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Acute buspirone abolishes the expression of behavioral dopaminergic supersensitivity in mice

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Abstract

Previous studies have shown that rats withdrawn from long-term treatment with dopamine receptor blockers exhibit dopaminergic supersensitivity, which can be behaviorally evaluated by enhanced general activity observed in an open-field. Recently, it has been reported that co-treatment with the non-benzodiazepine anxiolytic buspirone attenuates the development of haloperidol-induced dopaminergic supersensitivity measured by open-field behavior of rats. The aims of the present study were: 1) to determine, as previously reported for rats, if mice withdrawn from long-term neuroleptic treatment would also develop dopaminergic supersensitivity using open-field behavior as an experimental paradigm, and 2) to examine if acute buspirone administration would attenuate the expression of this behavioral dopaminergic supersensitivity. Withdrawal from long-term haloperidol treatment (2.5 mg/kg, once daily, for 20 days) induced a significant (30%) increase in ambulation frequency (i.e., number of squares crossed in 5-min observation sessions) but did not modify rearing frequency or immobility duration in 3-month-old EPM-M1 male mice observed in the open-field apparatus. Acute intraperitoneal injection of buspirone (3.0 and 10 but not 1.0 mg/kg, 12-13 animals per group) 30 min before open-field exposure abolished the increase in locomotion frequency induced by haloperidol withdrawal. These data suggest that the open-field behavior of mice can be used to detect dopaminergic supersensitivity, whose expression is abolished by acute buspirone administration.

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- Mice

In rats, abrupt withdrawal from long-term dopamine receptor blockers such as haloperidol (1-3), bromopride (4), metoclopramide (5) or sulpiride (6) enhances general activity observed in an open-field. This effect has been considered to be a consequence of the development of supersensitivity of central dopaminergic pathways. Indeed, behavioral supersensitivity is thought to result from receptor site proliferation in mesolimbic and striatal brain tissues in response to chronic dopamine receptor blockade (7,8). From a clinical point of view, dopaminergic supersensitivity has been proposed as a possible contributing factor to the development of tardive dyskinesia, a serious adverse mo-

tor effect of prolonged administration of antipsychotic drugs that is characterized by abnormal involuntary repetitive movements of the face and limbs that are purposeless in nature (for a review, see Ref. 9).

The effects of the non-benzodiazepine anxiolytic buspirone have been studied both on dopaminergic supersensitivity measured by rat behavior in an open-field and on tardive dyskinesia in humans. The similar results obtained in these two experimental situations have corroborated the alleged relationship between this movement disorder and dopaminergic supersensitivity. Indeed, although buspirone has high affinity for dopaminergic-binding sites (10) and reduces yawning and stereotypy induced by the dopaminergic agonist apomorphine (11), its withdrawal after repeated treatment fails to enhance the open-field behavior of rats (12). Buspirone has not been demonstrated to produce tardive dyskinesia in humans (13). On the contrary, Moss et al. (14) showed that repeated buspirone treatment decreased the severity of tardive dyskinesia, consistent with the inhibitory effects of buspirone co-treatment on the development of haloperidol-induced dopaminergic supersensitivity measured by open-field behavior (12).

The aims of the present study were twofold: first, to determine if, as previously reported for rats, mice withdrawn from long-term haloperidol treatment would develop dopaminergic supersensitivity evaluated by open-field behavior, and second, to investigate the effects of acute buspirone administration on the expression of this behavioral phenomenon.

Two experiments were performed using 3-month-old male EPM-M1 mice, an outbred stock. In the first experiment, animals were randomly divided into five groups, i.e., one control and four experimental groups of 12-13 mice each. Mice of the experimental groups were injected intraperitoneally (*ip*) with 2.5 mg/kg haloperidol (HAL) daily for 20 days and animals of the control group received the same number of injections of 0.9% NaCl (SAL) by the same route. Seventy-two hours after the last haloperidol or 0.9% NaCl injection, the animals received an *ip* injection of 0.9% NaCl or 1.0, 3.0 or 10 mg/kg buspirone (BUS). Thus, the treatments of the five groups of animals were as follows: SAL-SAL, HAL-SAL, HAL-BUS1, HAL-BUS3 and HAL-BUS10. Thirty minutes later, mice were placed individually in the open-field arena and behavioral parameters (i.e., ambulation and rearing frequencies and immobility duration) were observed for 5 min as proposed by Conceição and Frussa-Filho (11). Hand-operated counters and stopwatches were employed to score ambulation (the number of squares crossed) and rearing frequencies, and immobility duration (time of complete absence of paw movements), respectively.

In the second experiment, animals were randomly divided into four groups, i.e., one control and three experimental groups of 12 animals each. Mice of the experimental groups were acutely treated with 1.0, 3.0 or 10.0 mg/kg buspirone (*ip*) and animals of the control group received 0.9% NaCl. Thirty minutes later, mice were placed individually in the open-field arena for behavioral quantification as described above.

An experienced observer who was blind to the identity of the animals quantified the open-field behavior. Analysis of variance (ANOVA) followed by the Duncan *post hoc* test was used to study the open-field data. The level of significance was set at $P<0.05$.

As shown in Figure 1A, withdrawal from haloperidol treatment significantly increased ambulation frequency of HAL-SAL mice compared to SAL-SAL mice ($F(4,59) = 3.71$; $P<0.05$). Acute buspirone administration abolished this effect. Indeed, the ambulation frequencies presented by mice of the HAL-BUS3 and HAL-BUS10 groups were significantly lower than that exhibited by the HAL-SAL group. In addition, the ambulation frequencies of animals of the HAL-

BUS1, HAL-BUS3 and HAL-BUS10 groups were not different from those presented by the SAL-SAL group. The duration of immobility presented by the HAL-BUS3 and HAL-BUS10 groups was significantly higher than that presented by the HAL-SAL group ($F(4,59) = 2.57$; $P<0.05$). Finally, no experimental group presented differences in rearing frequency or immobility duration when compared to the SAL-SAL group.

As can be seen in Table 1, acute buspirone administration was also able to inhibit open-field behavior in mice that did not receive haloperidol before. Indeed, at the doses of 3.0 and 10.0 mg/kg, buspirone induced significant decreases in ambulation ($F(3,44) = 12.09$; $P<0.01$) and rearing ($F(3,44) = 22.40$; $P<0.01$) frequencies and a significant increase in immobility duration ($F(3,44) = 17.43$; $P<0.01$).

The major findings of the present study were that: 1) as previously reported for rats, mice withdrawn from long-term treatment with haloperidol develop dopaminergic supersensitivity measured by open-field behavior; 2) this behavioral supersensitivity was detected by the locomotion frequency parameter but not by two other open-field behavioral parameters, i.e., rearing frequency and immobility duration, and 3) the expression of this haloperidol-induced behavioral supersensitivity was abolished by acute buspirone administration.

As far as we know, this is the first demonstration that, like rats, mice develop dopaminergic supersensitivity as evaluated by spontaneous open-field behavior. This animal model may be of particular use since the hypothesis of dopamine supersensitivity has dominated the conceptual approaches to studying tardive dyskinesia over the last decades (15) and behavioral supersensitivity is still a useful animal model of tardive dyskinesia (for a review, see Ref. 9).

Within this context, the demonstration that mice also can be used as experimental subjects of this behavior paradigm raises the possibility of performing interesting studies using knockout mice.

In mice, ambulation frequency was the only behavior parameter able to detect dopaminergic supersensitivity, whereas previous studies in rats have shown that withdrawal from long-term haloperidol treatment not

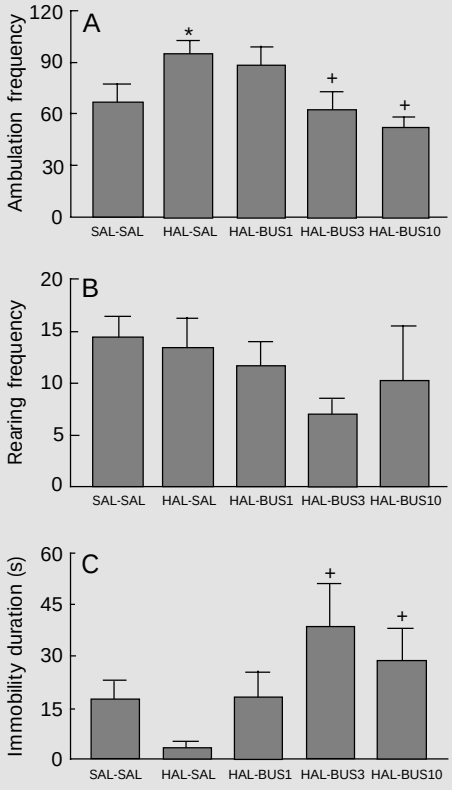

Figure 1. Effect of acute administration of buspirone (BUS) or 0.9% NaCl (SAL) on ambulation frequency (A), rearing frequency (B) and immobility duration (C) of mice withdrawn from long-term haloperidol (HAL - 2.5 mg/kg) or SAL treatment (once daily, for 20 days) and observed in an open-field. The doses of buspirone are reported as mg/kg. *P<0.05 compared to SAL-SAL group; +P<0.05 compared to HAL-SAL group (ANOVA followed by the *post hoc* Duncan test).

Table 1. Effect of acute administration of different doses of buspirone on open-field behavior.

Groups	Behavioral parameters		
	Ambulation frequency	Rearing frequency	Immobility duration
Saline	117.67 ± 5.92	40.25 ± 2.76	16.00 ± 3.62
Buspirone (1 mg/kg)	111.92 ± 6.74	34.67 ± 3.66	16.83 ± 6.11
Buspirone (3 mg/kg)	78.67 ± 8.05*	18.75 ± 3.10*	75.50 ± 12.55*
Buspirone (10 mg/kg)	57.58 ± 11.02*	10.67 ± 1.75*	109.00 ± 16.58*

The parameters evaluated were ambulation and rearing frequencies and immobility duration (s). Data are reported as means ± SEM.
*P<0.05 compared to the control (saline) group (ANOVA followed by the *post hoc* Duncan test).

only increases ambulation and rearing frequencies, but also decreases immobility duration. This concern notwithstanding, it should be noted that in those studies, ambulation frequency was the most effective behavioral parameter in detecting dopaminergic supersensitivity in rats (1,3,16). With respect to the immobility duration parameter, a trend towards supersensitivity (decreased duration presented by the HAL-SAL group when compared to the SAL-SAL group) was observed, suggesting that the phenomenon could be detected by this parameter in an experimental situation that produced a stronger supersensitivity (for example, if haloperidol treatment were more prolonged). Indeed, the above-mentioned trend was strengthened by the fact that acute buspirone administration at the doses of 3.0 and 10 mg/kg (HAL-BUS3 and HAL-BUS10 groups, respectively) increased the immobility duration compared to the SAL-HAL group but not compared to the SAL-SAL group. However, there was no trend towards supersensitivity concerning rearing frequency data. In this respect, Al-Khatib et al. (17) demonstrated that the nucleus accumbens and caudate putamen have a differential role in mediating ambulation and rearing of rats in the open-field test. Indeed, these investigators showed that the caudate putamen seems to play a greater role than the nucleus accumbens in the control of rearing. Species differences in the role of these two dopaminergic regions in the control of rearing could be a speculative hypothesis to explain the present data.

The finding that acute buspirone administration abolishes the expression of dopaminergic supersensitivity measured by open-field behavior extends previous studies which have demonstrated that buspirone co-treatment attenuated the development of dopaminergic supersensitivity (also measured by this behavioral paradigm) in rats withdrawn from haloperidol (12). Since buspirone shows high affinity for dopaminergic- and serotonergic-binding sites (10,18), it was suggested that the inhibitory effect of repeated buspirone co-treatment on the development of behavioral supersensitivity could result from peculiar actions of the drug on these two neurotransmission systems. Specifically, since buspirone seems to block preferentially presynaptic dopamine receptors as compared to the postsynaptic ones (19), it was suggested that the ability of chronic buspirone treatment to attenuate the development of behavioral supersensitivity could be related to the development of presynaptic dopamine receptor supersensitivity (leading to a decreased availability of dopamine in the synaptic cleft). In line with this possibility, while Tunnicliff et al. (20) showed that withdrawal from repeated treatment with buspirone led to marked reductions in the synthesis of dopamine in the rat striatum, we have shown that the drug potentiates yawning behavior induced by small doses of apomorphine (16), which is considered a behavioral parameter of nigrostriatal dopaminergic presynaptic function (21). In addition, we have demonstrated that withdrawal from long-term haloperidol treatment at doses that selectively block dopamine autoreceptors decreases open-field behavior, suggesting that the development of dopamine autoreceptor supersensitivity can attenuate the behavioral effects produced by postsynaptic dopamine receptor supersensitivity (22). Alternatively, the inhibitory effects of chronic buspirone on the development of behavioral dopaminergic supersensitivity could be related to modifications in the activity of 5-HT1a serotonergic receptors since there is convincing evidence that raphe serotonergic projections inhibit dopamine nigrostriatal function (see 23).

Regardless of the exact mechanism by which chronic buspirone attenuates the development of behavioral dopaminergic supersensitivity, the inhibitory effect of acute buspirone administration on the expression of this behavioral phenomenon seems to be

more probably related to the blockade of dopamine postsynaptic receptors. Indeed, as is the case for classical neuroleptics, acute buspirone administration reduces apomorphine-induced stereotyped behavior (11), which is thought to result from stimulation of postsynaptic dopamine receptors (24). Accordingly, while the present study demonstrates that the inhibitory effect of acute buspirone on the open-field behavior is not conditional upon prior haloperidol treatment, acute administration of neuroleptic drugs also inhibits spontaneous open-field behavior, an effect that is thought to result from blockade of postsynaptic dopamine receptors (6). In line with these observations, the symptoms of tardive dyskinesia are alleviated by the administration of higher neuroleptic doses (25).

Although extrapolation to clinical situations from animal data must always be made with caution, the present data, taken together with previous behavioral results, suggest that buspirone may alleviate tardive dyskinesia symptoms, as well as attenuate the development of this movement disorder.

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