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SHORT COMMUNICATION

[^{3}H]SCH 23390 Binding in Various Brain Regions of C57BL/6J Mice with Repeated Experience of Victory or Social Defeat in Agonistic Interactions

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Summary

The binding of [^{3}H]SCH 23390 has been studied in various brain regions of male mice with the experience of repeated victory (winners) or defeat (losers) gained over 10 (T10) and 20 (T20) days of daily agonistic confrontations. In the frontal cortex, B_{max} of [^{3}H]SCH 23390 binding sites was found to be increased in T10 losers and decreased in T20 losers when compared to the control mice. In the striatum, T10 and T20 winners had reduced values of [^{3}H]SCH 23390 binding sites than the ones in the control mice. The K_d was increased in the frontal cortex of T10 losers and T10 winners as well as in the amygdala of T20 losers. Reduced K_d values were found in the striatum of all experimental groups as well as in the amygdala of T20 winners. Thus, both specific changes relating to social behavior patterns and non-specific ones in [^{3}H]SCH 23390 binding were found in the brain regions of mice after 10 and 20 days of intermale confrontations.

Key words

Sensory contact model • Mice • Agonistic confrontations • Winners • Losers • [^{3}H]SCH 23390 binding

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Social defeats in mice (Puglisi-Allegra and Cabib 1990) and rats (Tidey and Miczek 1996) have been shown to lead to changes in the levels of dopamine (DA) and its metabolism in the brain regions related to the mesocorticolimbic dopaminergic system. Recent data obtained using positron emission tomography with [^{11}C]SCH 23,390 in patients with major depressive disorder and anger attacks (Dougherty *et al.* 2006) gave evidence of the participation of D_1 striatal receptors in the mechanisms of aggression. It was shown that [^{11}C]SCH 23,390 binding in the bilateral striata was significantly reduced in such patients.

A sensory contact model, which uses prolonged intermale confrontations, has revealed nonspecific changes in DA and its important metabolite 3,4-dehydroxyphenyleacetic acid (DOPAC) levels in hypothalamus and whole midbrain in males with prolonged experience of defeats (losers) or victories (winners) (Kudryavtseva 2000). In addition, the development of pharmacological desensitization of dopamine D_1 receptors have been found in losers and winners after 20 days of intermale confrontations (Kudryavtseva *et al.* 2008). To determine specific dynamic changes in D_1 receptors in the mice brain, which are induced by the experience of victories and defeats gained in intermale confrontations, it is necessary to study the kinetic characteristics of [^{3}H]SCH 23390 binding in losers and winners with 10 and 20 days of experience of agonistic confrontations.

Adult male C57BL/6J mice (10-12 weeks of

age), maintained at the Institute of Cytology and Genetics SD RAS (Novosibirsk, Russia), were kept under standard vivarium conditions with a natural light:dark cycle of 12:12 h. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

To generate aggressive and submissive behavior in male mice, the sensory contact model (Kudryavtseva 2000) was used. Animals of the same weight were placed by pairs in steel cages (28x14x10 cm) divided into halves by a perforated transparent partition permitting them to see, hear and sense the smell of the neighbor, whilst preventing physical contact. After three days of adaptation to the housing conditions and sensory contact, testing commenced. Every afternoon (15:00-17:00 h local time), the steel cover of the cage would be replaced by a transparent one, and 5 min later (the period necessary for the individuals' activation) the partition would be removed for 10 min to let the agonistic interaction begin. With each pair, the superiority of one of the opponents became evident within 2 or 3 tests in daily social encounter. One member was seen to attack, bite, and chase the other, who, in turn, had been displaying only defensive behavior (sideways, upright postures, and also "on the back" posture, and 'freezing') during the test. As a rule, in all our experiments, aggressive confrontations between males are discontinued by lowering the partition if the aggression had lasted more than 3 min. Every day after the test, each defeated male of 1 pair was paired with the winning member of another pair behind the partition in an unfamiliar cage. The aggressive males would remain in their own compartments. This procedure resulted in equal numbers of animals with opposite social status. Five experimental groups of mice were studied: T10 and T20 Winners – aggressive males, i.e. the mice who had been victorious all the way through 10 and 20 encounters, respectively; T10 and T20 Losers – submissive males, i.e. the mice who had been defeated all the way through as many encounters, respectively; and the control mice, that is, those who had been kept alone for 5 days. The latest were thought to be best as intact controls in the sensory contact model, because, in this case, the submissiveness of grouped males would be removed, and effects of social isolation would not yet be acquired (Kudryavtseva 2000).

Twenty four hours after the last encounter, the animals were decapitated; their brains were quickly removed and chilled rapidly on ice. The amygdala, frontal cortex and striatum were isolated, frozen in liquid nitrogen and stored at –70 °C (for less than 3 weeks) until used. The specific binding of [^{3}H]SCH 23390 (85 Ci/mmol, Amersham, UK) to D_1 receptors in the membranes of the dissected brain regions was investigated in radioligand assay with minor modifications (Nikulina *et al*. 1995). Brain specimens were homogenized in 20 volumes (w/v) of ice-cold potassium phosphate buffer (50 mM, pH 7.4) using glass homogenizers and centrifuged at 20,000 x g for 20 min. This procedure was repeated twice. Final pellets were resuspended in 100 volumes of potassium phosphate buffer (pH 7.4). Kinetic analysis was performed using selective antagonist of the D_1 receptors [^{3}H]SCH 23390 in 6-8 concentrations ranging between 0.0625 and 2 nM or 0.03125 and 4 nM. Nonspecific binding was detected by adding a displacing agent 10 μM unlabelled *cis*-flupentixol (Lundbeck, Denmark). All samples were incubated 60 min at 30 °C. The reaction was stopped by filtration of the suspensions through fine fiber glass filters GF/B (Whatman) under vacuum. The maximum number of receptor binding sites (B_{max}), dissociation constant (K_d) and SEMs were calculated by linear regression analysis (Cornish-Bowden 1976). The protein content was determined by the standard method of Lowry *et al*. (1951). Binding data of all experiment groups were compared using Student's *t*-test.

Table 1. K_d values (nM) of [^{3}H]SCH 23390 binding in brain regions of experimental groups of mice[a].

Brain regions	Control	T10 Losers	T20 Losers	T10 Winners	T20 Winners
Amygdala	0.25 ± 0.03	0.20 ± 0.02	0.35 ± 0.03* $^{+++}$	0.20 ± 0.04	0.14 ± 0.02**
Striatum	1.00 ± 0.04	0.77 ± 0.03***	0.80 ± 0.04**	0.43 ± 0.03***	0.81 ± 0.05** $^{+++}$
Frontal cortex	0.17 ± 0.03	0.67 ± 0.07**	0.19 ± 0.03^{+++}	0.37 ± 0.09*	0.24 ± 0.04

[a] n=12-19 mice in each group; * p<0.05; ** p<0.01; *** p<0.001 – *vs* Control; $^{+++}$ p<0.001 – T20 Losers *vs.* T10 Losers (or T20 Winners *vs.* T10 Winners)

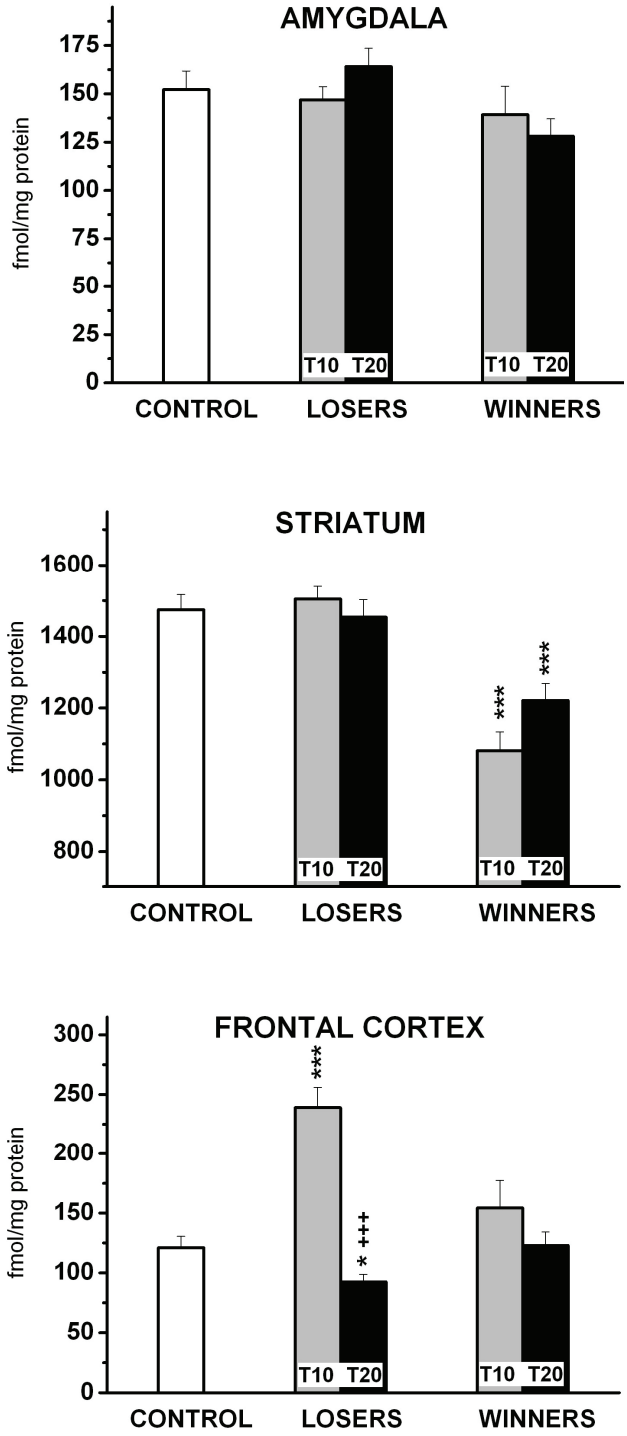

Fig. 1. [^{3}H]SCH 23390 binding sites in brain regions of loser, winner and control male mice. Data are presented as (mean ± SEM), n=12-19, the number of mice in each group. * $p<0.05$; *** $p<0.001$ – *vs* Control; $^{+++}$ $p<0.001$ – T20 Losers *vs* T10 Losers.

In amygdala, the brain region of mesolimbic dopaminergic system, no difference was found in B_{max} values of [^{3}H]SCH 23390 binding between control mice, losers or winners after either 10 or 20 days of agonistic confrontations (Fig. 1). At the same time, changes in K_d value have been observed after 20 social encounters with K_d being significantly higher in T20 losers and significantly lower in T20 winners compared to control mice (Table 1). The data indicate that the affinity of D_1 receptors in amygdala undergoes opposite changes during the formation of alternative behavior patterns in mice under social encounters – it increases in winners and decreases in losers.

In the striatum, which relates to the nigrostriatal dopaminergic system, both losers and control mice had the same B_{max} values, however, in T10 and T20 winners the B_{max} value was significantly reduced (Fig. 1). The K_d values were found to undergo changes in the striatum of losers and winners after both 10 and 20 days of agonistic confrontation experiences (Table 1). In T10 and T20 losers, the K_d was gradually reduced as compared with control mice, while in the winners it was reduced significantly more in T10 than in T20 winners. Reduced density of [^{3}H]SCH 23390 binding sites in the striatum of winners could be regarded as specific reduction of D_1 receptors sensitivity in this brain region induced by aggressive behavior pattern. Our pharmacological data on the administration of D_1/D_2 receptor antagonist haloperidol and D_1 receptor antagonist SCH 23390 support this assumption (Kudryavtseva *et al*. 2008).

In the frontal cortex, associated with the mesocortical dopaminergic system, major changes were observed in the losers rather than the winners. In this case B_{max} of [^{3}H]SCH 23390 binding sites was almost twice higher in T10 losers and significantly lower in T20 losers than in the control mice (Fig. 1). The dynamics of K_d in the frontal cortex of losers and winners was similar: the K_d values were increased after 10 intermale confrontations and decreased after 20 ones (Table 1). With stress escalation resulting from intermale confrontations an increased number of D_1 receptors was observed in the frontal cortex of T10 losers followed by a significant decrease of this value in T20 losers, suggesting the dynamic changes in the sensitivity of D_1 receptors in the losers' brain. Supportive of this assumption are our pharmacological data on the use of nonselective D_1/D_2 receptors antagonist *cis*-flupentixol (Kudryavtseva *et al*. 2008). It is to be emphasized that T20 losers manifest all the signs of depression-like state, as was shown earlier (Kudryavtseva *et al*. 1991, Kudryavtseva and Avgustinovich 1998). One may speculate that changes in [^{3}H]SCH 23390 binding observed in the losers' frontal cortex are specific and

conditioned by the development of depression-like state in mice. Other authors also pointed to depression-mediated changes primarily in the mesocorticolimbic dopaminergic system (Markou *et al*. 1998), but not in the striatum of depressed suicides (Bowden *et al*. 1997).

Therefore, one may conclude that there is a difference in [^{3}H]SCH 23390 binding in winners and losers, primarily in the frontal cortex and striatum. A prolonged experience of victories results in a reduction of [^{3}H]SCH 23390 binding sites in the striatum of mice accompanied by an enhancement of receptor affinity, which is particularly pronounced after 10 days of intermale confrontations. On the other hand, chronic social defeat experience leads to the specific changes in the number of [^{3}H]SCH 23390 binding sites in the losers' frontal cortex – an increase after 10 and decrease after 20 intermale confrontations, which is accompanied by fluctuations in D_1 receptor affinity at these times. Besides, the affinity of D_1 receptors in amygdala undergoes opposite changes depending on the social experience – it decreases in T20 losers and increases in T20 winners. Therefore, it seems probable that these changes are related both to the status achieved by mice suffering chronic social stress and to the duration of daily agonistic confrontations. Moreover, our results confirm the hypothesis of possible dynamic neurochemical changes depending on the duration of the psychoemotional stress and the depth of the behavioral pathology that develops (Kudryavtseva and Avgustinovich 1998).

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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