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Research report

# Prior fear conditioning does not impede enhanced active avoidance in serotonin transporter knockout rats

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## HIGHLIGHTS

- Fear conditioning does not impair signalled active avoidance learning in 5-HTT$^{-/-}$ or wildtype rats.
- The finding of improved active avoidance (AA) in 5-HTT$^{-/-}$ rats is replicated.
- Exposure to the CS used for AA in a novel context results in lower freezing in 5-HTT$^{-/-}$ rats.
- 5-HTT$^{-/-}$ animals show higher locomotion during post AA CS exposure in a novel context.
- Fear conditioning normalized the increased locomotion of 5-HTT$^{-/-}$ rats during post AA CS exposure.

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## ABSTRACT

Stressors can be actively or passively coped with, and adequate adaption of the coping response to environmental conditions can reduce their potential deleterious effects. One major factor influencing stress coping behaviour is serotonin transporter (5-HTT) availability. Abolishment of 5-HTT is known to impair fear extinction but facilitates acquisition of signalled active avoidance (AA), a behavioural task in which an animal learns to avoid an aversive stimulus that is predicted by a cue. Flexibility in adapting coping behaviour to the nature of the stressor shapes resilience to stress-related disorders. Therefore, we investigated the relation between 5-HTT expression and ability to adapt a learned coping response to changing environmental conditions. To this end, we first established and consolidated a cue-conditioned passive fear response in 5-HTT$^{-/-}$ and wildtype rats. Next, we used the conditioned stimulus (CS) to signal oncoming shocks during signalled AA training in 5-HTT$^{-/-}$ and wildtype rats to study their capability to acquire an active coping response to the CS following fear conditioning. Finally, we investigated the behavioural response to the CS in a novel environment and measured freezing, exploration and self-grooming, behaviours reflective of stress coping strategy. We found that fear conditioned and sham conditioned 5-HTT$^{-/-}$ animals acquired the signalled AA response faster than wildtypes, while prior conditioning briefly delayed AA learning similarly in both genotypes. Subsequent exposure to the CS in the novel context reduced freezing and increased locomotion in 5-HTT$^{-/-}$ compared to wildtype rats. This indicates that improved AA performance in 5-HTT$^{-/-}$ rats resulted in a weaker residual passive fear response to the CS in a novel context. Fear conditioning prior to AA training did not affect freezing upon re-encountering the CS, although it did reduce locomotion in 5-HTT$^{-/-}$ rats. We conclude that independent of 5-HTT signalling, prior fear conditioning does not greatly impair the acquisition of subsequent active coping behaviour when the situation allows for it. Abolishment of 5-HTT results in a more active coping style in case of novelty-induced fear and upon CS encounter in a novel context after AA learning.


*Abbreviations:* 5-HTT, serotonin transporter; AA, active avoidance; CS, conditioned stimulus; PTSD, posttraumatic stress disorder; MD, major depression; 5-HTTLPR, serotonin transporter linked polymorphic region; ENU, ethyl-N-nitrosurea.

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## 1. Introduction

Stress is recognized as one of the foremost contributors to the development of psychiatric disorders such as posttraumatic stress disorder (PTSD), major depression (MD) and anxiety disorders [1]. However, large inter-individual variation exists in vulnerability to stress; not all individuals who are faced with severe trauma succumb to anxiety or mood disorders [2]. The capability of an individual to appropriately adapt one's coping response to a stressor has a great influence on its potentially deleterious sequelae. Therefore, it has been proposed that varying levels of stress susceptibility may in part be mediated by differences in stress coping strategies [3]. Stress coping is defined as the actions an individual undertakes to reduce the impact of a stressor. Coping can be done either actively in an effort to remove the stressor, or passively by conserving energy while enduring a stressor [4]. It has been suggested that both styles can be adaptive or maladaptive and thus can confer resilience or vulnerability [5]. Whether a stress coping style is adaptive depends on its appropriateness to the exact environmental setting; stress coping *flexibility* has been proposed as an important factor in resilience [6,7].

Certain genetic factors modulating serotonergic neurotransmission are known to affect stress coping behaviour, and thereby influence vulnerability to stress-induced psychopathology. The short (s) allelic variant of the serotonin transporter linked polymorphic region (5-HTTLPR) is thought to compromise the availability of the serotonin transporter (5-HTT) in the brain (although conflicting evidence exists as well [8]). The s-allele is well known for increasing susceptibility to MD in conjunction with the presence of early life adversity [9,10], and to PTSD following severe trauma [11]. Since associations between 5-HTTLPR and these stress-related disorders have been found exclusively in the presence of previous stressful life experience it is likely that modulation of coping behaviour is key to understanding these gene x environment interactions [12].

Work in animals with genetically altered levels of 5-HTT has solidified the association between serotonin and stress coping strategy, though many of the underlying mechanisms remain unclear. 5-HTT$^{-/-}$ animals display impaired extinction memory, and thus prolonged expression of a passive stressor coping response (i.e., freezing) in a cued fear conditioning paradigm [13–15]. At the same time, 5-HTT abolishment was shown to improve the acquisition of an active stressor coping task, signalled active avoidance (AA) [16]. The discrepancy between impaired fear extinction and improved AA performance in 5-HTT$^{-/-}$ animals is peculiar, as overcoming the freezing response induced by the shock-predicting signal is a prerequisite to proactively respond to it. During initial unsuccessful AA trials, signal–shock pairings induce conditioned freezing. The animal then has to overcome this conditioning in order to subsequently avoid or escape the shock [17,18]. While successful fear extinction is dependent on updating the contingency of the conditioned stimulus (CS) by *passive* exposure to it, signalled AA learning allows the individual to re-evaluate the CS contingency by *actively* interacting with it.

Here, we further explore whether the updating of a passive coping stimulus contingency to an active one is modulated by 5-HTT expression. To this end, we assessed signalled AA performance in previously fear conditioned and sham conditioned 5-HTT$^{-/-}$ and wildtype rats, using the CS to signal incoming shocks during AA. We then measured stress coping behaviours in response to a novel environment and new CS encounter to evaluate the effects of 5-HTT genotype on the carry-over of the conditioned fear response to different environmental conditions (Fig. 1). Using fear conditioning, we induce a pre-existent behavioural freezing response to the CS. This is expected to reduce the ability to acquire an active coping response (i.e., impair AA learning), due to the animals having to overcome their acquired freezing response to the CS in order to respond actively to it. Therefore, we hypothesized that AA performance would decrease in both genotypes as a result of prior fear conditioning. We expected that 5-HTT$^{-/-}$ rats would be relatively resistant to these effects of prior fear conditioning, as they have previously been demonstrated to be resilient to stressor induced escape learning deficiencies [19]. Consequently, we expected a greater freezing response to the CS after AA learning in a novel context in wildtype animals, since improved AA learning of 5-HTT$^{-/-}$ rats would strengthen the active coping contingency of the CS and reduce their fear response.

## 2. Methods and materials

### 2.1. Animals

Serotonin transporter knockout rats (Slc6a41Hubr) were generated on a Wistar background by N-ethyl-N-nitrosurea (ENU)-induced mutagenesis [20] as described previously [21]. Experimental animals were derived from crossing heterozygous 5-HT transporter knockout (5-HTT$^{+/-}$) rats that were outcrossed for at least twelve generations with wild-type Wistar rats obtained from Harlan Laboratories (Horst, The Netherlands). Ear punches were taken at the age of 21 days after weaning for genotyping, which was done by Kbiosciences (Hoddesdon, United Kingdom). Since stress sensitivity in females is dependent on their oestrous cycle phase [22,23], we here restricted ourselves to the gender with the most stable stress response, i.e., males. Twenty homozygous knockout (5-HTT$^{-/-}$) and twenty wildtype animals were used for this experiment; half of each group received fear conditioning before signalled AA training, while the remaining animals received sham conditioning. All animals had *ad libitum* access to food and water and were housed in pairs in standard Makrolon type 3 open cages. A 12-hr light-dark cycle was maintained, with lights on at 08.00 AM. For consistency with previous experiments performed in this rat line (e.g. [14–16]), all behavioural experiments were performed between 08.00 AM and 18:00 PM. At the time of entering the experiments, the animals were between 12 and 20 weeks old. All experiments were approved by the Committee for Animal Experiments of the Radboud University, Nijmegen, The Netherlands, (application # RU-DEC 2013-149) and all efforts were made to minimize animal suffering and to reduce the number of animals used.

### 2.2. Fear conditioning

A 30.5 cm × 24.1 cm × 21 cm operant conditioning chamber (Model VFC-008, Med Associates) was used for fear conditioning and sham conditioning. The box was housed within a sound-attenuating cubicle and contained a white LED stimulus light, a white and near infrared house light as well as a speaker capable of producing an 85 dB 2.8 kHz tone. The metal grid floor of the apparatus was connected to a scrambled shock generator (model ENV-412, Med Associates) configured to deliver shocks at 0.6 mA intensity. Animals were habituated to the fear conditioning chamber for the duration of 10 min, 24 h prior to conditioning. For the conditioning and habituation, the apparatus was cleaned before and after each animal using a tissue slightly dampened with 70% EtOH. The house light was on during habituation and conditioning. For the fear conditioning itself, after a two minute habituation period, a 30 s 85 dB 2.8 kHz auditory stimulus (the CS) co-terminated with a 1 s 0.6 mA foot shock, followed by a 1 min inter-trial interval. A total of 5 of these tone – shock pairings were given. For the sham conditioning groups, the foot shock was omitted.