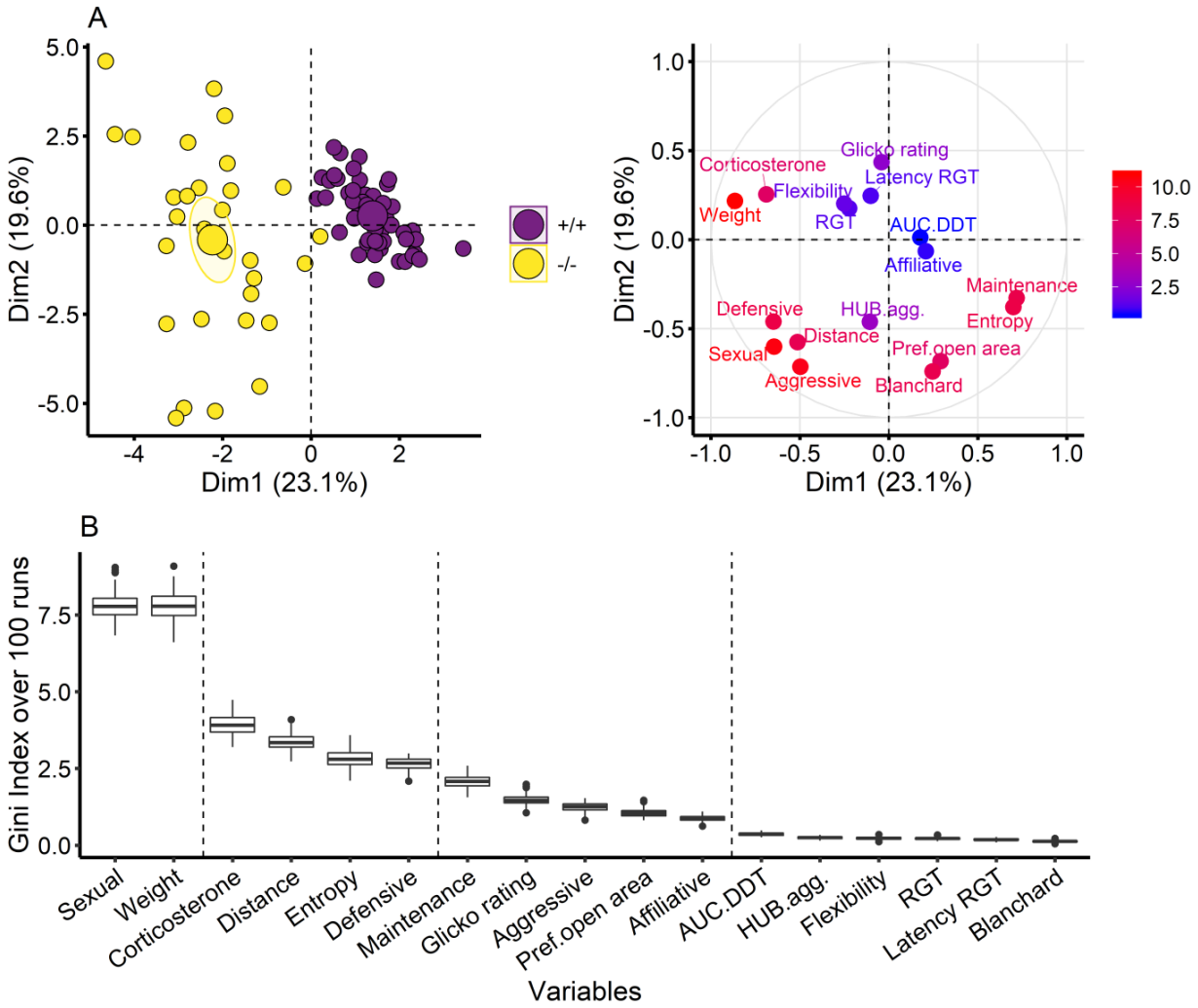

Figure 2. Principal component analysis and random forest classification. A- left. Dimension 1 and 2 of the principal component analysis, genotypes are separated along dimension 1. *Tph2*$^{+/+}$ in purple and *Tph2*$^{-/-}$ in yellow; large symbols show group centroids and ellipses show the 0.95 confidence interval. **A- right.** Contribution of the behavioral traits to dimensions 1 and 2 of the principal component analysis, higher contribution with warmer color (red) and lower contribution with colder color (blue). **B.** Gini index of the RF classification indicating for each trait its importance to discriminate between *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ animals. The dashed line indicates the groups of variables resulting from the k-means clustering of the Gini indexes over 100 RF 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; allogrooming, attending, huddling, sniffing), area under the curve in the delay discounting task (AUC.DDT), hub centrality in

aggression social network (HUB.agg), flexibility score in reversed-RGT (Flexibility), preference in last 20 minutes of rat gambling task (RGT), latency to collect pellet in RGT (Latency RGT), Blanchard dominance score (Blanchard). Panels A–B: +/+ n = 48, −/− n = 30. Figure from Alonso et al., 2023.

3.3 Effect of acute and moderate brain serotonin depletion on the behavioral profile of SD rats, published in the International Journal of Molecular Sciences (Alonso et al., 2024b)

In order to avoid the potential consequences of the developmental compensations inherent to the genetic models of constitutive depletion, we used an inducible knock-down rat model (tetO-shTPh2 rats) to investigate the effect of an acute and moderate decrease of brain serotonin on the behavioral profile of rats. We focused the behavioral profile on the cognitive dimensions, namely complex and risky decision making, flexibility, and social recognition memory. We used network analysis which originates from the field of psychopathology to transform the behavioral profiles into behavioral networks to explore changes of structure and connections between the cognitive dimensions of the profiles of different spontaneous decision-makers under lower serotonin conditions.

After three weeks of chronic doxycycline treatment the low-serotonin group showed a lower performance in the rat gambling task with a higher proportion of poor decision makers. Moreover, those low-serotonin poor decision makers showed a lower risk taking and a lower social recognition memory (Table 4). Applying the network analysis as an exploratory approach, we described the interactions between the cognitive dimensions within the profiles of the Control and low-serotonin groups without poor decision makers. In both groups, the decision making and the motivation for the reward (RGT-Lat) were highly connected and this connection was stable and unchanged between groups (Fig. 3AB). Other connections were impacted by serotonin decrease: the strong connection between the social preference and the short-term social recognition memory (SP-STM) was not seen and the connection between short-term social recognition memory and motivation for the reward (STM-Lat) was found (Fig. 3AB). The behavioral network of the low-serotonin poor decision makers presented strong connections between STM and all other cognitive dimensions and between social preference, decision making and social preference and risk taking (Fig. 3C, n = 5). Short-term social recognition memory was a central node in this strongly connected network (Fig. 3C).

In this last study we found that complex decision making ability was impacted by the acute and moderate serotonin decrease and that only low-serotonin poor decision makers expressed additional impaired cognitive abilities with social recognition memory a core symptom of the behavioral network.

Table 4: Control and *Tph2*-kd rats' performances and comparison with the Wilcoxon rank sum test and additional performances of the subgroup of low-serotonin poor decision makers, own representation for this doctoral work.

Trait	Test	Parameter	Control Median (IQR)	*Tph2*-kd Median (IQR)	Wilcoxon rank sum test W (p-value)	*Tph2*-kd-PDM Median (IQR)
Complex decision making	RGT	RGT score	91 (21)	88 (39)	2113 (0.045)	0 (10)
Sensitivity to reward	RGT	Latency to collect reward	1.7 (0.5)	1.7 (0.3)	1725 (0.94)	1.5 (0.3)
Cognitive flexibility	Rev-RGT	Flexibility score	28 (53)	20 (69)	1166 (0.78)	16 (8)
Cognitive risk-taking	PDT	AUC	0.8 (0.3)	0.6 (0.2)	863 (**0.007**)	0.5 (0.1)
Social preference	SRt	Ratio of interaction time	2.3 (0.8)	2.5 (0.9)	814 (0.67)	2.6 (0.1)
Short-term social recognition memory	SRt	Ratio of interaction time	1.5 (0.5)	1.5 (0.8)	856 (0.97)	0.9 (0.2)
Odor discrimination	Odor test	Ratio of interaction time	0.6 (0.1)	0.6 (0.2)	417 (0.79)	0.6 (0.1)

IQR: interquartile range, PDM: poor decision makers, AUC: area under the curve. Following a Bonferroni correction for multiple testing, the significance level in table 4 is 0.007, significant p-values are shown in bold.

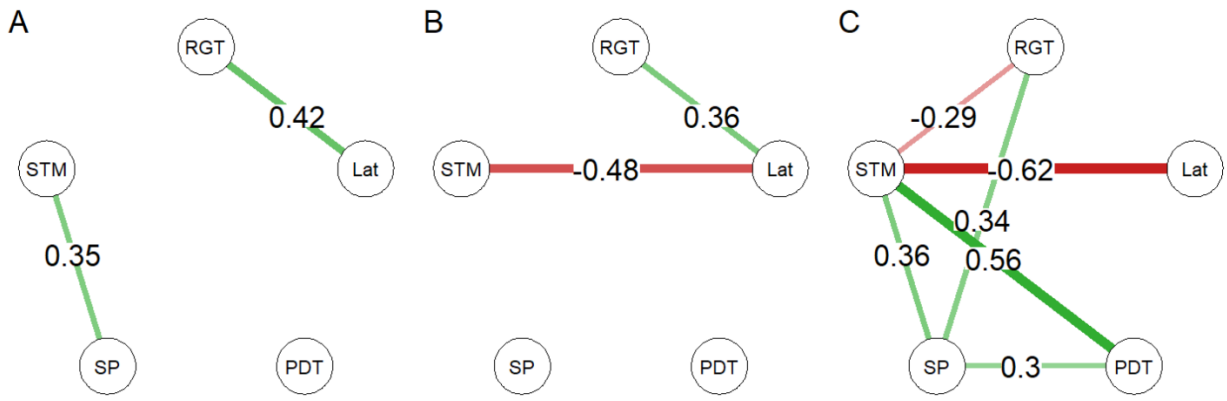

Figure 3. Network analysis of the behavioral profiles with Spearman's correlations. A- Control group without poor decision makers. **B-** Low-serotonin (*Tph2*-kd) group without poor decision makers. **C-** Low-serotonin poor decision maker subgroup (from the *Tph2*-kd group). Edges between cognitive functions are Spearman's correlations, green edges for positive correlations and red edges for negative correlations. Thickness of the edge represents the strength of the correlation. Correlations lower than 0.29 are filtered out. Decision making ability (RGT), motivation to collect reward (Lat), risky choices (PDT), recognition of social novelty (SP), recognition of social familiarity (STM). Control without PDM, n = 35, *Tph2*-kd without PDM, n = 29, *Tph2*-kd-PDMs, n = 5. Figure from Alonso et al., 2024.

4. Discussion

4.1 Short summary of results

In this doctoral work I have developed a multidimensional and differential profiling approach for preclinical studies with translational value. The primary objective was to develop a methodology to integrate several socio-cognitive and biological dimensions together for the establishment of individual-based bio-behavioral profiles of healthy rats. The second objective was to evaluate how brain serotonin would influence the identified bio-behavioral profiles in two genetical models and also in a complex environment of living using the same multidimensional and differential approaches and to identify reliable bio-behavioral markers of serotonin functioning. The final objective was to confirm the specific role of serotonin in the worsening of the behavioral profile of the most vulnerable individuals specifically.

As a proof of concept for the development and the validation of the method, I first applied this multidimensional and differential approach to describe and compare the behavioral profile of individuals of one inbred and one outbred rat strains: the Dark Agouti strain, a rare inbred strain of rats *vs*. the Wistar Han strain, a classical outbred strain of rats. Here I showed that despite genetic differences, both strains of rats spontaneously displayed comparable behavioral phenotypic variations at the individual level although in different proportions. For instance, in both strains, individuals spontaneously presented either one of the three typical decision making strategies seen in healthy populations of rats. This work replicated and augmented the description of the behavioral characteristics associated to each decision making type. The profiles associated to each decision making type were found comparable between strains. Finally, and critically, at the strain level, the multidimensional analysis revealed specific bio-behavioral characteristics with different baselines of reward sensitivity, impulsivity, anxiety and activity for each strain. These refined results are critical for the choice of a given strain of rats depending on the human mental conditions that is to be modeled.

The same multidimensional and differential approach was used to investigate the role of brain serotonin in the expression of those spontaneous behavioral profiles. Two different and novel rat models with genetic modifications of brain serotonin function were used. First using a rat’s strain with complete constitutive serotonin depletion in the brain

(*Tph2*$^{-/-}$), I revealed an unexpected contrasting role of serotonin in modulating behaviors in social *vs*. non-social contexts. While the absence of brain serotonin in these animals affected drastically the expression of spontaneous behaviors expressed in the unconstrained context of the home-cage, these same animals could correctly perform operant and somewhat complex cognitive tasks, when outside the home-cage. Within the group-housed social context of their home-cage the complete absence of brain serotonin impacted the *Tph2*$^{-/-}$ rats' quality and quantity of social interactions, their social organization and hierarchy but also patterns of space occupation. These animals exhibited higher levels of stress and experienced greater weight loss than controls. Interestingly, using machine learning analysis the most affected spontaneous dimensions in *Tph2*$^{-/-}$ rats appeared to be reminiscent of symptoms of mental disorders such as impulse control disorders and stress and anxiety disorders. In parallel, however, the same individuals with (still) no brain serotonin behaved at the same level of performance as control animals in classical testing, outside of the home cage when tested individually in operant chamber or open field.

Finally, using an inducible knockdown model (TetO-shTPH2), I showed that the acute and moderate decrease of serotonin (low-serotonin condition) led to an increase in the proportion of individuals showing poor decision making abilities (one of the three typical decision making type). Remarkably, this subset of individuals, the poor decision makers, was also the only one impaired on other dimensions namely short-term social recognition memory and economical risk-taking. For the first time in a preclinical setting, I used a behavioral network approach to illustrate the interactions between the cognitive dimensions of control and low-serotonin animals. Social cognition emerged as a core parameter of the behavioral profile of low-serotonin poor decision makers. These original results suggest that social cognition, modulated by serotonin, could be a potentially strong marker of vulnerability in rats, which could be eventually targeted as a therapeutic entry door in humans.

4.2 Interpretation of results

The strain differences that we have established will help in the choice between the Wistar Han (WH) and Dark Agouti (DA) strains in behavioral preclinical studies. Each laboratory usually has a traditional use of one strain over another (Lindsey and Baker, 2005). Change in rat strain may be necessary for technical reasons such as the creation of a

new genetically-modified line. Knowledge about strain specificities is essential (Butler-Struben et al., 2022) however, rat strain comparison studies and phenotyping studies remain sparse. One reason may be the difficulty to publish strain comparison results. In our case it was quite challenging despite the addition of two innovative methods: the multidimensional analysis and the home-cage monitoring system. According to our results, the WH strain should preferentially be used to investigate processes involved in reward related behavior, impulsivity, locomotor exploration and to test strategies that tend to the reduction of those phenotypes. Although the use of the DA strain of rats in behavioral studies is rare, its characterization was essential as the background strain of one of our genetic models. Moreover, we have shown the interesting results that DA rats present a more prosocial and anxious-like profile than WH and could be a very relevant strain to use to investigate anxiety-related dimensions and to test strategies that tend to the reduction of those phenotypes.

All strains of rats used in this doctoral work, namely the DA, WH and Sprague-Dawley, presented intra-strains' individual variations in behavior and the three different subpopulations of decision makers. Poor decision makers of each strains behaved as expected with regard to their decision making type in the other cognitive tasks and context conditions (Rivalan et al., 2013, 2009a). The conservation of this behavioral profile between genetically distinct strains of rats supports a strong translational power of this naturally existing profile in healthy populations.

Beyond the study of the spontaneously existing behavioral profiles of healthy subjects, the induction of a more extreme or pathological phenotype allows the study of the transition from vulnerability to pathology and therefore, to examine risk factors to pathology, new phenotypes and associated markers with the pathological phenotype. Inducing a moderate decrease of brain serotonin specifically impaired cognitive dimensions in the profile of poor decision makers. Low-serotonin poor decision makers lost their ability to socially habituate and to make risky decisions in probabilistic conditions. One interpretation would be that due to a lower serotonin level, they used preferentially a "short term" strategy prioritizing immediate and certain gains which can be in line with an increase of reward sensitivity towards whether food or social reward (El Rawas et al., 2020). Considering the strong centrality of social recognition memory in the behavioral network of low-serotonin poor decision makers, an alternative view of our

results would be that a decrease in serotonin availability, altered social recognition memory in the first place and, by diffusion through the behavioral network, had impacted the other functions, strongly connected to social recognition memory, namely risky decision making and latency to collect the reward. The hypothesis of the diffusion of a dysfunction between cognitive dimensions from social to more executive should be further tested. In humans, for instance, network approach studies have demonstrated the importance of social cognition for executive function and daily activity performance in the context of schizophrenia (Galderisi et al., 2018; Hajdúk et al., 2021). Further investigations of the properties of the behavioral network of poor decision makers in normal and “pathological” low-serotonin conditions are needed to better understand the role of social cognition in the transition from normal to impaired behavioral network and thus as a potential marker of the establishment of psychopathology. The combination of the differential, multidimensional and genetic approaches presented here revealed a novel emerging social marker of pathology in spontaneously vulnerable individuals.

Investigating a constitutive lack of brain serotonin, I was able to observe a strong pathological profile in *Tph2*$^{-/-}$ rats. From this profile, using machine learning methods, the most impaired dimensions were interestingly similar to clinical symptoms of impulse control disorders (disruptive, impulse control, and conduct disorders, compulsive sexual behavior disorder, and behavioral addictions) and stress and anxiety disorders (obsessive-compulsive, post-traumatic stress, and generalized anxiety disorders). The main discriminative markers of serotonin function were: (1) exacerbated sexual behaviors reminiscent of uncontrollable repetitive sexual behavior (World Health Organization, 2019), (2) strongly reduced maintenance (eating, drinking, grooming) behaviors and (3) increased weight loss reminiscent of neglect of personal care (American Psychiatric Association, 2013; World Health Organization, 2019), (4) increased defensive behaviors and (5) reduced home-cage occupation (entropy) reminiscent of hypervigilance, over-reaction to harmless stimuli disturbing daily life, avoidance of situations with negative expectation and repetitive checking behavior (Baldwin, 2013; Cludius et al., 2019; Dunsmoor and Paz, 2015; Gillig and Sanders, 2011; Weintraub et al., 2015). Finally (6) their increased corticosterone levels was reminiscent of cortisol disturbances and stress triggering impulses (Buchanan et al., 2020; Cackowski et al., 2014; Djamshidian et al., 2011; Grant and Potenza, 2004; Seo et al., 2016). All those markers and all the other impaired functions of *Tph2*$^{-/-}$ rats (discussed in more details in Alonso et al. 2023) were

specifically expressed in the visible burrow system. The use of this semi-natural home cage revealed the essential role of serotonin in the modulation of social and non-social daily life behaviors. However, in stand-alone tests no such role could be observed as *Tph2*$^{-/-}$ rats expressed preserved cognitive performance. This was highly unexpected and contrasted with the dominant literature indicating an essential role of serotonin in modulating decision making (Izquierdo, 2017; Koot et al., 2012), flexibility (Brigman et al., 2010; Clarke et al., 2004), cognitive impulsivity (Baarendse and Vanderschuren, 2012; Winstanley et al., 2006), and cognitive risk-taking (Ishii et al., 2015; Kirkpatrick et al., 2014) using the same classical tests as in our study. I proposed two explanations for these negative results. First, constitutive genetic models are known for their unexpected developmental compensations (Kreiner, 2015). In *Tph2*$^{-/-}$ rats, the lack of serotonin may have led to an increase in neuronal development (hyperinnervation) through the brain-derived neurotrophic factor pathway (Brivio et al., 2018; Kronenberg et al., 2016; Migliarini et al., 2013) which could have impeded stronger deficits. Second, I proposed that the inherent differences between the semi-natural home-cage and the classical setups (operant chambers and open field), which were historically developed to challenge one specific cognitive function at a time, had a strong impact on the cognitive demand required from the animal. While the more dynamic, experimenter-free setup would challenge several functions, including social functions, and allowing the expression of complex behaviors on the long term, and would require a higher cognitive effort from the individuals (Kondrakiewicz et al., 2019; Matusz et al., 2019; van den Bos et al., 2013b). Our data support the facilitating role of serotonin for behavioral adaptation in social environments through its influence on neural plasticity (Branchi, 2011; Kiser et al., 2012). *Tph2*$^{-/-}$ rats present a rich phenotype that can be further used to model everyday life features of several human disorders.

4.3 Embedding the results into the current state of research

We obtained profoundly different results with the two genetic rat models that we used, although the common point of the two models was brain serotonin. This allows us to elaborate about the overall role of brain serotonin in the modulation of individual profiles of behavior in rats. On one hand, the moderate destabilization of the serotonergic system in *Tph2*-kd induced individual-specific socio-cognitive impairments and changes in the behavioral network of a subset of individuals from the general population: the vulnerable

poor decision makers. On the other hand, the constitutive lack of brain serotonin induced a maladapted profile of behavior that could be observed within the home-cage environment for all individuals, independently of their decision making type. In both studies however, the social context was decisive for the expression of impairments and social cognition appeared particularly sensitive to the disturbance of the serotonergic system. Taken together, our results point towards an essential role of serotonin in the adaptation to the social environment through the ability to adaptively respond and interact with social stimuli. In human, social cognition attracts more and more interest as a trans-diagnostic construct and a marker of mental disorders (Lakhani et al., 2021). Social cognition impairments undercover for instance social reward sensitivity in schizophrenia patients, interpersonal accuracy in remitted patients with bipolar disorders and relatives (Bora and Özerdem, 2017; Espinós et al., 2019; Lee et al., 2019). Rehabilitation through social cognition trainings is being developed and promising (Jones and Harvey, 2020; Nijman et al., 2020). The development of robust measures of social cognition in clinical and healthy populations is deeply needed (Lakhani et al., 2021; Patin and Hurlemann, 2015). From the preclinical perspective, we must pursue the development of methods that can evidence and measure social cognition in animals to fuel clinical research on personalized preventive medicine. This will be essential to finally bridge the gap currently existing between clinical and preclinical research and contribute to improve therapeutic development.

A recent study combining transcriptomic mapping and neuroimaging in human and mice, linked patterns of brain-wide serotonin receptor expression to human individual variation in cognitive impulsivity, reward responsiveness and affective response (Salvan et al., 2023). This study suggests a serotonergic origin of inter-individual differences in human behavior. In the case of the rat poor decision makers a cortical-subcortical monoaminergic imbalance was reported along with reduced brain network activations during the Rat Gambling Task (Fitoussi et al., 2015). Which is in line with the hypothesis of serotonin being at the origin of the vulnerability of the poor decision makers as well as the molecular trigger of the transition from non-pathological to pathological states.

During this doctoral work, I have developed a semi-automatic version of the Visible Burrow System for rats (Alonso et al., 2019b) that is an adaptation of the non-automated Visible Burrow System created by Blanchard and colleagues (Blanchard et al., 2001).

The original semi-naturalistic enclosure was used as a home-cage to study the hormonal correlates of social hierarchy in rats. Blanchard and colleagues thoroughly described aggressive and defensive behavioral sequences associated with social ranking as well as non-social behavior dominance criteria (see Blanchard dominance score in the methods). In our system, the addition of an individual tracking system and an automatic video recording made it possible to collect numerous time-series, individualized locations and behavioral data. Home-cage monitoring exists since the 70's and has been growing faster the last 10 years with a jump in technology allowing the development of longitudinal studies (Kahnau et al., 2023; Nachev et al., 2021; Torquet et al., 2018). More recently the development of automated machine learning methods for analysis of animal behavior within the home-cage is revolutionizing the continuous detection of individuals and behaviors (de Chaumont et al., 2019; Kahnau et al., 2023; Lauer et al., 2022; Mathis et al., 2018; Múgica et al., 2022). Home-cage monitoring improves welfare for the animals and reproducibility of the results (COST Action CA20135, 2021; Kahnau et al., 2023). In our case the use of the visible burrow system allowed us to integrate context complexity (including social complexity) into our experimental design. Only in this dynamic environment *Tph2*$^{-/-}$ rats were unable to adjust their behavior to the inherent uncertainty of naturalistic group living conditions revealing strong behavioral markers of serotonin deficit. It would be enlightening to test the decision making ability, flexibility, and impulsivity of the *Tph2*$^{-/-}$ rats within home-cage settings (Huang et al., 2023). The multivariate analysis and supervised machine learning methods are essential to apprehend the complexity of animals' phenotypes and to identify markers of vulnerability or pathology. They allowed us to identify real-life markers of serotonergic function: sexual, maintenance and defensive behaviors, home-cage occupation, weight maintenance and corticosterone levels.

4.4 Strengths and weaknesses of the studies

Our goal with the multidimensional approach was to precisely define behavioral phenotypes and extract translational, novel, real-life (spontaneous and home-cage based) markers of serotonergic function. Beyond our study, the translation effort is important to unveil new (bio)markers and fuel the field of precision medicine (Menke, 2018). Our strategy to obtain the highest translational value as possible, was to include as much diversity as possible in our design (Devlin and Roberts, 2022). First the study of

interindividual differences sets the analysis in the direction of exploring the whole variability of the animal response. We also used a variety of complementary statistical analyses. Then we included a large diversity of tests to scrutinized several behavioral dimensions and in different testing environment. The use of the visible burrow system was critical to this work. Semi-natural housing allows the expression of a large repertoire of natural behavior and the discovery of phenotypes otherwise ignored, contrary to brief assessments outside of the home-cage influenced by random elements and with limited face validity (Kas and Van Ree, 2004). Ethologically relevant setups for rodents will help closing the gap between preclinical and clinical research (Shemesh and Chen, 2023). The direct comparison of classical and home-cage testing has shown very informative (Körholz et al., 2018; Mieske et al., 2023), in our case it was essential to the interpretation of our data.

In this doctoral work, we confirmed the vulnerability of poor decision making individuals to a modification of brain functioning. Poor decision makers constitute a very valuable model of vulnerability. However, they represent a minority of the population. This implies that the sample size may be hard to reach when working with this subpopulation. Preparing the experiment with power analysis and pilot experiment is crucial. The choice of the rat strain is decisive as the proportion of poor decision makers depends on it. So far, the best strain to use to have the highest proportion of poor decision makers (in % of the population) remains the Wistar Han, before Dark Agouti and Sprague Dawley strains with much less poor decision makers.

The use of the HPLC method was technically challenging. We did not succeed to obtain data of sufficient quality to include the individual serotonin levels as a variable in our multivariate and network analyses. Brain samples were limited, repeated and non-invasive sampling is not possible for serotonin measurements.

In this doctoral work we investigated the involvement of serotonergic function in the expression of behavioral profiles in male rats. In the last decade, the inclusion of females in rodent studies has exploded and soon will become the standard. Sex differences in decision making have been evidenced in animals and human with serotonergic neurobiological correlates (Truckenbrod et al., 2023; van den Bos et al., 2013a, 2012) and the higher vulnerability of females is documented (Radke et al., 2021). The study of the behavioral profile of poor decision makers must be extended to female individuals. It

is of prime importance to investigate at the preclinical level the sex differences evidenced in clinical and non-clinical populations (Leger and Neill, 2016; Paletta et al., 2023).

4.5 Implications for future research

In this doctoral work, we observed strong behavioral markers associated with disruption of the serotonergic system in social context and related to social cognition. Future research on the role of serotonin in animal behavior should strongly focus on social cognition features. The use of ethologically relevant tests, longitudinal designs and multidimensional approach are highly recommended for their translational power.

The network approach to psychopathology is one of the newest ways to conceptualize and study mental disorders. It considers that the interactions between the symptoms are causally responsible for the emergence of the pathology (Borsboom, 2017; Gauld, 2020). For the first time to our knowledge in an animal study, we used network analysis as a behavioral profiling method. It yielded interesting results and innovative hypotheses about the interactions between behavioral impairments. Combining network analysis of behavioral profile with a longitudinal design and high throughput home-cage testing is a very promising approach.

The multidimensional approach was thoroughly described in this thesis and our publications. Data and scripts developed in part II and III are openly available online. The approach is flexible and can be adapted to other similar designs. The blueprint of the visible burrow system is also openly available (Alonso et al., 2019a).

5. Conclusions

In this doctoral work, I have developed a behavioral, statistical and home-cage method for the description and comparison of multidimensional and differential behavioral profiles in healthy and genetically modified rats. Using this multidimensional, naturalistic and differential approach, I was able to compare two strains of rats and to identify the dimensions that characterized each strain the most. Applying the multidimensional, naturalistic and differential approach to two genetically modified rat models targeting the synthesis of central serotonin, I identified strong behavioral markers associated with the disruption of the serotonergic system. First, in rats with a constitutive lack of central serotonin the expression of everyday life behaviors was compromised in the group-housed naturalistic home-cage. The dimensions most critically impaired resembled symptoms of impulse control disorder and stress and anxiety disorder. When tested in classical stand-alone tests, the cognitive abilities of the same rats were surprisingly preserved. Secondly, in rats with an induced and moderate decrease of central serotonin the proportion of poor decision makers increased and these individuals specifically presented impairments in other cognitive abilities. Social recognition memory was the central node of their maladapted behavioral network. From these two interventional studies and thanks to the variety of analyses, I could conclude on a primary role of the serotonergic system in modulating social cognition and on the importance to further study behavioral markers of social cognition as translational markers of vulnerability. Multidimensional and differential analysis and the home-cage semi-naturalistic setup were key to this discovery offering a way to access the complexity of individual profiles of behaviors and generating a very high translational value for this doctoral work.

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Statutory Declaration

“I, Lucille, Alonso, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “Multidimensional description of behavioral phenotypes – Role of serotonin in individual profiles of behavior” “Multidimensionale Beschreibung von Verhaltensphänotypen – Dir Rolle von Serotonin für individuelle Verhaltensprofile”, independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

Declaration of your own contribution to the publications

Lucille Alonso contributed the following to the below listed publications:

Publication 1 in an international journal:

Alonso L, Peeva P, Ramos-Prats A, Alenina N, Winter Y, Rivalan M, *Inter-individual and inter-strain differences in cognitive and social abilities of Dark Agouti and Wistar Han rats*, Behavioural Brain Research, 2019. doi:10.1016/j.bbr.2019.112188

Alonso L conceived the study, planned and designed the experiments with Rivalan M
Alonso L performed behavioral experiment with Ramos-Prats A
Alonso L performed sacrifice and brain extraction with Peeva P and Ramos-Prats A
Alonso L developed data analysis methodologies with Rivalan M
Alonso L analyzed and interpreted the data with Peeva P, Alenina N and Rivalan M
Alonso L created all the figures and tables of the publication based on her statistical analysis in R.
Alonso L wrote the manuscript with Alenina N and Rivalan M
All co-authors reviewed and edited the manuscript
Alonso L responded to the reviewers with Rivalan M in agreement with all other co-authors

Publication 2 in an international leading journal:

Alonso L, Peeva P, Stasko S, Bader M, Alenina N, Winter Y, Rivalan M, *Constitutive depletion of brain serotonin differentially affects rats' social and cognitive abilities*, iScience, 2023. doi:10.1016/j.isci.2023.105998

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Publication 3 in an international leading journal:

Alonso L, Peeva P, Fernández-del Valle Alquicira T, Erdelyi N, Gil Nolskog Á, Bader M, Winter Y, Alenina N, Rivalan M. 2024. *Poor Decision Making and Sociability Impairment Following Central Serotonin Reduction in Inducible TPH2-Knockdown Rats*, International Journal of Molecular Sciences, 2024. doi:10.3390/ijms25095003

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Signature, date and stamp of first supervising university professor / lecturer

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Inter-individual and inter-strain differences in cognitive and social abilities of Dark Agouti and Wistar Han rats

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Dark Agouti

ABSTRACT

Healthy animals displaying extreme behaviours that resemble human psychiatric symptoms are relevant models to study the natural psychobiological processes of maladapted behaviours. Using a Rat Gambling Task, healthy individuals spontaneously making poor decisions (PDMs) were found to co-express a combination of other cognitive and reward-based characteristics similar to symptoms observed in human patients with impulse-control disorders. The main goals of this study were to 1) confirm the existence of PDMs and their unique behavioural phenotypes in Dark Agouti (DA) and Wistar Han (WH) rats, 2) to extend the behavioural profile of the PDMs to probability-based decision-making and social behaviours and 3) to extract key discriminative traits between DA and WH strains, relevant for biomedical research. We have compared cognitive abilities, natural behaviours and physiological responses in DA and WH rats at the strain and at the individual level. Here we found that the naturally occurring PDM's profile was consistent between both rat lines. Then, although the PDM individuals did not take more risks in probability discounting task, they seemed to be of higher social ranks. Finally and despite their similarities in performance, WH and DA lines differed in degree of reward sensitivity, impulsivity, locomotor activity and open space-occupation. The reproducibility and conservation of the complex phenotypes of PDMs and GDMs (good decision makers) in these two genetically different strains support their translational potential. Both strains, present large phenotypic variation in behaviours pertinent for the study of the underlying mechanisms of poor decision making and associated disorders.

1. Introduction

Inter-individual variability in behaviour is a natural phenomenon that applies to all behavioural dimensions. In the laboratory, however, these phenotypic variations are often perceived as inconvenient and are usually masked by averaging of the data. Considering the spectrum nature of brain disorders, most psychiatric symptoms can be conceptualized as extreme manifestations of different behavioural traits [1]. Thus, the identification of animals spontaneously exhibiting extreme behaviours that resemble human psychiatric symptoms offers the opportunity to study the natural psychobiological processes underlying maladapted behaviours [2,3].

Utilizing this dimensional approach to the analysis of the Rat Gambling Task (RGT), a rat version of the human Iowa Gambling task, we and others have consistently identified three types of decision makers spontaneously existent in healthy groups of Wistar Han (WH) and Sprague Dawley rats [4–8]. Whereas the majority of rats develop a strong preference for the most advantageous options in the RGT (good decision makers [GDMs]), a smaller group prefers the least advantageous options (poor decision makers [PDMs]) and some show no clear preference (intermediate phenotype [INT]) [6].

Compared to GDMs, healthy PDMs were found to co-express several cognitive impairments and reward-based deficits similar to symptoms observed in human patients with substance abuse disorder, pathological gambling disorder, attention-deficit hyperactivity-disorder (ADHD) or suicidal behaviour [6,8,9]. Healthy PDMs were more prone to take risks

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in potentially dangerous environments, showed higher motivation to obtain a reward and greater anticipatory (motor) impulsive responses, were more inflexible and chose less advantageously in the RGT due to their over-valuation of the high-reward/high-risk options compared to GDMs [8]. Their social abilities and spontaneous level of activity (*e.g.* arousal) are, however, still unknown [10,11]. At the biological level, PDMs also present a particular profile compared to GDMs. PDMs show different use of distinct regions of the prefrontal cortex (PFC) to solve the RGT [7], and a decreased c-fos activation in the PFC-subcortical network normally used by the GDMs [5]. Moreover, PDMs display an opposite pattern of serotonin turnover compared to GDMs, with higher turnover rate in the PFC (*i.e.* infralimbic cortex) but lower turnover rate in subcortical areas (*i.e.* basolateral amygdala) [5], making serotonin a promising candidate responsible for the co-expression of the traits constitutive of the PDM psychobiological profile.

Serotonin plays a critical role in executive functioning (decision making, impulse control, flexibility, attention), mood control, sociality and emotional state [9,12–19], and is a privileged therapeutic target for treating pathologies associated with poor decision making such as substance abuse, ADHD, suicidal behaviour, impulsive control disorders (*i.e.*, eating disorders, gambling), psychopathy and other aggression related disorders [20–22]. Although more than one behavioural domain has rarely been tested in the same individual, other studies have reported equivalent deleterious effects after dietary, genetic or pharmacological reductions of central serotonin function on group (*vs.* inter-individual) performance in decision making [23,24], motor impulsivity [25] and cognitive inflexibility [26], but also in social recognition [27], aggression [28] and social hierarchy [29,30].

In order to evaluate the functional role of the serotonergic system in the expression of the vulnerable behavioural profile in rats, we plan to use in follow up study an animal model of congenital central serotonin depletion [31]. The background strain of this newly created rat line is the Dark Agouti (DA) strain. However, historically, DA rats have been mainly used in physiological studies, and have only rarely been tested for their cognitive abilities [32] and never for their social skills. We also wanted to confirm that this inbred strain of rats naturally displayed comparable behavioural phenotypic variability to WH rats [33].

Therefore, the goal of this study was to evaluate the conservation of the GDM and PDM profiles between the WH and DA strains by establishing the bio-behavioural profile of the DA rats, examining the same behavioural traits naturally exhibited by the WH rats and selected for their relation with the serotonergic system. We also used this opportunity to test the reproducibility of previous results obtained from a different laboratory with the WH strain, and to extend the behavioural profile of the PDMs to yet untested serotonin-sensitive tasks such as probability based decision making and social behaviours. We compared cognitive abilities, natural behaviours and physiological responses in DA and WH rats using several tests. These tests included the RGT, the reversed-RGT, the Delay discounting task (DDT), the Probability discounting task (PDT), the Fixed-interval and Extinction schedule of reinforcement (FI-EXT), a semi-automated version of the Visible Burrow System (VBS), the Social Recognition test (SRt), and the Elevated Plus maze (EPM). The results were analysed at both the group (strain) and individual (within strain) levels. Finally, by performing a random forest analysis, we were able to highlight key traits to discriminate one strain from the other and discuss the relevance of using each strain in different types of studies.

2. Material and Methods

2.1. Animals

In this study, we used 42 male WH rats (Charles River, Germany) and 42 male DA rats (Max Delbrück Center for Molecular Medicine, Berlin). They arrived at our animal facility at between six and nine weeks of age. Rats of the same strain were housed in pairs in standard rat cages (Eurostandard Type IV, 38 cm × 59 cm) in two temperature-controlled rooms (22 °C and 50% humidity) with inverted 12 h light cycles (lights on at 20:00 in room 1 or 01:00 in room 2). The two different light cycles allowed us to maximize the use of four operant cages with two groups of 12 animals tested either in the morning or in the afternoon (*i.e.* 24 animals per day). To habituate the animals to their new environment, they were left undisturbed for at least a week after arrival. Thereafter, they were handled daily by the experimenter. Two weeks before the beginning of the training phase, rats were marked, subcutaneously and in the ventral left lower quadrant, with a radio-frequency identification (RFID) chip (glass transponder 3 × 13 mm, Euro I.D.) under short isoflurane anaesthesia. Rats were between 9 and 12 weeks of age when first trained in the operant cages. Rats had *ad libitum* access to food and water. During operant training and testing, rats were maintained at 95% of free-feeding weight by food restriction. One DA rat was excluded from the RGT and reversed-RGT analysis since it did not show sampling behaviour at the start of the test and a strong side bias over the entire duration of the tests. One DA extreme outlier (< mean − 2*SD) was excluded from the weight analysis after VBS housing.

2.2. Ethics

All procedures followed national regulations in accordance with the European Communities Council Directive 2010/63/EU. The protocols were approved by the local animal care and use committee and run under the supervision of the animal welfare officer of the animal facility of the Charité University Medicine.

2.3. Behavioural tests

Training and testing started 1 h after the beginning of the dark phase. Animals were habituated to the experimental room conditions for 30 min. The order of the tests and inter-test pauses was chosen to minimize any interference of one test on another (Fig. 1A). All animals included in this study were tested under the same experimental conditions for each individual test (animals tested in a pilot condition in the EPM or SRt were not included for these tests) and following the same order of tests (except for 12 WH rats which performed the DDT before VBS housing). Finally and due to time constrains the last experimental groups were tested following a shorter protocol: in the RGT, reversed-RGT, VBS, EPM and DDT [one month duration], but not in the SRt, FIEXT and PDT [another month duration]. The number of animals used in each test is indicated in the figures.

2.3.1. Operant system and tests

All operant training and testing was done in four operant cages (Imetronic, Pessac, France) controlled by a computer. The operant cages contained a curved wall on one side equipped with one to four nose-poke holes, depending on the test. On the opposite wall, a food magazine was connected to an outside pellet dispenser. 45 mg sweet pellets (5TUL, TestDiet, USA) were used. A clear partition with a central opening in the middle of the cage, ensured an equal distance to all nose-poke holes from this central opening.

2.3.1.1. Complex decision making in the Rat Gambling Task (RGT). The training and testing procedures have been described previously [6]. The operant cages had four nose-poke holes on the operant wall. Training 1 started with the four nose-poke holes lit; a single nose poke by the rat led to the delivery of one pellet, and the lights in the non-selected holes were then turned off until the food magazine was visited thereafter all holes were lit again. Daily training continued until rats obtained 100 pellets in a 30 min session (cut-off criteria). During Training 2, two consecutive nose pokes at the same hole were required to obtain one pellet; this training continued until rats obtained 100 pellets in a 30 min session. After Training 2 and for all subsequent testing phases, rats

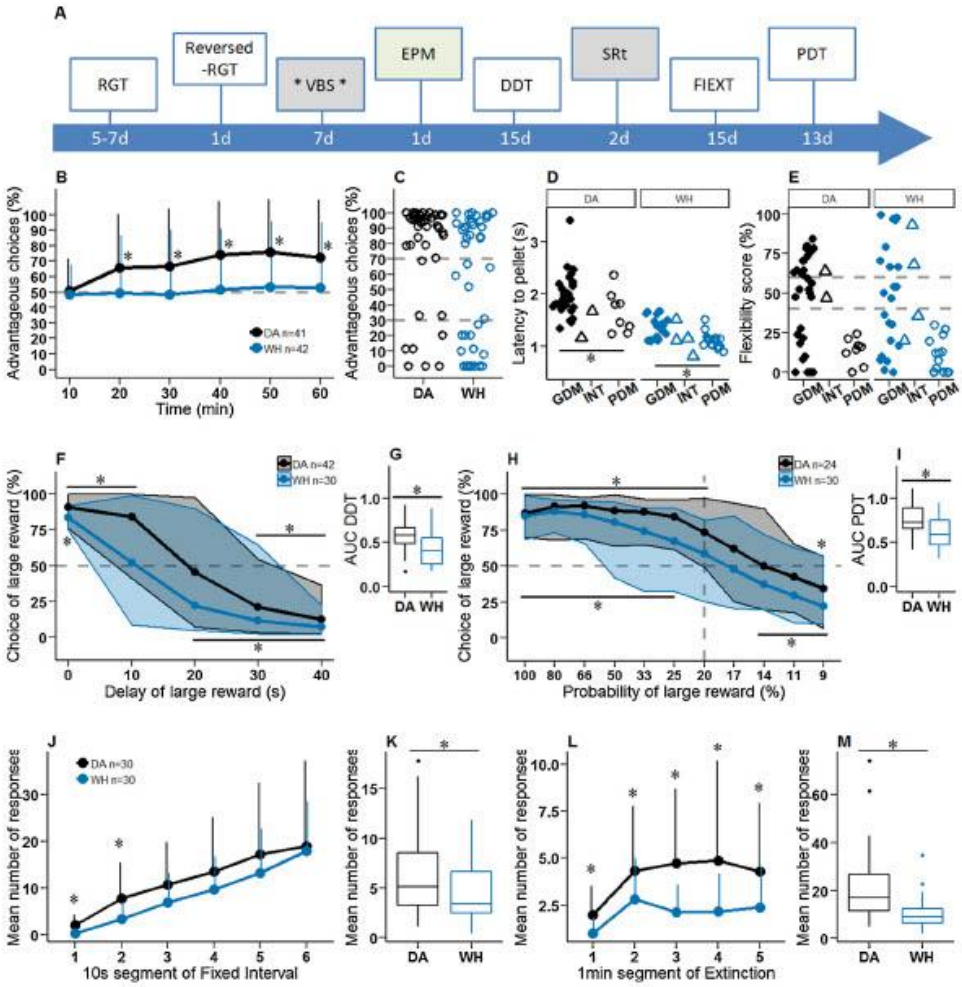

Fig. 1. Order and duration of testing and cognitive abilities of Dark Agouti (DA) and Wistar Han (WH) rats in the RGT, reversed-RGT, DDT, PDT and FIEXT. A Order and duration of testing. RGT: Rat gambling task. VBS: Visible burrow system, with faeces collection (asterisks) before and after VBS housing. EPM: Elevated plus maze. DDT: Delay discounting task. SRt: Social recognition test. PDT: Probability discounting task. Cognitive tasks are in white and social tasks are in grey. d: day. B Advantageous choices in the RGT. Data are mean + SD, one sample *t*-test *vs.* 50%. C Individual (mean) scores during the last 20 min of the RGT. The dashed line at 70% and 30% of advantageous choices visually separates good decision makers (GDMs), intermediates (INTs) and poor decision makers (PDMs). D Motivation for the reward in the RGT, with filled circles representing GDMs, triangles representing INTs and empty circles representing PDMs; Wilcoxon rank sum test, GDM *vs.* PDM. E Flexibility scores in the reversed-RGT. F Choice of the large reward option as a function of the delay of reward delivery. Lines indicate the medians, and areas shaded in grey (DA) or blue (WH) indicate the 5th–95th percentiles. The dashed line indicates the 50% chance level. The asterisk denotes significant difference (Wilcoxon sign test) from 50% choice for DA (*above curve) and WH (*below curve). G Area under the curve for the DDT; Wilcoxon rank sum test, DA *vs.* WH. H Choice of the large reward option as a function of the probability of reward applied. Lines indicate the median, and areas shaded in grey (DA) or blue (WH) indicate the 5th–95th percentiles. The vertical dashed line shows the indifference point (20% chance of receiving 5 pellets). The asterisk shows significant difference (Wilcoxon sign test) from 50% choice for DA (*above curve) and WH (*below curve). I Area under the curve for the PDT. Wilcoxon rank sum test, DA *vs.* WH. J Mean number of nose pokes during the 1 min FI expressed for the 10 s segments + SD. K Mean number of nose pokes during all 1 min FI (last 4 days). L Mean number of nose pokes during the 5 min EXT expressed for the 1 min segments + SD. Wilcoxon rank sum test, DA *vs.* WH. M Mean number of nose pokes during all EXT (last 4 days). DA in black and WH in blue. Panels A-E: DA, n = 41; WH, n = 42; panels F-G: DA, n = 42; WH, n = 30, panels H-I: DA, n = 24; WH, n = 30 and panels J-M: DA, n = 30; WH, n = 30. * $p < 0.05$.

always had to make two consecutive nose pokes at the same hole for a valid choice. Training 3 was a single 15 min session in which two pellets were delivered after a choice was made, up to a maximum of 30 pellets. A forced training (Training 4) was given to counter any side preference developed during the training procedure. This training was given when a rat had chosen the holes of one side of the operant wall in more than 60% of choices during the last session of Training 2 (holes on one side considered together). During the first phase of Training 4, only the two nose-poke holes on the non-preferred side were lit, and choosing any of them led to the delivery of one pellet. After the collection of the first 15

pellets, the second phase of Training 4 started with all four holes lit. Choosing one hole from the side preferred in Training 2 was rewarded (with one pellet) in only 20% of the cases, whereas choosing from the other (least-preferred) side was rewarded in 80% of the cases. The cut-off criterion was set at a maximum of 50 pellets or 30 min. This training phase usually took between five and seven days, and the RGT was performed the next day.

During the test, the four nose-poke holes were lit and each hole was associated with an amount of reward and a possible penalty (time-out). Two holes on one side were rewarded with two pellets and associated with unpredictable long time-outs (222 s or 444 s; probability of occurrence 50% and 25%, respectively); over the long term, these options were disadvantageous. Two holes on the other side were rewarded with one pellet and associated with unpredictable short time-outs (6 s or 12 s; probability of occurrence 50% and 25%, respectively); over the long term, these options were advantageous. The theoretical gain of pellets for the advantageous options was five times higher than for the disadvantageous options at the end of the test (*i.e.*, 60 min) [6]. After a choice, the reward was delivered and the selected hole remained lit until a visit to the magazine or for the duration of the time-out. During this time, all the nose-poke holes were inactive. The cut-off criterion was 250 pellets.

The percentage of advantageous choices during the last 20 min of the RGT was used to identify GDMs and PDMs. GDMs were defined as choosing > 70% advantageous options and PDMs as choosing < 30% advantageous options. Intermediate animals (INTs) chose between 30% and 70% advantageous options and did not show a steady preference for only one type of option at the end of the test. To visualize progression of preference during the RGT, advantageous choices were plotted for 10 min time intervals. In a previous study, fast and slow GDMs were described based on how rapidly they developed a preference for the advantageous options [5]. Fast GDMs chose > 70% advantageous options during the first 20 min of the test, whereas slow GDMs stayed < 70%. The motivation to obtain a reward (reward sensitivity) was indicated by the mean latency to visit the feeder after a choice. To insure that decisions during testing were based on knowing all options, we always checked before data analysis, that the animals had sampled each type of reward and penalty associated with each type of option before they established a preference (or the animal was excluded from the analysis).

2.3.1.2. Cognitive flexibility in the reversed-RGT. Animals were tested in the reversed-RGT 48 h after performing the RGT [6]. For this test, the contingencies associated with the four holes during the RGT were spatially reversed by switching the sides for the advantageous and disadvantageous options. A test lasted 60 min (or a cut-off of 250 pellets).

A flexibility score was calculated as the preference for the same preferred options during the reversed-RGT and the RGT, which meant choosing holes at the location of the non-preferred option during the RGT. For INTs and GDMs, the flexibility score was determined from the percent of advantageous choices during the last 20 min. For PDMs, the flexibility score was determined from the percent of disadvantageous choices during the last 20 min.

Flexible rats had flexibility scores > 60%, undecided rats had flexibility scores between 60% and 40%, and inflexible rats had flexibility scores < 40%.

2.3.1.3. Cognitive impulsivity in the Delay Discounting Task (DDT). For this task, the two outside nose-poke holes (25 cm apart) were used. The operant cages were otherwise identical to the other tests. During the DDT, one nose-poke hole (NP1) was associated with a small (one pellet) immediate reward (during training and test phases); the second nose-poke hole (NP5) was associated with a large (five pellets) immediate (training phase) or delayed (test phase) reward. The protocol was adapted from Rivalan et al. [8], in which levers were used instead of nose-poke holes.

During training, the large and small rewards were delivered immediately after the corresponding choice (0 s delay) to NP5 or NP1 respectively. The absence of delay before reward delivery allowed the rats to develop a preference for the five pellet reward (NP5). After a choice, the selected hole stayed lit for 1 s. The magazine and house lights were turned on during a 60 s time-out. A session lasted for 30 min or until 100 pellets were delivered. A > 70% preference for the large reward option on two consecutive sessions with ≤ 15% difference was required to start the test. At least three training sessions were performed. During the test, choosing NP5 induced the delivery of the large reward after a fixed delay, and NP5 stayed lit for the duration of the delay. After the delivery of the large reward, the magazine and the house lights were turned on for a time-out (60 s minus the duration of the delay). The delay was fixed for one day and one session, it was increased by 10 s from 0 s to 40 s, between days, after a stability criterion (≤ 10% variation of choice of the large reward during two consecutive sessions) was met. The test sessions lasted for 60 min or until 100 pellets had been delivered. The preference for the large delayed reward was calculated as the mean percentage of NP5 choices during the two stable sessions. Individual area under the curve (AUC) was measured to estimate cognitive impulsivity. The choices for the large delayed reward were normalized to the choice for the large delayed reward during the training phase (0 s delay) and plotted against the normalized delays on the x-axis (from 0 to 1). The AUC was calculated as the sum of the areas of the trapezoids formed by the individual data points and the x-axis following the formula $(x2-x1)[(y1+y2)/2]$, [34]. The total number of nose pokes during the last training session was used as an index of the activity during this test.

2.3.1.4. Cognitive risk-taking in the Probability Discounting Task (PDT). For this task, the two outside nose-poke holes (25 cm apart) were used. The operant cages were otherwise identical to the other tests. During the PDT, one hole (NP1) was associated with a small and certain reward (one pellet) and the second hole (NP5) was associated with a large but uncertain reward (five pellets) [24].

During training, choosing NP5 always delivered the large reward (probability P = 100%). This allowed rats to develop a preference for NP5. NP1 always delivered one pellet. The reward was delivered 4 s after a choice was made in one of the nose-poke holes, and the hole stayed lit until pellet collection. The reward delivery was followed by a 15 s time-out during which the magazine light was on. A session lasted 25 min or until 200 pellets were delivered. A ≥ 70% preference for the large reward was required to start the test. At least three training sessions were performed. During the test, the delivery of the large reward was associated with a set probability (P = 80%, 66%, 50%, 33%, 25%, 20%, 17%, 14%, 11%, or 9%). The probability was fixed for one day and decreased every day. A session lasted 25 min or until 200 pellets were delivered. For each individual, the AUC was calculated as in the DDT. The preference for the large reward was normalized to the preference during training and plotted against the probability values expressed as odds, with odds = (1/P)-1 and normalized (x-axis from 0 to 1) [35].

2.3.1.5. Motor impulsivity in the Fixed-interval – Extinction (FI-EXT) schedule of reinforcement. For this task, only the central nose-poke hole was used. The operant cages were otherwise identical to the other tests. The 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 [8]. The 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 seven FI of variable duration depending on the session and one EXT of 5 min; this pattern was repeated two times within a single session. 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. The mean number of nose pokes was 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. As described earlier [36], the data from the first FI of the session and the first FI after the first EXT were excluded because they deviated from the other intervals.

2.3.2. Social behaviour in the Visual Burrow System (VBS)

The VBS (Supplementary Figs. 12–13 and supplementary video) consisted of an open area (61 × 43 cm, 2000P, Tecniplast, Italy) extended to the top by high Forex PVC foam and Plexiglas (Modulor, Germany) walls and connected through two transparent tunnels to a burrow system placed into a second cage (59 × 38 cm, EU type IV, Tecniplast, Italy). The burrow system was made of infrared transparent black plastic and consisted of a large chamber, a small chamber and a tunnel system (25 cm × 53 cm). Throughout the test, the burrow system remained in the dark. Food and water were available in the open area. A grid of 32 RFID detectors (PhenoSys, Berlin,) was placed below the VBS in order to automatically determine individual animal positions using the program PhenoSoft (PhenoSys, Berlin). An infrared camera (IP-Camera NC-230WF HD 720p, TriVision Tech, USA) above the VBS recorded a 30 s video every 10 min (CamUniversal, CrazyPixels, Germany). The software PhenoSoft ColonyCage (PhenoSys, Berlin) was used to identify individuals in the videos. Six rats were housed in the VBS for seven days in a humidity- and temperature-controlled room (temperature 22 °C–24 °C, humidity 45% to 50%). The behaviours expressed by the animals were scored on the videos of the last two days of VBS housing during the first 4 h of each dark and light phase (100 videos) using a scan sampling method [37]. Four classes of behaviours were scored: affiliative, aggressive, defensive and maintenance (details in Table 1). The behaviours with a median of < 5 occurrences per strain were grouped for the analysis. The body weight of the animals was measured before and after VBS housing. Although wounds were rarely observed during this study, they were counted and documented at the end of VBS housing. The activity (distance travelled) and the place preference were extracted using the software PhenoSoft analytics (PhenoSys, Berlin). The time spent in the open area of the VBS was measured using the data collected from the grid of detectors.

2.3.3. Faeces collection for corticosterone measurements

Faeces collection took place one day before and immediately after VBS housing. At the same time of day, all rats were simultaneously housed in individual cages with food, water and clean bedding. They spent up to 4 h in their cages. Every 30 min, faeces produced were collected in microtubes and stored at −20 °C until corticosterone extraction. Next, the samples were thawed and 0.1 g of faeces was added to 0.9 ml of 90% methanol, agitated for 30 min and centrifuged at 3000 rpm for 15 min. A 0.5 ml aliquot of the supernatant was added to 0.5 ml water; this extract was stored at −20 °C. Corticosterone measurements were performed with an enzyme immunoassay (EIA) following the method of Lepschy et al., [41] in the laboratory of Dr. Dehnhard at the Leibniz Institute of Zoo and Wildlife Research, Berlin. The antibody was purchased from Rupert Palme (University of Veterinary Medicine, Vienna, Austria), and has been described in detail in [42]. The intra assay coefficients for two biological samples with low and high concentration were 6.2% and 8.4% respectively. The respective inter assays were 10.4% and 13.4%. Briefly, a double antibody technique was used in association with a peroxidase conjugate, generating a signal quantitatively measurable by photometry. The concentration of corticosterone was expressed in µg per g of faecal material as an indicator of stress level in an individual. The change in corticosterone level (%) was calculated from the values obtained before and after VBS housing.

2.3.4. Social preference and recognition in the SRt

The protocol was adapted from Shahar-Gold et al., [43]. The test took place in a square open field (50 cm), with a small cage placed in one corner (Supplementary Fig. 14A). The intruder animals were older WH rats with a prior habituation to the procedure. A video camera placed above the open field was used to record the experiment. Each rat was tested on two consecutive days. On the first day, the subject was placed in the open field containing the empty small cage in a corner for a habituation period of 15 min (Hab). The intruder was then placed in the small cage, and the subject could freely explore the open field for 5 min (E1). Subsequently, the small cage with the intruder was removed

Table 1
Ethogram of the behaviours scored during VBS housing. Based on Burman et al., Rademacher et al., and Whishaw, Ian Q and Kolb Bryan [38–40].

Category	Behaviour	Definition
Affiliative	Allogrooming	Gentle grooming of another rat which 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
Aggressive	Aggressive grooming	Vigorous grooming of another rat while pinning it
Aggressive	Attack bite	Sudden bite toward neck and back of another rat
Aggressive	Attack jump	Sudden jump toward another rat
Aggressive	Following	Rat runs after another one
Aggressive	Fight	Rough-and-tumble of two animals
Aggressive	Lateral attack	Arched-back posture oriented towards another rat, often including shoving and piloerection
Aggressive	Mutual upright posture	Both rats are standing in front of each other with vertical movements of the forepaw
Aggressive	Pinning	Being above another rat and maintaining it with the forepaw usually lying on its back
Aggressive	Struggle at feeder	Rats are pushing each other to have the place at the feeder
Aggressive	Struggle in tunnels	Rats are pushing each other to pass in the tunnel, struggling with the paws.
Defensive	Flight	Rapid movement away from another rat
Defensive	Freezing	Being immobile or maintaining a specific posture (crouching)
Defensive	Lateral defence	Exposing the flank to another rat.
Defensive	Supine posture	Lying on the back (exposure of the belly) because of another rat
Defensive	Upright defence	Exposing the belly to another rat in a half-erect posture
Maintenance	Drinking	Drinking water
Maintenance	Eating	Eating food
Maintenance	Grooming	Self-grooming, when a rat is cleaning itself with rapid little nibbles

from the open field, and the subject remained alone in the open field for 10 min. The encounter procedure was repeated two more times with the same intruder (E2, E3). On the second day, the first 15 min of habituation were followed by a fourth encounter (E4) of 5 min with the same intruder as on day 1. After this encounter, a break of 30 min took place, during which the subject remained alone in the open field. The last encounter then took place with an unfamiliar intruder placed in the same small cage for 5 min (Enew). The time spent in close interaction, including when the subject's head was in contact with the grid or within 1 cm of the grid and the nose directed to the grid, was measured for each encounter (E1, E2, E3, E4 and Enew) and for the first 5 min of Hab. The social preference was calculated as the ratio of the interaction time in E1 and the interaction time during Hab. The short-term social recognition memory index was defined as the ratio of the interaction time in E1 and the interaction time in E3. The long-term social recognition memory index was calculated by dividing the interaction time in Enew by the interaction time in E4.

2.3.5. Exploration in the Elevated Plus Maze (EPM)

The apparatus (made of black painted wood) consisted of two open arms (50 cm × 15 cm), alternating at right angles with two closed arms enclosed by 40 cm high walls. The four arms opened onto a central area (15 cm × 15 cm). There was a small ridge along the edge of the open arms (1 cm wide). The whole maze was elevated 60 cm from the ground. A video camera mounted above the maze and connected to a computer outside the experimental room was used to observe and record animal's behaviour. Light intensity in the open arms was 15 lx.

The experimenter placed a rat in the central area of the maze facing a closed arm. The rat was allowed to freely explore the maze for 10 min. The time spent and entries in the open and closed arms were measured. Risk taking was evaluated as time and number of visits in the last third of the open arms, constituting the more risky areas [6].

2.4. Statistical analysis

R (3.5.1) and R studio (1.1.456) free software was used for the statistical analyses [44]. For each test, two levels of analysis were considered: first, the inter-strain comparison, where whole populations of WH *vs.* DA were compared, including INT animals; and second, the intra-strain comparison, where GDMs *vs.* PDMs were compared within each strain (excluding the INT animals).

Several non-parametric tests were used: a) the Fisher's exact test was used to compare the number of GDM and PDM in WH and DA groups; b) the Wilcoxon sign test (RVAidememoire package) [45] was used to compare the performance of the animals to the indifference level (DDT, PDT and SRt); c) the Wilcoxon rank sum test was used to compare groups of animals (DA *vs.* WH, GDM *vs.* PDM, and cluster groups between them), and whenever appropriate a continuity correction was applied to the data with the Wilcoxon rank sum test; and d) the non-parametric ANOVA with permutation for repeated measures (lmPerm package) [46] was used to compare groups of animals along different time points. The one sample *t*-test was used to compare performance with the indifference level in the RGT. For the global discrimination between strains, we used a random forest (RF) classification with leave-one out validation (randomForest package) [47]. The traits included in this analysis were the variables from the different tests. Seventeen traits were used: percentage of advantageous choices during the last 20 min (RGT score); flexibility score; mean latency to visit the feeder after a choice (latency RGT); AUC in DDT; activity in DDT; AUC in PDT; mean number of responses in FI; mean number of responses in EXT; activity in VBS housing; time open VBS; number of aggressive, affiliative and maintenance behaviours in VBS test; weight variation in VBS housing; corticosterone variation in VBS housing; social preference ratio; and short-term recognition ratio. Missing values (NA) were not tolerated by the model; therefore, some animals and variables had to be excluded from the analysis (for example, two animals did not produce faeces during faeces collection and the EPM was not included). n = 22 WH and n = 24 DA were included in the RF analysis.

3. Results

3.1. Cognitive and social abilities in DA and WH rats

3.1.1. Decision-making abilities in the RGT

At the beginning of the test (first 10 min), rats of both strains chose the advantageous and disadvantageous options equally (Fig. 1B). After 10 min and until the end of the test, the average performance of the DA rats moved toward the most advantageous options (20 min: one sample *t*-test for DA: 0.95 CI [55, 76.6], p = 0.005), while the average performance of the WH rats remained at chance level for the entire duration of the test. However, at the end of the test (the last 20 min), large individual differences in choice became clear (Fig. 1C). In both strains, a majority of the rats preferred the most advantageous options at the end of the test (> 70% advantageous choices during the last 20 min of test; good decision makers or GDMs); a smaller proportion preferred the most disadvantageous options (< 30% advantageous choices; poor decision makers or PDMs) and a minority of the animals showed intermediate performance (INTs). Of the DA rats, 79% were GDMs (n = 31), 19% were PDMs (n = 8) and 5% were INTs (n = 2); of the WH rats, 50% were GDMs (n = 22), 40% were PDMs (n = 16) and 10% were INTs (n = 4). The proportion of GDMs, INTs and PDMs between strains was not statistically different (Fisher's exact test, p = 0.082), only the proportion of GDMs *vs.* non-GDMs (INTs and PDMs) was higher in the DA than the WH (Fisher's exact test, p = 0.039). These observations could explain why the average performance of the DA rats was above the 50% indifference level while that of the WH rats was not. The development of choice preferences during the test of the GDMs on one hand and of PDMs on the other hand were similar between strains (Supplementary Fig. 1A).

In both strains, "fast" and "slow" GDMs could be identified (Supplementary Fig. 1B). In the DA rats, the majority of the GDMs were the "fast" type (74%, n = 23/31), choosing significantly and consistently the advantageous options at 20 min of testing. In the WH rats, only half of the GDMs were of the "fast" type (59%, n = 13/22).

3.1.2. Motivation for reward in the RGT

The latency to collect a reward after making a choice in the RGT was shorter in the WH rats (median 1.1 s) than in the DA rats (median 1.8 s; Fig. 1D, Wilcoxon rank sum test, W = 1613, p < 0.001). This difference was not due to the different proportions of GDMs and PDMs. In both strains, the PDM rats were faster than the GDM rats at collecting the reward (Fig. 1D, Wilcoxon rank sum test, WH: W = 284, p = 0.001; DA: W = 181, p = 0.047). Interestingly, the WH GDMs had the same latency as the DA PDMs (Fig. 1D).

3.1.3. Cognitive flexibility in the reversed-RGT

The flexibility score indicates the propensity of an individual in the reversed-RGT to keep choosing (inflexibility) the same outcome as in the previous RGT or not choosing it (flexibility). All animals considered, DA and WH rats presented similar levels of cognitive flexibility (Fig. 1E; median 47% and 24% for DA and WH, respectively). In both strains and as expected for WH, all PDMs made highly inflexible choices in the reversed-RGT (low flexibility score; Fig. 1E). PDM rats kept choosing the hole(s) previously preferred (in the RGT), despite the outcomes of such choices now being different than in the RGT (Supplementary Fig. 2). In both strains, GDM rats had either high, intermediate or low flexibility scores (Fig. 1E). The proportion of GDMs with a high flexibility score (flexible GDMs) was 39% in DA and 31% in WH. Flexible GDMs progressively (trial after trial) switched their spatial preference from the nose-poke holes previously associated with the advantageous options (in the RGT) to the nose-poke holes currently associated with

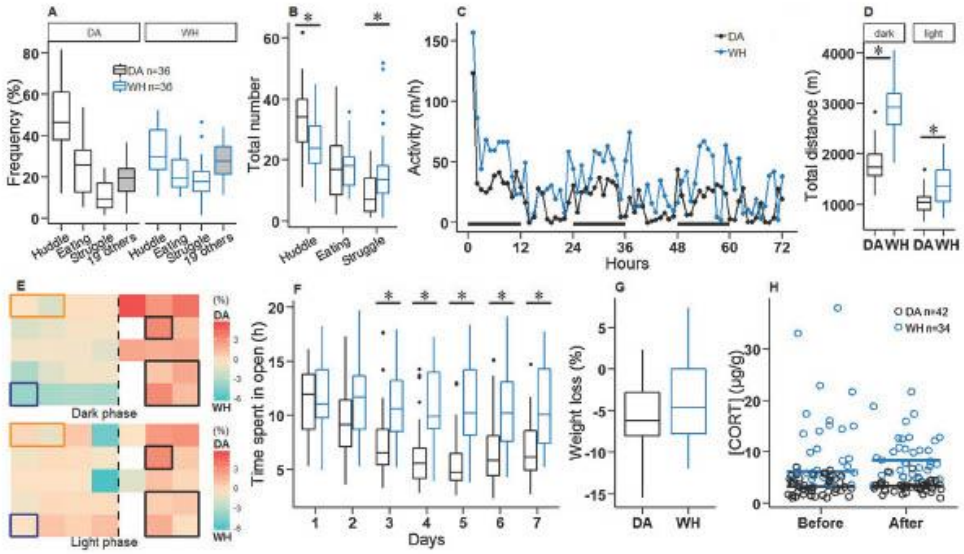

Fig. 2. Daily activity, behavioural and biological measures of Dark Agouti (DA) and Wistar Han (WH) rats during the Visible Burrow System (VBS) housing. A Relative frequency of occurrence of behaviours in the VBS. White boxes represent a unique type and grey boxes represent a composite behaviour category. "Struggle" = "struggle at feeder". "19 Others" comprised the 19 behaviours (all behaviours minus the three main behaviours) scored during the VBS video analysis but which had a median < 5 in each strain due to their rare occurrence. B Occurrence of the three main types of behaviours observed in the VBS (50 min observation). C Typical locomotor activity of one DA and one WH individual during the first three days in the VBS. Bars indicate dark phase. D Total distance travelled during the dark and light phases over seven days in the VBS. E Difference in place preference (%) between DA and WH during the dark and light phases over seven days of VBS housing. Red indicates a preference of the DA relative to WH for each of the 32 zones of the VBS (corresponding to the 32 RFID detectors located beneath the VBS cage). Rectangles indicate the locations of feeder (orange), water bottle (blue), and small and large chambers in the burrow area (black). The vertical dashed line indicates the separation between the open area (left side) and the burrow area (right side). F Total time spent in the open area. G Weight loss after VBS housing. H Concentration of corticosterone in faeces before and after VBS housing. Horizontal bar: median of each group. DA in black and WH in blue; Panels A-G: WH, n = 36; DA, n = 36 and panel H: WH, n = 34; DA, n = 42. * $p < 0.05$, DA *vs.* WH, Wilcoxon rank sum test except panel F ANOVA with permutations for repeated measures. The VBS test was conducted with n = 6 individuals in the cage at a time.

the advantageous options (Supplementary Fig. 2). 22% of DA GDMs and 18% of WH GDMs had no clear preference for either advantageous or disadvantageous options during the reversed-RGT. Finally, 39% of DA GDMs and 50% of WH GDMs showed an inflexible pattern of choices similar to the PDM rats (Fig. 1E) and kept choosing the hole(s) previously preferred in the RGT (Supplementary Fig. 2).

3.1.4. Cognitive impulsivity in the DDT

In both strains, increasing the delay of delivering a highly palatable large reward decreased the preference for this option (Fig. 1F; Wilcoxon sign test, delay 0 s: DA 0.95 CI [85.1, 93.0], $p < 0.001$, WH 0.95 CI [81.4, 88.7], $p < 0.001$; delay 10 s: DA 0.95 CI [73.1, 93.0], $p < 0.001$). The sooner an individual rejects the large reward that is increasingly delayed, the more impulsive it is. On average, the DA rats preferred an immediate one-pellet reward over a delayed five-pellet reward when the delay reached 30 s (Wilcoxon sign test, 0.95 CI [19.4, 26.5], $p < 0.001$). Similarly, on average, WH rats preferred an immediate one-pellet reward over a delayed five-pellet reward when the delay reached 20 s (Wilcoxon sign test, 0.95 CI [10.8, 31.3], $p = 0.001$). Interestingly, although the preference for the high-reward option at a delay of 0 s was very strong in both strains (91% in DA and 84% in WH), the performance was significantly different between strains (Fig. 1F; Wilcoxon rank sum test with continuity correction, $W = 891$, $p = 0.002$). After normalizing performances to the preference at a delay of 0 s, the comparison of the AUC indicated that WH rats lost the preference for the high-reward option earlier than DA rats when the delay was added (Fig. 1G; Wilcoxon rank sum test, $W = 923$, $p < 0.001$). Within strains (and as expected for WH) [8], GDMs and PDMs had the same switching point and AUCs (Supplementary Fig. 3A and B).

3.1.5. Cognitive risk taking in the PDT

In both strains, decreasing the probability of delivery of the most rewarding option (five pellets) also decreased the preference for this option (Fig. 1H; Wilcoxon sign test, probability 100%: DA 0.95 CI [73, 91.2], $p < 0.001$; WH 0.95 CI [80, 90], $p < 0.001$). A delivery probability of 20% for the five-pellet option is the point of indifference at which both options (certain – one pellet *vs.* uncertain – five pellets) are, on average, equivalent. If an animal prefers the certain option (one pellet) over the uncertain option (P = 80% to 20% – five pellets), it indicates an aversion to uncertainty. If an animal prefers the uncertain option (P = 20% to 9% – five pellets) over the certain option (one pellet), it indicates risk taking. DA rats lost their preference for the (uncertain) high-reward option when probability dropped to 17% (Wilcoxon sign test, 0.95 CI [50.8, 72.8], $p = 0.063$). WH rats lost their preference when probability dropped to 20% (Wilcoxon sign test, 0.95 CI [40.8, 66.7], $p = 0.361$). Comparison of the AUCs indicated that DA maintained a higher preference for the high reward with the decrease of reward probability than WH (Fig. 1I; Wilcoxon rank sum test, $W = 516$, $p = 0.006$). In both strains, the AUCs were comparable between GDMs and PDMs (Supplementary Fig. 3C and D).

3.1.6. Anticipatory and perseverative behaviour in the FI-EXT schedule of reinforcement

DA anticipatory activity was higher, particularly during the first 20 s of the FI (Fig. 1J; non-parametric ANOVA with permutation, 1st

segment $p < 0.001$, 2nd segment $p = 0.004$). The mean number of nose pokes was higher in DA rats than in WH rats for the 1 min FI (Fig. 1K; Wilcoxon rank sum test with continuity correction, $W = 589.5$, $p = 0.039$). DA rats nose poked more than WH rats during the 5 min EXT (Fig. 1M; Wilcoxon rank sum test with continuity correction, $W = 690$, $p < 0.001$), and this was the case during all the 1 min segments of EXT (Fig. 1L; non-parametric ANOVA with permutation, 1st segment $p = 0.002$, 2nd segment $p = 0.045$, 3rd segment $p < 0.001$, 4th segment $p = 0.001$, 5th segment $p = 0.015$). Within strains, DA PDMs ($n = 7$) nose poked significantly more than DA GDMs during EXT (Supplementary Fig. 4B; Wilcoxon rank sum test with continuity correction, $W = 35$, $p = 0.043$); however, this was not observed in WH.

3.1.7. Natural behaviours expressed in the VBS

In both strains, the behaviours most frequently observed in the VBS were huddle, eating and struggle at feeder (with median number of occurrences > 5 in 100 30s-videos on the last two days of VBS housing; Fig. 2A). The 19 other scored behaviours (allogrooming, attending, drinking, grooming, aggressive grooming, attack, embracing, fight, following, mounting, mutual upright posture, pinning, struggle at water, struggle in tunnel, flight, freezing, lateral defence, supine posture and upright defence) were seen more rarely (median number of occurrences < 5 in 100 30s-videos on the last two days of VBS housing) and are grouped in the composite category "19 others" in Fig. 2A (for further details, see Supplementary Fig. 5). Considering the three most frequent behaviours, DA rats huddled more and struggled (at the feeder) less than WH rats (Fig. 2B; Wilcoxon rank sum tests with continuity correction, huddle: $W = 984$, $p < 0.001$; struggle at feeder: $W = 313.5$, $p = 0.005$). Strains did not differ in their number of bouts of eating. The occurrences of huddle, eating and struggle at feeder were similar between PDMs and GDMs in both strains (Supplementary Fig. 6).

3.1.8. Total distance travelled in the VBS

Both DA and WH rats changed their activity (*i.e.*, distance travelled) with the light/dark phase (Fig. 2C) and were more active during the dark phase (Fig. 2C). Over all days, locomotion in WH rats was higher than in DA rats during both dark and light phases (Fig. 2D; dark phase: Wilcoxon rank sum test, $W = 45$, $p < 0.001$; light phase: Wilcoxon rank sum test, $W = 313$, $p < 0.001$). During the dark phase, the WH PDMs were more active than the WH GDMs (Supplementary Fig. 7; Wilcoxon rank sum test, $W = 60$, $p = 0.005$).

3.1.9. Place preference in the VBS

DA rats preferred to stay more in the burrow area than the WH, both during the dark phase (Fig. 2E, top panel; Wilcoxon rank sum test, $W = 105$, $p < 0.001$) and during the light phase (Fig. 2E, bottom panel; Wilcoxon rank sum test, $W = 371$, $p = 0.001$). The two strains had different occupation of the VBS, the DA preferring to stay in the burrow area during both phases (Supplementary Fig. 8; dark phase: Wilcoxon rank sum test, $W = 1069$, $p < 0.001$, light phase: Wilcoxon rank sum test, $W = 1049$, $p < 0.001$) while the WH preferred the open area during the dark phase and had no preference for burrow or open area during the light phase (Supplementary Fig. 8; dark phase: Wilcoxon rank sum test, $W = 39$, $p < 0.001$). Furthermore, during the light phase, while in the open area, WH rats were mostly present in the entry zones of the burrow area (Fig. 5E) where they were seen to sleep most of the time. At the inter-individual level, the WH GDMs preferred to stay in the burrow more than the WH PDMs during the dark phase (Supplementary Fig. 8; Wilcoxon rank sum test, $W = 195$, $p = 0.038$) and the same tendency was observed in DA rats (Supplementary Fig. 8).

3.1.10. Total time spent in the open area of the VBS across days

The DA rats spent less time in the open area starting from day 3 (non-parametric ANOVA with permutation, day 3 $p < 0.001$) than WH rats (Fig. 2F). There was no difference in the time spent in the open area across day between DA GDMs and DA PDMs, whereas in WH the PDMs tended to spend more time in the open than GDMs starting on day 3 (Supplementary Fig. 9).

3.1.11. Weight loss during VBS housing

Before being housed in the VBS (and in general), DA rats were smaller and lighter than WH rats (Supplementary Fig. 10A; Wilcoxon rank sum test with continuity correction $W = 0$, $p < 0.001$). During their stay in the VBS, DA and WH rats lost the same relative weight (Fig. 2G). However, DA GDMs lost more weight than DA PDMs (Supplementary Fig. 10B; Wilcoxon rank sum test with continuity correction, $W = 35$, $p = 0.039$).

3.1.12. Corticosterone (metabolite) levels after VBS housing

At baseline (before VBS housing), the concentration of corticosterone in DA rats was lower than in WH rats (Fig. 2H; Wilcoxon rank sum test $W = 206$, $p < 0.001$). After VBS housing, the corticosterone levels in DA and WH rats were unchanged (Fig. 2H). In both strains, corticosterone levels were not different between GDMs and PDMs, neither before nor after VBS housing (Supplementary Fig. 11).

3.1.13. Social preference and social recognition memory in the SRt

In the SRt, both strains exhibited a clear preference for social *vs.* non-social cues and an accurate short-term social recognition memory. Rats spent more time exploring the unfamiliar social partner during the encounter 1 (E1) than an unfamiliar non-social cue (empty box) during the habituation phase (Hab, Fig. 3A; Wilcoxon rank sum test with continuity correction, WH: $W = 576$, $p < 0.001$; DA: $W = 258.5$, $p < 0.001$). Exploration time was twice as long in E1 as in Hab (Fig. 3B; social preference ratio E1/Hab > 1, Wilcoxon sign test DA: 0.95 CI [1.3, 2.8], $p = 0.030$ and WH: 0.95 CI [2.2, 3.4], $p < 0.001$). WH rats had a higher social preference ratio than DA rats (Wilcoxon rank sum test, $W = 121$, $p = 0.016$). The third time WH and DA rats encountered the same animal (E3), the time spent exploring this animal was significantly reduced compared to their first encounter (E1), indicating effective short-term social recognition memory (Fig. 3A; Wilcoxon rank sum test with continuity correction, WH: $W = 484.5$, $p < 0.001$; DA: $W = 225$, $p = 0.018$). Due to experimental limitations, long-term social recognition memory could not be evaluated, although it is likely that both strains did have such memory. In both strains, the social preference ratio and short-term memory ratio did not differ between GDMs and PDMs (Supplementary Fig. 14C and D).

3.1.14. Exploration in the EPM

DA rats expressed very different behaviour in the EPM compared to WH rats. DA rats very rarely (or never) visited the open arms of the maze (Fig. 3C; Wilcoxon rank sum test with continuity correction, $W = 5.5$, $p < 0.001$) and if then only for a very short time (Fig. 3D; Wilcoxon rank sum test with continuity correction, $W = 3$, $p < 0.001$) compared to WH rats. Only one DA individual visited the part of the maze that was furthest from enclosing walls (the last third of the open arms), as opposed to all the individuals in WH (data not shown). DA and WH rats had the same number of visits to closed arms (Fig. 3E). Within strains, no differences were observed between PDMs and GDMs for the parameters of total number of visits to open arms, total time spent in open arms or total number of visits to the last third of the open arms (Supplementary Fig. 15).

3.1.15. Inter-individual differences within DA and WH

In both strains, GDMs and PDMs showed similar tendencies in all tests (see Table 2 for details). In both strains, PDMs were faster to collect the reward than GDMs in the RGT, and all showed higher cognitive inflexibility in the reversed-RGT. In the VBS, the WH PDMs were more active during the dark phase, did not prefer the burrow area during the dark phase and spent more time in the open area on day 4

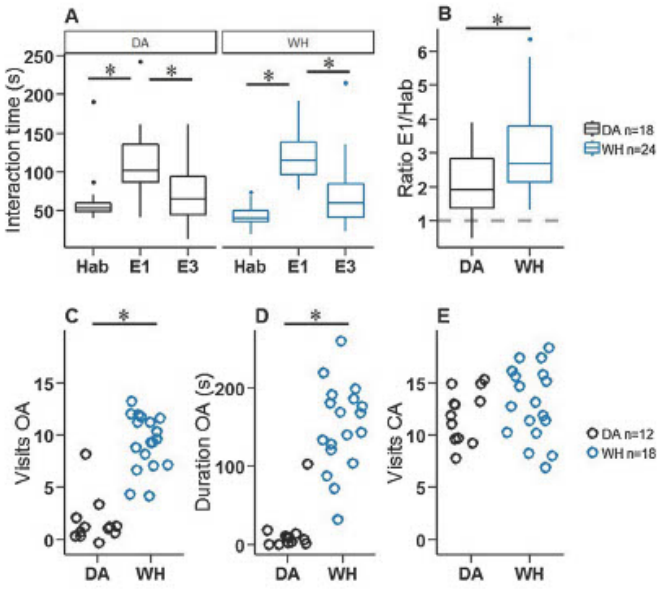

Fig. 3. Social preference, social short-term recognition and exploration of the EPM in Dark Agouti (DA) and Wistar Han (WH) rats. A Interaction times during the social recognition test. Hab: non-social cue (empty box) present during the habituation phase; E1: first encounter with intruder (unfamiliar); E3: third encounter with same intruder (familiar); Wilcoxon rank sum test Hab *vs.* E1 and E1 *vs.* E3. B Social preference represented as the ratio of exploration times in E1 and in Hab, DA *vs.* WH (Wilcoxon rank sum test). C Total number of visits to the open arms (OA), DA in black and WH in blue. D Time spent in the OA, DA *vs.* WH (Wilcoxon rank sum test). E Total number of visits to the closed arms (CA). Maximum exploration time was 10 min. DA in black and WH in blue, Panels A-B: DA, n = 18; WH, n = 24 and panels C-E: DA, n = 12; WH, n = 18.* $p < 0.05$.

than the WH GDMs. In the VBS, the DA PDMs lost less weight than the DA GDMs (Table 2).

3.2. Identification of the key variables discriminating WH from DA strain

We performed a random forest (RF) classification with a leave-one-out cross-validation (LOOCV) to quantify the efficiency of each of the previously described cognitive and social functions to distinguish WH and DA strains from each other. The RF was run using the behavioural and biological variables described above (See 2.4. Statistical analysis). In brief, the decision trees of the RF with LOOCV led to the prediction of the strain of each given individual by comparing its performance (for each variable) to the performance of the other individuals for which the strain was known. For WH and DA variables, the prediction of the strain was high, with an accuracy of 84% (± 0.72 SD over 10 runs). The importance of each variable to accurately differentiate the strains was given by the Gini index of the RF (Fig. 4A). The most discriminating variables were the AUC of the DDT and the distance travelled in the

Table 2
Behaviours of the GDMs and PDMs in DA and WH strains. d.n.s: data not shown.

Trait	Test	Parameter	GDM vs. PDM within strain	Figure
Sensitivity to reward	RGT	Latency to collect reward	Both strains: PDMs faster than GDMs	Fig. 1D
Cognitive flexibility	Rev-RGT	Flexibility index	Both strains: All PDMs and 1/3 GDMs inflexible	Fig. 1E
Cognitive impulsivity	DDT	AUC-DDT	No difference	Supp. 3B
	DDT	Switch point	No difference	Supp. 3A
Cognitive risk taking	PDT	AUC-PDT	No difference	Supp. 3D
	PDT	Switch point	17% for DA GDMs, 25% for DA PDMs (n = 6). 25% for WH GDMs, 33% for WH PDMs.	Supp. 3C
Anticipatory activity	FI	Mean number of nose pokes	No difference	Supp. 4A
Perseverative activity	EXT	Mean number of nose pokes	DA PDMs (n = 7) poked more than DA GDMs	Supp. 4B
Affiliative behaviour	VBS	Occurrences	No difference in huddle	Supp. 6
Aggressive behaviour	VBS	Occurrences	No difference (in struggle at feeder, struggle in tunnel, mutual upright posture and pinning)	Supp. 6 and d.n.s.
Defensive behaviour	VBS	Occurrences	No difference in supine posture	d.n.s.
Maintenance behaviour	VBS	Occurrences	No difference in grooming, eating and drinking	Supp. 6 and d.n.s.
Distance travelled	VBS	Total distance (dark phase)	WH PDMs were more active during the dark phase than WH GDMs	Supp. 7
Place preference	VBS	Place preference	WH PDMs had less burrow occupation during the dark phase than WH GDMs. DA PDMs tended to have less burrow occupation than DA GDMs.	Supp. 8
Time in open	VBS	Time spent in open per day	WH PDMs spent more time in open on day 4 than WH GDMs (non-parametric ANOVA with permutations, day 4 $p = 0.023$)	Supp. 9
Stress response	VBS	CORT variation	No difference	Supp. 11
Weight loss	VBS	Weight loss	DA PDMs lost less weight than DA GDMs	Supp.10B
Social preference	SRt	Ratio interaction times E1/Hab	No difference	Supp.14C
Short-term recognition	SRt	Ratio interaction times E1/E3	No difference	Supp.14D
Exploration EPM	EPM	Visits to open arm	No difference	Supp. 15

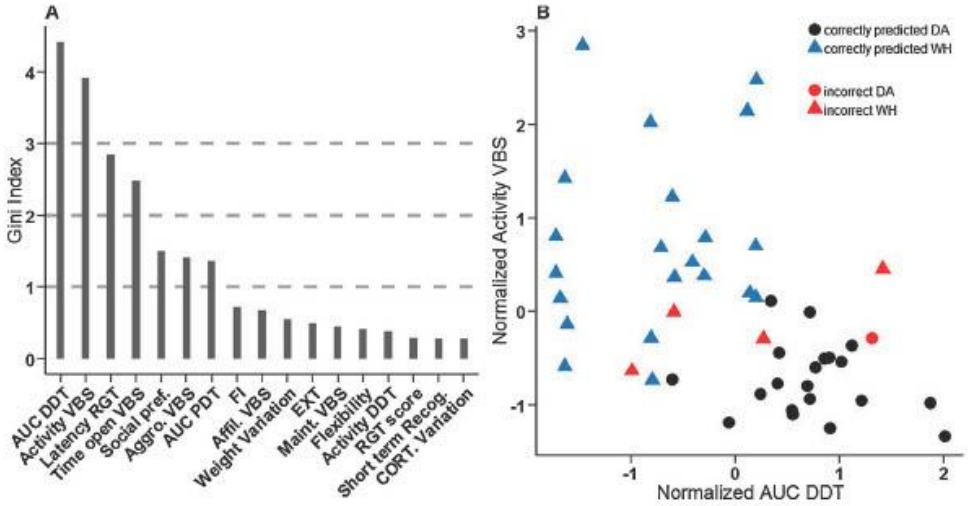

Fig. 4. Discriminating classification of the DA and WH. A Gini index for each trait used for the random forest (RF) classification. Dashed lines are included to sort the variables in groups of importance. pref. = preference, Aggro. = aggressive, affil. = affiliative, maint. = maintenance, CORT = corticosterone. B RF classification for the two most discriminating variables. DA, n = 24, in black; WH, n = 22, in blue. Symbols show predicted strain by the RF. DA: dot, WH: triangle. Red symbols indicate an incorrect prediction.

VBS (Gini index > 3), followed by the latency to collect a reward in the RGT and the total time spent in the open area in the VBS (3 > Gini index > 2; Fig. 4A). Of lesser significance were the social preference index in the social preference test, the AUC of the PDT, and the number of aggressive behaviours in the VBS (2 > Gini index > 1). As an example, one run of the RF classification including the two most discriminating variables (the distance travelled in the VBS and the AUC of the DDT) attributed the correct strain to 41 rats out of a total of 46 rats (Fig. 4B).

4. Discussion

4.1. Behavioural performance of PDMs and GDMs from DA and WH strains

One of the advantages of the RGT is the possibility it offers to uncover which decision-making strategy each individual of a healthy population of rats will spontaneously use to cope with complex and uncertain choice options. Here we found that, similar to WH, each individual DA could be classified in one of the three typical categories, GDM, PDM or INT [6]. Although not significant, the higher number of GDMs found in DA rats compared to WH rats could explain their more advantageous performance as a group (averaged performance) during the RGT compared to the WH, which on average stayed at chance level for the entire duration of the test. In a follow-up study, we will evaluate the effect of a lack of central 5-HT on the animals' decision-making abilities in the RGT. Thus, the large number of GDMs in healthy DA individuals will help us to quantify the effect of this genetic manipulation, which is expected to shift the behavioural profile from GDM to PDM.

Interestingly and independent of strain, we found that all PDM rats behaved as expected with regard to their decision-making type [6]. They were more sensitive to reward than GDMs and were unable to flexibly adjust their behaviour during the reversed-RGT. For humans, a new computational modelling of the analogous Iowa Gambling Task called Outcome-Representation Learning predicts that poor decision making of drug users could be due to higher reward sensitivity and more exploratory behaviour (in cannabis users), lower punishment sensitivity (in abstinent heroin users) and higher inflexibility perseverance (in abstinent amphetamine users) [48]. The expression of the same key features between PDMs of genetically distinct strains of rats and, to a certain extent, to results found in humans [49,50] suggests a strong conservation of this potential endophenotype within and between species.

As seen in previous studies in WH rats but now also in DA rats, the GDMs were not a single homogeneous group of rats [5,8]. While some (50%–75%) were faster than others in choosing the advantageous options during the RGT (at only 20 min of test), in the reversed-RGT only one-third of GDMs were able to flexibly adjust their behaviour.

In addition, differences were not observed between PDMs and GDMs in either strain in cognitive impulsivity (DDT) or risk-based decision-making tests (PDT). Although the result of the DDT was expected [8], the lack of difference in the PDT between PDMs and GDMs was more surprising. Indeed, in another version of the RGT (*i.e.*, the rGT) with repeated test sessions and punishment given as quinine pellets, poorer decision-making abilities were correlated with higher risk taking in the PDT [24]. The differences between the experimental procedures of each study and in the definition of what constituted poor decision making (in rGT, all rats were "GDMs", but some individuals made poorer decisions than others) may be the reasons for the discrepancies between these results. However, it is noteworthy that in the human literature a loss of control over risk (probability)-based choices is not characteristic of all PDM-associated psychiatric disorders. Patients with pathological gambling [51], alcohol dependence [52], schizophrenia [53] and autism [54] are more risky decision makers than patients with obsessive-compulsive disorder [55], pathological buying disorder, Huntington's disease [56] or suicidal attempts [57]. These and our results indicate that preference for high-risk (probabilistic) options may be a marker of pathology rather than a marker of vulnerability to diseases, as discussed by Bernhardt et al. [58], in humans. Risk-taking may preferentially be observed in "ill-induced" PDMs than in healthy PDM rats.

In a follow up study, a deeper analysis of the heterogeneity of the GDMs could reveal stronger differences between subgroups of GDMs and PDMs and uncover related neurobiological markers.

In the FI-EXT test, we only witnessed increased motor impulsivity in

DA PDMs during EXT, and did not witness this in either FI or EXT in WH. This inconsistent result in WH rats compared to our previous study may be due to the use of a different manipulandum (nose-poke holes instead of levers) for the operant response [8]. Very few studies have investigated the consequences of this difference in operant responding. Although Mekarski [59] defended nose poking to be a more innate behaviour than lever pressing, it has also been shown that escalation behaviour is better achieved with lever pressing and not nose poking in mice [60].

We also explored if PDM and GDM rats differed in their social skills. In the VBS, compared to GDM rats, PDM rats expressed a higher level of activity, less occupation of the burrow during dark phases, longer time spent in the open area of the cage (WH PDMs), and limited weight loss (DA PDMs). In the VBS, these features characterize dominance in rats (along with the number and location of wounds, which were not witnessed in this study) [61], suggesting a more dominant status for PDM rats than for GDM rats. Along the same line, Davis et al. [62], found that individual dominance correlated with higher motivation for rewards and higher exploration of risky zones in the EPM. These are also two known characteristics of PDM rats [6]. Interestingly, PDMs were not more aggressive or less affiliative in the VBS than GDMs and presented a similar interest for the social cue in the SRt. Buwalda et al. [63] showed that the level of aggression in the resident-intruder paradigm and in the VBS were not correlated with dominance. Social hierarchy is a dynamic feature [64] that depends on the outcome of agonistic and non-agonistic interactions [65] but on other dimensions too such as privileged access to resources [66,67] and lower sensitivity to stressors [68]. In humans, excessive aggression is a disruptive symptom widely distributed among psychiatric disorders. Studies have shown that decision making and aggression-related behaviours could share biological markers, such as monoamine oxydase A (MaoA), serotonin transporter (SERT), and tryptophan hydroxylase (TPH) 1 and 2 [69,70]. In further studies, we will use the rich semi-natural and around-the-clock experimental conditions of our VBS housing to explore more specifically which social domains and how social hierarchy develop along with decision-making abilities and serotonin manipulations.

The reproducibility and conservation of the socio-cognitive and behavioural phenotypes of GDM and PDM individuals in the two genetically different strains of WH and DA rats highlight the promising translational value of these complex phenotypes, not only between strains but likely also between species (*e.g.*, rats and humans). Following the Research Domain Criteria framework (RDoC), which promotes the exploration of cross-species endophenotypes for better translational value of preclinical studies [71,72], this study presents the PDM rats as a promising animal model for the identification of the specific biological circuits underlying equivalent patterns of deficits which can be observed in patients (or healthy relatives) and independent of their disorder categories. Both DA and WH rat strains offer interesting individual variation in behaviour, allowing the use of both strains for the study of the underlying mechanisms of poor decision making and associated disorders. It will be possible to examine the risk factors responsible for the transition from vulnerability to pathology by comparing the expression of each of the PDM-associated traits and how the neural substrates of this phenotype overlap or differ in ill-induced *vs.* healthy PDMs.

4.2. Strain differences between DA and WH

Beside the inter-individual differences within strains, we found at the group level that WH rats were, on average, more sensitive to reinforcement and more impulsive in the DDT, but less prone to take risks in the PDT compared to DA rats. In the DDT and PDT, WH rats dismissed both the delayed and uncertain option more rapidly than the DA rats in favour of the immediate or certain option, although this meant that the option associated with the largest reward (absolute value) was abandoned for a one-pellet option. Since the WH rats were more sensitive to reward than DA rats in RGT, the earliest switch of WH compared to DA, was not due to reduced motivation. The late switching point of the DA compared to the WH, at the same delay (*e.g.* 10 s) or the same probability, could have been driven by their lower "interest" for the reward (Fig. 1F–H). If the DA would give a lower subjective value to the reward, than the WH, the discounting effect of a given delay or probability could be reduced to these animals compared to WH and make them tolerate longer delays or probabilities than the WH before wanting to pursue the other option. The discounting factor (delay or probability) appeared to have a stronger impact on the subjective evaluation of rewards by WH rats, and WH rats had a lower tolerance to uncertain situations when rewards were involved compared to DA rats. In the VBS, WH rats were more aggressive, more active (higher distance travelled) and spent more time in the open area of the VBS than DA rats.

In biomedical research, the WH line is one of the two most commonly used strains of rats (with the Sprague Dawley rats) [73]. This research included studies investigating reward-related disorders such as drug addiction [74,75] and poor impulse control-related disorders such as substance abuse, eating disorders, ADHD or manic disorders [76,77]. WH rats are also used in studies on reward processing and valuation [78], and have been found to have a high tendency for compulsive and impulsive behaviours [79,80].

In contrast, DA rats made more perseverative responses in the FI-EXT test in anticipation of a reward and during extinction phases, indicating either a lower tolerance to frustrating inactive phases of the test or higher motor impulsivity compared to WH. Knowing that the conditions for this test may not have been optimal (as the low level of activity may be due to the requirement for nose-pokes instead of lever presses) and that such higher motoric response was not similarly observed in the training phase of the DDT (as both variables were correlated) [8], we prefer not to place too much emphasis on this result. Finally, DA rats were more affiliative in the VBS, preferred hiding in the burrows and were more fearful of the open arms of an EPM. They also had a weaker social preference in the SRt, which could be due to the avoidance of the centre of the open field during the first 5 min of habituation in this test. These results could confirm a specific fear of the elevated and widely open spaces, as discussed elsewhere [81,82]. It would be interesting to test the DA rats in the zero maze test (comparable to the EPM but sometimes qualified as less anxiogenic) [83] to further study their complex profile.

With DA rats presenting a more compulsive, anxious and prosocial phenotype, this strain seems promising for studies on anxiety-related disorders. For example, patients diagnosed with anxiety disorder are extremely fearful/anxious of real-life threats (as opposed to unreal life-threatening concerns of OCD patients); they can express un-ritualized compulsive behaviours and, in the case of social anxiety disorder (social phobia), a subcategory of anxiety disorder, they show strong social contact avoidance and/or seek to reduce their social fear (DSM-5) [84]. Anxiety indeed appears to be a trait often witnessed in inbred lines of mice [33].

Biological substrates could support the behavioural differences. It would be very interesting to further explore the genetic and neurophysiological differences between DA and WH. As said above, DA and WH shared alternating traits that have been associated with the serotonergic system, and especially hyposerotonergia. Mapping and quantification of the expression of the serotonin autoreceptor 5-HT1A and receptor 5-HT4 could support respectively their specific anxiety [85] and reward sensitivity profiles [86]. Also, the central levels of expression of SERT which influence the 5-HT turnover would be crucial to evaluate. In DA the lower sensitivity to reward, impulsivity, aggression and social preference and higher anxiety might indicate a lower level of SERT expression compared to WH [87].

Finally, and despite their remarkable differences, DA and WH rats also shared similar traits. For example, they presented higher levels of huddling, eating and struggling at the feeder than other behaviours during VBS housing, and equivalent corticosterone levels and weight

loss after VBS housing.

4.3. Prediction of the strain differences with RF analysis

Although we identified specific traits on which DA and WH strains spontaneously differed in performance, using a RF classification method we determined which of these traits were more characteristic of one strain than the other. These were the ability to wait for a reward in the DDT, the motivation to collect a reward in the RGT, and the level of activity and time spent in the open area of the VBS. The RF classifier was less able to accurately differentiate strains based on the expression of their affiliative and maintenance behaviours, weight variation, decision making or flexibility. The RF classification results were similar to those obtained after a principal component analysis (Supplementary Fig. 16).

In other words, the most critical difference between WH and DA rats related to behavioural control when facing a (delayed or non-delayed) reward as it can be seen in the DDT (cognitive impulsivity) and the RGT (reward seeking), respectively. Based on this observation, it could also be argued that the increased time the WH rats spent in the open area of the VBS was driven by the presence of the only food source of the cage being in this area, although this zone was also potentially the most aversive zone of the cage.

5. Conclusion

In this study, we compared several abilities of DA and WH rats at the group and the individual levels using multiple cognitive tests, a social naturalistic set-up and assays of physiological responses.

Both the dimensional and group approaches provided new insights for the preferential use of each strain in future neuropsychopharmacological studies and further advanced our knowledge of the complex phenotype of healthy PDM and GDM. At the group level, we identified specific traits by which these genetically distinct strains spontaneously differed most (AUC of the DDT, distance travelled in the VBS, latency to collect a reward in the RGT and total time spent in the open area in the VBS). The WH and DA strains could preferentially be used to model reward sensitivity and impulsivity on one side and compulsivity and anxiety-related behaviours on the other side.

At the individual level, we could reproduce previous findings in WH rats and generalize them to the DA strain. Each PDM individual of either strain displayed a similar naturally occurring combination of behavioural traits, including a higher sensitivity to reward, higher cognitive inflexibility and higher social rank, but no cognitive impulsivity in delay- or probability-based decision-making tasks, no deficits in social recognition and no differences in corticosterone response to stressors. The multi-domain profile of the PDM individuals should be suitable to reveal bio-behavioural specificities highly relevant for the study of human mental illnesses. In a follow-up study, we will directly interfere with the rats' central serotonergic system and evaluate the impact of this intervention on the concomitant modulation of the PDM-associated traits.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bbr.2019.112188.

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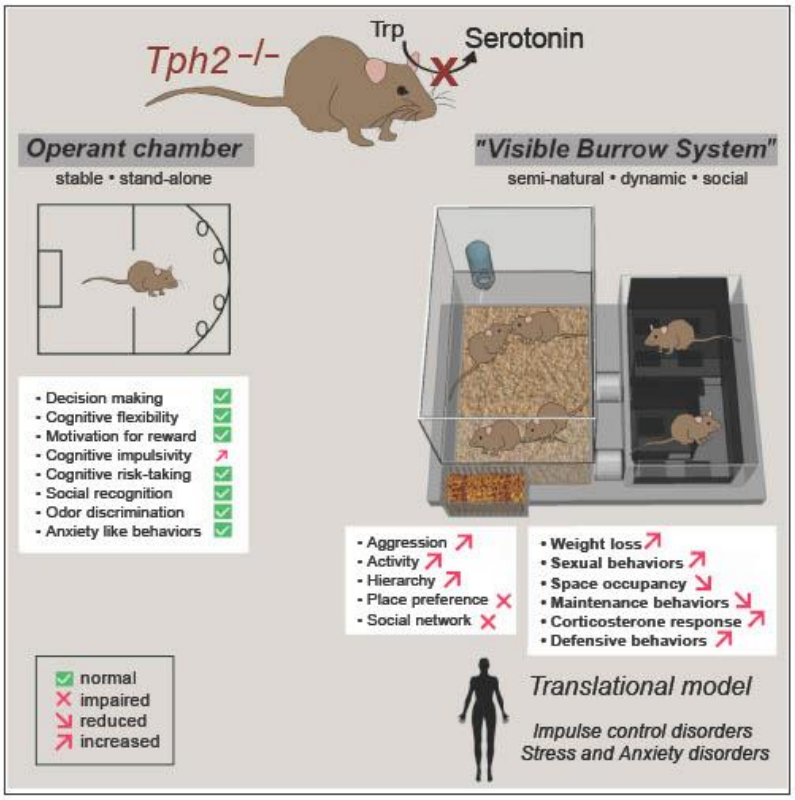

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Highlights

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

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

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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

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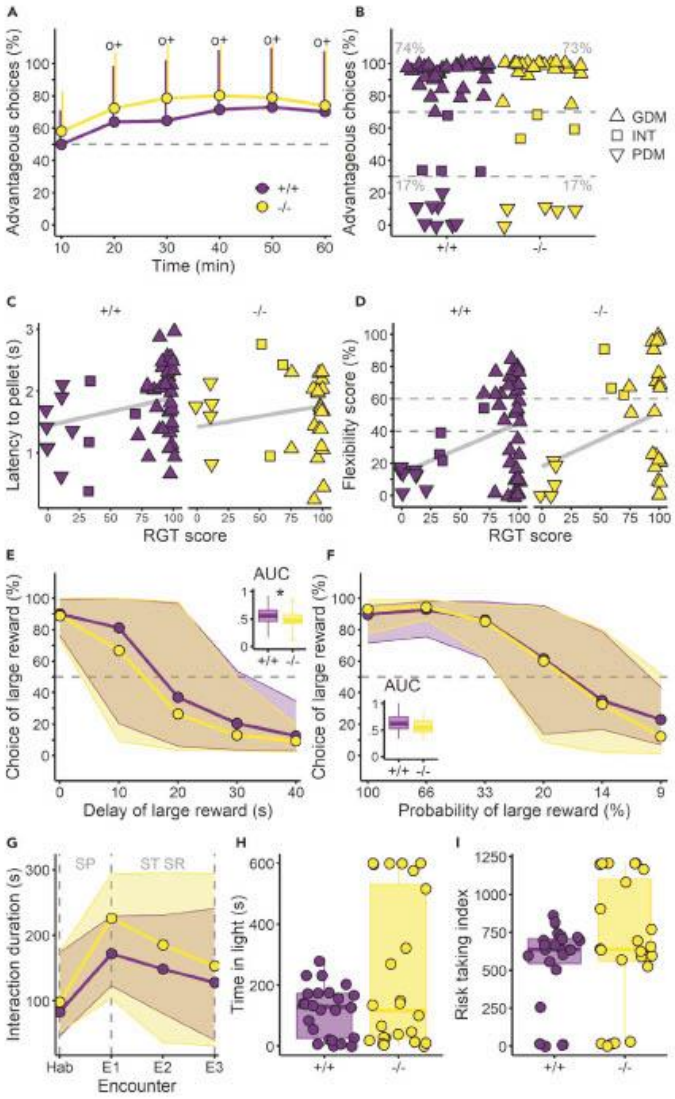

Figure 1. Cognitive abilities of the *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ rats

(A) Advantageous choices in the rat gambling task (RGT). Lines indicate mean +SD, one-sample t-test compared to 50% with $^{\circ}$ 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*: $F(1, 94) = 69$, $p < 0.001$].

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

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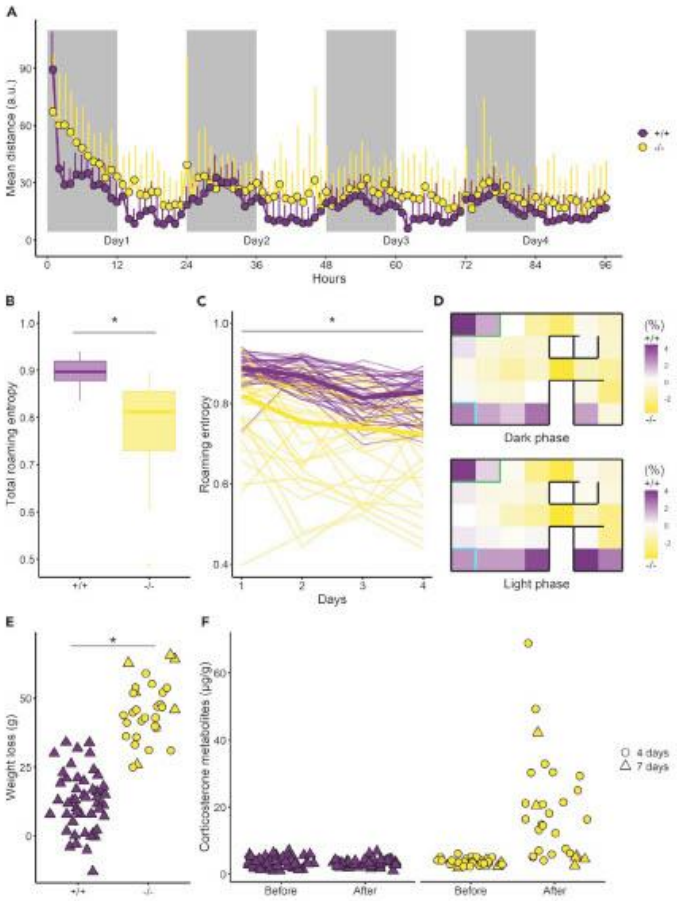

Figure 2. Activity, roaming entropy, and place preference of the *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ rats in the automated visible burrow system (VBS)

(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 before −/− 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.

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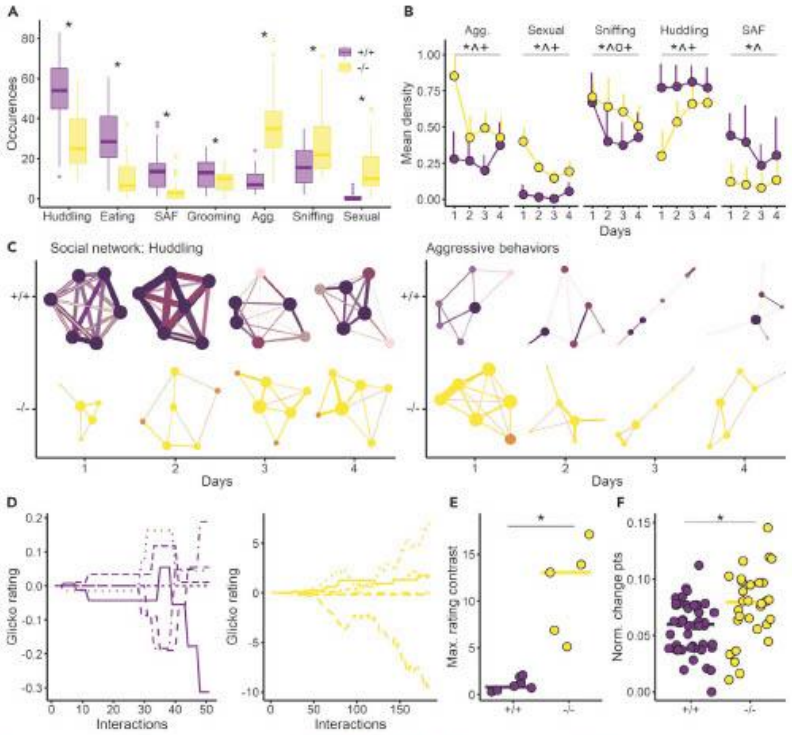

Figure 3. Social abilities and dominance of *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ in the automated VBS

(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 +SD, lmer genotype: *p value <0.001, lmer genotype x day: ^ p value <0.01, lmer day: ° p value <0.001 for +/+ and $^{+}$ 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 viewing 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

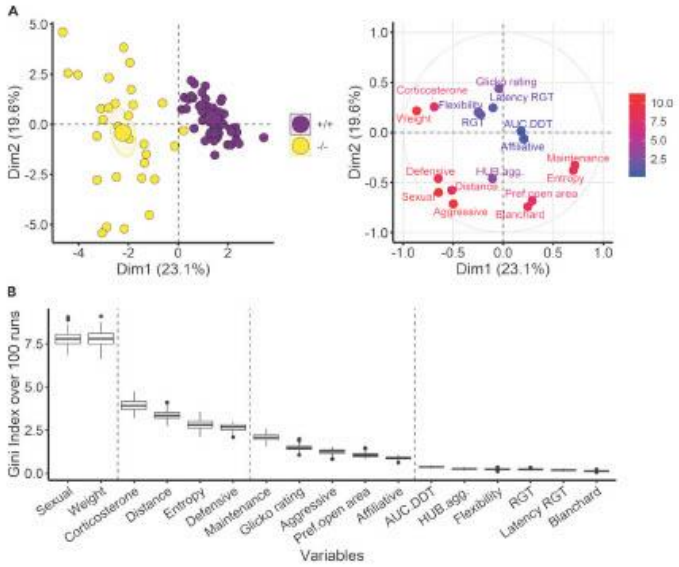

Figure 4. Principal component analysis (PCA) and random forest (RF) classification

(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.

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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.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-5α-pregnane-3β,11β,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-Tph2em2Mcwi
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, France[123]) and TPH2 rats[54] 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 (EurostandardType 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% +/−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 Guidelines[124] (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 training[53] 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 ½ and ¼ 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 ½ and ¼ 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 ≥70% on two following sessions with ≤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 ≤ 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 delay[125]; 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., 2012[42] previously described in Alonso et al., 2019[53] 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 ≥70% on two following sessions with ≤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 ≤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 odds[126] 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 design[127] (with unique time-out duration) is possible to 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 place 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 male 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 x 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 tests 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 index[131] 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 days[132] 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 score[141] 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: $RE_{i,d} = -\Sigma (p_{i,j,d} \log p_{i,j,d}) / \log(k)$ 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, 0.1g 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 BSA[122] 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 *^°+, 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.

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Article

Poor Decision Making and Sociability Impairment Following Central Serotonin Reduction in Inducible TPH2-Knockdown Rats

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Abstract: Serotonin is an essential neuromodulator for mental health and animals' socio-cognitive abilities. However, we previously found that a constitutive depletion of central serotonin did not impair rat cognitive abilities in stand-alone tests. Here, we investigated how a mild and acute decrease in brain serotonin would affect rats' cognitive abilities. Using a novel rat model of inducible serotonin depletion via the genetic knockdown of tryptophan hydroxylase 2 (TPH2), we achieved a 20% decrease in serotonin levels in the hypothalamus after three weeks of non-invasive oral doxycycline administration. Decision making, cognitive flexibility, and social recognition memory were tested in low-serotonin (Tph2-kd) and control rats. Our results showed that the Tph2-kd rats were more prone to choose disadvantageously in the long term (poor decision making) in the Rat Gambling Task and that only the low-serotonin poor decision makers were more sensitive to probabilistic discounting and had poorer social recognition memory than other low-serotonin and control individuals. Flexibility was unaffected by the acute brain serotonin reduction. Poor social recognition memory was the most central characteristic of the behavioral network of low-serotonin poor decision makers, suggesting a key role of social recognition in the expression of their profile. The acute decrease in brain serotonin appeared to specifically amplify the cognitive impairments of the subgroup of individuals also identified as poor decision makers in the population. This study highlights the great opportunity the Tph2-kd rat model offers to study inter-individual susceptibilities to develop cognitive impairment following mild variations of brain serotonin in otherwise healthy individuals. These transgenic and differential approaches together could be critical for the identification of translational markers and vulnerabilities in the development of mental disorders.

Keywords: tryptophan hydroxylase 2; TPH2; serotonin; tetracycline responsive system; inducible knockdown; vulnerability; network analysis; rat

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1. Introduction

Mental health is a dynamic process and alternation between phases of deterioration and improvement of health is one hallmark of mental illness. In regard to the current lack of specific and universal treatments of mental disorders [1], identifying individual-specific targets to prevent a transition from adaptive to pathological mental states is essential [2].

Along the continuum between adaptive and maladaptive behavioral dimensions, vulnerable individuals at a higher risk for the development of psychiatric disorders would present a combination of interconnected preserved and impaired behaviors [3]. Following the network approach to mental disorder, a higher connectivity within such networks of symptoms is an objective characteristic of vulnerability to pathology [4]. In humans, making repeated poor decisions in everyday life is known to lead to long-term disadvantageous outcomes and a general deterioration of mental health. A poor decision-making ability is one common symptom of most human mental disorders [5]. In the Iowa Gambling Task, a test of decision making under everyday uncertain conditions of choices, approximately 30% of non-clinical populations present similar decision-making deficits as clinical populations [6–8]. In previous studies, we identified a subpopulation of healthy rats whose primary deficit consists of less advantageous strategies of choice under uncertain and complex conditions of choice as tested in the Rat Gambling Task (RGT) [9,10]. Healthy poor decision makers are consistently found between labs [11,12], across strains (WH, DA [9] and SD (this paper and [11,12])), and across species (mouse [13], primate and monkey [14]). In rats, poor decision makers present a unique combination of preserved and impaired neurological and behavioral characteristics [9,10,15–17]. They express high reward-seeking and risk-seeking behavior, inflexibility, and a tendency to dominant behavior together with normal control of cognitive impulsivity, economical risk taking, and a normal social everyday life [9,16]. In addition, poor decision makers exhibit an imbalance in brain monoaminergic neurotransmitters and a smaller and weaker cortical–subcortical brain network activated during the RGT [18]. With such a vulnerable profile it is, however, not known if poor decision makers are indeed more vulnerable to an acute biological change in the way that it would impair their phenotype more than the phenotype of other individuals.

Central serotonin is an essential neuromodulator for mental health and a promising transdiagnostic marker of mental illness. Selective serotonin reuptake inhibitors can be prescribed following adverse life events in order to boost the central serotonergic system and improve coping processes when facing, for example, grief, unsolicited work termination, or seasonal affective disorder [19–21]. In animals, the models of choice to cause an acute biological perturbation are genetic On–Off inducible models [22]. The new TetO-shTPH2 transgenic rat [23,24] is a knockdown model that targets serotonin synthesis to create a mild acute drop in central serotonin. The application of Doxycycline (Dox) in the drinking water of TetO-shTPH2 rats induces the expression of shRNAs against messenger RNA of tryptophan hydroxylase 2 (TPH2), which results in a decrease of up to 25% in brain serotonin levels [23]. In this study, we used TetO-shTPH2 rats to test the impact of acute moderate brain serotonin imbalance on cognition and depending on individual spontaneous decision making type. We focused on complex decision making and risky based decision making, cognitive flexibility, and social recognition memory which are serotonin-dependent transdiagnostic symptoms of psychiatric disorders. We explored how these functions interacted with each other using a novel behavioral network analysis. Following our hypothesis of increased vulnerability in poor decision makers, we expected the moderate drop in serotonin function to impair more specifically their behavior and that it would also reflect on the properties of their behavioral network.

2. Results

In this study, we performed a battery of behavioral tests comparing Dox-treated Te-tO-shTPH2 rats (Tph2-kd) rats with a control group (Tph2-wt) consisting of Dox-treated wild-type Sprague Dawley (SD) rats and untreated TetO-shTPH2 (TetO-water) rats (Figure 1).

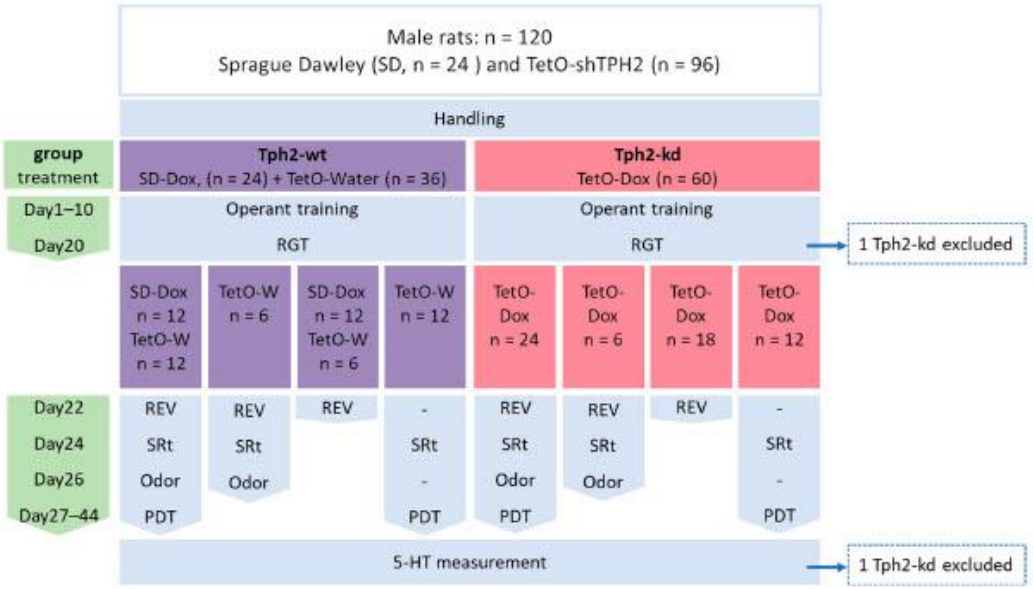

Figure 1. Flow chart for reporting attrition and experimental design. Dox treatment started at Day1 with operant training. Five behavioral tests were run: Rat Gambling Task (RGT) with n = 60 Tph2-wt and n = 60 Tph2-kd; reversed-RGT (REV) with n = 48 Tph2-wt and n = 48 Tph2-kd; social recognition task (SRt) with n = 42 Tph2-wt and n = 42 Tph2-kd; odor discrimination test (Odor), a control measure of the olfactory ability with n = 24 Tph2-wt and n = 24 Tph2-kd; and probability discounting task (PDT) with n = 36 Tph2-wt and n = 36 Tph2-kd. Two Tph2-kd rats were excluded from the study: one animal excluded from RGT, reversed-RGT, and subsequent decision-making subgroups analysis because it did not perform correctly in the RGT (its motivation was low and it paused during the test; its pattern of choice did not fit any known pattern); and one animal was excluded from the whole analysis because the serotonin (5-HT) level was higher than mean + 2 standard deviations.

In both groups, each animal showed either one of the three typical decision-making strategies observed in the RGT (Figure 2A). All animals started the test at 50% of advantageous choices (Figure 2A, Kruskal–Wallis rank sum test, chi-squared = 10.2, df = 5, *p*-value = 0.067, after 10 min: Tph2-wt-gdm 56 + 17 (mean + sd), Tph2-wt-int 52.5 + 17, Tph2-wt-pdm 38.5 + 24, Tph2-kd-gdm 59.6 + 17, Tph2-kd-int 52 + 11, Tph2-kd-pdm 39.2 + 18). For good decision makers (upward triangles) the percentage of advantageous choices increased until reaching a very high preference at the end of the test (above 70%). For poor decision makers (downwards triangles) the percentage of advantageous choices decreased until reaching a very low preference at the end of the test (below 30%). For intermediate individuals (squares) the percentage of advantageous choices stayed at approximately 50% until the end of the test (between 70% and 30%). More disadvantageous choices (RGT score < 30%) were made by Tph2-kd rats than by Tph2-wt rats (Figure 2B left, Wilcoxon rank sum test, W = 2112.5, *p*-value = 0.045). The difference between the Tph2-wt and Tph2-kd groups was found in the proportion of individuals in each decision-making category, with a higher proportion of poor decision makers in Tph2-kd than the Tph2-wt group (Figure 2B right, Fisher's exact test, *p*-value = 0.044). The distribution density of each group similarly illustrated the decrease in individuals showing good decision making strategy after an acute serotonin drop in Tph2-kd animals (Figure 2B right). The latency to collect the reward was dependent on the decision-making group (Figure S1, p.anova, RGT score, F(1,115) = 38, *p*-value < 0.001) and independent of the treatment group (Figure S1, p.anova,

treatment, F(1,115) = 0.14, *p*-value < 1.103). Behavioral flexibility was similarly expressed in Tph2-wt and Tph2-kd animals (Figure 2C). Good decision makers and intermediate decision makers expressed low to high flexibility indexes, whereas poor decision-making animals of both groups expressed low flexibility indexes (Figure 2C).

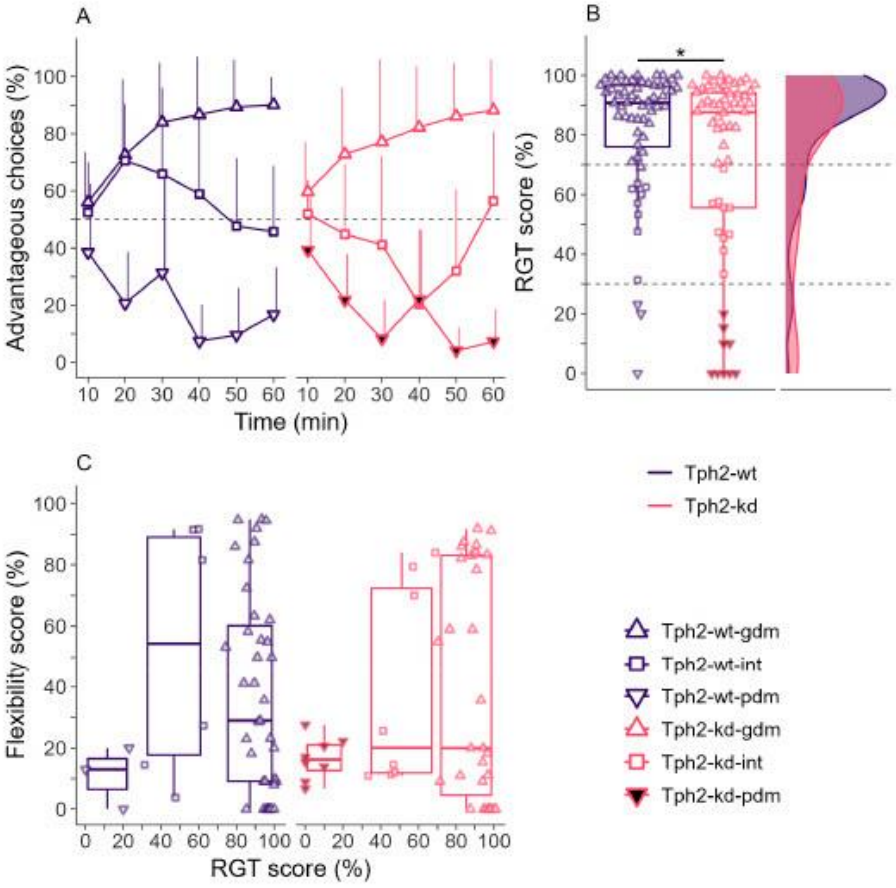

Figure 2. (**A**) Advantageous choices in the Rat Gambling Task (RGT) over time (10 min bins). Lines indicate mean + sd, dashed line shows 50% chance level. (**B**) **Left:** Individual (mean) scores during the last 20 min of the RGT (RGT score). Wilcoxon rank sum test, * *p*-value < 0.05. The dashed lines at 70% and 30% of advantageous choices visually separate good decision makers (gdm, upward triangle, above 70% of advantageous choices in the last 20 min), intermediates (int, square, between 30S% and 70% of advantageous choices in the last 20 min), and poor decision makers (pdm, downward triangle, below 30% of advantageous choices in the last 20 min). Individual data over boxplots. **Right:** Distribution density of final scores for Tph2-wt and Tph2-kd groups. (C) Flexibility scores in the reversed-RGT corresponding to the preference for the new location of the preferred option in the RGT for gdm (upward triangle), int (square), and pdm (downward triangle). The flexibility score is the preference for the location of the non-preferred option during the RGT. Individual data over boxplots. Boxplots classically represent the median, 25th and 75th percentiles, and 1.5 IQR. Panels (**A**,**B**): Tph2-wt, n = 60 (gdm, n = 49, int, n = 8, pdm, n = 3), Tph2-kd, n = 58 (gdm, n = 39, int, n = 10, pdm, n = 9). Panel (**C**): Tph2-wt, n = 48 (gdm, n = 39, int, n = 6, pdm, n = 3), Tph2-kd, n = 47 (gdm, n = 31, int, n = 8, pdm, n = 8).

The decreasing probability of obtaining the large reward induced a discounting effect on the preference for the large reward (Figure 3A left, p.anova, *probability*, F(4,256) = 153, *p*-value < 0.001). This discounting effect was stronger in the low-serotonin poor decision-maker group than in the other groups, as shown by the area under the curve (AUC), which was lower for the low-serotonin poor decision maker group (Figure 3A right, Kruskal–Wallis rank sum test, chi-squared = 13.02, df = 5, *p*-value = 0.023). All groups showed interest in the social partner (social preference, SP), as indicated by the increase in the interaction time during the first social encounter (E1) compared to habituation (Hab, Figure 3B left). Rats also formed a short-term social recognition memory (STM) of the social partner, indicated by the decrease in interaction time between E1 and the third encounter (E3, Figure 3B left, p.anova, *encounter*, F(3,234) = 202, *p*-value = 0). However, low-serotonin poor decision makers showed a lower decrease in interaction time from E1 to E3 (Figure 3B left, p.anova, *encounter* × *treatment* × *RGT*, F(3,234) = 3, *p*-value = 0.018) and the STM ratio for this group was not different from 1, unlike the other groups (Figure 3B right, Wilcoxon signed rank test with continuity correction, V = 9, *p*-value = 0.786), indicating a lack of social recognition. The odor discrimination ability was similar between the Tph2-wt, Tph2-kd, and decision making subgroups (Figure S2).

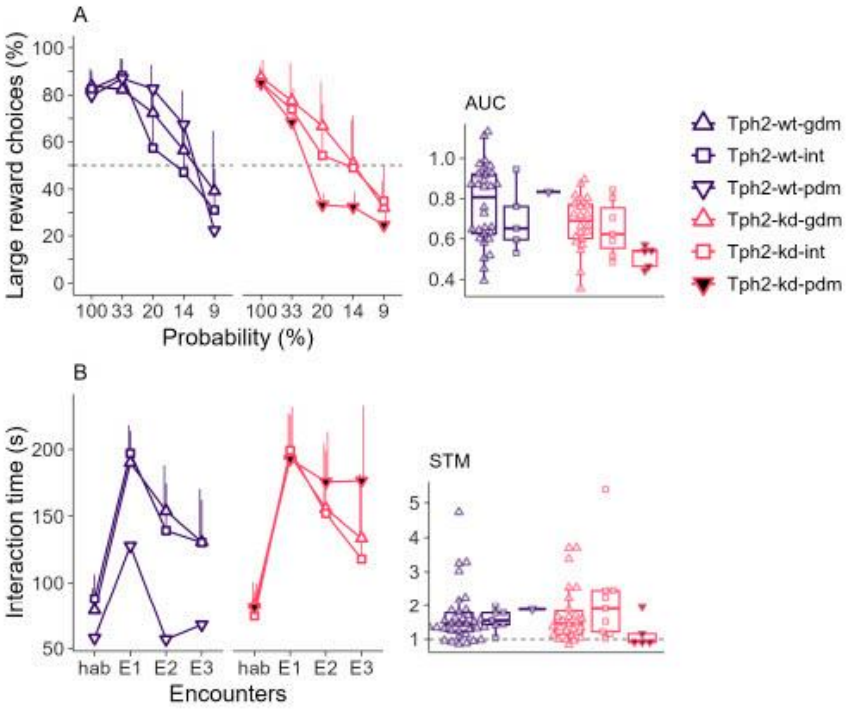

Figure 3. **(A) Left:** Choice of the large reward option as a function of the probability of reward delivery in the probability discounting task (PDT). Lines indicate mean + sd, dashed line shows 50% chance level. **Right:** Area under the curve (AUC) per individual. **(B) Left:** Duration of interaction in the social recognition task (SRt). Lines indicate mean + sd. Habituation with empty cage (Hab), successive encounters with same conspecific placed in the small cage (E1–3). **Right:** Short-term memory ratio (STM) between E1 and E3. Boxplots classically represent the median, 25th and 75th percentiles, and 1.5 IQR. Panel **(A)**: Tph2-wt, n = 36 (gdm, n = 30, int, n = 5, PDMs, n = 1), Tph2-kd, n = 34 (gdm, n = 22, int, n = 7, pdm, n = 5). Panel **(B)**: Tph2-wt, n = 42 (gdm, n = 34, int, n = 7, pdm, n = 1), Tph2-kd, n = 40 (gdm, n = 26, int, n = 9, pdm, n = 5).

We applied a network analysis to the data to understand the relationships between the different functions of the behavioral profile of low-serotonin poor decision makers compared to other Tph2-kd and control animals. In both the Tph2-kd and control groups, without poor decision makers, decision making (RGT score) and motivation for the reward (Lat, i.e., latency to collect reward) were strongly connected (Figure 4A,B). However, other strong pairwise connections differed between groups: in the control group, a strong connection between social preference and short-term recognition memory (SP-STM) was found, while in the Tph2-kd group, a strong connection between short-term recognition memory and motivation for reward (STM-Lat) was found (Figure 4A,B). The network of low-serotonin poor decision makers (n = 5) revealed a central position of STM with strong connections between STM and all other functions (Figure 4C).

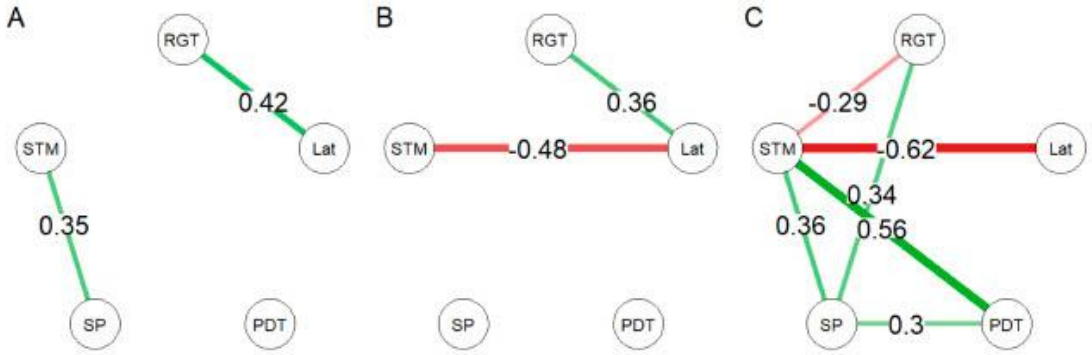

Figure 4. Network analysis of the behavioral profiles with Spearman's correlations. (**A**) Tph2-wt and (**B**) Tph2-kd groups without poor decision maker animals and (**C**) low-serotonin poor decision makers (from the Tph2-kd group). Edges between cognitive functions are Spearman's correlations, green edges for positive correlations and red edges for negative correlations. Only strong correlations ($r > 0.3$) are indicated and thickness of the edge the strength of the correlation. RGT: Decision-making ability; Lat: motivation to collect reward; PDT: impulsive choices; SP: recognition of social novelty; STM: recognition of social familiarity. Panel (**A**): Tph2-wt without poor decision makers, n = 35. Panel (**B**): Tph2-kd without poor decision makers, n = 29. Panel (**C**): low-serotonin poor decision makers, n = 5.

After the behavioral tests, we examined how effectively the Dox treatment reduced serotonin metabolism in Tph2-kd rats. The Dox treatment induced on average a 21% decrease in serotonin levels in the Tph2-kd rats compared to the control group (sd = 23, Figure 5A, Wilcoxon rank sum test with continuity correction, W = 2259, *p*-value < 0.001). The 5-HIAA levels were also decreased by 25% on average (sd = 23, Figure 5B, Wilcoxon rank sum test with continuity correction, W = 2825, *p*-value < 0.001). As expected, the tryptophan levels were stable between groups (Figure 5C, Wilcoxon rank sum test with continuity correction, W = 2034, *p*-value = 0.1141). The ratio of 5-HIAA/TRP indicated a consistent decrease in serotonin metabolism in Tph2-kd animals independent of the duration of the treatment (Figure S3, p.anova, treatment, $F(1,107) = 75$, *p*-value < 0.001, duration, $F(1,7) = 0.23$, *p*-value = 0.719) and showed variation in the 5-HIAA/TRP decrease between batches. Due to a technical problem, for batch 12, 5-HT could not be measured correctly; nevertheless, the 5-HIAA/TRP ratio showed the effect of the treatment for these animals (Figure S3).

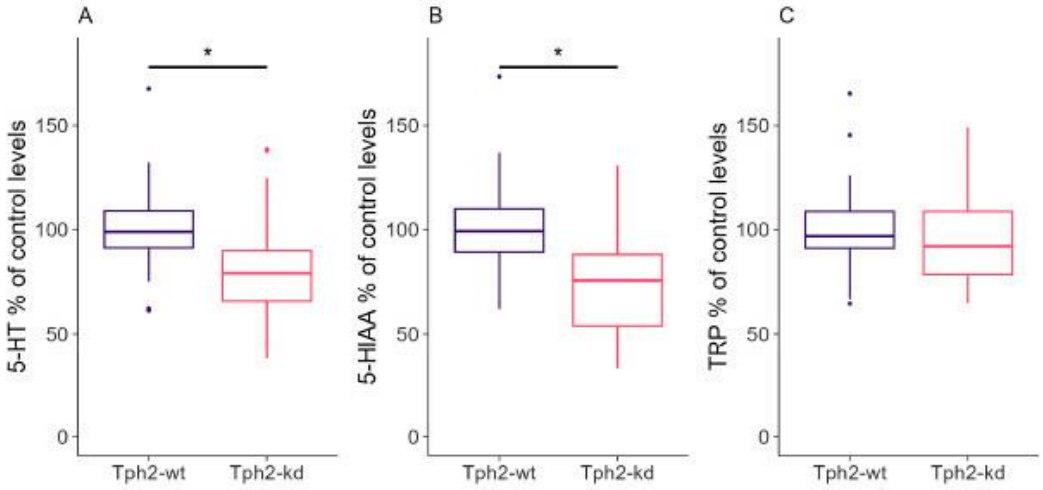

Figure 5. Levels of (**A**) serotonin (5-HT), (**B**) 5-HIAA, and (**C**) Tryptophan (TRP) in the hypothalamus. Values were normalized to control (Tph2-wt) individuals within each batch; one batch was excluded from (**A**) due to a technical problem in serotonin detection. Boxplots classically represent the median, 25th and 75th percentiles, and 1.5 interquartile range (IQR). * p-value < 0.05. Panel (**A**): Tph2-wt, n = 54; Tph2-kd, n = 54. Panels (**B**,**C**): Tph2-wt, n = 60; Tph2-kd, n = 59.

3. Discussion

In this study, an acute and mild reduction of central serotonin in Teto-shTph2 rats resulted in an increased number of individuals making disadvantageous choices in conditions of uncertainty of the RGT. The link between serotonin function and poor decision making was previously shown using equivalent tests of the RGT in systemic dietary or pharmacological approaches [25,26]. In this study, looking at spontaneous individual differences in decision-making strategies, we demonstrated that a reduction in central serotonin levels in previously healthy individuals did not affect all animals uniformly but only a subgroup of them. This discrepancy between studies on the impact of serotonin dysfunction—in all individuals (other studies) versus only some individuals in the population (current study)—could be attributed to the attention we gave here to individual differences as well as the advantages of using the refined model of TetO-shTph2 rats. This rat model offers a temporary, moderate, and physiologically relevant variation in serotonin levels, triggering impairments in the most spontaneously vulnerable individuals. This serotonin drop is brain specific and can be modulated during a specific time window due to the inducible and reversible nature of the model [24]. Therefore, this model prevents the confounding impact of developmental compensatory mechanisms of knock-out models [15] and other off-target effects of classical non-genetic approaches.

As expected, low-serotonin poor decision makers presented a unique combination of cognitive impairments otherwise preserved in wild-type poor decision makers [9] or in the low-serotonin good and intermediate decision makers. Low-serotonin poor decision makers presented deficits in social recognition memory and probability-based decision making, in addition to the typical hypersensitivity to reward and cognitive inflexibility, traits commonly observed in wild-type poor decision makers [9,16]. This combination of deficits in vulnerable individuals was specific to the acute, moderate, and brain-specific decrease in serotonin function.

In the social recognition test, although low-serotonin poor decision makers exhibited the typical social preference for novel subjects, they maintained a longer investigation time for familiar subjects, indicating a lack of habituation possibly due to a deficit in short-term

memory and social recognition of familiarity. Serotonin signaling is known to control social recognition memory [27–30] and to be critical for the adaptation of social behaviors in the home-cage environment [15,31]. In conditions of degraded serotonin function, vulnerable individuals might present increased difficulties in the integration and transmission of social cues to adjust behavior. In the probability discounting task, at the indifference point (P = 20), although both available options were mathematically equivalent in the total amount of food they provided, low-serotonin poor decision makers, but not the other groups, switched preference for the more certain option where a (small) reward is always delivered. This behavior indicated an increased intolerance to the risk of missing a reward. Interestingly, in the RGT, the poor decision makers' hypersensitivity to immediate reinforcement was found to be one key driver of their choice in the test [16]. While typically healthy poor decision makers are not impaired in the probability discounting task (PDT) and are able to choose following the absolute amount of reward of each option in this task [9,15], here, associated with a drop in serotonin function, the poor decision makers presented an increased focus on short-term, immediate, and certain rewards vs. long-term rewards. This is in line with the role of serotonin in the anticipation of a future reward and the encoding of reward value [32–35].

Interestingly, despite the known effect of serotonin on behavioral flexibility [36–38], in this study behavioral flexibility was not worsened in the low-serotonin group compared to controls for each decision making subgroup. Perhaps the decrease in serotonin function should be more pronounced and/or the reversal task more complex for an effect of serotonin on behavioral flexibility to be evident. It would be interesting to test the Tph2-kd rats in other behavioral flexibility tests, such as reversal learning [39,40], and in more complex and ethological conditions [41] to better understand the specific role of central serotonin in behavioral flexibility.

Here, we showed that a moderate, realistic, and brain-specific decrease in serotonin levels have a particular effect on a subset of vulnerable individuals only. In these animals, low serotonin levels affected their ability to socially habituate and to make decisions in uncertain (probabilistic) conditions by adopting a "short term" strategy focused on immediate and certain gains and in line with an increase in sensitivity to rewards. Considering the rewarding effect of social interactions [42], beyond impaired recognition, sustained interest for a social partner could reflect a generalization of high motivation for reward from food to social interactions. The role of serotonin in modulating social and non-social cognition is well documented [25,43]. However, it is not yet known on which function(s) serotonin could have a primary impact and if impairments then propagate to other functions. Considering the centrality of social recognition memory in the behavioral network of the low-serotonin poor decision makers, an alternative view of our results could be that serotonin alters primarily social cognition and, by diffusion, alters the other connected cognitive functions within the network, especially risky probability-based decision making. This would not be the first time that the importance of studying social cognition as a potential origin point for the modulation of other executive and seemingly non-social functions has been pointed out. In humans, for instance, network approach studies have emphasized the importance of social cognition for executive function and the ability to perform daily essential activities in schizophrenia patients [44,45]. Although we did not assess decision making before inducing the decrease in serotonin, it could be assumed that a pre-existing vulnerability of the poor decision making profile could have made them more sensitive to the decrease in serotonin function and induce the impairments seen in this study. This could explain why other low-serotonin non-poor decision makers were not similarly affected by the mild central serotonin decrease. Also, following our exploratory network analysis, we propose to further investigate the properties of the behavioral network of poor decision makers in normal and "pathological" low-serotonin conditions. Longitudinal experiments in a group-housed complex semi-natural environment, for example, will increase the ethological validity of the behavioral network analysis by informing about the temporal relationships between specific traits of poor decision makers. This would contribute to

the understanding of the role of social cognition in the transition to a pathological state, as a target to prevent the establishment of a psychopathology and challenge the role of serotonin as a triggering factor of psychopathology contributing to the current debate on the serotonin hypothesis of psychiatric disorders [46–48]. Further studies should apply the combination of the differential, multidimensional, and genetic approaches presented here to model, at the individual level, the transition phenomenon from an adaptive to a pathological state and to reveal the emerging markers of pathology in spontaneously vulnerable individuals.

In this work, some limitations can be pointed out. First, we only studied male rats. Sex differences in decision making, however, exist in both animals [49,50] and humans with strong serotonergic neurobiological correlates [51]. It is of prime importance to extend our study to female rats in order to evaluate the impact of a serotonin decrease on the cognitive functions at the group level and on the interaction between functions, especially in female "low-serotonin poor decision makers". It was shown recently that sex differences in decision making may be underpinned by distinct cognitive mechanisms [52], which are critical for the study of vulnerability. Second, levels of 5-HT, 5-HIAA, and tryptophan could vary between batches of animals (Figure S3), although procedures were implemented to reduce variability. Those procedures are reported in the Materials and Methods. Thirdly, administering the drug in drinking water is not the most precise method, but it remained the best option for the chronic behavioral study we present here. To ensure the dose of Dox remained as consistent as possible, we adjusted the concentration of Dox according to the actual body weight and drinking volume of the animals.

4. Materials and Methods

4.1. Animals

We used 96 male TetO-shTPH2 transgenic rats of Sprague Dawley background (SD) and 24 male SD rats (Figure 1). Control animals (Tph2-wt) included 36 TetO-shTPH2 rats treated with water (TetO-water) and 24 SD rats treated with Dox (SD-Dox). Tph2-knockdown (Tph2-kd) group included the 60 TetO-shTPH2 rats treated with Dox (TetO-Dox). Number of animals for each test and exclusion criteria are reported in Figure 1. Animals were tested by group of twelve individuals called a batch and consisting of six controls and six Tph2-kd. Eight out of ten batches of animals were tested at the same time in pairs. Pairs of batches are indicated in Figure S1.

Animals were born at the Max Delbrück Center for Molecular Medicine, Berlin, and transferred to the animal facility of Charité—Universitätsmedizin Berlin between nine and ten weeks of age. They were housed in standard rat cages (Eurostandard Type IV, 38 cm × 59 cm) in pairs of animals receiving the same treatment (TetO-water, SD-Dox or TetO-Dox). Cages were maintained in temperature-controlled rooms (22 °C–24 °C and 45%–55% humidity) with inverted 12 h light–dark cycles. Animals had ad libitum access to water and to standard maintenance food (V1534-000, Ssniff, Soest, Germany). During operant training and testing, they were maintained at 95% of their free-feeding weight. After their daily operant testing, rats were fed up to 40 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 and drug treatment.

4.2. Treatment

Animals received Dox from the first day of operant training and until the end of the protocol in the drinking water of the cage (Figure 1). The Dox solution was prepared every three days at a dosage of 40 mg/kg of body weight. The concentration of the solution was adapted to the average water consumption and body weight of the animals. For the batches tested in pairs, Dox solutions were prepared from the same supply bottle by the same experimenter and at the same time to apply the same conditions to both batches.

4.3. Sacrifice and Brain Collection

Two days after the last test, whatever the length of the protocol, rats were anesthetized via an intraperitoneal injection of Ketamine (100 mg/kg) and Xylazine (10 mg/kg) under isoflurane anesthesia. Pairs of batches were sacrificed by the same experimenters over two days. Animals were transcardially perfused with phosphate-buffered saline. Brain parts were immediately collected, snap-frozen on dry ice, and stored at −80 °C until further use. Orbitofrontal area, prefrontal area, hippocampus, hypothalamus, and raphe were dissected. Brain tissue was weighed after freezing. After the first pilot batches tested in pair (11 and 12), the time of the sacrifice was controlled to prevent circadian effects on the measurements. Sacrifice started one hour after the start of the dark phase except for batch 12, for which it started five hours before the dark phase.

4.4. HPLC Analysis

For the determination of the dosages of monoamines and their metabolites in brain tissue, frozen tissues were homogenized in 300 µL lysis buffer containing 10 µM ascorbic acid and 1.8% perchloric acid using a FastPrep system (VWR, Darmstadt, Germany). Samples were centrifuged for 30 min at 13,000 rpm. Supernatants were transferred in Eppendorf tubes and stored at −80 °C until HPLC measurement. Tissue levels of TRP, 5-HT and its metabolite 5-HIAA were analyzed using high sensitive HPLC with fluorometric detection (Shimadzu, Tokyo, Japan). Sample separation took place at 20 °C on a C18 reversed-phase column (OTU LipoMareC18, AppliChrom Application & Chromatography, Oranienburg, Germany) using a 10 mM potassium phosphate buffer, pH 5.0, containing 5% methanol with a flow rate of 2 mL min^{-1}.

Calculation of substance levels was based on external standard values. Amounts of 5-HT, 5-HIAA, and TRP were measured in hypothalamic samples and normalized to the wet tissue weight for statistical analysis. Individual concentrations of 5-HT, 5-HIAA, and TRP were normalized per batch to the mean of the control individuals and are presented as percentage.

4.5. Behavioral Testing

Animals were grouped in batches of 12 animals (6 Tph2-wt and 6 Tph2-kd) and 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 1 h after start of dark phase and within less than 3 h (5 h for the social recognition test).

4.5.1. Operant System

The four operant cages (Imetronic, Marcheprime, France) contained on one side a curved wall equipped with two or four nose-poke holes, 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 Cat#1811155, TestDiet, St. Louis, MO, USA). A separator with a 10 × 10 cm aperture was placed in the middle of the cage. The same light conditions were applied to the four cages.

4.5.2. Rat Gambling Task (RGT)

For the RGT, operant cages were equipped with four nose-poke holes arranged in two pairs (5 cm between holes) on each side of the curved wall (12.5 cm between pairs). The training and testing procedures [9,10,15,16] were adapted to the treatment period required by the tetO system. The training started at day 1 of the Dox treatment (Figure 1). First, rats learned to poke into the nose-poke holes and retrieve the associated reward (1 pellet) into the magazine. Training 1 was completed when 100 pellets had been collected in 30 min (cut-off). Training 2 consisted of poking two consecutive times into the same hole to obtain a reward (1 pellet). Training 2 was completed when 100 pellets had been collected in 30 min

(cut-off). Two consecutive nose-pokes into the same hole were considered as a choice for all operant tests. Then, training 3a consisted of a short session during which a choice to any hole was rewarded with 2 pellets within 15 min (cut-off and 30 pellets maximum). After that, a forced training [9] was given to those rats who had a preference above 60% for one of the two pairs of holes in 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; the two holes on the preferred side were inactive and not lit. Choosing the active holes induced the delivery of one pellet. After the collection of 15 pellets, the second part of the forced training started with the four holes active and lit. Choosing holes of the formerly preferred side induced the delivery of one pellet with a probability of 20%, whereas choosing the formerly non-preferred side induced the delivery of one pellet with a probability of 80%. The cut-off was 50 pellets or 30 min. The whole training was completed in seven to ten days. Rats were fed ad libitum from training completion until day 18 of the treatment (Figure 1). On day 19, a session of training 2 was performed in order to check the behavior of the rats and any side preference. A forced training was applied on the same day to the rats with a preference for one side superior to 80% (n = 4 animals). This criterion was refined depending on which side the preference was developed for the last three cohorts, the preference threshold was 80% for the side that would be advantageous during the test and 70% for the side that would be disadvantageous (n = 3 animals).

Testing took place on the twentieth day of treatment for 60 min. Two nose-poke holes on one side were rewarded with a large reward (two pellets) and associated with unpredictable long time-outs (222 s and 444 s with the probability of occurrence ½ and ¼, respectively). This was the disadvantageous option, leading to a lower maximum gain of pellets in 60 min. Two nose-poke holes on the other side were rewarded by one pellet and associated with unpredictable short time-outs (6 s and 12 s with the probability of occurrence ½ and ¼, respectively). This was the advantageous option, leading to a maximal gain of pellets within 60 min. The percentage of advantageous choices for the last 20 min of RGT was used to identify the sub-types of decision makers: good decision makers (GDMs) with more than 70% of advantageous choices, poor decision makers (PDMs) with less than 30% of advantageous choices, and intermediate animals in between. Computed over the 60 min of testing, the percentage of advantageous choices per ten-minute interval 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 magazine after a choice.

4.5.3. Reversed-RGT

The animals were tested in the reversed-RGT 48 h after the RGT. For this test, the two disadvantageous options were spatially switched with the two advantageous options [9,15,16]. A flexibility score was calculated as the preference during reversed-RGT for the location of the non-preferred option during the RGT. Flexible rats had more than 60% of such choices during the last 20 min, undecided rats had between 60% and 40% choices, and inflexible rats had less than 40% choices.

4.5.4. Probability Discounting Task (PDT)

For the PDT, operant cages were equipped with two nose-poke holes on each outer side of the curved wall (25 cm between holes). The protocol was adapted to reduce its overall duration [15] due to the pharmacological treatment. One hole (NP1) was associated with a small and sure reward (1 pellet), the second one (NP5) was associated with a large and uncertain reward (5 pellets) [25]. The delivery of the large reward was dependent on the probability applied that changed from high to low during the experiment: P = 1, 0.33, 0.20, 0.14, and 0.09. The probability was fixed for a day and increased the next day only after reaching the stability criterion. At least three training sessions (P = 1) were performed and a percentage of choice of the large reward ≥70% on two following sessions with ≤15% variation was required to start the test. During testing sessions (P < 1), the stability criterion was ≤10% variation of choice of the large reward during two consecutive sessions.

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 area under the curve (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 the training (P = 1) and plotted versus the probabilities expressed as odds, with odds = (1/P) − 1 [53].

4.5.5. Social Recognition Task (SRt) [15]

The test took place in a square open field (50 × 50 cm). A small clean and empty cage was placed in one corner of the open field shielded by walls to avoid the test rat hiding behind the cage. The unfamiliar conspecifics were older Wistar Han rats, accustomed to the procedure. A video camera on top of the OF recorded the experiment. The subject was placed in the OF containing the empty cage 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 five minutes (E1). After that the small cage with the conspecific was removed from the open field, a second clean and empty cage was used to fill the space, 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). The time spent in close interaction with the unfamiliar rat was measured for each encounter and for the first five minutes 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 memory was calculated as the ratio of the interaction time in E1 and E3.

4.5.6. Odor Discrimination Test [15]

The test took place in a square OF (50 × 50 cm). Two plastic petri dishes filled with either used or fresh bedding were placed in two opposite corners of the OF. Except for the first group, the dishes were taped onto the OF flour to avoid animals pushing them around. A video camera above 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 by trained observers using JWatcher (version 1.0) [54] and the preference for the used bedding (social odor) was calculated.

4.6. Statistical Analysis

The free software R (version R-3.6.1) and R studio (version 1.1.456) were used for the statistical analyses [55]. We used the Wilcoxon rank sum test to compare the Tph2-wt and Tph2-kd groups, the Kruskal–Wallis rank sum test to compare the decision maker groups against each other (GDMs, INTs and PDMs of Tph2-wt and Tph2-kd groups), Fisher's exact test to compare the number of GDMs and PDMs in each groups, and the Wilcoxon sign test (Package RVAideMemoire) [56] to compare the performance of the animals to a theoretical value in PDT, SRt, and odor discrimination test. We used ANOVA with permutations (package lmPerm) [57], which is suited for small groups and non-parametric data to compare the multiple groups and decision maker groups (dm-group) over several time points (probability, encounter), with animal as error factor.

4.7. Network Visualization of Behavioral Data

We used a network analysis method (Package qgraph) [58] to visually represent the strength of connections between pairs of behaviors, as behavioral networks of the groups and subgroups. According to the network approach of psychopathology, the visual representation of the connectivity between symptoms could inform about the potential dynamic existing between them in pathological context. In this study, we used this visualization to explore how the five tested cognitive functions connected to each other depending on their treatment and decision making profile. We were also interested to see if one function

would appear to be more central (in terms of number and weight of ties to other nodes) than the others.

The five parameters constituting the nodes of the networks were the RGT score, mean latency to visit the magazine after a choice during RGT, AUC of the PDT, social preference, and short-term social recognition memory. They represented complex decision-making ability, motivation for reward, risky decision-making ability, social preference, and social recognition memory, respectively. The reversed-RGT and odor discrimination tests were not performed by all animals and were not included in the networks. The network analysis was performed using the qgraph() function (type of graph "association") where correlations are used as edge weights between two nodes. Although partial correlations are usually preferred to correlations as they account for the relationships of the network for each pairwise link, it was not possible in our analysis to apply Spearman's partial correlation to the Tph2-kd poor decision maker group as the number of individuals equaled the number of functions. We used the Spearman's correlations (package Hmisc) for all groups. To simplify the description and visualization of the networks, only correlations above 0.28 were represented, and the networks were calibrated from 0 to 0.7. We calculated the strength centrality for each node, which is the centrality of a node taking into account the number and the weight of edges connecting to the node [59]. With only one control poor decision maker individual, no network could be computed for this subgroup.

5. Conclusions

The destabilization of the serotonergic system, using the TetO-shTph2 model, led to specific cognitive and social impairments in only a subset of vulnerable individuals from the general population. Besides their poor abilities to choose long-term advantageous feeding options, the low-serotonin poor decision makers expressed poor social recognition memory and a strong risk aversion in a probability-based decision-making task, whereas the individuals with a more adapted strategy were not affected by the temporary serotonin decrease in any other tests. The behavioral network of low-serotonin poor decision makers was deeply affected by the decrease in central serotonin. Taken together, these findings may suggest that social recognition memory is a key factor dependent on serotonin function and is at the core of a larger cognitive network. With this study, we show the high potential of the Teto-shTph2 rat to model in further specific studies the transition processes of psychopathological deterioration associated with a central serotonin drop.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The original data presented in the study and scripts are openly available at https://github.com/alonsolucille/TetO-shTPH2_rats.

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Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

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Repositories

Alonso L, Peeva P, Stasko S, Bader M, Alenina N, Winter Y, Rivalan M. 2021. Constitutive depletion of brain serotonin differentially affects rats' social and cognitive abilities. *Dataset on Zenodo* doi:10.5281/zenodo.7526514

alonsolucille (Lucille Alonso) · *Dataset and code on GitHub* https://github.com/alonsolucille

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