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Relationship between striatal levels of interleukin-2 mRNA and plus-maze behaviour in the rat

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Abstract

Our previous experiments have shown that adult male Wistar rats can differ systematically in elevated plus-maze (EPM) behaviour, which was related to the neurotransmitter serotonin in the ventral striatum. The EPM serves as a model of anxiety-like behaviour, and there is evidence that interleukin (IL)-2 in the brain may be related to anxiety-like behaviour, and that IL-2 interacts with the striatal serotonergic system. We asked whether EPM behaviour may also be related to constitutive levels of cytokines in the striatum. Based on open arm time in the EPM, male Wistar rats were divided into sub-groups with either low or high anxiety-like behaviour. Then, IL-1 β , IL-2, IL-6, and tumour necrosis factor (TNF)- α cDNA levels were measured post mortem in striatal tissues using semi-quantitative, competitive, reverse transcription polymerase chain reaction. Rats with high anxiety-like behaviour in the EPM showed significantly higher levels of IL-2 mRNA compared to those with low anxiety-like behaviour, but did not differ significantly in expression of IL-1 β , IL-6, and TNF- α mRNA. These results provide new evidence indicating that specific cytokine patterns in the striatum may be associated with EPM behaviour in adult male Wistar rats.

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The elevated plus-maze (EPM) is a widely used behavioural paradigm that presumably measures fear-motivated avoidance behaviour and which has been extensively assessed as a model of anxiety [11]. During a typical EPM test, animals will actively avoid the open arms in favour of the closed arms. Work with selectively bred Wistar rats [9] and outbred Wistar rats [10] has shown that these animals, although identical in strain, sex, and age, can differ systematically in anxiety-like behaviour in the EPM. These behavioural differences were related to the neurotransmitter serotonin in the ventral striatum [17], a brain region which is critical for motivated behaviour, and a transmitter which is critical for anxiety [7]. Moreover, there is evidence that cytokines may influence the release of biogenic amines such as serotonin in the nucleus accumbens of the ventral striatum, and that the profile of changes is cytokine-specific [19].

Cytokines have been shown to affect behaviour, e.g.

interleukin (IL)-2 induces behavioural changes in novelty-induced locomotion [21], and spatial memory [14]. However, only very few studies have analyzed anxiety-like behaviour and IL-2, with yet inconsistent results. In animal models of anxiety, repeated systemic IL-2 administration was ineffective [1,14], whereas in patients with anxiety disorders decreased IL-2 production compared to normal controls was observed [12]. Conversely, it is unknown whether constitutive cytokines (defined here as constantly present) in specific brain areas may be related to anxiety-like behaviour.

Here, we asked whether plus-maze behaviour may be related to cytokine mRNA levels in the striatum. Based on the percentage of open arm time in the EPM, male adult Wistar rats were divided into sub-groups with low (LA), or high (HA) anxiety-like behaviour [10]. Then, IL-1 β , IL-2, IL-6, and tumour necrosis factor (TNF)- α cDNA levels were measured post mortem in striatal tissues.

Thirty-four male outbred Wistar rats (Harlan Winkelmann, Borchon, Germany), weighing 270–330 g at the

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beginning of the experiment were used. They were housed in groups of five per cage under standard laboratory conditions with food and water ad libitum. The housing room was maintained on a 12 h light/dark cycle (lights on: 07:00–19:00 h). Ambient temperature was 23 ± 1 °C. All animals underwent gentling and handling daily (5 min each) for 3 days prior to testing. All experiments were conducted in the light cycle (10:00–16:00 h), and in accordance with the ethical regulations for animal experimentation at the University of Marburg.

According to our routine procedure [10], the animals were first exposed to an open field on two consecutive days (10 min each; data not shown). Four days later, the rats were tested once (5 min) in the EPM (50 cm above the floor), which was illuminated by four red bulbs (28 lux in the centre). The EPM was made of plastic and consisted of two opposite open arms (50 × 10 cm), two opposite closed arms (50 × 10 cm; with 40 cm high walls), and a middle section (10 × 10 cm). Behaviour was recorded by a video system. The following behavioural measures were scored from videotape: (1) the time spent on open arms; (2) the number of entries into all arms; (3) the number of rearings. Based on the percentage of time spent on the open arms relative to total arm time (open arm time/total arm time × 100), the animals were divided into HA ($n = 17$) and LA ($n = 17$) rats by median split [10,17].

Twenty-four hours after EPM testing all animals were anaesthetized with sodium pentobarbital (Narcoren®; Merck GmbH, Hallbergmoos, Germany, 1.5 ml/kg, i.p.), decapitated, and both brain hemispheres were quickly removed. Immediately after excision, the striatal tissue samples, which comprised dorsal and ventral striatum, were weighed and frozen in liquid nitrogen. Until RNA extraction with RNA-Clean® (ASG, Heidelberg, Germany), the samples were stored at -70 °C. Extracted RNA was stabilized with 40 units/μl RNasin® (Promega, Madison, WI). For semi-quantitative, competitive reverse transcription polymerase chain reaction (RT-PCR), a multispecific competitor fragment for rat cytokines was used [18]. Before amplification of IL-1β, IL-2, IL-6, and TNF-α, the housekeeping gene β-actin was amplified to check the efficiency of the reverse transcriptase reaction. When needed, a correction was introduced in order to start with the same amount of cDNA in each tube (cDNA equivalent of about 5 ng total RNA). After PCR, the samples were separated in a 1% agarose gel, ethidium bromide stained, digitalized, and analyzed with the Gelscan Software (BioSciTec, Marburg, Germany). For quantification, only bands with similar intensity on the grey scale between the competitor fragment and the cytokine of interest were used.

Behavioural measures and mRNA expression were analyzed double-blind. Unpaired *t*-tests were applied for behavioural analyses and tissue weight comparisons. Mann–Whitney *U*-tests, Wilcoxon tests, or Spearman correlations were used for all analyses involving cytokine mRNA expression. All *P* values are two-tailed and taken as

statistically significant when <0.05 . Data are expressed as mean ± SEM.

In the EPM (Fig. 1), where the percentage of time spent on the open arms was used to assign rats to either the HA or LA sub-group, HA rats showed a range of 0–46% of time spent on the open arms compared to LA rats with a range of 47–85%. In contrast, HA and LA rats did not show significant differences in rearing activity, or in the number of arm entries in the EPM ($P > 0.10$).

The mean weight of the striatal samples was similar in HA (58.94 ± 1.73) compared with LA rats (60.29 ± 2.34 ; $P > 0.10$). The levels of cytokine cDNA in the striatum varied intra-individually and inter-individually analyzing all rats (Table 1). IL-1β mRNA expression was significantly higher compared to all other cytokine mRNA levels (IL-2, IL-6, TNF-α; $P < 0.001$). Additionally, IL-2 mRNA was significantly higher than IL-6 mRNA, and TNF-α mRNA ($P < 0.01$), whereas IL-6 and TNF-α showed comparable amounts of cDNA ($P > 0.10$). Analyses of the sub-groups showed that HA rats expressed significantly higher levels of IL-2 mRNA compared to LA rats ($P = 0.022$). HA rats also showed a trend for higher levels of IL-1β ($P = 0.073$). In contrast, there were no differences between HA and LA rats with regard to IL-6 mRNA, or TNF-α mRNA ($P > 0.10$; Fig. 2).

When correlating behaviour with cytokine mRNA using all rats ($n = 34$), significant negative relationships between the percentage of open arm time and IL-2 ($r = -0.39$, $P = 0.023$), or IL-1β ($r = -0.34$, $P = 0.047$) were found. In contrast, the number of arm entries and the number of rearings were not significantly correlated with any of the cytokine mRNAs ($P > 0.10$).

The aim of the present study was to analyze if EPM behaviour is related to the expression of striatal cytokine mRNA. Higher IL-2 mRNA levels were expressed in HA rats compared with LA rats. In contrast, IL-1β, IL-6, and TNF-α mRNA levels were not significantly different

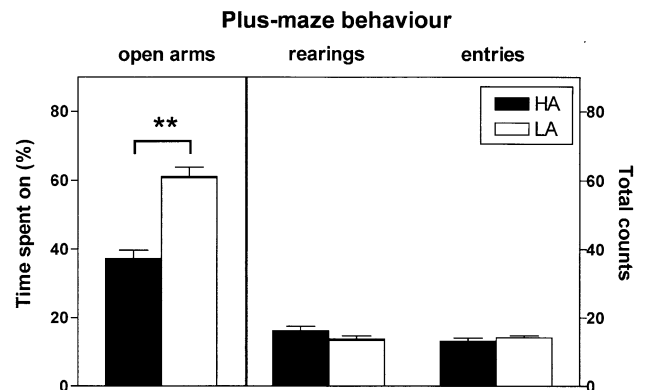


Fig. 1. Based on the percentage of open arm time, the animals were divided by median split into sub-groups with high (HA; dark bars) and low anxiety-like behaviour (LA; white bars). Percentage of open arm time (open arms), rearing activity (rearings), and number of arm entries into all arms (entries) for HA vs. LA animals are shown. Data are expressed as mean + SEM. *** $P < 0.001$.

Table 1
Expression of cytokine cDNA (pg) in the striatum of 34 adult male Wistar rats

	Mean	SEM	Range	
			Minimum	Maximum
IL-1 β	0.0403	0.0040	0.0096	0.0994
IL-2	0.0207	0.0039	0.0001	0.0792
IL-6	0.0078	0.0016	0.0004	0.0389
TNF- α	0.0069	0.0019	0.0007	0.0599

between the two sub-groups, suggesting that levels of IL-2 mRNA expression in the striatum are associated with anxiety-like behaviour in male Wistar rats. When performing correlational analyses using all rats, significant negative correlations between the percentage of open arm time and IL-2, and to a lesser extent IL-1 β , were found. These results confirm previous evidence of immunological differences between rats of the same sex, age, and strain [5], providing first evidence that striatal cytokines may be related to plus-maze behaviour in the rat. However, future work will be necessary to investigate whether these expression effects are also linked to differences in protein production, and whether such effects are anatomically specific to the striatum. Preliminary data show that hippocampal IL-1 β mRNA levels do not differ between HA and LA rats (unpublished data).

In the present study we found cytokine-specific differences in HA and LA sub-groups, showing that only the percentage of open arm time, but neither vertical rearing nor horizontal locomotion in the EPM, were related to striatal cytokine mRNA levels. Moreover, behavioural analyses showed no significant differences between HA and LA rats in rearings (vertical activity), or in the number of arm entries (horizontal activity) in the EPM. This indicates that our EPM criteria can gauge a behavioural trait in Wistar rats which is related to cytokine mRNA in the brain.

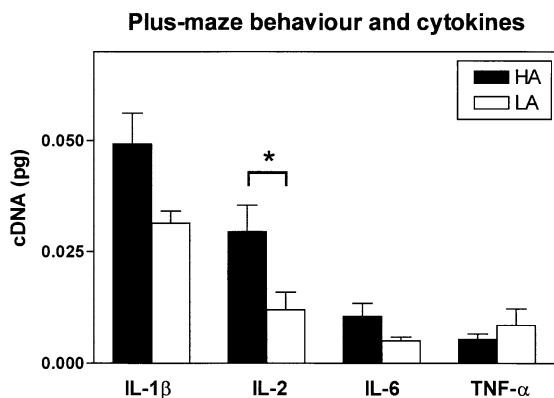


Fig. 2. Based on the percentage of open arm time, rats with high (HA; dark bars) compared to low anxiety-like behaviour (LA; white bars) expressed significantly higher levels of IL-2 mRNA (* $P = 0.022$), and showed a trend for higher levels of IL-1 β ($P = 0.073$). IL-6 mRNA or TNF- α mRNA did not differ between the two sub-groups. Data are expressed as mean + SEM.

The origin of striatal cytokine mRNA is not clear. Blood–brain barrier passage of circulating IL-2 or infiltrating lymphocytes could serve as a possible source of both IL-2 protein and mRNA. Centrally, for example, the occurrence of CNS-specific forms of IL-2 mRNA transcripts and protein, and genes for IL-2 receptor β have been detected in brain regions like striatum, hippocampus, and cortex [6,8,15]. Although the cellular origin of IL-2 is not known, astrocytes have been suggested as one possible source [6].

Next to IL-2, our results also provide some evidence for a role of striatal IL-1 β mRNA in relation to EPM behaviour. The differences in striatal cytokine mRNA levels between HA and LA rats were lower for IL-1 β (57%) than for IL-2 (145%), and in contrast to IL-2, there were no significant group differences of IL-1 β mRNA. However, the correlational analysis showed that IL-1 β mRNA was also negatively related to the percentage of open arm time. There is evidence that i.c.v. administration of IL-1 β may have anxiogenic effects [4]. Therefore, not only IL-2, but also IL-1 β function should deserve specific attention in future studies.

Apart from the possible physiological source of cytokine mRNA, the mechanism has to be investigated which led to the differences between animals. One can speculate that the mRNA levels reflected either constitutive differences between subjects, or that they were due to transient impacts (like prior behavioural testing, handling, or the procedure of tissue removal) which might have different effects on HA and LA rats. On the basis of the present data, this question can not be answered. It should be pointed out, however, that a previous study showed that neither an acute nor a repeated stressor exposure affected brain cytokine mRNA [16]. We therefore suggest that the present cytokine mRNA differences were due to constitutive rather than transient mechanisms.

Previously, we have shown that anxiety-like behaviour in the EPM was negatively related to serotonin levels in the ventral striatum [17], a neurotransmitter which is important for anxiety [7], and a brain region which is critical for motivated behaviour. Interestingly, Song et al. [19] showed that administration of IL-2 into the nucleus accumbens, which is part of the ventral striatum, suppressed levels of the serotonergic metabolite 5-hydroxyindoleacetic acid, indicating that it may have inhibited serotonergic activity. In the present study, we found higher levels of IL-2 mRNA expression in HA rats. We do not know whether this higher IL-2 expression is also linked to higher cytokine protein production in HA rats, but if so, one could suggest this as a mechanism underlying the previously found differences in ventral striatal serotonin levels [17], which in turn may have mediated the distinctive behavioural profiles in the EPM.

Previous animal studies showed that IL-2 can affect various types of motivated behaviour [14,21], but they did not provide evidence for a relationship with anxiety-like behaviour [1,4,14]. This lack of effect may be due to the sites of administration used, which were systemical [1,14]

or i.c.v. [4]. Since the present study points at a role of cytokines in the striatum, further studies should be done where anxiety-motivated behaviour is investigated using striatal administration of cytokines. This call for further animal work is also supported by clinical evidence which showed that IL-2 administration may have effects on motivation and emotion since depressive-like symptoms were induced by IL-2 in tumour patients [20].

Cytokines in the brain have been suggested as mediators of 'sickness behaviour', a presumptive strategy of the organism to fight infection and disease, which comprises adaptive physiological (like fever) and behavioural (i.e. motivational) changes [13]. Similar changes are also evident in humans during infection, which were determined by quality of life measures [2]. The present data may be of relevance in this context, since evidence for a role of cytokines was obtained in a brain site which is known for its importance in motivated behaviour [3]. Thus, one can hypothesize that striatal cytokine function may modulate individual expression of motivated behaviour in the intact organism, and thus determine the type or degree of individual behavioural responsiveness to bodily challenges (like infection).

In conclusion, the present findings complement and extend existing knowledge on differential physiological characteristics of anxiety models in rats. We suggest levels of IL-2 mRNA expression in the striatum to be related with plus-maze behaviour in adult male Wistar rats. It is hypothesized that striatal serotonergic mechanisms may interact with cytokines in the determination of behavioural patterns in the EPM, which may have implications for motivational behaviour in the healthy organism, and in sickness behaviour.

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