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Research report

Modulation of behavioural profile and stress response by 5-HTT genotype and social experience in adulthood

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ABSTRACT

Behavioural profiles can be shaped by genotype and environmental factors during early phases of life. The aim of this study was to investigate whether anxiety-like behaviour, exploration and adrenocortical stress responses can be modulated by genotype and social experiences in adulthood. Male mice lacking the serotonin transporter gene which is under scrutiny for anxiety disorders were compared with heterozygous and wildtype controls. Concerning social experiences, the males of all three genotypes were provided with a winner or a loser experience in a resident-intruder paradigm on three consecutive days. Anxiety-like behaviour and exploration were recorded in the dark-light, elevated plus-maze and open-field test. To non-invasively assess adrenocortical activity, corticosterone metabolites were determined from feces. The main findings were: Repeated social experience, irrespective of winning or losing, elevated levels of anxiety-like behaviour and decreased exploration. In losers a distinct effect of genotype occurred, with homozygous knockout males showing more anxiety-like behaviour and less exploration than the other genotypes. In winners no genotype-dependent variation was found. Genotypes did not differ in basal stress hormone secretion. There was, however, a main effect of social experience with higher activation of the stress hormone system in losers than in winners. This effect was strongest in the heterozygous genotype. In conclusion, our data show that anxiety circuits retain their plasticity throughout adulthood and can be shaped by genotype and social experiences during this phase of life. Moreover, responsiveness towards negative life experiences is influenced significantly by the 5-HTT genotype.

1. Introduction

Individual differences in traits of anxiety and even in the etiology of anxiety disorders can be modulated by both, environmental and genetic factors [24]. Regarding the molecular genetic basis, candidate genes have been identified which are associated with anxiety disorders (e.g., [15]). Of particular interest for this study was the serotonin transporter (5-HTT) which plays a key-role in serotonergic neurotransmission by removing the serotonin that is released into the synaptic cleft [6]. In humans, a repeat length polymorphism in the transcriptional control region of the 5-HTT gene (SLC6A4) was found, resulting in allelic variation of 5-HTT expression and function, and associated traits of negative emotionality including anxiety, depression and aggressiveness [3,33,34]. The generation of mice with a targeted disruption of the 5-HTT allows to investigate the consequences of its diminished or absent function. In fact, 5-HTT knockout mice were shown to display increased anxiety-like behaviour and 'behavioural despair' (e.g., depression-related behaviour) [26,47,68].

Interestingly, phenotypic consequences of the 5-HTT polymorphism in humans and monkeys and 5-HTT knockout mice seem to critically depend on adverse and stressful environmental influences during early development [24]. Humans with one or two copies of the short allele of the 5-HTT promoter polymorphism have been reported to exhibit more depressive symptoms, and suicidality behaviour than individuals homozygous for the long allele but only in relation with stressful life events ([8]; but see: [53]). Heterozygous 5-HTT knockout mice display increased anxiety- and depression-related behaviours compared to wildtypes but only when they had received low maternal care [7].

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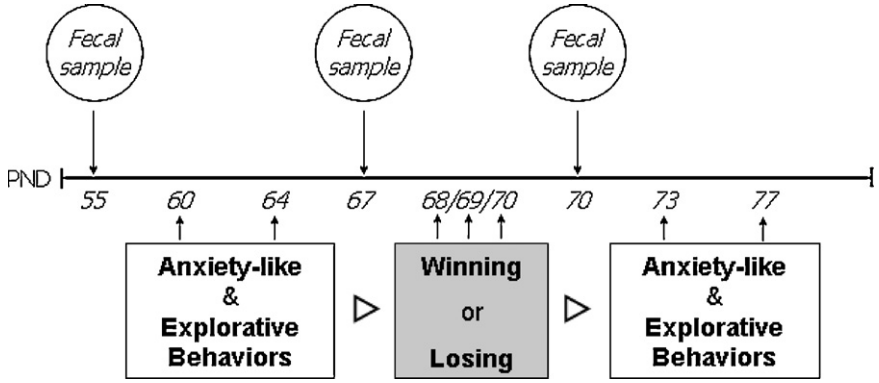

Fig. 1. Experimental design. Males of all three genotypes were provided with winner and loser experiences on postnatal days (PND) 68, 69, and 70. Before and after these experiences anxiety-like and explorative behaviours were assessed in a battery of tests. On PND 55, 67, and 70 fecal samples for investigation of corticosterone metabolites were collected.

Anxiety and fear circuits seem to be particularly vulnerable to environmental influences in times when synaptic connections are developing and refining, i.e., when brain circuits are highly plastic [9]. Accordingly, research on environmental influences on anxiety-related traits has mainly focused on early phases so far (e.g., [40,45,63]). However, these circuits seem to retain their plasticity in adulthood, as is indicated by the efficacy of psychotherapy and pharmacotherapy in later life [24]. Moreover, in rodents exposure to an enriched environment in adulthood decreases anxiety and fear [52]. On the other hand, a single social defeat in adulthood is related to increases in anxiety-like behaviour; at the same time the functionality of hippocampal serotonergic 5-HT_{1A} receptors is decreased [5].

Based on the knowledge that anxiety circuits seem to remain plastic during adulthood and that social defeat can enhance anxiety-like behaviour, the aim of the present study was to investigate possible involvement of the 5-HTT in maintaining this plasticity during adulthood in a genotype- and social experience-dependent way. Therefore, we focused on the modulation of anxiety-like behaviour by different social experiences in adulthood in wildtype as well as in heterozygous and homozygous 5-HTT knockout mice. We hypothesised that repeated losing (social defeat) as well as repeated winning (social victory) would influence anxiety-like behaviour, but in a differential way (hypothesis 1) and that these effects might be modulated by 5-HTT genotype (hypothesis 2). Since the endocrine stress response of winners and losers can differ significantly [57,66], we also expected winning and losing to differentially influence adrenocortical activity (hypothesis 3) and this hormonal response to be modulated by genotype (hypothesis 4).

Table 1
Description of behavioural patterns.

Behaviour	Definition
Winner behavioural patterns	
Attack	Rushing and leaping at another mouse with biting.
Escalated fight	Physical struggle between two mice which is initiated by a bite and usually involves further biting, kicking, wrestling, and rolling over and over. In-between, mice locked jaws. (A score was given for each escalated fight from the onset until the mice separated.)
Chasing	A mouse follows another mouse, while the head of the chasing mouse is directed to the backside of the other individual. The maximum distance between the animals is one body length. After stopping in forward motion the behaviour starts again.
Rushing	*Chasing* subsequent to an agonistic interaction (biting, attack or escalated fight).
Biting	A mouse contacts the body of another mouse with its mouth, making that mouse react with winced movement of either single extremities or the tail or the whole body.
Loser behavioural patterns	
Avoiding	Directed movement away from another mouse at a walking or running pace.
Flee	*Avoiding* subsequent to an agonistic interaction (biting, attack or escalated fight).
Defensive upright posture	Rearing up on the hind paws and keeping still, with the head up in the air, and the forepaws rigidly stretched out toward another mouse.
Defensive sidewise posture	Rearing up on the hind paws and keeping still, with shoulder and flank presented to another mouse.

Note: For description of behavioural patterns see also Marashi et al. [41,42].

2. Materials and methods

2.1. Animals and housing conditions

5-HTT knockout mice (5-HTT KO) [4] backcrossed into a C57BL/6J genetic background for >10 generations derived from our local stock. Parents were bred in pairs of heterozygous knockout males and females, and resulting offspring were of three different genotypes. To distinguish between wildtype (5-HTT+/+), heterozygous knockout (5-HTT+/−) and homozygous knockout (5-HTT−/−) mice, genomic DNA was extracted from tail tissue (sampling was performed on day 21 ± 1 of life). PCR amplicons of 225 bp (5-HTT+/+), 272 bp (5-HTT−/−) or both (5-HTT+/−) were identified by agarose gel electrophoresis.

Table 2
Validation of winner or loser experiences.

Genotype	Social experience		
	Winning	Losing	Unclear
(a) Confrontation of 5-HTT males with males from the strain C3H			
+/+[a]	10	0	1
+/−[b]	8	0	1
−/−[c]	6	0	5
(b) Confrontation of 5-HTT males with males from the strain NMRI			
+/+[a]	0	9	0
+/−[b]	0	10	3
−/−[c]	0	8	0

[a] Wildtype mice.
[b] Heterozygous knockout mice.
[c] Homozygous knockout mice.