Amphetamine-induced anxiety-related behavior in animal models

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Abstract:
The purpose of this study was to examine the anxiety-related effects of acute and repeated amphetamine administration using the elevated plus maze (EPM) and light/dark box tests in mice. D-amphetamine (2 mg/kg ip, 30 min after injection) had a significant anxiogenic effect only in the EPM test, as shown by specific decreases in the percentage of time spent in the open arms as well as in the percentage of open arm entries. Tolerance to this anxiogenic action developed after 8 days of daily d-amphetamine administration (2 mg/kg, ip). An anxiolytic effect was observed after the ninth injection, i.e. there were specific increases in the percentage of time spent in the open arms and in the percentage of open arm entries. L-type voltage-dependent calcium channel antagonists: nimodipine (5, 10 and 20 mg/kg, ip), flunarizine (5, 10 and 20 mg/kg, ip), verapamil (5, 10 and 20 mg/kg, ip), and diltiazem (5, 10 and 20 mg/kg, ip) were also injected prior to an acute low dose of d-amphetamine or to each injection of subchronic d-amphetamine. Our results revealed that calcium channel blockers dose-dependently attenuated both an anxiogenic effect of d-amphetamine and the development of tolerance to this effect. Our results suggest that neural calcium-dependent mechanisms are involved in the anxiety-related responses to acute and subchronic amphetamine injection that may lead to addiction relapse in human users.

Key words:
d-amphetamine, anxiety, calcium channel antagonists, elevated plus maze, light/dark box, mice

Abbreviations: CaM kinase II – Ca²⁺/calmodulin-dependent protein kinase II, CCAs – calcium channel antagonists, DAT – dopamine transporter, EPM – elevated plus maze, HPA – hypothalamic-pituitary-adrenal axis, MDMA – 3,4-methylenedioxy-methamphetamine, VDCC – voltage-dependent calcium channels, 5-HT – serotonin

Introduction

Animal models of human anxiety are typically employed to search for anxiolytic drugs with therapeutic potential. Both in humans and rodents, anxiety is a common symptom in physical dependence and withdrawal syndrome of many drugs of abuse, including psychostimulant drugs. Among amphetamine abusers, psychiatric disorders, such as anxiety, panic attacks and mania are commonly reported [37], especially among first time amphetamine users [10]. Withdrawal from amphetamine has been also associated with states of increased anxiety and dysphoria in human addicts. Anxiolysis after prolonged administration can be one of the main effects underlying psychostimulant dependence and drug relapse.

Controversies over amphetamine effects in animal models can be encountered in the literature. An anxiogenic-like effect of amphetamine in the plus maze test has been previously shown in rats [29] and in mice [20]. However, some authors reported that amphetamine-
amine failed to alter indices of anxiety in mice [22] or that the drug produced anxiolytic-like action in rats [6]. The discrepancy among different studies might be related, at least in part, to the dose used, time between injections and testing, route of administration, strain differences and behavioral test employed [18].

The mechanism of amphetamine influence on anxiety-related behavior is complex. It can be associated with enhanced release of different neurotransmitters, and can engage a variety of brain structures. It is well established that amphetamine increases dopamine, noradrenaline and serotonin (5-HT) neurotransmission by acting on monoamine transporters, causing an increase in the cytoplasmic levels of these monoamines and leading to an increase in their release from the terminals [38]. Different administration schedules of amphetamine lead to different behavioral consequences, neurochemical changes and gene expression patterns in a variety of brain areas [27]. Anatomically, the behavioral expression of anxiety is associated with a set of interrelated limbic and cortical structures: the septo-hippocampal system, amygdala, hypothalamus, and periaqueductal gray matter of the midbrain [1]. Among these areas, the central nucleus of the amygdala and its efferent projections are thought to play a pivotal role in anxiety processes and are involved in the acquisition, consolidation and expression of conditioned fear [36]. Thus, activation of the amygdala results in behavioral and physiological responses associated with anxiety, whereas lesions of this structure reduce this effect [17]. Moreover, a synthetic amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), was reported to exert a marked effect on c-fos expression in this brain region [26].

Generally, there is a good evidence for the neuropharmacological and neuroanatomical parallels between rodent emotionality and human anxiety. Although the recent studies have already described pharmacological and neurochemical mechanisms of the reinforcing and locomotor effects of amphetamine, conflicting evidence exists about the exact mechanisms underlying the influence of its acute and chronic exposure on anxiety-like behavior. The present experiments were intended to further investigate the effect of amphetamine on anxiety in the elevated plus maze (EPM) and light/dark box tests in mice. Firstly, an attempt was made to clarify the effects of acute administration of d-amphetamine on anxiety level. In the second step, we evaluated the anxiety-related response after subchronic injection of d-amphetamine in order to reveal the possible development of tolerance to the anxiogenic action of a low dose of the drug. The range of doses of d-amphetamine was chosen according to published data [21, 22]. Additionally, in view of previous findings showing that calcium ions and voltage-dependent calcium channels (VDCCs) are important in several aspect of drug reward, addiction and anxiety-related behavior [2, 23], we investigated the influence of calcium channel antagonists (CCAs) on both acute and subchronic effects of d-amphetamine, in order to determine the role of calcium homeostasis in anxiety-related profile evoked by exposure to d-amphetamine. For this purpose, L-type VDCC antagonists of various classes including nimodipine, flunarizine, verapamil and diltiazem were used. The results of the present studies are discussed in the context of influence of amphetamine treatment on anxiety-related responses, and may be important for our understanding the calcium-dependent mechanisms underlying dependence on psychostimulant drugs.

Materials and Methods

Animals

The experiments were carried out on naive male Swiss mice (delivered by the Farm of Laboratory Animals, Warszawa, Poland) weighing 20–25 g at the beginning of the experiments. The animals were maintained under standard laboratory conditions (12-h light/dark cycle, room temperature 21 ± 1°C) with free access to tap water and laboratory chow (Bacutil, Motycz, Poland), and were adapted to the laboratory conditions for at least one week. Each experimental group consisted of 7–12 animals. All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and to the European Community Council Directive for the Care and Use of Laboratory Animals of 24 November 1986 (86/609/EEC), and were approved by the local ethics committee.

Drugs

The compounds tested were: d-amphetamine sulfate (Sigma, St. Louis, MO, USA), nimodipine (RBI, Natick, MA, USA), verapamil (Isoptin, Knoll, Germany),
diltiazem hydrochloride (RBI, Natick, MA, USA), and flunarizine dihydrochloride (Sigma, St. Louis, MO, USA). Verapamil was diluted to an adequate concentration using saline (0.9% NaCl). Other drugs were dissolved in saline and refer to the salt forms. All agents were administered intraperitoneally (ip) in a volume of 10 ml/kg. Control groups received saline injections at the same volume and by the same route.

The EPM procedure

Anxiety responses were measured in the EPM test. The procedure was similar to the method of Lister [22]. The experimental apparatus is shaped like a “plus” sign and consists of a central platform (5 × 5 cm), two open arms (30 × 5 cm) and two equal-sized closed (30 × 5 × 15 cm) arms opposite to each other. The maze is made of dark Plexiglas, elevated to a height of 50 cm above the floor and illuminated by a dim light. The test consisted in placing a mouse in the central platform facing a closed arm and allowing it to freely explore the maze for 5 min. Entry into one arm was recorded when an animal placed all four paws past the line dividing the central square from the open arms. The test arena was wiped with a damp cloth after each trial. The number of entries into the open and closed arms and the time spent in open arms were measured by an observer blind to the drug treatment. Anxiolytic activity was indicated by increases in time spent in open arms or in number of open arms entries; anxiogenic effects were characterized by decreases in these measures. The percentage of time spent on the open arms was calculated, as the percentage of open arm entries. Additionally, the number of entries into the closed arms was recorded as an indicator of motor activity of animals in this test.

Light/dark box test

The test was performed according to the method described by Costall et al. [5]. In brief, the mouse light/dark box (60 × 30 × 25 cm) consisted of two parts, a lit (850 lx) open white compartment and a darkened (0 lx) closed black compartment with a volume ratio of 3:1. The compartments were separated by a black Plexiglas partition containing a 12 × 12 cm opening. Each mouse was placed individually in the center of the white area facing away from the dark chamber and allowed to explore both compartments freely for 5 min. After each test, the box was cleaned with a 70% ethanol solution and wiped dry. Behaviors measured included: times spent in light (white) and dark (black) compartment, number of transitions between the two compartments as well as latency to first leave (escape) from the light compartment. Among these measures, number of dark box entries and the latency to first leave can be valuable indicators of locomotor activity in this animal model.

Treatment

During acute treatment, the animals were allocated to the group receiving d-amphetamine (2 and 4 mg/kg, ip) or saline, and mice of each group were tested 30 min after injection. The exploratory behavior in the maze was recorded for 5 min in the EPM test and light/dark test.

Because a significant anxiogenic effect in the EPM test was observed in the mice that had been treated with amphetamine (2 mg/kg), in the second experiment animals were randomly allocated to group receiving ip injections of d-amphetamine (2 mg/kg) or saline for 8 days. On the ninth day, these animals were treated with the same dose of d-amphetamine or saline (for a control group), and were tested 30 min after the last injection in order to see if tolerance to the anxiogenic effect developed after the longer treatment period.

The next experiment was designed to investigate whether CCA application modifies the influence of d-amphetamine on anxiety level. For this purpose, distinct groups of acute d-amphetamine-treated mice were injected with nimodipine (10 and 20 mg/kg, ip), flunarizine (10 and 20 mg/kg, ip), verapamil (10 and 20 mg/kg, ip), diltiazem (10 and 20 mg/kg, ip) or saline, 15 min prior to d-amphetamine (2 mg/kg, ip) or saline administration. In the second step of this experiment, distinct groups of mice receiving subchronic d-amphetamine injections were pretreated with nimodipine (5 and 10 mg/kg, ip), flunarizine (5 and 10 mg/kg, ip), verapamil (10 and 20 mg/kg, ip), diltiazem (10 and 20 mg/kg, ip) or saline, 15 min prior to each d-amphetamine or saline injection, for 8 days. On the ninth day, these mice were challenged with 2 mg/kg of amphetamine or saline, and their exploratory behavior in the maze was recorded 5 min after injection.

Statistics

The data are expressed as the means ± SEM. The statistical analyses were performed using one-way analysis of variance (ANOVA). Post-hoc comparison of the means was carried out with the Tukey test for multi-
ple comparisons, when appropriate. The confidence limit of $p < 0.05$ was considered statistically significant.

**Results**

In the light/dark box test, d-amphetamine at the dose of 2 mg/kg (ip) caused only a decrease in the percentage of light compartment entries [$F(2, 27) = 4.53, p = 0.02$], affecting neither the percentage of time spent in this compartment [$F(2, 27) = 2.572, p = 0.095$] nor the dark box entries or the latency to first leave. D-amphetamine at the highest dose of 4 mg/kg (ip) did not have any significant effect on any measure (Tab. 1).

It can be seen from Figure 1 and 2 that, in the EPM test, an acute ip dose of d-amphetamine (2 mg/kg) significantly decreased the percentage of time spent in the open arms and percentage of open arm entries in animals that were tested 30 min after injection, indicating an anxiogenic effect ($p < 0.001$; $p < 0.05$, respectively). The highest dose of d-amphetamine (4 mg/kg, ip) caused only a decrease in the percentage of time spent in the open arms ($p < 0.001$) without affecting the percentage of open arm entries (Fig. 1). It is important to note that d-amphetamine at both doses used did not modify locomotor activity of mice, i.e.

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Amphetamine 2 mg/kg</th>
<th>Amphetamine 4 mg/kg</th>
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</thead>
<tbody>
<tr>
<td>% Time spent in light box</td>
<td>57.20 ± 7.34</td>
<td>74.87 ± 11.65</td>
<td>43.03 ± 10.35</td>
</tr>
<tr>
<td>% Entries into light box</td>
<td>38.40 ± 6.48</td>
<td>13.34 ± 6.94*</td>
<td>36.30 ± 6.15</td>
</tr>
<tr>
<td>Entries into dark box</td>
<td>5.50 ± 1.61</td>
<td>1.40 ± 0.89</td>
<td>4.30 ± 1.14</td>
</tr>
<tr>
<td>Latency (in s)</td>
<td>49.10 ± 14.53</td>
<td>28.20 ± 19.47</td>
<td>36.20 ± 6.10</td>
</tr>
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</table>

Tab. 1. Mean (±SEM) percentage of time spent in the light box, percentage number of entries into the light box, number of dark box entries, and latency to first leave measured in the light/dark box test, 30 min after an acute ip injection of d-amphetamine (2 and 4 mg/kg) or saline in mice; n = 10. * $p < 0.05$ vs. saline control group, Tukey test.

**Fig. 1.** Mean (±SEM) percentage time spent in open arms and percentage open arm entries in the EPM, 30 min after an acute ip injection of d-amphetamine (2 and 4 mg/kg) or saline in mice; n = 7-12. * $p < 0.05$ and *** $p < 0.001$ vs. saline control group, Tukey test.

**Fig. 2.** Influence of calcium channel antagonists on the anxiogenic effect of an acute d-amphetamine injection (2 mg/kg, ip). Nimodipine (10 and 20 mg/kg, ip), verapamil (10 and 20 mg/kg, ip), diazepam (10 and 20 mg/kg, ip) or saline were administered 15 min prior to d-amphetamine (2 mg/kg, ip) or saline injection and tested 5 min later in the EPM test; n = 7-12. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ vs. saline-ip treated and amphetamine-test group; # $p < 0.05$ and $## p < 0.001$ vs. saline control group, Tukey test.
number of closed arm entries, after an acute (Tab. 2) or subchronic administration remained unchanged (Tab. 3).

Figure 2 shows the influence of CCA pretreatment on acute d-amphetamine-induced changes in the behavioral performance in the EPM [ANOVA for the percentage of time spent in the open arms: F(9, 64) = 27.285, p < 0.0001; ANOVA for the percentage of open arm entries: F(9, 64) = 8.346, p < 0.0001]. The post-hoc Tukey test indicated that pretreatment with nimodipine (20 mg/kg, p < 0.001), flunarizine (20 mg/kg, p < 0.01 for the percentage of time, p < 0.05 for the percentage of entries), verapamil (20 mg/kg, p < 0.001; 10 mg/kg, p < 0.05 for the percentage of entries), and diltiazem (20 mg/kg, p < 0.01) significantly reversed the anxiogenic-like effect of acute d-amphetamine (2 mg/kg) as compared with saline-pretreated amphetamine control group (Fig. 2).

In the next experiment, the animals tested 30 min after the ninth daily injection of d-amphetamine (2 mg/kg), showed a significantly increased time of the open arms as well as an increased number of entries to these arms compared with the acute amphetamine group (p < 0.001, Fig. 3), suggesting that tolerance developed to the anxiogenic effect of d-amphetamine. Moreover, pretreatment with the CCAs before every daily injection of subchronic d-amphetamine (2 mg/kg) also influenced the anxiety-related response and the development of tolerance [ANOVA for the percentage of time spent in the open arms: F(10, 84) = 16.397, p < 0.0001; ANOVA for the percentage of open arm entries: F(10, 84) = 6.038, p < 0.0001]. Actually, nimodipine (5 and 10 mg/kg), flunarizine (5 and 10 mg/kg), verapamil (5 and 10 mg/kg) and diltiazem (5 and 10 mg/kg) completely abolished the anxiolytic-like effect of subchronic d-amphetamine revealed as the decrease in the percentage of time spent in open arms (p < 0.01 for verapamil 10 mg/kg and p < 0.001 for other CCAs used). However, the CCAs only at the dose of 5 mg/kg, provoked a decrease in the percentage of open arm entries after subchronic amphetamine (p < 0.01 for verapamil and dil-
 MDMA (3,4-methylenedioxymethamphetamine, “ecstasy”) on anxiety are unclear. Recent experimental findings indicated that acute treatment with this drug might provoke both anxiogenic and anxiolytic effects depending upon the dose range employed or the test used, with an anxiogenic-like effect revealed in the light/dark box test and the EPM test in mice [24–26]. After subchronic MDMA administration at low doses, the anxiogenic-like effect in the EPM test in mice was even more marked while the highest dose produced anxiolytic-like activity, suggesting a possible dual pharmacological property of MDMA on anxiety [25].

Our results have shown that a single injection of a low dose of d-amphetamine had a significant anxiogenic effect in mice in the EPM test, but not in the light/dark box test of anxiety, without affecting their locomotor activity. The higher dose of d-amphetamine caused a less significant anxiogenic effect in this test. Probably, two behavioral tests employed in the present study do not reflect the same aspects of the animal anxiety-related behavior. These findings are in accordance with the recent studies in which an anxiogenic-like action has been described using a variety of behavioral paradigms [20, 29]. Moreover, tolerance to this effect developed rapidly when the lower, anxiety-related behavior provoked by d-amphetamine was administered repeatedly. The recent results from light/dark box, open field, and EPM tests also demonstrated that the repeated administration of a high dose of dl-amphetamine significantly decreased the level of anxiety-like behavior in rats [12]. Similarly to d-amphetamine, results from animal studies investigating the effects of MDMA (3,4-methylenedioxyamphetamine, “ecstasy”) on anxiety are unclear. Recent experimental findings indicated that acute treatment with this drug might provoke both anxiogenic and anxiolytic effects depending upon the dose range employed or the test used, with an anxiogenic-like effect revealed in the light/dark box test and the EPM test in mice [24–26]. After subchronic MDMA administration at low doses, the anxiogenic-like effect in the EPM test in mice was even more marked while the highest dose produced anxiolytic-like activity, suggesting a possible dual pharmacological property of MDMA on anxiety [25].

In the second set of our experiments, CCAs acting at the L-type VDCCs were administered prior to both acute administration and each subchronic dose of d-amphetamine. It was revealed that the anxiogenic effect of d-amphetamine and the development of tolerance were blocked dose-dependently by nimodipine, flunarizine, verapamil and diltiazem, demonstrating the role of calcium ions and calcium channels in anxiety-related behavior provoked by d-amphetamine injections in mice.

The EPM test is probably the most widely used animal model of anxiety [22, 29]. This test has been proven to be extremely useful in detecting both anxiolytic and anxiogenic effects of drugs. Clearly detectable changes occur during the first 5 min of exposure to the maze [9]. Generally, this model is based on the natural aversion of rodents to heights and open spaces. Thus, anxiolytic effects are characterized by an increased number (percentage) of entries into open arms, and increased percentage of time spent in open arms; anxiogenic effects are characterized by decreases in these measures. Nonspecific locomotor effects of drugs are measured by changes in behavior in the closed arms or in the total number of entries made in any arm during the test session.

The exact mechanism by which amphetamine affects anxiety behavior is far from being completely understood. Recently, a growing body of evidence suggested that dopaminergic mechanisms, especially through the target structures of the mesolimbic dopamine system originating from the ventral tegmental area (i.e. amygdala, prefrontal cortex and nucleus accumbens) were also significant for different aspect of anxiety [30]. Many effects of amphetamine, including its locomotor and reinforcing effects are attributed to the ability of amphetamine to induce dopamine efflux through the dopamine transporter (DAT) in basal ganglia [38]. This enhancement in amphetamine-induced dopamine efflux depended upon the presence of extracellular Ca2+ and was inhibited by the blockade of N- and L-type VDCC.

Besides the dopaminergic system, the lateral septum is another area that mediates the anxiogenic effect of drugs (including acute amphetamine, especially at high doses), through the 5-HT1A receptors, as a result of a tonic modulation of 5-HT release. However, reciprocal inhibition between the dorsal hippocampus and the lateral septum in mediating behavior in the EPM has also been suggested [3, 4]. Among possible candidates, the 5-HT system is the most
likely to be involved. Classic theories propose that an excess 5-HT increases anxiety whilst manipulations that reduce this transmission are anxiolytic [11]. Behavioral dysfunction has been observed in the EPM after generalized depletion of central 5-HT. On this basis, it is reasonable to conclude that calcium-dependent alterations both interrelated brain mesolimbic dopamine and serotonin activity may be involved in the development of amphetamine-induced behaviors in the EPM.

Rapid development of tolerance to the anxiogenic effect of amphetamine, i.e. a behavioral response opposite to the acute drug effect, is likely to be due to the pharmacodynamic mechanisms that involve the progressive recruitment of processes opposing the acute effect of the drug. It has already been suggested that behavioral disinhibition in the EPM, i.e., the increased exploration of the open arms, can be interpreted to reflect an alleviation of anxiety [28]. The findings have shown that repeated daily treatment with psychostimulants can produce sensitization to their locomotor stimulatory effects [2, 34]. Since the open arm exploration normally is inhibited, the increase in time spent and entries to open arms in the EPM test, following subchronic drug administration reflects amphetamine-induced behavioral disinhibition in the amphetamine-sensitized mice when challenged with amphetamine. Our results have revealed that both acute pretreatment and daily co-treatment with CCAs counteracted the acute anxiogenic action of amphetamine and the expression of the disinhibitory response to amphetamine in the sensitized mice. Taking into account these results, we can suggest that the behavioral sensitization and inhibition observed in the EPM are controlled by the same calcium-dependent neural mechanisms. This mechanism may also participate in the amphetamine-induced modification of anxiety behavior revealed in our studies.

In order to further explore the calcium-dependent mechanisms discussed above, it is important to note that considerable evidence exists for central VDCC involvement in some effects of drugs of abuse, especially in their reinforcing properties [2, 19, 33]. Recent investigations suggest that disturbance of calcium homeostasis in neurons, especially the increased influx of calcium ions due to up-regulation of L-type VDCCs, is involved in the drug dependence after long-term exposure [16]. Generally, pharmacological studies have demonstrated that modulation of calcium influx through L-type VDCCs also modifies many behavioral effects of amphetamine. In fact, the enhancement in amphetamine-mediated dopamine efflux following repeated amphetamine requires extracellular Ca²⁺ and is blocked by inhibitors of Ca²⁺/calmodulin-dependent protein kinase II (CaM kinase II) [14]. Some authors reported an increase in CaM kinase II activity in striatal synaptosomes following treatment of rats with repeated but not acute amphetamine [13]. Repeated treatment with amphetamine results in an activation of N- and L-type Ca²⁺ channels, which mediates the entry of extracellular Ca²⁺ into cells. The increased cellular Ca²⁺ appears to activate CaM kinase II, which mediates the enhanced amphetamine-induced efflux [32]. The triggering of exocytotic release of neurotransmitters is primarily mediated by the L-type VDCC, and Ca²⁺ influx via L-type VDCC is necessary for psychostimulant-induced behavior and neurochemical changes. It has been revealed that the antagonists of L-type VDCC inhibit only subchronic, but not acute, psychostimulant-induced neurochemical and behavioral changes [31], so these channels play an important role in long-term neuronal plasticity. The mechanisms, by which the CCAs affect psychostimulant effects, like tolerance, sensitization and withdrawal syndrome already described, are complex and not fully understood. Therefore, the influence of CCAs on the drug effects associated with addiction, including anxiety-related behavior, may be the consequence of their interaction with hypersensitive or up-regulated neural calcium channels associated with the development of amphetamine tolerance.

In order to further discuss the influence of calcium homeostasis on anxiety-related behavior, it can be mentioned that some modest anxiolytic-like effects of certain CCAs can be observed, especially under conditions of high degree of behavioral inhibition due to increased fear [7]. For instance, L-type VDCCs in the amygdala play a role in cued fear conditioning [35]. It is also possible to speculate about the involvement of the hypothalamic-pituitary-adrenal (HPA) axis in these anxiolytic-like effects of calcium channel blockers. Indeed, in the brain limbic areas, like the hippocampus, frontal cortex and amygdala, an increased corticosterone secretion resulting in the stimulation of serotonin 5-HT₁A receptors, can increase the amplitude of sustained L-type VDCC and enhance calcium influx into neurons within these limbic areas implicated in anxiety behavior [15]. Furthermore, withdrawal from drug administration may cause an increased ac-
tivity of the HPA axis associated with anxiogenic-like behavior. As the involvement of serotonergic pathways in anxiety-related action of drugs, including psychostimulants, has been mentioned above, it is worth noting that also the anxiolytic-like effects of CCAs could be modulated by the 5-HT1A receptor agonists [8], suggesting some influence of calcium channels and calcium ions on serotonin transmission. Therefore, it is important to note that none of the CCAs used in our study, given acutely or repeatedly, had any effects in naïve mice or provoked any anxiety-related effects in the EPM paradigm by themselves.

An important objective of this study was to further examine the anxiety-related behavioral consequences of acute and subchronic amphetamine in the mouse tests of anxiety. First, it has been confirmed that d-amphetamine is capable of producing significant anxiogenic effect after an acute injection of a low dose, in the EPM test. After subchronic administration, tolerance develops to this anxiogenic action. Secondly, consistent with some findings pointing out the involvement of calcium fluxes in the response to amphetamine, we have established that administration of CCAs acting at L-type VDCCs, prior to an acute or every subchronic injection of amphetamine, attenuated both anxiogenic effects of acute dose as well as the development of tolerance to this effect after subchronic amphetamine administration in mice. Taking all these results together, it seems plausible to speculate about an influence of calcium homeostasis, i.e. intracellular calcium ion concentration and calcium-dependent molecular events, and the L-type VDCCs in the adaptive changes underlying anxiety behavior. Further experiments are necessary in order to identify the precise neural mechanisms associated with this interaction, and its relevance to drug abuse. As anxiety is one of major signs in patients after abrupt withdrawal of psychostimulant drugs, identifying the mechanism involved in anxiety-related responses could help develop new therapies of addiction.

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