The serotonin transporter modulates decisional anhedonia

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Abstract

Serotonin transporter gene variance has long been considered an important factor contributing to depression. However, meta-analyses yielded inconsistent findings recently, asking for further understanding of the link between the gene and depression-related symptoms. One key feature of depression is anhedonia. Anhedonia is not one united feature but involves consummatory, anticipatory, and decisional aspects. These different aspects of anhedonia can be particularly well investigated in rodents. While data exist on the effect of serotonin transporter gene knockout in rodents on consummatory and anticipatory anhedonia, with mixed outcomes, the effect on decisional anhedonia has not been investigated thus far in rats. Here, we tested whether serotonin transporter knockout (5-HTT$^{-/-}$) contributes to decisional anhedonia. To this end, we established a novel touchscreen-based task employing a 'go/go' task for decision-making under ambiguity. Both genotypes of rats were able to reach 70% accuracy across three consecutive sessions. However, 5-HTT$^{+/+}$ rats performed more optimal decision-making compared to 5-HTT$^{-/-}$ rats during the course of discriminative conditioning for the conditioned stimulus associated with high reward value at the beginning. 5-HTT$^{+/+}$ rats gradually increased response time to generalization stimuli across the three generalization test sessions, while 5-HTT$^{-/-}$ rats tended to decrease response time. The monotonic graded responses towards the stimuli did not alter for both genotypes. The results suggest that the association between the serotonin transporter gene and anhedonia may dynamically change over time when processing ambiguous information. This may explain the inconsistent findings on the association between the serotonin transporter gene and various aspects of depression in humans.

Keywords: anhedonia; decision-making; ambiguity, generalization; serotonin transporter knockout rat

Introduction

According to the World Health Organization (WHO), depression is the leading cause of disability worldwide, and a major contributor to the global burden of disease. More than 264 million people worldwide suffer from depression (James et al. 2018). Some people are more vulnerable to develop depression than others (Caspi et al. 2010) and this vulnerability is shaped by multiple factors. The serotonin transporter (5-HTT) gene polymorphism presents one of these factors, specifically the short allelic variant of the serotonin-transporter-linked polymorphic region (5-HTTLPR). Some genetic meta-analyses studies reported statistical evidence for a relation between the 5-HTTLPR short allele and depression in the presence of life stress (Bleys et al. 2018; Karg et al. 2011) although others did not find this relationship (Border et al. 2019; Culverhouse et al. 2018). The discrepancies ask for a more behavioral approach and thus the identification of mechanisms that may link the 5-HTT gene to depression (Ormel et al. 2019). By regulating the function of the 5-HTT gene in animals, such as knocking out 5-HTT, it is possible to shed light on the nature of the potential relation between the 5-HTT gene and various aspects of depression.

Anhedonia is one of the essential features of depressive disorder, and may bridge the link between the 5-HTT gene and depression. Anhedonia has been conceptualized in multiple aspects, including consummatory ("liking"), anticipatory (“wanting”) and decisional (“choosing”) anhedonia (Treadway and Zald 2011; Der-Avakian and Markou 2012; Ho and Sommers 2013; Wu et al. 2017). While studies targeting anhedonia mostly address consummatory ("liking") and anticipatory ("wanting") anhedonia, decisional "choosing" anhedonia is less often considered. Treadway et al. proposed that decisional anhedonia of "choosing" refers to the ability to balance costs and benefits when selecting among multiple positive/rewarding options provided at the same time is impaired (Treadway and Zald 2011; Denk et al. 2005). However, most real-world decisions are based on ambiguous information (Huettel et al. 2006; Lauriola and Levin 2001). This type of decision-making is much related to emotion, not only in humans (Rustichini 2005) but also in animals (Nguyen et al. 2020). While positive or rewarding emotions may be experienced when the decision turns out to be a good one, untrustworthy ambiguous information triggers aversive emotions (FeldmanHall et al. 2018). To measure trustworthiness of information during decision-making we propose to add the aspect of “believing” to the concept of decisional anhedonia during decision-making under ambiguity. Hereby, to measure this, we designed a reward-only "go/go" decision-making task requiring rats to make decisions under ambiguity. Notably, some animal studies place ambiguous information interpretation experiments into the concept of cognitive/judgment bias, according to which subjects interpret ambiguous information as either negative or positive (Roelofs et al. 2016). We argue that if all information that is available is positive or rewarding, no negative interpretation is possible. Under such conditions, the hedonic value and trustworthiness of the information becomes critical in decision-making. Accordingly, reward-only ambiguous information interpretation experiments allow us to investigate the aspect of "believing" in decisional anhedonia.

To test the relationship between the down-regulation of 5-HTT and anhedonia, 5-HTT$^{-/-}$ rodents have been subjected to various anhedonia-related measurements. In an experiment where

animals could freely choose in their homecage between water and a sucrose solution, 5-HTT knockout rats (Olivier et al. 2008), but not 5-HTT knockout mice (Kalueff et al. 2006) show increased consummatory ("liking") anhedonia. In fixed ratio and progressive ratio tasks where rewards were presented to reinforce behavior, anticipatory ("wanting") anhedonia-like behavior has been observed in 5-HTT$^{-/-}$ mice (Sanders et al. 2007). Controversy, 5-HTT$^{-/-}$ rats showed anticipatory hedonic-like or addiction-like behavior when rewards were not presented to reinforce behavior (Nonkes and Homberg 2013; Nonkes et al. 2010). The controversy suggests that 5-HTT$^{-/-}$ rodents pay less effort to what they will gain if stimulus-associated-reward remains stable, but more effort to what they lost if stimulus-associated-reward is devalued. It also indicates that 5-HTT deficiency in rats might affect the processing of reward predicting conditioned stimuli. However, it is unclear whether and how 5-HTT deficiency affects the processing of the value properties of stimuli (e.g. reward value) and/or the physical properties of stimuli (e.g. ambiguity, influencing "believing" in decisional anhedonia). This insight is critical as anhedonia does not just involve hedonic capacity but also responsivity to pleasant stimuli (Der-Avakian and Markou 2012).

In this study, we sought to set out whether 5-HTT deficiency in rats affects decisional anhedonia in a reward-only ambiguous information interpretation study. To this end, we introduced a novel reward-based “go/go” decision-making task in a touchscreen operant box. The task allowed us to assess the ability of the animals to 1) discriminate between two reward-related conditioned stimuli (CSs) to measure the value property of stimuli, and 2) generalize the learned information from CSs to generalization stimuli (GSs) to measure the physical property (ambiguity) of stimuli. During CSs discrimination, rats were provided chances to acquire the maximum amount of rewards by paying effort to memorize the discrepancy of CSs and make optimal decisions based on the single CS temporarily presented. During stimulus generalization, rats paid efforts to categorize the GSs into the two CSs and to make decisions based on the stimulus temporarily presented.

Materials and Methods

Subjects

Ten wild-types (5-HTT$^{+/+}$) and ten serotonin transporter knockout (5-HTT$^{-/-}$) male Wistar rats aged between 68 and 84 days were served as subjects. The sample size was calculated based on our previous study(Nonkes et al. 2013). All rats were generated through homozygous breeding. The homozygous parents were derived from heterozygous 5-HTT knockout rats that had been outcrossed for at least 10 generations with commercial Wistar rats (Homberg et al. 2007). All animals were housed in pairs under a reversed day/night cycle (light on from 19:00 to 7:00). From 7:00 to 19:00, animals were housed under dim red light conditions. The animals had *ad libitum* access to chow and water in their home cages. All experimental procedures were approved by the Central Committee on Animal Experiments (Centrale Commissie Dierproeven, CCD, The Hague, The Netherlands).

Apparatus

Training and testing were performed in 10 operant chambers (Med Associates, USA) placed within sound-attenuated boxes. Each chamber was equipped with a pellet dispenser that delivered 45 mg sucrose pellet (TestDiet, USA) to a magazine when triggered. The boxes contained a magazine equipped with a light to indicate pellet delivery. A houselight for illuminating the chamber was mounted on the same wall above the magazine. The chambers contained a touchscreen positioned opposite to the pellet dispenser. The screen was equipped with a metal plate containing three windows. See Fig. 1A for an illustration of a rat in the chamber facing the touchscreen presenting a stimulus image in the middle and choice images on both sides. The highest intensity of illumination in the operant chambers was about 35 lux (Lutron, LX-1108, TW). Computers were installed with K-limbic software which controlled the equipment and collected data from subjects' responses.

Behavioral procedures

General

Each day before starting the experiment, rats stayed in a separate room for about 2 hours for habituation. At the same time, their chow was removed from their homecages. Rats had *ad libitum* access to chow in their homecages after behavioral training and testing. All rats were handled for 3 days before instrumental conditioning. Details about the conditioning is described as below:

Instrumental conditioning

Rats were freely exploring in the touchscreen box a session per day. Each session consisted of 30 trials. Each trial lasted for 30 seconds and trials were separated by a random inter-trial interval (ITI) between 15 and 30 seconds. During each trial, a small white circular disc (2.88π cm^2) was presented on the screen in the middle of the central window. Each time the rat touched the circular disk, a sucrose pellet was dispensed into the magazine as a reinforcer. This trial was marked as a successful trial. Rats could constantly touch the disc and pellets were dispensed continuously within the period of a trial. If a rat did not touch the disc during the trial period, the house-light was illuminated for 5 seconds starting at the end of the trial. Rats received sequential instrumental conditioning (see below) once they completed at least 10 successful trials in the last session.

Sequential instrumental conditioning

Rats in this stage were trained to associate touching a conditioned stimulus (CS) and a reporter stimulus (RS) located at different positions of the screen sequentially with acquiring rewards. The CSs were either a white square of 25.2 or 10.8 cm^2 (70% or 30% size of 36 cm^2) and therefore were named CS70 and CS30 respectively. The RS was the Chinese character '靈'. Fig. 1B represents the trial sequence. Each trial contained three sequential phases. For example, CS70 was presented in phase 1, and if it was touched, RS was presented randomly on the left or right side in phase 2. Randomization might eliminate position bias. If the RS was touched, two sucrose pellets were dispensed immediately in phase 3, and therefore this trial was named CS-2. Completion of a trial with reward as an outcome was marked as a successful trial. CS-1 trials were similar to CS-2 trials, except that rats were rewarded with one sucrose pellet in each

successful trial and the CS was replaced by CS30. Each trial lasted for 40 seconds and trials were separated by a random range of ITIs lasting between 15 seconds and 30 seconds. Rats were subjected to 20 CS70 trials and 20 trials CS30 per session in total and received one session per day. The order of each trial type was randomly assigned. CSs associated one or two sucrose pellets were counterbalanced across groups so that half of the CS-1 and CS-2 trials was presented with CS70 and the other half was presented with CS30. After receiving five consecutive sessions, rats were shifted to the next stage of discrimination conditioning (see below).

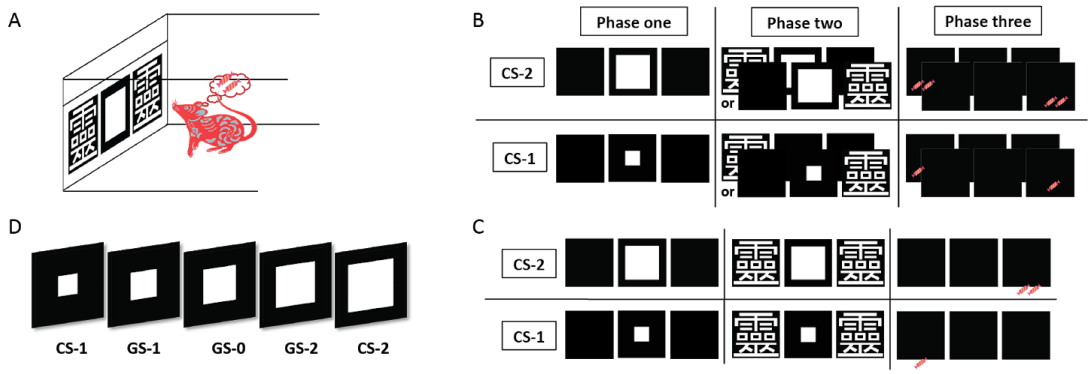


Fig. 1. Schematic view of the experimental procedures of decision-making under ambiguity. (**A**) A schematic view of a rat in an operant chamber with a touch screen. (**B**) The sequence of events during CS-1 and CS-2 instrumental conditioning trials. Rats were allowed to touch the white square image (phase one), which was followed by the random presentation of a reporter stimulus (RS) of 靈 on either the left or the right side of the screen (phase two). Then, rats were allowed to touch this single RS, leading to the delivery of one sucrose pellet for CS-1, and two sucrose pellets for CS-2, trials (phase three). (**C**) The sequence of events during the CS-1 and CS-2 discriminative conditioning trials. If rats touched the white square image (CS) trials required a touch on the left-side RS only. Similar to the stage of instrumental conditioning, in the stage of discriminative conditioning a correct response produced one sucrose pellet in CS-1 trials, and two sucrose pellets in CS-2 trials (phase three). An incorrect response initiated a correction trial. (**D**) A representation of all the stimuli during the generalization phase. CS-1 and CS-2 are the same as above (see figures 1B and 1C). The white square image of the GS-1 is larger than of the CS-1 while the white square image of the GS-2 stimulus is smaller than of the CS-2. The size of GS-0 is between the GS-1 and GS-2.

Discriminative conditioning

In this stage, rats had to pay efforts to memorize the discrepancy between CS70 and CS30 to acquire the rewards. To earn reward, they had to make decisions based on the CS temporarily presented within a trial. Each trial contained three sequential phases. Fig. 1C represents a trial sequence. For example in a CS-2 trial, the CS70 was presented in phase 1, and if it was touched, two RSs were presented on both left and right sides in phase 2. Subsequently, if the RS was

presented on the right side of the screen was touched, two sucrose pellets were immediately dispensed in phase 3. Completing a trial with acquiring a reward was marked as a successful trial. The sequence of phases for CS-1 trials was similar as for CS-2 trials, except that a different CS was presented in phase 1, and rats were rewarded with one sucrose pellet in a successful trial by touching the RS presented on the left side of the screen (see Fig 1C, CS-1). Correction trials were applied if rats touched the wrong RS or omitted the trial. During correction trials the same trial was repeated until rats made the correct response. Both the CS and the position of the target RS were counterbalanced across the rats. Rats were subjected to 40 trials (20 CS-1 and 20 CS-2 trials) per session. The order of the two CS trials was randomized in each session and no more than four consecutive trials were the same. Rats received one session per day until they successfully completed at least 70% of trials in each session of the last three consecutive sessions.

Generalization tests

The animals received three identical generalization sessions, conducted on three separate days. To make sure the discrimination accuracy was not declined before each generalization test, the discrimination accuracy of the CSs was tested between the generalization tests. If accuracy was less than 70%, rats were exposed to more sessions of discriminative conditioning until the accuracy was higher or equal to 70% at the last two sessions before the next day of the generalization test. In the generalization test, two CSs and three GSs were introduced. The square size of the GSs was 40% (GS40), 50% (GS50), and 60% (GS60) of 36 cm^2, respectively. The sequence of GS trials was similar to CS trials except that no reward outcome was delivered in phase 3 to reduce a reward conditioned effect on the ambiguity of the GSs. Fig. 1D presented the CS and GS trials applied during the generalization test. CS30 was presented in CS-1 trials and CS70 was presented in CS-2 trials, and GS40, GS50, and GS60 were named GS-1, GS-0, and GS-2, respectively. Each session consisted of 72 trials, in which GS40, GS50, GS60 each were presented during 12 trials and CS30, CS70 each during 18 trials. The 72 trials were divided into 12 blocks resulting in 6 trials per block. The inter-block interval was one minute. The order of the trials in each block was a CS followed by a GS to guard against the sequential effects of the same stimulus. The CSs and GSs were evenly distributed in each block and counterbalanced across blocks.

Data analysis

Data were statistically analyzed using JASP (version 0.11.1) for t-tests and logistic regression. Linear mixed-effect regression modeling was run in software R (version 3.6.2) and package lme4 (Bates et al. 2015) to reduce the probability of false positives (Aarts et al. 2014). Genotype, stimuli, and session were entered as fixed effects and subjects and date of the experiment were entered as random effects in the model. Visual inspection of the residual plot was used to check the heteroskedasticity of data distribution between fitted data and residuals. The P-value was derived from Wald chi-square tests using the package *car* (Fox and Weisberg 2018) for the mixed-effect analysis. P-values less than 0.05 were defined as significant.

Results

Discriminative conditioning

Rats had to pay efforts to memorize the discrepancy between CS30 and CS70 in order to acquire the maximum number of rewards by making optimal decisions when a single CS was temporally presented. Their decisions resulted in a discrimination performance during a session, which we termed the discrimination accuracy. It is defined as the percentage of correct trials in a session (correction trials were not included). The performance of discrimination accuracy across the sessions is presented in Fig. 2A. A Student t-test indicated that 5-HTT$^{-/-}$ and 5-HTT$^{+/+}$ rats needed a similar amount of sessions to reach the 70% accuracy of pooled CSs for consecutive three sessions ($t_{(18)}$=-1.204, p=0.244, d=0.538). CSs were associated with either one sucrose pellet or two sucrose pellets. To acquire the maximum number of pellets, rats had to acquire how to make optimal decisions across sessions during both CSs trials. Logistic regression was performed to assess rats' performance of discrimination accuracy for each of the CS-1 and CS-2 trials. For the CS-2 trials associated with two sucrose pellets (see Figure 2B), the logistic regression model was statistically significant ($\chi^2_{(251)}$=104.150, p<0.001, McFadden's R^2=0.315). Both session and genotype had a significant effect on the outcome of 70% accuracy (p<0.001 and p=0.036 respectively). Increasing sessions was associated with an increased likelihood of 70% accuracy for CS-2 trials per session, but 5-HTT$^{-/-}$ genotype reduced the probability of 70% accuracy to 50.1%. For the CS-1 trials associated with one sucrose pellet (Figure 2C), the logistic regression model was also statistically significant ($\chi^2_{(251)}$=7.574, p=0.023, McFadden's R^2=0.033) and sessions had a significant effect on the outcome of 70% accuracy (p=0.007), but no significant effect of genotype was observed (p=0.968). Although the accuracy for pooled CSs across 3 consecutive sessions was 70%, rats achieved an accuracy higher in CS-2 than in CS-1 trials (see Figure 2D, $\chi^2_{(1)}$=168.880, p<0.001). There were no other significant effects (genotype: $\chi^2_{(1)}$=0.176, p = 0.674; session: $\chi^2_{(1)}$=0.171, p=0.679; genotype*session: $\chi^2_{(1)}$=0.0315, p=0.859).

In summary, 5-HTT$^{+/+}$ rats performed more optimal decision-making compared to 5-HTT$^{-/-}$ rats during the course of discriminative conditioning for CS-2 trials. Yet, both genotypes were able to reach 70% accuracy criterion of pooled CSs with across three consecutive sessions at the end.

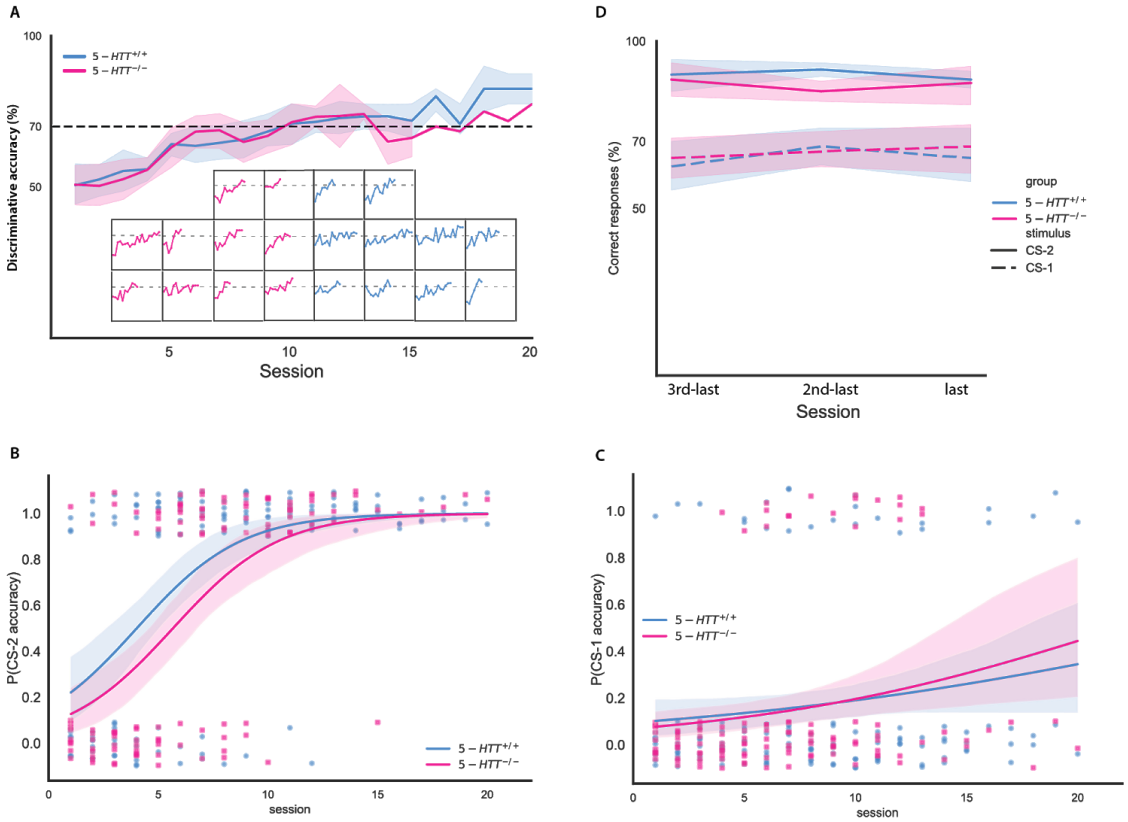


Fig. 2. **Discriminative conditioning.** (A) Solid lines represent the mean of discrimination accuracy of pooled CSs in each genotype. The transparent stripes are 95% confidence intervals. Figures embedded in panel A are the individual performance data for discrimination accuracy of pooled CSs in every session. (B) As sessions progressed the probability of reaching 70% accuracy increased for CS-2. The probability of reaching 70% accuracy for the CS-2 was lower in 5-HTT$^{-/-}$ versus 5-HTT$^{+/+}$ rats. (C) The probability of reaching the discrimination criterion for the CS-1 increased as the session progressed. There were no significant differences between 5-HTT$^{-/-}$ versus 5-HTT$^{+/+}$ rats for CS-1. (D) Blue and red solid curves represent the mean accuracy for CS-2 and blue and red dashed curves the mean accuracy for CS-1. The transparent strips around the mean estimators are 95% confidence intervals. Accuracy in CS-2 was higher than in CS-1 for both genotypes.

Generalization tests

Rats pay efforts to generalize the learned information from CS30 and CS70 to GS40, GS50 and GS60 based on the physical property (similarity or ambiguity) among the stimuli by making decisions on the stimulus currently presented on the screen. If the physical property of a GS is close to one of the CSs, the decision on the GS is typically closer to the CS, resulting in a monotonic graded response to the stimuli (Ghirlanda and Enquist 2003). Fig. 3A and 3B present a linear mixed-effect model showing that there is a significant effect of stimulus similarity on decision-making. Thus, as the physical property (similarity) was closer to CS-2 (associated with

two sucrose pellets), the decisions were more robust ($\chi^2_{(4)}$=7733.518, p<0.001). Furthermore, the individual performance of stimulus generalization shows monotonic graded responses (see Suppl Fig. 1). However, no significant genotype or stimulus*genotype effect was observed (genotype: $\chi^2_{(1)}$=0.0022, p=0.962; stimulus*genotype: $\chi^2_{(4)}$=3.0004, p=0.558). As shown in Fig. 3C and 3D, session and session*genotype effects were also not significant (session: $\chi^2_{(1)}$=0.0619, p= 0.803; session*genotype: $\chi^2_{(1)}$=0.265, p=0.606). On the other hand, rats' responses to the stimuli were also more robust as their physical property was closer to CS-1 (see Suppl Fig. 2A, $\chi^2_{(4)}$=719.627, p<0.001). Again, no significant genotype or stimulus*genotype effect was found (genotype: $\chi^2_{(1)}$=0.491, p=0.483; stimulus*genotype: $\chi^2_{(4)}$=6.081, p=0.193). The session and sessions*genotype effects were also not significant (see Suppl Fig. 2B, session: $\chi^2_{(1)}$=0.807, p=0.369; session*genotype: $\chi^2_{(1)}$=0.516, p=0.472).

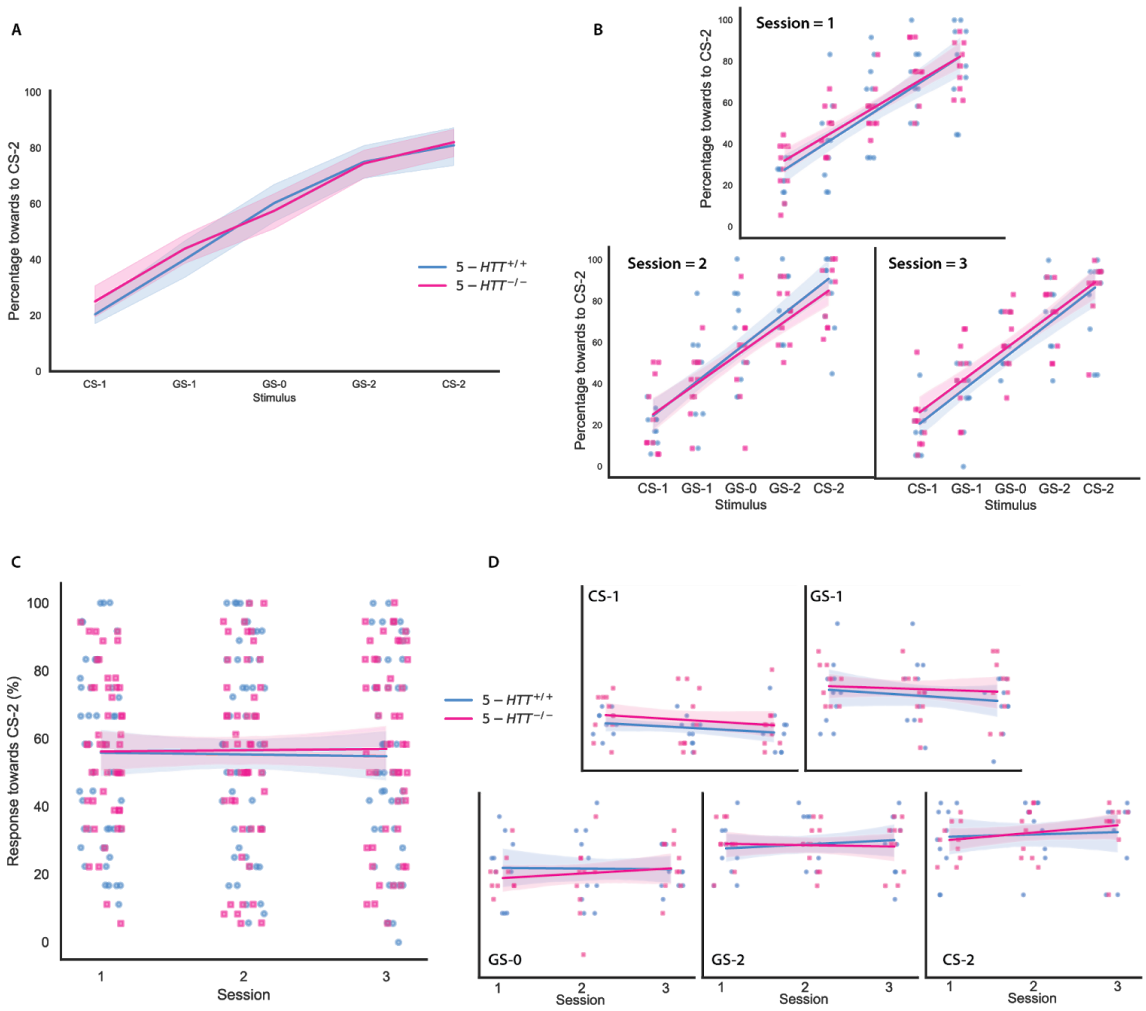
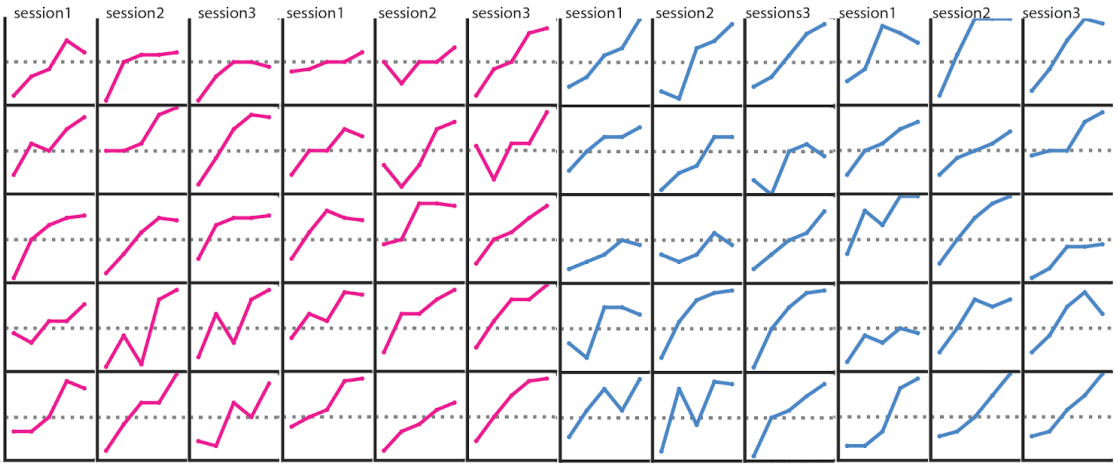
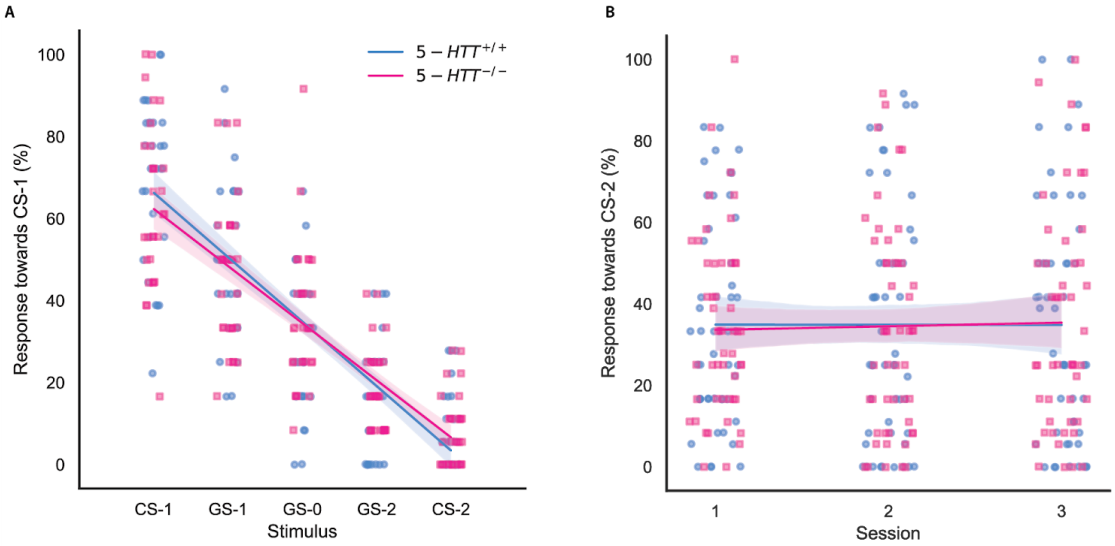


Fig. 3. Stimulus generalization. The "believing" aspect of decisional anhedonia was similar in 5-HTT$^{-/-}$ and 5-HTT$^{+/+}$ rats. (A) The percentage of responses towards CS-2 increased with the

similarity of the GS closer to the CS-2. Solid lines represent the mean percentage of performance. Stripes represent 95% confidence intervals. (B) The percentage of responses towards CS-2 in each session. (C) The interaction between session and genotype. The solid lines are the linear regression with the 95% confidence intervals around. (D) The interaction between session and genotype in each stimulus.



Suppl Fig. 1. Stimulus generalization in individuals. A single line represents a generalization curve of a rat per session. Red lines represent the performance of 5-HTT$^{-/-}$ rats, blue lines represent the performance of 5-HTT$^{+/+}$ rats.



Suppl Fig. 2. Stimulus generalization. The "believing" aspect of decisional anhedonia was similar in 5-HTT$^{-/-}$ and 5-HTT$^{+/+}$ rats. (A) The percentage of responses towards CS-1 increased

with the similarity of the GS closer to CS-1. The solid lines are the linear regression with the 95% confidence intervals around. (B) The interaction between session and genotype. The solid lines are the linear regression with the 95% confidence intervals around.

Before taking optimal actions on the stimulus, response time (RT) plays a crucial role in information processing both in rats and humans (Blokland 1998; Donders 1969). A linear mixed-effect model showed a significant effect of the stimuli on the RT (see Fig. 4A, $\chi^2_{(4)}$=57.917, p<0.001). As shown in Fig. 4B, there was a significant effect of genotype ($\chi^2_{(1)}$=11.432, p < 0.001), but no stimulus*genotype interaction effect ($\chi^2_{(4)}$=1.936, p=0.747). There was no significant effect of session ($\chi^2_{(1)}$=0.299, p=0.584). However, a significant effect of session*genotype was observed (see Fig. 4C; $\chi^2_{(1)}$=21.1967, p<0.001). A follow-up analysis show that there was also a significant effect of sessions in 5-HTT $^{+/+}$ rats ($\chi^2_{(1)}$)=10.210, p=0.001) but while a trend was found for 5-HTT$^{-/-}$ rats ($\chi^2_{(1)}$=3.391, p=0.0656), revealing that as sessions progressed 5-HTT$^{+/+}$ rats took more time to react while 5-HTT $^{-/-}$ rats tended to take less time.

As shown in Fig 4D, the significant effect of session*genotype was observed for GS-2 ($\chi^2_{(1)}$=5.435, p=0.0197). However, follow-up analysis showed that no significant effect of session was observed in 5-HTT $^{-/-}$ rats ($\chi^2_{(1)}$=2.242, p=0.134), but a trend for 5-HTT$^{+/+}$ rats ($\chi^2_{(1)}$=3.193, p=0.0740). Furthermore, a trend towards ansession*genotype interaction effect for GS-0 ($\chi^2_{(1)}$=2.916, p=0.0877) was obtained, but not for GS-1 ($\chi^2_{(1)}$=0.623, p=0.430), CS-2 ($\chi^2_{(1)}$=0.0701, p=0.791) and CS-1 ($\chi^2_{(1)}$=2.0236, p=0.155).

Taken together, 5-HTT$^{+/+}$ rats gradually increased RT across the three generalization test sessions while 5-HTT$^{-/-}$ rats tended to decrease RT.

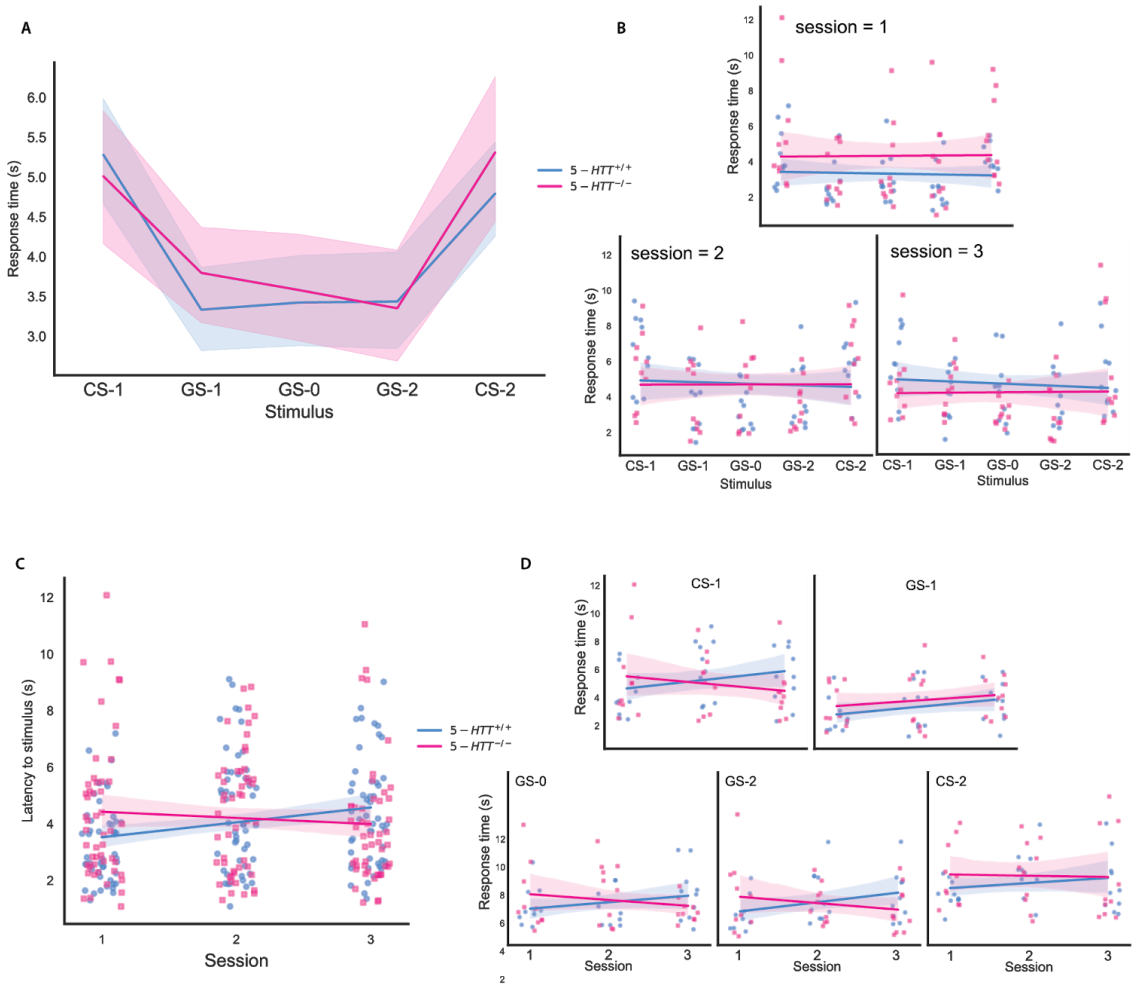


Fig. 4. Stimulus response time (RT). 5-HTT$^{-/-}$ rats gradually decreased, over sessions, RT to GSs compared to 5-HTT$^{+/+}$ rats who showed an increase in RT over sessions. (A) The stimuli effect on RT was significantly different. Solid lines represent the mean of RT. Stripes are 95% confidence intervals. (B) Linear regression of RT for each stimulus in each session. (C) The interaction between session and genotype. The solid lines are the linear regression with the transparent stripes of 95% confidence intervals around. (D) The interaction between session and genotype in each stimulus.

Discussion

We found that both 5-HTT$^{-/-}$ and 5-HTT$^{+/+}$ rats were able to reach 70% accuracy across three consecutive sessions. Also, the number of sessions needed to reach such criterion was similar between the two genotypes. However, 5-HTT$^{+/+}$ rats performed more optimal decision-making compared to 5-HTT$^{-/-}$ rats during the course of discriminative conditioning for the conditioned stimulus associated with high reward value at the beginning. Further, we found that 5-HTT$^{+/+}$

rats gradually increased response time to generalization stimuli across the three generalization test sessions, while 5-HTT$^{-/-}$ rats tended to decrease response time. The monotonic graded responses towards the stimuli did not alter for both genotypes.

Decision-making of "choosing" was conceptualized as a crucial aspect of anhedonia for example, which is important to understand how risk factors may contribute to depression (Treadway and Zald 2011). Here, by using a touch-screen based “go/go” strategy of decision-making under ambiguity, we were trying to demonstrate that 5-HTT deficiency in rats results in decisional anhedonia in the aspect of “believing”. As discussed shortly in the introduction, decision-making under ambiguity was also conceptualized in the framework of cognitive/judgment bias, but with the focus on negative versus positive interpretations of ambiguous information (Roelofs et al. 2016). We previously summarized the cognitive bias studies in rodents, showing that there are two combinations of outcomes possible in "go/go" experiments: “reward/reward” and “reward/punishment" (Nguyen et al. 2020). In case of the "reward/reward" combination, for which no specific term was used under the framework of cognitive bias to distinguish it from the "reward/punishment" paradigm (Roelofs et al. 2016), the detection strength of negative bias is weakened. This is because the outcomes are positively valenced. We, therefore, argue that a reward-only "go/go" experiment for decision-making under ambiguity is better conceptualized as one aspect of decisional anhedonia, namely "believing". Furthermore, the aspect of "choosing" in decisional anhedonia was proposed by Treadway et al., in which the outcome in the experimental design was also only positively valenced (Treadway and Zald 2011). We think that the focus on specific behaviors rather than the theoretical concept of either anhedonia or cognitive bias might lead to significant advances in the understanding of depression and other reward-related deficits in neuropsychiatry (Der-Avakian and Markou 2012).

The finding that during discriminative conditioning, all 5-HTT$^{-/-}$ and 5-HTT$^{+/+}$ rats reached 70% accuracy across the last three consecutive sessions indicates that both genotypes were able to pay efforts to discriminate between the two CSs with similar accuracy. However, 5-HTT$^{-/-}$ rats displayed a lower probability to reach CS-2 (two sucrose conditioned stimulus) accuracy across the sessions compared to 5-HTT$^{+/+}$ rats, suggesting that 5-HTT$^{-/-}$ shows decisional anhedonia (or less pleasure) for CS-2 during the early stage of learning. It could also be possible that their higher flexibility distracts 5-HTT$^{-/-}$ rats from focusing on optimal decision-making, resulting in less accuracy at the beginning of discrimination conditioning (Nonkes et al. 2013). However, for responses to CS-1 (two sucrose conditioned stimulus), no such effect was detected. The discrepancy of accuracy between CS-1 and CS-2 might be because 5-HTT$^{+/+}$ rats were more engaged into "habit behavior" when responding to CS-2. Potentially the animals more readily develop habitual behaviour when the reward is high. In similar experiments, different accuracy criterions were reported (for example, accuracy for each of the two CSs is higher than 60% or accuracy for two CSs pooled is higher than 80%), but the differences of the accuracy in each of these two CSs were not reported (Hales et al. 2016, 2017; Krakenberg et al. 2019b). Whether this difference can be eliminated through continuous training may need further research. Addressing whether animal models for anhedonia could reach similar accuracy for the two CSs may contribute to the framework of decisional anhedonia in depression. For instance, depressive patients are more accurate in making decisions (Alloy and Abramson 1979; Moore

and Fresco 2012) without being over-controlled by a preference for the outcome of higher positive value.

Both stimulus generalization and stimulus RT were analyzed during the generalization stage. 5-HTT$^{-/-}$ and 5-HTT$^{+/+}$ rats categorized the GSs and CSs similarly during decision-making under ambiguity. This indicates that the ablation of 5-HTT in rats didn't change the capacity of generalization to process the ambiguity of the stimuli. The capacity of generalization between 5-HTT$^{+/+}$ and 5-HTT$^{-/-}$ mice was also similar in a touch-screen based task where the discrepancy of physical property (ambiguity) was the location of visual stimuli (Krakenberg et al. 2019a). However, the RT to the stimulus was not reported in this experiment. A spatial generalization test did show that 5-HTT$^{-/-}$ mice exhibited a tendency towards an increase in RT to the generalization arm (Kloke et al. 2014). In our study, we found that 5-HTT$^{+/+}$ rats increased RT with the progression of sessions, while 5-HTT$^{-/-}$ rats tended to decrease RT. The stimulus generalization and stimulus RT findings suggest that 5-HTT$^{-/-}$ rats may respond gradually more trustworthy to the stimuli, to finally categorize the ambiguous stimuli in the same way as 5-HTT$^{+/+}$ did. The initial less trustworthy response to the ambiguous stimuli in 5-HTT$^{-/-}$ rats may reflect an aversive emotion (FeldmanHall et al. 2018), in line with the anxious phenotype of these animals (Olivier et al. 2008). However, the 5-HTT$^{+/+}$ rats behaved in the opposite way and gradually responded less trustworthy (or more suspicious) to the stimuli. Despite these genotype differences in RT, both genotypes paid similar efforts to categorize the stimuli as shown through the monotonic curves.

Previous *in vivo* studies in rodents support the notion that serotonergic neurons encode the time for reward-related information processing. Serotonergic neurons have been found to fire tonically when the animal is waiting for the reward and then phasically when the animal received a reward (Li et al. 2016). In addition, optogenetic activation of serotonergic neurons has been found to promote waiting (Fonseca et al. 2015). 5-HTT$^{-/-}$ mice show under resting-state conditions a reduced firing rate of serotonergic neurons (Gobbi et al. 2001; Lira et al. 2003), which may lead to changes in waiting or the perception of reward. These findings indicate that serotonin controls the time needed for reward-related information processing and represents an interesting substrate for the mechanistic understanding and treatment of decisional anhedonia before taking action.

The present work contains some limitations that are important to consider when interpreting the findings. First, the draw-back of the 70% accuracy criterion applied during the discrimination trials is that it might not have had enough power to detect genotype differences in trials measuring the responses to CS-1. However, responses to CS-2 were found to be different between the genotypes. Furthermore, different sizes of the stimulus images resulted in different light intensities in the touchscreen box. The maximum light intensity was only about 35 lux. It is not clear whether changes in light intensity affected the accuracy of discrimination.

In summary, our results suggest that individuals characterized by inherited 5-HTT down-regulation may be at risk for depression because their initial reduced bias to high reward value as well as their initial hesitance to trust that generalization stimuli are predicting reward value may decrease reward approach behavior. On the other hand, repeated exposure to such

generalization stimuli can overcome this initial hesitance, and may, therefore, provide an avenue to decrease the risk for depression. Potentially, the latter may also help to explain the inconsistent findings from human studies on the association between the 5-HTT gene and depression.

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Conflict of Interest

All authors declare no conflict of interest.

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