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Dose-effect analysis of 6-APB (“Benzofury”) on fear memory, motor behavior, and reward

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Abstract

Rationale 6-(2-aminopropyl)benzofuran (6-APB) is a recreational drug that is structurally similar to MDMA. Very little is known about the behavioral effects of 6-APB or its dose-effect relationship in vivo. As the popularity of new psychoactive substances (NPS), such as 6-APB, increases due to their unscheduled status and easy availability, it is important to understand the potential benefits and/or harms of such substances.

Objectives The current study aims to establish a dose-effect curve of 6-APB on fear memory, pain sensitivity, motor coordination, and addiction-related behavior in mice.

Methods Male and female hybrid C57BL/6Jx129S1/SvImJ mice were used to examine the effects of 0.01 to 5 mg/kg of 6-APB on Pavlovian fear conditioning; flinch, jump, vocalize; Accelerating Rotarod; behavioral sensitization, conditioned place preference, and conditioned responding.

Results We found that 6-APB elicits effects at very low doses (0.01 and 0.1 mg/kg), while impairing short-term fear memory and long-term context memory at a dose of 5 mg/kg. We also observed that 6-APB produces modest locomotor sensitization and may produce addiction. Finally, there is evidence of reduced shock reactivity; this was not likely due to analgesia and more likely due to ataxia produced by the drug.

Conclusions 6-APB produces a behavioral profile broadly similar to MDMA, with amnesic effects emerging at higher doses than MDMA and a larger gap between potential therapeutic doses and doses that produce cognitive deficits. These findings suggest 6-APB may not be the low-risk therapeutic alternative to MDMA currently sought in clinical contexts, though further research is warranted.

Keywords Benzofury · 6-APB · Novel psychoactive substance · MDMA derivative

Introduction

The psychedelic entactogen±3,4-Methylenedioxymethamphetamine (MDMA) has become increasingly popular as a potential aid in therapeutic settings. However, there are well-warranted concerns about its behavioral side effects,

such as memory impairments, addiction, and potential for both acute (e.g., hyperthermia) and long-term toxicity (e.g., serotonin depletion; Clemens et al. 2005; Itzhak et al. 2003; Pantoni et al. 2022; Schenk and Newcombe 2018). We have previously reported that MDMA’s antidepressant and amnesic effects in mice are elicited at doses similar to those used recreationally and therapeutically in humans (Pantoni et al. 2022). Likewise, neurotoxic effects may begin at doses only about three times those used recreationally and clinically (Mueller et al. 2013; Pantoni and Anagnostaras 2019). Because of these concerns, searching for similar alternatives is warranted.

New psychoactive substances (NPS) have gained popularity among recreational drug users due to their similarities to illicit drugs and their unregulated status (Brandt et al. 2020; Chan et al. 2013; Simmler and Liechti 2018). As psychedelic substances continue to show promise in

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therapeutic settings, it is necessary to evaluate NPS alternatives. Most of these non-controlled substances have little or no formal behavioral research examining the potential harm or benefits that may come with their use. Evaluation of NPS may lead to useful therapeutics and aid in the regulation of harmful substances.

One class of emerging NPS is the benzofurans, including 6-(2-aminopropyl)benzofuran (6-APB) or “Benzofury.” 6-APB was created as a potential alternative to 3,4-methylenedioxyamphetamine (MDA) and MDMA due to its structural similarity, but by reducing and reorienting oxygen atoms it was predicted to lack serotonin neurotoxicity (Chan et al. 2013; Monte et al. 1993; Rickli et al. 2015). However, it did not gain popularity among recreational users until around 2010 (Chan et al. 2013). Recreational users have reported having entactogen-like effects, such as increase in empathy, openness, and overall sociality, that are similar to MDMA (Brandt et al. 2020; Chan et al. 2013; Welter et al. 2015). 6-APB is illegal in some countries, including Germany, Italy, China and the United Kingdom, but as of 2025 remains unregulated in the United States, except in Florida (The Florida Statutes 2025; Ledberg 2015). Pharmacologically, 6-APB has been shown to act as an agonist at the 5-HT_{2A} and 5-HT_{2B} receptors, in a manner somewhat consistent with classical serotonergic psychedelics (Dawson et al. 2014; Iversen et al. 2013; Rickli et al. 2015). In addition, *in vitro* studies have found that 6-APB dose-dependently increases the release of dopamine, serotonin, and norepinephrine via transporter reversal in a manner similar to an amphetamine (Brandt et al. 2020; Iversen et al. 2013; Rickli et al. 2015). Brandt et al. (2020) conducted microdialysis comparing benzofurans to both MDMA and MDA, and they found that 6-APB has stronger activity at the dopamine transporter (DAT), serotonin transporter (SERT), and norepinephrine transporter (NET) compared to MDA and MDMA. Due to its higher efficacy at these transporters, 6-APB might cause a behavioral effect at lower doses than MDMA. Taken together, these studies suggest that 6-APB may be a more potent entactogen with stronger stimulant properties than MDMA.

Pavlovian fear conditioning is an efficient method to examine drug effects on learning and memory in rodents (Anagnostaras et al. 2000, 2010; Carmack et al. 2014; Pantoni et al. 2022; Wood and Anagnostaras 2011; for a brief review, see Anagnostaras et al., 2010). In prior research,

we have found that, in mice, MDMA produces amnesia on this task at doses similar to those that seem to produce antidepressant effects, as well as those used recreationally and therapeutically in humans (i.e., around 1 mg/kg; Pantoni et al. 2022).

Flinch, vocalize, jump (FVJ) is a pain threshold sensitivity test that measures a rodent’s response to a series of ascending foot shocks. Studies have shown that mice that are given analgesics will elicit these three behaviors (flinch, vocalize, and jump) at a higher shock threshold (Anagnostaras et al., 1999; Kimble 1955; Kovacsics and Gould, 2010).

To assess motor coordination and potential ataxia, we used the Accelerating Rotarod task, which measures gross motor skill acquisition and retention (Anagnostaras et al., 2000; DeLorey et al., 1998a; Lee et al. 2023).

Lastly, we tested for behavioral sensitization, conditioned place preference, and drug-environment conditioning to establish an understanding of 6-APB’s potential for addiction. Behavioral sensitization is an increase in responsiveness to a drug after repeated administration over time (Anagnostaras et al. 1996; 2002; Carmack et al. 2017; Pantoni et al. 2022; Robinson and Berridge 1993).

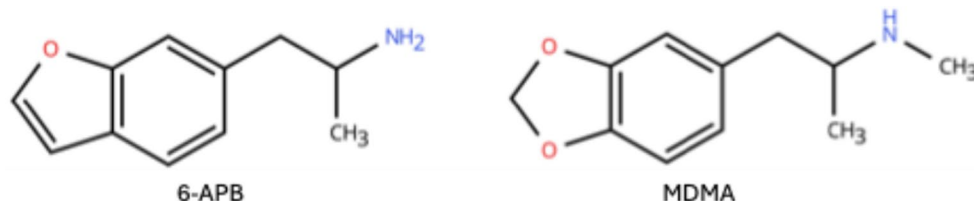
No studies to our knowledge have examined the effects of 6-APB on behavior such as fear memory, pain sensitivity, motor coordination and memory, or addiction-related behavior. The present study looks at a large range of doses of 6-APB and its effects on behaviors related to memory, analgesia, motor coordination, and addiction. Because evidence suggests that 6-APB is similar to MDMA (Fig. 1) and more potent at producing increases in dopamine and norepinephrine, we expected it to produce memory deficits as MDMA did in our previous work (Pantoni et al. 2022), as well as place preference and sensitization similar to psychostimulants. However, its increased potency could potentially dissociate these effects at lower doses, making it more therapeutically useful than MDMA.

Methods

Subjects

A total of 218 hybrid C57BL/6Jx129S1/SvImJ (129B6; Jackson Laboratory, West Sacramento, CA, USA) male ($n = 110$) and female ($n = 108$) were used. Mice were weaned

Fig. 1 Chemical structure of 6-APB and MDMA. Made with MolDraw (Ugliengo et al. 1993; Ugliengo 2006)



at approximately 3 weeks of age and were group housed (2–5 mice per cage) with unlimited access to food and water. Mice were at least 10 weeks old at the beginning of testing. Each mouse was handled for three days (1 min/day) prior to testing. The vivarium was on a 14:10-h light: dark schedule and all testing occurred during the light phase. All experimental methods and animal care procedures were conducted in accordance with the NRC *Guide for the Care and Use of Laboratory Animals* (8th ed.) and were approved by the University of California Institutional Animal Care and Use Committee.

Drug

6-APB HCl (CAS No. 286834-85-3; Cayman Chemical, Ann Arbor, MI, USA) was dissolved in 0.9% saline and was given intraperitoneally (i.p.). The doses were either 0 (saline control), 0.01, 0.1, 1, or 5 mg/kg (salt weight) and given 10 ml/kg.

Fear conditioning

The VideoFreeze system (Med Associates Inc., St. Albans, VT, USA) was used as described previously (Anagnostaras et al., 2010; Carmack et al. 2014; Pantoni et al. 2022) and consists of a Skinner box within a sound-attenuated chamber. Each box had stainless-steel rod floors and side walls, white acrylic rear walls, and clear polycarbonate front walls. Four mice were tested concurrently in individual conditioning chambers (32 × 25 × 25 cm). Groups were counterbalanced by sex and conditioning chamber. During training, the mice ($n=107$; male=54, female=53) were injected 15 min prior with their randomly assigned dose: 0 (saline control, $n=22$), 0.01 ($n=21$), 0.1 ($n=22$), 1 ($n=21$), or 5 ($n=21$) mg/kg, i.p.

The training context consisted of an illuminated chamber with moderate (~80 lx) white light and near-infrared light (980 nm) and was cleaned and scented with 7% isopropanol. Training began with a 2-min baseline followed by one tone-shock pairing, consisting of a 30-sec pure tone (2.8 kHz, 85 dBA) that co-terminated with a 2-sec scrambled, AC constant current foot shock (0.75 mA, RMS). Ninety seconds after the shock was administered, a 5-min short-term memory test was conducted. Movement during the 2-min baseline and the 2-sec foot shock was used to measure locomotor activity and shock reactivity, respectively, while percent freezing after the tone-shock pairing measured on-drug short-term memory.

One week after training, the mice returned to the same context, without drug, for 5-min. Freezing behavior during this test served as a proxy for measuring long-term context memory. One day later, mice were placed into an alternate context, off-drug, for 5-min. The alternate context consisted

of a black triangular teepee with black polycarbonate flooring (both transparent to infrared light) covering the rod floor. No white light was on in the chambers, only a near-infrared light (980 nm), creating a dark environment. The chambers were cleaned and scented with a 5% vinegar solution. Tone testing consisted of a 2-min baseline, followed by the presentation of 3, 30-sec tones identical to the training tone each separated by 30-sec. Freezing behavior during the tone presentations was used to measure long-term tone memory.

Flinch, vocalize, jump (FVJ)

The chamber used was the same as the training and context test described above. Rod floors were exposed so the mice could receive the series of foot shocks. The box was cleaned with 10% ethanol, a different scent than the mice experienced in fear conditioning. Two weeks following fear conditioning, a subset of the same mice ($n=28$; males=14, females=14) used to test fear conditioning were used to measure FVJ. Based on prior research of MDMA administration (Pantoni et al. 2022), mice were injected 15 min prior and were assigned the same dose as fear conditioning: 0 (control, $n=7$), 0.01 ($n=6$), 0.1 ($n=5$), 1 ($n=5$), or 5 ($n=5$) mg/kg, i.p.

One mouse was placed in the conditioning box at a time. Two-sec foot shocks started at 0.05 mA and increased by 0.05 mA every 20 s until all three behaviors (flinch, vocalize, and jump) were observed. Flinch was defined as any observable reaction to the shock (typically lifting or shaking their paws or orienting toward the grid floor). Vocalize was operationalized as an audible squeak when shocked. Jumping was characterized as all of the animal's paws leaving the grid floor (Anagnostaras et al., 1999; Kim et al., 1991; Sneddon et al., 2023). A blind observer scored FVJ by the lowest current (i.e., threshold) that elicited each behavior.

Accelerating Rotarod

The Rotarod consists of a 10-cm-diameter rubber-coated cylinder with 5 slots that accelerate at 3 to 30 RPM across 5-min. A new set of mice ($n=50$; males=25, females=25) were given saline or 6-APB 15 min prior to the initial training trial. Three sessions were completed within 90 min of injections to ensure the drug was still on board. Mice were allowed to get accustomed to the stationary Rotarod prior to training. Groups were counterbalanced by sex and randomly assigned a dose: 0 (saline control, $n=10$), 0.01 ($n=10$), 0.1 ($n=10$), 1 ($n=10$), or 5 ($n=10$) mg/kg, i.p.

One week later, mice were given the same dose of 6-APB and were tested for rotarod memory retention. They were given one session of the same protocol of 3 to 30 RPM

across 5-min during training. On the next day, mice were given the same test off drug.

Behavioral sensitization, conditioned place preference, and conditioned responding

Seven mice were tested concurrently in individual place preference chambers (Med Associates Inc., Georgia, VT, USA). As described previously (Carmack et al. 2014, 2017; Pantoni et al. 2022), chambers (43 × 43 × 31 cm) consisted of two visually and tactilely distinct sides, separated by a black wall (transparent only to infrared light) with a removable insert in the middle of the wall. The two sides were visually and tactilely different from one another; the floor was made up of either steel rods or wire mesh, while the walls were either decorated with stickers or white translucent material. Chambers were counterbalanced by the different context combinations and by drug-paired and unpaired side assignments. Each chamber was cleaned with diluted (10%) glass cleaner (Zep Inc., Atlanta, GA, USA) between trials. Activity Monitor software (Med Associates Inc.) used the interruption of multiple rows of infrared beams to identify mouse position in order to score locomotion (distance), rearing, and stereotypy (counts of small repetitive movements in the same location).

A new set of mice ($n=33$; males=17, females=16) were randomly assigned to a drug dose: 0 (control, $n=13$), 1 ($n=9$), and 5 ($n=11$) mg/kg, i.p. These doses were selected because of previously reported results of an increase in locomotor activity at 1 mg/kg (Brandt et al. 2020) and an impairment of memory at 5 mg/kg we observed during the fear conditioning task. Groups were counterbalanced by sex and testing chamber. Two mice in the 1 mg/kg group were 2.5 SD above the mean and therefore were excluded.

Mice were habituated to their assigned testing chamber for 15-min per side, two consecutive days prior to training. Four days following habituation, mice were trained for five consecutive days. On each training day, mice were first injected with saline and then immediately placed onto the unpaired side for 30-min, and then removed, injected with 6-APB, and immediately placed on the paired side for another 30-min. Locomotor and stereotyped activity on the paired side was scored and behavioral sensitization was calculated as the difference between day 5 versus day 1.

One day after training day 5, mice were tested for off-drug conditioned place preference. The inserts that previously divided the paired and unpaired side were removed and mice were placed in the entryway between the two sides of the chamber (direction of entry was counterbalanced) and were allowed access to both sides for 15-min. Locomotor activity and time spent on each side was scored and place

preference was calculated as the difference between time spent on the paired side versus the unpaired side.

Forty-eight hours after training day 5, all mice received two back-to-back challenge tests with saline and an intermediate dose of 6-APB (2.5 mg/kg). Mice were injected with saline and then immediately placed into their assigned paired side for 15-min, then were removed and injected with 2.5 mg/kg of 6-APB, and placed back into their paired side for 30-min. Locomotor activity and time spent on the drug-paired side scored to evaluate the presence of conditioned responding to the drug-paired side (saline challenge) and/or sensitized responding to an intermediate dose of 2.5 mg/kg 6-APB (2.5 mg/kg challenge).

Statistical analyses

Data were analyzed using univariate or multivariate analyses of variance (ANOVA/MANOVA) to identify overall group differences. Post hoc comparisons were performed after finding a significant group effect using Fisher's protected least significant difference (PLSD) tests compared to the control group. Group size generally followed prior experience with each task; power analysis was conducted with G*Power 3.1.9.6 (Faul et al. 2007, 2009). Power for observed differences generally exceeded 0.9, unless otherwise noted; in general it appeared appropriate group sizes were used, although in future studies, we could slightly increase the group sizes in CPP studies to improve power. Finally, unlike high doses of MDMA (see supplemental data in Pantoni et al. 2022), there was no evidence of sex differences in any measures (p values > 0.2), so male and female data were collapsed.

Results

Fear conditioning

Pavlovian fear conditioning was used to examine the effects of 6-APB (0.01–5 mg/kg, i.p.) on fear learning and memory. Mice were trained on-drug using a single tone-shock pairing to evaluate short-term fear memory and on-drug locomotion. Percent freezing was scored and used to measure memory. 6-APB did not affect baseline locomotion compared to the controls [$F(4, 102)=0.8$, $p=0.6$; Fig. 2A]. On-drug shock reactivity showed a significant effect of dose [$F(4, 102)=2.6$, $p=0.04$, Fig. 2A]. Only 0.01 mg/kg ($p\leq 0.05$) and 5 mg/kg ($p<0.01$) showed a significant decrease in activity during the 2-sec shock compared to the control group (all other p values > 0.1). 6-APB dose-dependently effects freezing during the post-shock short-term memory test [$F(4, 102)=7.3$, $p<0.0001$; Fig. 2B]; only mice on

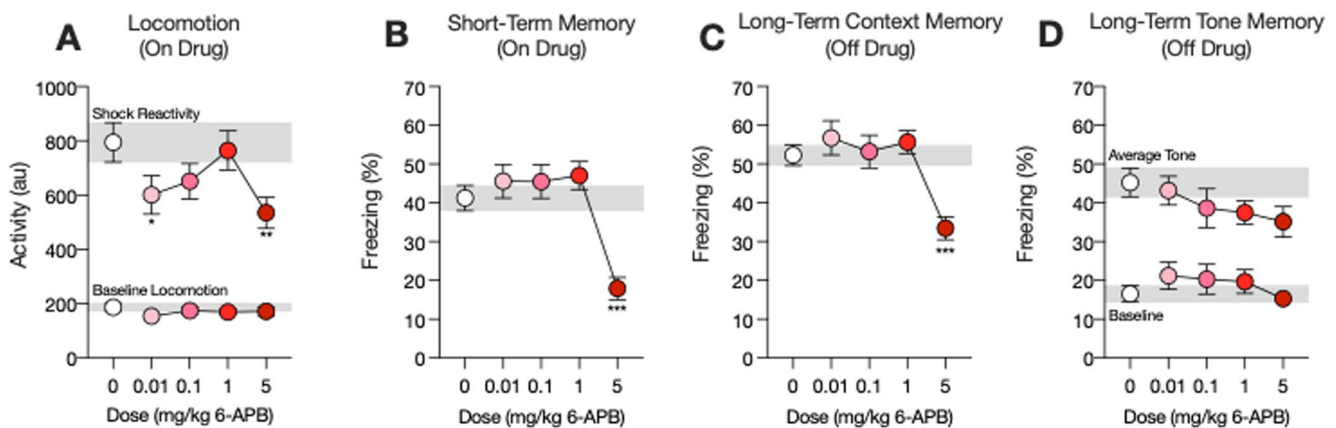


Fig. 2 Effects of 6-APB on fear learning and memory. **(A)** Two-minute baseline locomotor activity during on-drug training and 2-s foot shock. No groups differed from the control group during both baseline and foot shock. Both 0.01 and 5 mg/kg had significantly reduced shock reactivity. **(B)** On-drug short-term fear memory following shock measured by percent freezing. Only 5 mg/kg significantly impaired short-term fear memory. **(C)** Off-drug long-term context memory was measured by percent freezing 1 week following training. Mice given 5 mg/kg during training have significantly diminished context memory

5 mg/kg showed a significant decrease compared to saline control ($p < 0.001$). One week later, an off-drug context and a tone test examined long-term fear memory. 6-APB dose-dependently modulated freezing in context memory [$F(4, 102) = 7.3$, $p < 0.0001$; Fig. 2C] with 5 mg/kg significantly reducing memory ($p < 0.001$). For the tone test, a two-way ANOVA (dose $\times$ test period) found no significant main effect of dose [$F(4, 102) = 1.65$, $p = 0.17$], a main effect of test period [$F(1, 102) = 188.1$, $p < 0.0001$], and no dose $\times$ test period interaction [$F(4, 102) = 0.76$, $p = 0.55$]. Thus, the tone increased freezing relative to baseline, but 6-APB had no significant effect.

Flinch, vocalize, jump

FVJ was utilized to examine the potential analgesic effects that were suggested by the shock reactivity results in fear conditioning. Mice were injected 15-min prior to being placed in a chamber and were observed during ascending levels of foot shocks. After each mouse flinched, vocalized, and jumped they were placed back in their home cages. A two-way ANOVA (dose $\times$ behavior type) was performed. There was a main effect of dose [$F(4, 69) = 3.1$, $p = 0.02$] and behavior type [$F(2, 69) = 176.1$, $p < 0.0001$], but no interaction [$F(8, 69) = 0.98$, $p = 0.46$]. However, post-hoc analysis found that only 0.1 mg/kg decreased ($p < 0.05$) while 5 mg/kg increased ($p = 0.02$; Fig. 3A) shock threshold only for jumping behavior compared to the control group. These results suggest altered motion rather than analgesia, as only jumping was affected.

relative to saline controls. **(D)** Off-drug long-term tone memory was measured by percent freezing and was tested one day after context testing. There was no significant difference during baseline and average of the tones from saline controls. Each point represents the mean ± 1 standard error. The gray bar indicates standard error range for the comparison saline control group. Asterisks identify significant comparisons against the saline control group using Fisher's PLSD (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$)

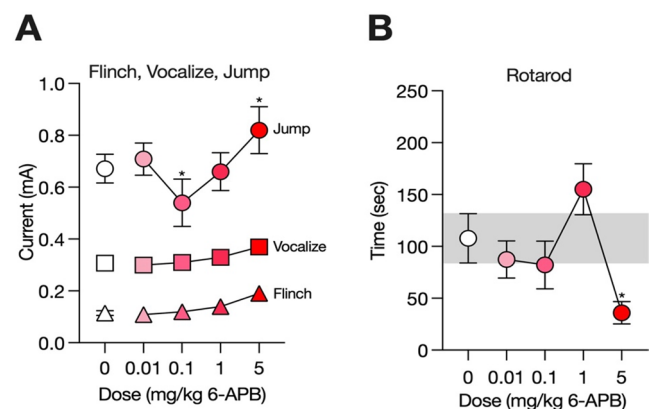


Fig. 3 Effects of 6-APB on pain thresholds and motor coordination. **(A)** When comparing each behavior across doses, only mice given 0.1 and 5 mg/kg differed significantly from the control group only during jumping. This suggests 6-APB specifically impaired jumping rather than producing analgesia. **(B)** Motor coordination was assessed with Rotarod. Only during session 1 was there a significant difference between control and 5 mg/kg. See supplemental for other sessions and retention test. Asterisks identify significant comparisons against the saline control group using Fisher's PLSD (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$)

Accelerating Rotarod

We used an Accelerating Rotarod to examine potential ataxia and motor learning impairments induced by 6-APB (0.01–5 mg/kg). Mice had three consecutive sessions of training within 90-min and were tested for retention of motor learning both on and off drug a week later. Duration of staying on the Rotarod was recorded. During training,

all mice improved how long they could stay on from Session 1 to Session 3 ($p < 0.0001$; Supplementary Fig. 1). We also saw a significant difference between our control group and 5 mg/kg dose, but only during the first session ($p = 0.045$; power = 0.7, Fig. 3B). This impairment of locomotion is consistent with other measures examining movement at the 5 mg/kg dose. There also seems to be some evidence of an enhancement-like effect at the 1 mg/kg dose, although this was not significant ($p > 0.05$, power = 0.1). One week later, we tested for retention of motor learning both on and off drug, but found no significant differences across our doses from our control groups (Supplementary Fig. 1), indicating that we did not see an impairment of motor learning.

Behavioral sensitization, conditioned place preference, and conditioned responding

Potential 6-APB (1 and 5 mg/kg) addiction-related rewarding behaviors were analyzed using tests of behavioral sensitization, conditioned place preference, and conditioned responding. Mice were trained for 5 days in a two-sided chamber, on each day they were first injected with saline and placed into their assigned unpaired side for 30-min, then injected with 6-APB and placed into their opposing paired side. Locomotion, stereotypy, and verticality on the on-drug paired side were scored, and a two-way ANOVA was performed with significant day $\times$ dose interaction for stereotypy and locomotion [$F(8,116)$ values > 4.5 , p values < 0.0001] but not verticality [$F(8,116) = 1.2$, $p = 0.3$]. There was an overall main effect of dose on locomotion and stereotypy across five days of training [locomotion, $F(2, 29) = 3.4$, $p < 0.05$, power = 0.61; stereotypy, $F(2, 29) = 3.8$, $p < 0.05$, power = 0.67; Fig. 4A and C], but not in verticality, perhaps due to low power [$F(2, 29) = 2.1$, $p > 0.05$; power = 0.4; Fig. 4E]. Mice on the 5 mg/kg dose showed an increase in locomotion on Day 3, 4, ($p = 0.01$) and 5 ($p = 0.05$). Compared to saline controls, mice that were given 1 mg/kg only showed a significant increase in locomotion ($p = 0.02$) on the last day of training, while showing a significant increase in stereotypy on Day 1, Day 4, and Day 5 (p values ≤ 0.01). Mice given 5 mg/kg began to show a significant increase in both locomotion ($p \leq 0.001$) and stereotypy ($p \leq 0.005$) beginning on the third day of training and persisted until the last day of training. Interestingly, mice given 5 mg/kg significantly decreased verticality counts compared to controls on Day 1 and Day 2 (p values ≤ 0.01). Lastly, there was some evidence of behavioral sensitization in stereotypy on Day 5 versus Day 1 [$F(2, 29) = 3.8$, $p = 0.03$; Fig. 4D], and when looking at doses, only the 5 mg/kg dose was significantly different compared to the saline control ($p = 0.02$, all other p values > 0.5).

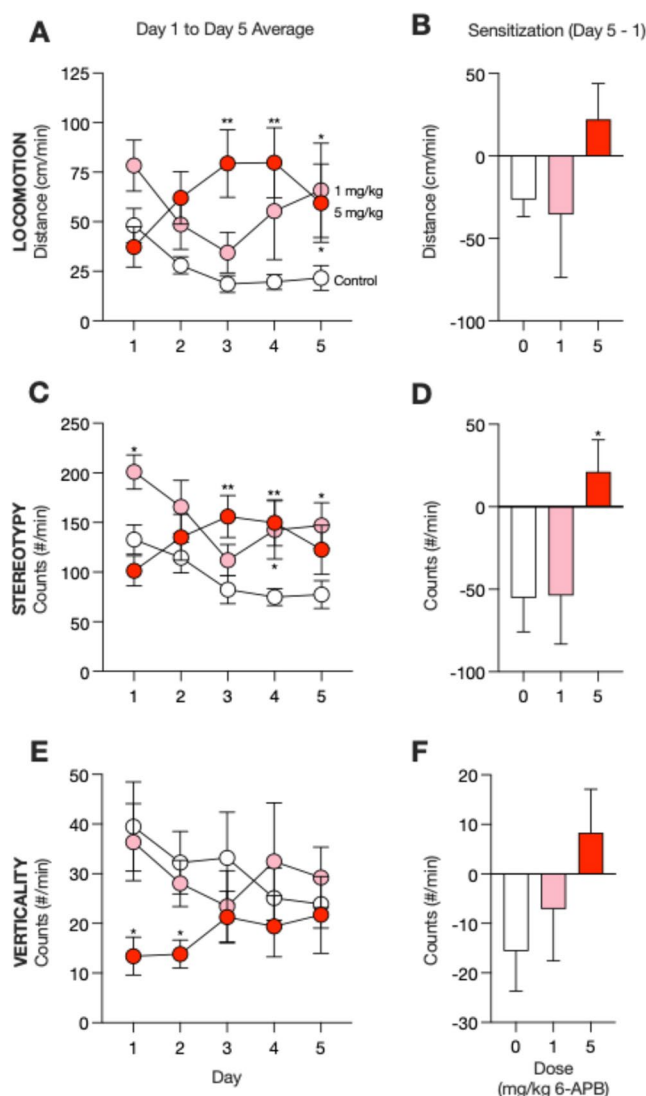


Fig. 4 Effects of 1 mg/kg and 5 mg/kg 6-APB during CPP training. (A) Average locomotion across all 5 days of training. Mice on 5 mg/kg increase locomotion over the 5 days of training. (B) Training Day 5-Day 1 to assess sensitization in locomotion. No significant sensitization. (C) Average stereotypy across 5 days of training. We see the same pattern in Fig. 3A as we do in 3 C, mice on 5 mg/kg increase stereotypy behaviors across 5 days of training, whereas mice on 1 mg/kg decreased stereotypy across training. (D) Training Day 5-Day 1 to assess sensitization of stereotypy. Mice trained with 5 mg/kg were significantly different from control mice, but mice trained with 1 mg/kg were not significantly different. (E) Average verticality across 5 days of training. Mice given 5 mg/kg were significantly impaired during the first two days of training, then increases after. (F) Training Day 5-Day 1. Asterisks identify significant comparisons against the saline control group using Fisher's PLSD (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$)

Twenty-four hours after the last training day, mice were tested off-drug for conditioned place preference. The middle divider was removed to allow free access to both sides, and place preference was measured by the difference in distance traveled and time spent on the drug-paired side compared to the unpaired side. There were no significant group

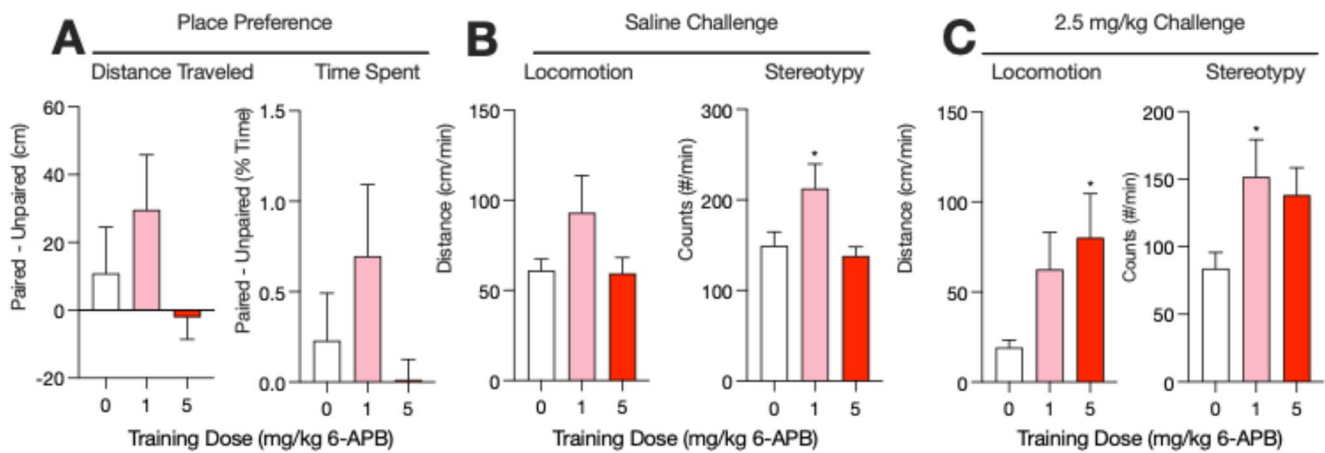


Fig. 5 Testing of conditioned place preference and conditioned and sensitization responding following training. **(A)** Place preference measured by distance traveled (left) and time spent (right) on the drug-paired side versus unpaired side. There were no significant group differences and none of the groups exhibited a preference for either side. **(B)** Saline challenge 24 h after CPP testing on both locomotion (left) and stereotypy (right). Mice that were trained on 1 mg/kg showed con-

differences in distance traveled [$F(2, 29)=1.5$, $p=0.2$; Fig. 5A, left] or time spent on either side [$F(2, 29)=1.6$, $p=0.2$; Fig. 5A, right].

Forty-eight hours after the last training day, mice were challenged with saline and a moderate dose of 6-APB (2.5 mg/kg) on the paired side. Locomotion and stereotypy in response to the saline and moderate dose challenge were scored to evaluate conditioned and sensitized responding, respectively. There was a significant group difference in stereotypy during the saline challenge [$F(2, 29)=4.8$, $p\leq 0.01$; Fig. 5B], but not locomotion. Mice given 1 mg/kg significantly differed in stereotypy from saline controls ($p\leq 0.01$, all other p values > 0.5). Additionally, there were significant group differences in locomotion [$F(2, 29)=3.4$, $p=0.05$] and stereotypy [$F(2, 29)=3.5$, $p=0.05$] during the 2.5 mg/kg challenge (Fig. 4C). Compared to the saline controls, there was a significant difference in locomotion in mice given 5 mg/kg only during the 2.5 mg/kg challenge ($p\leq 0.01$, all other p values > 0.5), whereas mice given 1 mg/kg significantly differed in stereotypy ($p=0.02$; although mice on 5 mg/kg trended towards significance, $p=0.051$). There were no overall group differences or individual differences verticality scores during either challenge, therefore the results were not graphed.

Discussion

As psychedelics gain popularity in therapeutic and recreational settings, it is important to investigate their derivatives to see if there are any potential advantages to conventional substances such as LSD, psilocybin, ketamine, or MDMA.

ditioned responding in stereotypy. **(C)** Moderate dose challenge following saline challenge on locomotion (left) and stereotypy (right). Mice trained on 5 mg/kg displayed an increase in both locomotion compared to controls, whereas mice trained on 1 mg/kg only shows a significant difference in stereotypy. Asterisks identify significant comparisons against the saline control group using Fisher's PLSD (* $p<0.05$, ** $p<0.01$, and *** $p<0.001$)

6-APB is structurally similar to MDMA, an illicit drug that has been found to be an effective but potentially unsafe therapeutic for post-traumatic stress disorder (Kupferschmidt, 2024b). However, little research has been done to examine the effects of 6-APB, a MDMA analogue that may be less toxic than MDMA (Chan et al. 2013; Monte et al. 1993; Rickli et al. 2015).

We first examined short- and long-term memory using Pavlovian fear conditioning, with training on-drug and testing one week later off-drug. We found that only a high dose of 6-APB (5 mg/kg) significantly impaired short-term fear memory on drug, while one week later, off-drug, long-term context memory was also deficient only at that dose. Together, this seems to suggest that 6-APB produces amnesic effects on both short-term and long-term fear memory only at fairly high (5 mg/kg) doses. We also examined baseline locomotion prior to the tone-shock pairing and shock reactivity as potential confounds to impaired fear conditioning. There was no significant difference in baseline locomotion, however there was a significant decrease in shock reactivity at 0.01 and 5 mg/kg. Further investigation using the flinch, vocalize, jump task suggests that 6-APB does not have an analgesic effect. However, this task was developed around opioid analgesia, and as such may not detect drug effects selectively affecting suffering (without affecting nociception or pain perception), such as the relief in suffering caused by many antidepressants. Hence, it is possible that 6-APB could induce a state incompatible with suffering, and therefore produce an apparent amnesia of fear conditioning (e.g., Loeser 2000). This is beyond the scope of this study, but should be considered. Nonetheless, in terms of nociception, this data was inconsistent with an

antinociceptive effect. In observing the mice walking on the steel rod floors during fear conditioning, mice on 6-APB seemed to have a low body posture and difficulty walking on the rod floors on 5 mg/kg. This suggests that 6-APB may have an ataxic effect that hinders the ability to jump or rear. In order to further evaluate this potential motor impairment, we examined gross motor skill acquisition and retention with the Accelerating Rotarod. Mice on the high dose of 6-APB (5 mg/kg) had an impairment only on the first trial, suggesting that this dose produces an acute motor impairment but does not affect motor skill acquisition. Thus, further exploration of this motor impairment is required, as this result suggests that high doses of 6-APB could be quite intoxicating.

For the most part, we did not see effects of 6-APB similar to psychostimulants in reward-seeking behaviors. There was no significant conditioned place preference and weak sensitization, but mice trained with 1 mg/kg displayed conditioned responding during the saline challenge in measures of stereotypy; a lack of conditioned responding to 5 mg/kg could be due to a memory impairment previously observed in fear conditioning. These findings imply 6-APB has some potential to produce addiction, but the findings are fairly similar to what we found with MDMA (Pantoni et al. 2022) and dissimilar to those of classical psychostimulants (Carmack et al. 2010; Wood et al. 2007; Wood and Anagnostaras 2009). Brandt et al. (2020) demonstrated that 6-APB significantly elevates extracellular dopamine levels in rat nucleus accumbens using microdialysis. The nucleus accumbens has been widely accepted as the brain region that is associated with reward circuitry and drug abuse. More investigation into 6-APB and other addiction-related paradigms is needed in order to determine if 6-APB is habit forming, but it is likely similar to MDMA in this respect, i.e., of lower risk of producing addiction than amphetamine (Pantoni et al. 2022; Pantoni and Anagnostaras 2019).

6-APB seems to impair short-term and long-term memory around 5 mg/kg, which is a higher dose compared to MDMA, where memory impairment begins at 3 mg/kg (Pantoni et al. 2022). According to one study, 6-APB was able to elicit a significant elevation of dopamine in the nucleus accumbens of rat brains at just 0.3 mg/kg (Brandt et al. 2020). This finding is consistent with our studies, as we saw some behavioral effects even at 0.01 mg/kg. This suggests that there is a larger gap between potential therapeutic doses and amnesic doses of this compound as compared to MDMA, which increases its potential usefulness in applied therapeutic settings.

Regarding the potential benefits and harms of this substance, more research is still needed, but 6-APB appears to be similar to MDMA in many aspects (see Pantoni et al. 2022). However, 6-APB seems to be more potent and

potentially carries an added motor impairment. Due to its reported entactogen-like effects, it might serve as a prospective substitute for MDMA when looking specifically at memory impairment (Brandt et al. 2020; Chan et al. 2013; Welter et al. 2015). However, since there is an ataxic effect that inhibits the ability to jump and rear at very low doses, 6-APB could pose a greater risk to users. In all, the present data does not provide enough evidence to conclude that 6-APB could be a useful therapeutic, suggesting it is not the desired low risk alternative that is currently sought after in therapeutic contexts.

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Author contributions K.W. wrote the manuscript, prepared all figures, helped with study design, and conducted the experiments. K.V. assisted with experimental protocols and aided in experiments. L.C. assisted in experiments. J.K. assisted with in experimental protocol. S.A. helped design and plan the studies. All authors reviewed the manuscript.

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Data availability Data is provided within the supplementary information files.

Declarations

Clinical trial number Not applicable.

Competing interests The authors declare no competing interests.

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