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Research report

The attribution of incentive salience to an appetitive conditioned cue is not affected by knockout of the serotonin transporter in rats

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HIGHLIGHTS

- Attribution of incentive salience to conditioned stimuli & motivation for reward were studied.
- Serotonin transporter knockout rats were compared with wild-type counterparts.
- Knockout did not affect conditioned stimulus salience attribution.
- Knockout animals showed an increased motivation for reward.

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ABSTRACT

Understanding the neurobiological basis underlying individual differences in conditioned stimulus (CS) sensitivity is pertinent, given that excessive conditioned responses to CSs is a key feature of anxiety-related disorders and drug addiction. We have previously shown that behaviour of serotonin transporter knockout (5-HTT$^{-/-}$) rats-mimicking the common 5-HTT promoter polymorphism in humans–is strongly driven by Pavlovian CSs. To investigate whether the knockout rats attribute greater incentive salience to CSs, we tested the 5-HTT$^{-/-}$ rats and their wild-type counterparts in the sucrose-reinforced sign-versus goal-tracking task. We also assessed whether motivational properties of the unconditioned stimulus (sucrose pellet) are involved in the individual differences under investigation, by testing the animals in a sucrose-reinforced progressive ratio schedule of reinforcement. We found no genotype differences in sign-versus goal-tracking behavior, despite that progressive ratio responding was increased in 5-HTT$^{-/-}$ rats. In conclusion, the high CS sensitivity in 5-HTT$^{-/-}$ rats cannot be explained by enhanced incentive salience attribution to the CS as measured by the sign- versus goal-tracking paradigm. Rather, 5-HTT$^{-/-}$ rats may be more sensitive to the motivational properties of the unconditioned stimulus.

1. Introduction

Behaviour is strongly driven by Pavlovian conditioned stimuli (CSs). These are stimuli that predict unconditioned stimuli (USs) that have emotionally and/or motivationally relevant aversive or rewarding properties. CSs may elicit 'automatic' conditioned responses (CRs), which help organisms to respond quickly and properly to environmental stimuli. Whereas CRs are highly adaptive, sometimes they go awry and can trigger pathological conditions like anxiety-related disorders [1] and drug addiction [2,3]. Understanding the neurobiological mechanisms contributing to excessive CRs is essential to further our insight into these neuropsychiatric disorders.

Pavlovian CSs are associated with complex psychological properties. First, they attract attention and thereby trigger approach (in case of a rewarding CS) or avoidance (in case of an aversive CS) behaviour. Secondly, CSs can become 'wanted' in the sense that individuals will work to get them, and they can even reinforce learning a new instrumental response to get them (i.e., they act as conditioned or secondary reinforcers) [4]. This feature can motivate organisms in such a way that they engage into reward-seeking or punishment-avoidance behaviour for a long period of time in the absence of the rewarding or aversive US itself.

Abbreviations: 5-HTT, serotonin transporter; BP, breaking point; CR, conditioned response; CS, conditioned stimulus; US, unconditioned stimulus; FR, fixed ratio; ITI, intertrial interval; PR, progressive ratio.

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Of interest, there are large individual differences in sensitivity to CSs. These individual differences have been extensively studied in the so-called sign-versus goal-tracking task. In this task, some animals approach and interact with the CS before collecting the reward (sign-trackers), whereas others directly approach the reward location without approaching or paying attention to the CS (goal-trackers). Sign-trackers attribute more incentive salience to CSs, making the CSs more effective reinforcers in sign-than in goal-trackers [5].

The neurobiological basis of individual differences in CS sensitivity may be, at least in part, related to serotonin, given that serotonin is implicated in individual differences in CRs [6–14]. For instance, the low activity short (s) allelic variant of the common serotonin transporter promoter polymorphism (5-HTTLPR) in humans, which hypothetically is associated with increased extracellular serotonin levels due to reduce serotonin reuptake, is associated with attentional vigilance and gaze bias toward negatively [15] and positively valenced stimuli [16,17]. In line, we have shown that behaviour of serotonin transporter knockout (5-HTT$^{-/-}$) rats is strongly driven by Pavlovian CSs [9], and that these animals show impaired extinction of conditioned fear and reward-seeking behaviour [7,18]. These findings prompted us to hypothesize that besides dopamine, serotonin mediates individual differences in sensitivity to CSs as measured in the sign-versus goal-tracking task.

To test this hypothesis we subjected 5-HTT$^{-/-}$ rats and their wild-type controls to the sign-tracking versus goal-tracking task and studied their behaviour during acquisition (revealing individual differences in CRs) and extinction (indicative for new learning). Furthermore, to assess whether motivational properties of the US are involved in the individual differences under investigation, the animals were tested in a sucrose-reinforced progressive ratio schedule of reinforcement. We used 5-HTT$^{-/-}$ rats as animal model, because they are characterized by a constitutive increase in extracelullar serotonin levels (Homberg et al., 2007), model the 5-HTTLPR s-allele in humans [19], and because the sign-versus goal-tracking task has been developed for rats [20].

2. Methods

2.1. Animals

All experiments were in compliance with national regulatory principles and approved by the Committee for Animal Experiments of the Radboud University Nijmegen Medical Centre,Nijmegen, The Netherlands. All efforts were made to reduce animal suffering and the number of experimental animals. Experimental animals (Slc6a4^{1-Hubr}) [21] were derived from crossing heterozygous 5-HTT knockout (5-HTT$^{+/-}$) rats that were outcrossed for atleast 10 generations with wild-type Wistar rats (Harlan Laboratories, The Netherlands) at the central animal facility of the Radboud University. Male animal facility reared 5-HTT$^{-/-}$ and 5-HTT$^{+/+}$ offspring was used for the experiments described below. The animals were 10 weeks of age at the start of the experiment.

Animal housing took place in a temperature ($21 \pm 1\,^{\circ}C$) and humidity-controlled room (60% relative humidity) with background music and a ventilation system based upon overpressurization (15-fold). The room was on a 12 hr reversed light–dark cycle, with lights on at 20:00 p.m. (maximum light intensity:60 lx; minimal light intensity:0 lx; transition period:30 min.). All rats were socially housed (2 animals per cage) under conventional housing conditions in Macrolon type III open cages with sawdust bedding and a shelter. Cages were changed every week, always after experimental sessions. Animals had *ad libitum* access to acidified tap water (pH value 2.6–2.9; weekly change of water bottles) except during the experimental sessions, and were food deprived for 21 h prior to the experimental sessions. After the daily experimental sessions the animals received 2 h of *ad libitum* access to food (V1534, ssniff Spezialdiäten, Soest, Germany). This food restriction schedule resulted in a nominal loss of body weight as well as well-motivated animals in the experimental paradigms. All rats were extensively handled for 5 days before the start of the experiments. Experimental sessions (1 session/day) were performed from Monday to Friday between 9 a.m. and 17 p.m. The experimenter was blind to the genotype of the rats.

2.2. Apparatus

All behavioural tests were conducted in four identical operant conditioning chambers ($24.1 \times 20.5 \times 29.2$ cm ($l \times w \times h$); MED Associates, St. Albans, VT, USA) equipped with a red house-light located on the upper right corner of the left wall, and a food cup for 45 mg sucrose pellet delivery and two retractable levers on either side of a food cup incorporated in the right wall of the chamber.

2.3. Experimental paradigms

2.3.1. Sign-versus goal-tracking experiment

Eight 5-HTT$^{+/+}$ and eight 5-HTT$^{-/-}$ animals were tested in an adapted variant of the sign-versus goal-tracking paradigm described in detail by Flagel et al., [20]. In brief, during two pre-acquisition sessions animals received 50 sucrose pellets on a random interval schedule (30s mean inter-trial interval; ITI) to familiarize them with pellet retrieval from the food cup. Subsequently, rats received 20 acquisition sessions during which sign-versus goal-tracking behaviour of the animals was examined. During these experimental sessions animals continued to receive sucrose pellets on the random interval schedule as described above, but prior to each pellet presentation one lever was extended for 8s (CS+; left or right, counter balanced within groups). Thus, the pellet was delivered directly after the retraction of the CS+ lever**.** In addition, the second lever (CS−) was presented for 8s on a random interval 30s schedule, but explicitly unpaired with the sucrose pellet presentation. This second lever served as a control to examine the animal's tendency to approach and contact a lever in general. Importantly, interaction (∼depression of the lever) with the CS+ (or CS−) lever was recorded but didn't have any programmed consequences. As such, animals received a sucrose pellet irrespective of whether they interacted with the CS+ or not.

Rats were given a total of 29 CS+ trials and 29 CS− trials in a randomized order. Following the above described 20 acquisition sessions animals received 8 extinction sessions in which they again received a total of 58 trials (29 CS+ trials, 29 CS− trials) but now no pellet was presented after CS+ presentation.

The total number and latency of lever (CS+ and CS−) and food cup contacts–as detected by interruption of an infrared sensor beam in the food cup–during CS presentation and inter-trial interval (ITI) were recorded using Med Associates (St. Albans, VT, USA)software and analyzed using MATLAB 8.2 (MathWorks, Natick, Massachusetts, USA) by means of a custom written script.

2.3.2. Progressive ratio schedule of reinforcement experiment

Nine 5-HTT$^{+/+}$and nine 5-HTT$^{-/-}$ rats were tested in a variant of the progressive ratio schedule of reinforcement paradigm as described in full length by Richardson and Roberts [22]. In short, during two sessions animals were trained on a fixed ratio (FR) 1 schedule of reinforcement. During these sessions animals had to choose during distinct trials between a rewarding lever (RL; left or right, counterbalanced within groups) and an unrewarding lever (UL). Successful session completion required fifty correct trials, i.e., animals had to make 50 RL responses. Trials commenced with