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Effect of repeated experience of victory and defeat in daily agonistic confrontations on brain tryptophan hydroxylase activity

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Abstract The rate-limiting enzyme of serotonin biosynthesis, tryptophan hydroxylase (TPH), was studied in brain areas of male mice with repeated experience of victory (winners) or defeat (losers) gained in 10 daily agonistic confrontations. A reduction of TPH activity in the midbrain and an increase in the hypothalamus was demonstrated for winners compared with controls. In contrast, repeated defeat in social confrontations was associated with higher TPH activity in the striatum and hypothalamus in losers compared with controls. Agonistic interactions did not affect TPH activity in the amygdala, nucleus accumbens or hippocampus in either winners or losers. The sensory contact technique used in this work for generating winners and losers may be productive in the analysis of TPH gene regulation.

Key words: Tryptophan hydroxylase; Aggression; Submission; Serotonin; Mouse; Agonistic behavior; Sensory contact technique

1. Introduction

There is ample evidence that a rise in brain serotonin (5-HT) activity attenuates agonistic behavior in animals (for reviews, see [1,2]) whereas pharmacological manipulation leading to lower brain levels of 5-HT can elicit aggressive behavior [3]. These findings favor the concept of the major inhibitory role of 5-HT in aggressive behavior [4–6]. However, direct measurements of 5-HT or 5-HIAA, its major metabolite, in CSF or in the brain tissue of animals that have just displayed aggressive behavior indicate that the level has either remained the same or become lower or higher (for review, see [7]). These measurements are therefore noninformative as far as the role of 5-HT in aggressive behavior is concerned.

The key rate-limiting enzyme of 5-HT biosynthesis, tryptophan hydroxylase (TPH), is a direct marker of brain serotonergic function (for review, see [8]). The aim of this study was to examine TPH activity in the midbrain (serotonergic cell bodies), limbic areas, and neostriatum (serotonergic terminals) of male mice with repeated experience of victory (winners) or defeat (losers).

2. Materials and methods

2.1. Animals

Adult male CBA/Lac mice, maintained at the Institute, were used. The animals were housed under standard vivarium conditions and a natural light regime; food and water were available ad libitum. Males were weaned at the age of one month and housed in one-litter groups of 8–10 individuals in plastic 36×23×12 cm cages. Mice used in the experiments were 10–12 weeks of age.

2.2. Technique for generating aggressive and submissive behavior in male mice

To generate aggressive and submissive behavior in the mice, the sensory contact technique [9] was used. The males were weighed and caged individually for 5 days to remove group effects. Animals of approximately the same weight were then placed by pairs in steel cages (28×14×10 cm) divided into halves by a perforated transparent partition, permitting them to see, hear and smell the neighbor whilst preventing physical contact. After 2 days of adaptation to the housing conditions and sensory contact, testing commenced. Every afternoon (14:00–17:00 h local time), the steel cover of the cage was replaced by a transparent one and, 5 min later (the period necessary for individuals' activation) the partition was removed for 10 min to allow agonistic interaction. Undoubted superiority of one of the partners was evident within 2 or 3 tests of daily social encounter with the same opponent. One member of each pair was seen to attack, bite, and chase the other who displayed only defensive behavior (sideways, upright postures, and also 'on the back' or 'freezing') during the test. Then, every day after the test, each defeated male of a pair was paired with the winning member of another pair behind the partition in an unfamiliar cage. The aggressive males remained in their own compartments. This procedure resulted in equal numbers of winners and losers. Three experimental groups were studied:

Winners	aggressive males that were victorious in 10 daily agonistic confrontations;
Losers	submissive males that were defeated in 10 daily agonistic confrontations;
Controls	males housed individually for 5 days. They were thought to be best as intact controls, because, in this case, the submissiveness of grouped males would be removed, and the repeated experience of aggression would not yet be acquired [9].

Twenty-four hours after the last agonistic confrontation, the animals were decapitated; their brains were removed and chilled rapidly on ice. The neostriatum, hippocampus, midbrain with pons, nucleus accumbens with the olfactory tubercles, hypothalamus, and amygdala were dissected according to [10], rapidly frozen, and stored at −60°C until use, but not longer 3 three weeks.

2.3. Tryptophan hydroxylase assay

The samples were homogenized with 5 volumes of 50 mM Tris-acetate buffer (pH 7.5) containing 1 mM dithiothreitol (Sigma). The homogenates were centrifuged at 20 000×*g* per for 30 min (+4°C). Enzyme activity was determined in the supernatant in the presence of L-tryptophan (0.8 mM, Sigma, St. Louis, MO) and 6,7-dimethyl-5,6,7,8-tetrahydropteridin (0.5 mM, Sigma) by a fluorescence microassay described in detail [11] and expressed in pmol of 5-hydroxytryptophan per mg of protein per min.

2.4. Statistics

Data were subjected to one- and two-factor (TPH activity; factor A, social status; factor B, brain region) ANOVAs using the STATGRAF/PC program package. Further comparison of the groups was performed using Tukey's tests.

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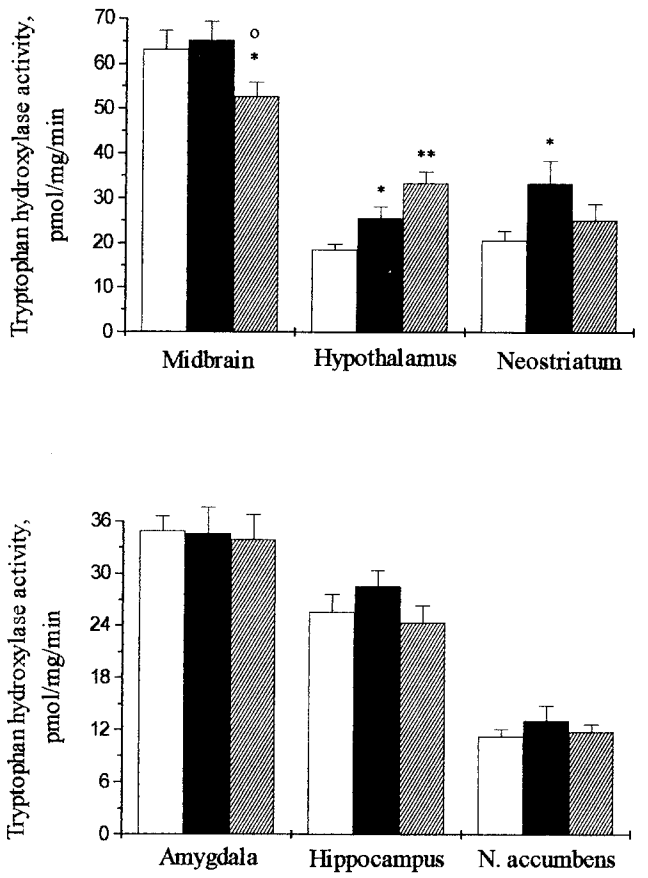

Fig. 1. Activity of tryptophan hydroxylase in brain areas of the control mice (open columns), losers (black columns) and winners (hatches columns). Data are presented as means and standard errors. Number of animals in each group is 9–10. $^{*}P<0.05$, $^{**}P<0.01$ vs. control, $^{\circ}P<0.05$ vs. losers.

3. Results

TPH activity in the winners, losers, and controls is depicted in Fig. 1. Two-factor ANOVA revealed significant main effects for social status ($F(2,176)=4.4$, $P<0.01$), brain area ($F(5,176)=106.83$, $P<0.0001$) and status × brain area interactions ($F(10,176)=2.26$, $P<0.02$). Follow-up analyses indicated significantly lower midbrain TPH activity in winners compared to losers ($F(1,18)=5.56$, $P<0.03$) and controls ($F(1,18)=4.22$, $P<0.05$). In the hypothalamus, TPH was significantly higher in both losers ($F(1.18)=5.81$, $P<0.03$) and winners ($F(1,18)=12.78$, $P<0.002$) than in controls, with winners and losers not differing significantly ($F(1,18)=0.874$, n.s.). Repeated defeats increased TPH activity in the neostriatum of losers compared with controls ($F(1.17)=5.92$, $P<0.03$). No significant differences in neostriatum TPH were found between winners and losers ($F(1,16)=1.78$ n.s.), or winners and controls ($F(1,17)=1.14$, n.s.). Positive or negative experience of agonistic confrontations did not affect TPH activity in the amygdala: winners vs. controls $F(1,18)=0.08$, n.s.; winners vs. losers $F(1,18)=0.032$, n.s.; controls vs. losers $F(1,18)=0.003$, n.s. Also, there were no significant differences in hippocampus TPH activity between winners and controls ($F(1,18)=0.23$, n.s.), winners and losers ($F(1,17)=2.44$, n.s.), or controls and losers ($F(1,17)=1.18$, n.s.). Neither did experience of victory or defeat affect TPH activity in the nucleus accumbens: winners vs. controls $F(1,18)=0.137$, n.s.; winners vs. losers $F(1,18)=0.493$, n.s.; losers vs. controls $F(1,18)=0.887$, n.s.

4. Discussion

The present study revealed changes in the activity of the key rate-limiting enzyme of 5-HT biosynthesis, TPH, in mice subjected to repeated victories or defeats in daily agonistic confrontations. The experience of repeated victory was associated with a specific reduction in TPH activity in the midbrain of winners compared with losers and controls (Fig. 1). This result suggests that continual aggression leads to lower TPH activity in the brain area which contains most neurons synthesizing 5-HT and TPH (for reviews, see [8,12]). Also, lower neostriatal TPH activity has been shown for winners versus losers and controls following more than 20 days of agonistic confrontations [13]. Lower TPH activity indicates that serotonergic function in aggressive males is low. Similar findings have recently been reported in the midbrain of Norway rats as a function of level of defensive aggression to man: lower TPH activity in this brain area has been observed in aggressive wild rats compared to docile domesticated rats [14].

The present results also show higher neostriatal TPH activity in losers versus controls. Previous work has shown higher 5-HT levels in the amygdala and 5-HIAA levels (or the 5-HIAA/5-HT ratio) in the olfactory bulbs and hippocampus after 10 days of repeated defeat experience [15]. It is well known that various kinds of repeated stress raise the brain level of tryptophan, which in turn enhances 5-HT synthesis [8]. Significantly lower basal TPH activity was found in 5-HT terminal areas (amygdala, nucleus accumbens and hippocampus), compared to midbrain. However, our results show that TPH activity in these areas does not change as a function of repeated agonistic confrontations. Interestingly, similarly higher TPH activity was discovered in the hypothalamus of both losers and winners (versus controls). This finding is therefore most probably related to status-independent chronic stress associated with daily agonistic confrontations. In this context, it has been shown that corticosterone acts as a genetic inducer of the synthesis of new molecules of TPH [16]. Thus, the present data provide evidence that different brain areas have different roles to play in agonistic behavior and that, in these areas, serotonergic processes differ depending on whether repeated victories or defeats have been experienced.

The TPH gene encodes the rate-limiting enzyme TPH in the raphe neurons of the midbrain. Moreover, polymorphism of the TPH gene correlates with serotonergic function and behavior [17]. It is important that the sensory contact technique [9], allowing changes in brain TPH activity to be detected, can be used in the analysis of TPH gene regulation. This technique should therefore facilitate studies of the mechanisms controlling the expression of the TPH gene and help to uncover its role, at least in aggressive and submissive behavior.

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