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The winner and loser effect, serotonin transporter genotype, and the display of offensive aggression

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ABSTRACT

Aggressive behaviour results from a complex interplay between genetic and environmental factors. Key modulators of aggression include the serotonergic system on the molecular level and experience in prior aggressive contests as an environmental factor. The aim of this study was to elucidate the effects of fighting experience on the display of offensive aggressive behaviour in adult male mice varying in serotonin transporter (5-HTT) genotype. 5-HTT +/+, 5-HTT +/− and 5-HTT −/− mice were given either a winning or a losing experience on each of three consecutive days and were subsequently observed for their offensive aggressive behaviour as residents against a docile intruder from the C3H strain in a resident–intruder paradigm. The main findings were: There was no significant difference between the amount of offensive aggressive behaviour displayed by the genotypes. Winners showed more engagement with the intruder, attacked him faster and exhibited overall higher aggression scores than losers. There was no significant genotype × social experience interaction: winning and losing had a similar effect on offensive aggressive behaviour in all three 5-HTT genotypes. We conclude that social experience in terms of having been a winner or having been a loser rather than the 5-HTT genotype determines the behaviour towards a docile intruder.

1. Introduction

Aggression in its maladaptive and escalated form is a major burden on the health and well-being of populations and exacts an economic toll from nations [1]. Aggressive traits and behaviours are the result of a complex interplay between genetic and environmental factors [2]. On the molecular level, serotonin (5-HT) has been implicated in the neural control of the expression of aggressive behaviour in a wide variety of animal species, more than any other neurochemical system [3–6]. However, it is not clear if the 5-HT system generally dampens aggression or if the neurotransmitter plays opposite roles in adaptive and escalated forms of aggressive behaviour [3,7–9].

Among several other functional components of the 5-HT system, the 5-HT transporter (5-HTT) has been linked to aggressive behaviour [10–12]. The 5-HTT is a key regulator for central serotonergic activity. That is, it functionally inactivates 5-HT molecules by active transport from the extracellular space to the 5-HT terminals and thus determines the magnitude and duration of postsynaptic receptor-mediated signalling [13,14]. The transcriptional activity of the human 5-HTT gene is modulated by a length polymorphism in the transcriptional control region (5-HTTLPR) with the short variant (*s*) being associated with a lower transcriptional activity and therefore a reduced amount of 5-HTT protein compared to the long (*l*) gene variant [10]. Low 5-HTT function induced by the *s* allele of the 5-HTTLPR has been associated with anxiety- and depression-related personality traits as well as with neuropsychiatric diseases [15–17]. Individuals carrying one or two copies of the *s* allele are more likely to develop major depression following stressful life events [18–21] but see also [22]. Thus, the 5-HTTLPR seems to moderate the response to environmental influences, potentially facilitating the development of mental illness [11].

In modelling the neurobiological implications of the 5-HTTLPR, mice with a partial or complete inactivation of 5-HTT function are an indispensable tool for measuring effects of 5-HTT depletion [23,24]. The loss of functional 5-HTT results in more than 50 different phenotypic changes, such as increased anxiety [25–29] and reduced aggressive behaviour [30,31]. Interestingly, some of these alterations

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can be shaped by environmental influences such as an adverse early environment or negative life experiences during adulthood, probably modelling the 5-HTTLPR-by-stress risk factor for behavioural pathology reported in humans [28,29,32–34].

So far, gene × environment interaction studies in the 5-HTT knockout mouse model have mainly focused on anxiety and depression-related behaviours, while the aggressive behaviour of the mouse model has been largely neglected in this context. Nevertheless, aggressive behaviour should be of special interest, since several disorders, including depression, personality disorders and drug abuse, which are associated with 5-HTT gene variants in humans, can also manifest in inappropriate aggression [35]. Moreover, there seems to be a systematic relationship between offensive aggression in laboratory rodents and angry aggression in humans. Although cognitive representations of emotions and motives associated with angry aggression in humans are more elaborate and differentiated, there are a number of detailed correspondences between human anger/aggression and conditions that produce offensive aggression in laboratory rodents, especially regarding its antecedents, its response characteristics, and its outcomes [36].

The majority of stressful stimuli involved in human psychopathologies are of a social nature. Indeed, social stressors probably constitute the most frequent and persisting sources of stress [37]. Furthermore, social status appears strongly associated with the number of stressful events experienced [38]. Animal models of social stress were found to have both face and predictive validity in modelling the implications of stressful social stimuli in psychopathologies in humans, with most involving the establishment of clear relationships of dominance/subordination in agonistic encounters [37,39]. While social defeat is one of the most stressful social stimuli in most species, dominant animals also can experience considerable amounts of stress [37,40]. Winning as well as losing agonistic encounters can strongly, and differentially, affect both the physiology and subsequent behaviour of the participants, with winners being generally more active and aggressive than losers in future fights [35,41]. Therefore, experience in prior aggressive contests has also emerged as an important environmental modulator of aggressive behaviour in animals [35,41–43].

Against the background that the 5-HTT genotype can be significantly involved in the processing of stressful life experiences, the aim of the present study was to investigate whether the 5-HTT genotype is also involved in the modulation of aggressive behaviour by previous fighting experience. For this purpose, wildtype mice (5-HTT +/+) as well as heterozygous (5-HTT +/−) and homozygous (5-HTT −/−) 5-HTT knockout mice were given the social experience of either being a winner, or being a loser, and were afterwards analysed for their offensive aggressive behaviour.

In line with the literature [41,43], we hypothesised that animals with repeated experiences as winner would show increased aggression scores compared with animals with repeated experiences as loser (hypothesis 1). Based on the findings of reduced aggression in 5-HTT −/− mice [30,31], we further expected a main effect of genotype with lowest levels of offensive aggressive behaviour in 5-HTT −/− mice (hypothesis 2). Moreover, we hypothesised that the 5-HTT genotype would interact with social experience in modulating aggressive behaviour (hypothesis 3), because 5-HTT depletion in humans as well as laboratory mice has been shown to interact significantly with environmental factors in shaping the behavioural profile [18,28,32]. In mice, this includes also fighting experience, which can result in genotype-dependent differences in the anxiety- and explorative behaviour of the 5-HTT knockout mouse model, suggesting a similar relationship for aggressive behaviour [29]. Since changes in aggressive behaviour by previous fighting experience are often related to increases or decreases in circulating steroid hormone levels [40,41,44–46], we also monitored adrenocortical activity as well as testosterone titres. Firstly, we expected adrenocortical activity to be differentially influenced by winning and losing and these influences also to have an impact on the stress response in future fights (hypothesis 4). Since a previous study indicated a 5-HTT genotype-dependent modulation of the adrenocortical stress response to agonistic experiences [29], we also expected the changes in corticosterone levels to be modulated by genotype with the effects of winning and losing being most pronounced in mice with impaired 5-HTT function (hypothesis 5). Finally, we expected testosterone titres to be influenced by 5-HTT genotype after different social experiences (hypothesis 6).

2. Methods

2.1. Animals and housing conditions

5-HTT +/+, 5-HTT +/− and 5-HTT −/− mice [47], backcrossed into a C57BL/6J genetic background for >10 generations, originated from the internal stock of the Department of Behavioural Biology at the University of Münster, Germany. The original breeding stock was obtained from the Department of Psychiatry at the University of Würzburg, Germany. Breeding pairs each consisted of a male and a female 5-HTT +/− mouse and resulting offspring were thus 5-HTT +/+, 5-HTT +/−, and 5-HTT −/− mice. Genotyping was accomplished using ear tissue to extract genomic DNA, amplified by PCR. Genotypes were identified by agarose gel electrophoresis of DNA-fragments of either 225 bp (5-HTT +/+), 272 bp (5-HTT −/−) or both (5-HTT +/−).

In total, 111 male mice (37 5-HTT +/+, 38 5-HTT +/−, and 36 5-HTT −/−) were used for the behavioural investigations (deviations from these sample sizes were for technical reasons). Pups were weaned on postnatal day (PND) 21 ± 1 and maintained in sibling groups of two to five animals of the same sex. Only in rare cases were age-matched males from different litters housed together. From PND 61 ± 3 of age all mice were housed individually to provoke isolation-induced aggressiveness necessary for the following resident intruder paradigm (RIP) and to exclude a possible influence of social interactions with conspecifics on the offensive aggressive behaviour.

To generate winning experiences and to assess aggressive behaviour (see Section 2.3), 20 males of the C3H strain (obtained from Harlan Winkelmann GmbH, Borchen, Germany) served as subordinate opponents, since C3H mice are characterised by a low level of intermale aggression [48]. To further minimise the probability of increased aggression resulting from prolonged isolation, C3H males were housed in groups of three.

To generate losing experiences 12 males of the NMRI strain served as opponents (obtained from Harlan Winkelmann GmbH, Borchen, Germany), since this strain is characterised by a high level of intermale aggression [49]. To further stimulate aggressiveness and therefore allow for a high success rate when generating experiences as a loser, NMRI males were housed individually during the whole experimental phase. At the time of the experiments C3H males, as well as NMRI males, were at least 60 days of age.

All experimental mice, as well as the C3H and NMRI opponents, were housed in standard Macrolon cages type III (38 cm × 22 cm × 15 cm) with a paper towel and sawdust as bedding material (Allspan, Höveler GmbH & Co. KG, Langenfeld, Germany). To guarantee that experimental males and NMRI males defended their cages as their territory against an intruding opponent, cages were not cleaned at least for four days prior to testing. The housing room was maintained at a 12 h light/dark cycle (lights on at 08:00 a.m.) and a temperature of 22 ± 3 °C. Commercial mouse diet (Altromin 1324, Altromin GmbH, Lage, Germany) and water were available *ad libitum*. Tests were conducted between 08:00 a.m. and 10:00 a.m..

The present work complies with current regulations covering animal experimentation in Germany and the EU (European Communities Council Directive 86/609/EEC). All experiments were announced to the local authority and were approved by the 'Animal Welfare Officer' of the University of Münster (reference number: 8.87–50.10.46.08.151).