Cannabis and Cannabinoid Research
Volume 7, Number 2, 2022
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DOI: 10.1089/can.2021.0066

# ABHD6 Controls Amphetamine-Stimulated Hyperlocomotion: Involvement of $CB_1$ Receptors

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**Abstract**

**Introduction:** Activation of cannabinoid 1 receptors ($CB_1$Rs) by endocannabinoids (eCBs) is controlled by both eCB production and eCB inactivation. Accordingly, inhibition of eCB hydrolyzing enzymes, monoacylglycerol lipase (MAGL) and $\alpha/\beta$-hydrolase domain containing 6 (ABHD6), enhances eCB accumulation and $CB_1$R activation. It is known that inhibition of MAGL regulates select $CB_1$R-dependent behaviors in mice, including locomotor behaviors and their modulation by psychostimulants, but much less is known about the effect of inhibiting ABHD6 activity on such behaviors.

**Methods:** We report a new mouse line that carries a genetic deletion of *Abhd6* and evaluated its effect on spontaneous locomotion measured in a home cage monitoring system, motor coordination measured on a Rotarod, and amphetamine-stimulated hyperlocomotion and amphetamine sensitization (AS) measured in an open-field chamber.

**Results:** ABHD6 knockout (KO) mice reached adulthood without exhibiting overt behavioral impairment, and we measured only mild reduction in spontaneous locomotion and motor coordination in adult ABHD6 KO mice compared to wild-type (WT) mice. Significantly, amphetamine-stimulated hyperlocomotion was enhanced by twofold in ABHD6 KO mice compared to WT mice and yet ABHD6 KO mice expressed AS to the same extent as WT mice. A twofold increase in amphetamine-stimulated hyperlocomotion was also measured in ABHD6 heterozygote mice and in WT mice treated with the ABHD6 inhibitor KT-182. It is known that amphetamine-stimulated hyperlocomotion is not affected by the $CB_1$R antagonist, SR141617, and we discovered that the enhanced amphetamine-stimulated hyperlocomotion resulting from ABHD6 inhibition is blocked by SR141617.

**Conclusions:** Our study suggests that ABHD6 controls amphetamine-stimulated hyperlocomotion by a mechanistic switch to a $CB_1$R-dependent mechanism.

**Keywords:** ABHD6; amphetamine; $CB_1$R; locomotion

## Introduction

Amphetamine affects neurotransmission in limbic brain areas involved in locomotor behaviors and motivation.[1,2] Repetitive use of large doses of amphetamine in humans contributes to subjective reward and positive reinforcement and may lead to addiction, impaired cognitive function, and psychosis.[3,4] In rodents, acute treatment with amphetamine triggers hyperlocomotion, and repeated administration of amphetamine leads to progressive enhancement of its response, coined amphetamine sensitization (AS).[5,6] Multiple molecular mechanisms mediate amphetamine's effect on neurotransmission, including (1) an increase in dopamine signaling through trace amine receptors and kinase pathways, (2) reversal of dopamine transporter function that depletes dopamine vesicles, and (3) a recently discovered action potential-dependent mechanism.[7,8] Few studies have tested the involvement of endogenous signaling lipids in the molecular mechanism that mediate amphetamine's effect of brain function.

The endocannabinoid (eCB) signaling lipids, arachidonoylethanolamine (AEA, anandamide) and

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2-arachidonoylglycerol (2-AG), activate presynaptic cannabinoid 1 receptor ($CB_1R$) that regulates neurotransmitter release (including the release of glutamate and gamma-aminobutyric acid [GABA]),[9,10] metabolism, and phenotype.[11,12] Thus, activity-dependent increases in eCB production and treatments with cannabinoid agonists, such as $\Delta^9$-tetrahydrocannabinol, activate $CB_1$Rs expressed by different neuronal subpopulations that mediate multiple behaviors, including locomotor behaviors and their modulation by psychostimulants. Specifically, blockade of eCB–$CB_1R$ signaling affects spontaneous locomotion and motor coordination.[13–15] Conversely, enhanced eCB–$CB_1R$ signaling achieved by inhibiting fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) that increase AEA and 2-AG levels, respectively, affects locomotion and motor coordination.[16–19] Of note, a crosstalk modulatory response exists between eCB–$CB_1R$ signaling and dopamine signaling. Enhanced dopamine signaling increases eCB levels in the striatum and cerebral cortex.[4,20,21] While $CB_1R$ blockade does not influence amphetamine-stimulated hyperlocomotion, it does prevent amphetamine self-administration and AS.[22–24] Thus, while the involvement of $CB_1R$, FAAH, and MAGL in motor behaviors and their modulation by amphetamine are known, the role of $\alpha/\beta$-hydrolase domain containing 6 (ABHD6) in such behaviors remains unknown.

ABHD6 hydrolyzes monoacylglycerol lipids with a preference for 2-AG and is expressed at the postsynaptic terminals, close to 2-AG synthesis, and in opposition to presynaptic $CB_1R$ and MAGL.[25–28] ABHD6 controls 2-AG accumulation, ensuing activation of $CB_1R$ when neurons are excited by neurotransmitters, suggesting that ABHD6 controls 2-AG–$CB_1R$ signaling predominantly when neurons are highly active and firing.[26] Thus, evidence indicates that MAGL and ABHD6 may play different roles in eCB signaling. In this study, we report a new mouse line that lacks *Abhd6* expression and tested whether this genetic deletion affects spontaneous locomotion, motor coordination, and amphetamine-stimulated hyperlocomotion and AS.

## Methods and Materials

### Subjects

We studied $Abhd6^{-/-}$, $Abhd6^{+/-}$, and WT littermates of both sexes and on a C57BL/6J background, weighing 18–29 g and between 10 and 14 weeks of age. $Abhd6^{-/-}$ mice were backcrossed every 6 months with C57BL/6J mice from the Jackson Laboratory (Massachusetts) to maintain genetic background. Animals were group-housed in a temperature-controlled facility (73 ± 2°F, 45% humidity, and regular 12-h light/12-h dark cycle, lights on at 7 AM), with food and water *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee of University of Washington and follow guidelines of National Institutes of Health.

### Drugs and chemicals

Amphetamine, ethanol, and dimethyl sulfoxide from Sigma-Aldrich (Missouri). Alkamuls EL-620 was from Rhodia (New Jersey). Saline was from Aqualite System (Illinois). SR141716 was provided by National Institute on Drug Abuse Drug Supply Program (Maryland) and was dissolved in vehicle (1:1:1:17 ratio of dimethyl sulfoxide/alkamuls EL-620/ethanol/saline) and was administered i.p.

### Membrane proteome

Brain tissues from WT and ABHD6 KO mice (8 weeks of age) were extracted immediately following decapitation and cortical and striatal regions were dissected, flash frozen in liquid nitrogen, and stored at −80°C. Tissues were thawed on ice, Dounce homogenized in ice-cold lysis buffer (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES] pH 7.2, 1 mM $MgCl_2$, 2 mM dithiothreitol [DTT], and 10 U/mL benzonase), incubated on ice for 15 min, and centrifuged (2500 × *g*, 3 min, and 4°C) to pellet debris. Supernatants were centrifuged (100,000 × *g*, 45 min, 4°C; Beckman Coulter TLA-55 rotor) to pellet the membrane fraction. Pellets were resuspended in ice-cold buffer containing 20 mM HEPES, pH 7.2, and 2 mM DTT; total protein was determined using the DC protein assay (Bio-Rad, California), and samples flash frozen and stored at −80°C until use.

### Gel based ABPP

Membrane proteome was thawed on ice, treated with 250 nM MB064 for 15 min at 37°C, and quenched with 4 × Laemmli sample buffer (Bio-Rad). Sample (10 $\mu$g protein) was resolved using sodium dodecyl sulphate–polyacrylamide gel electrophoresis by running on a 10% polyacrylamide gel (Bio-Rad). In-gel fluorescence was detected using the Cy3 (602/50 filter) and Cy5 (700/50) channels on a CemiDoc MP imaging system (Bio-Rad) and subsequently stained with Coomassie for total protein quantification. Images were quantified using ImageJ software.

### Spontaneous locomotion measured with the PhenoTyper system

Mice were placed in Noldus PhenoTyper instrumented cages (Wageningen, Netherlands; 45 × 45 cm area supplied with food and water) and their spontaneous locomotion tracked for 48 h. using 12-h light and dark phases.[29,30] Data were analyzed using the Noldus software and results presented as total distance traveled in meters using 2-h interval bins.

### Motor coordination and learning measured with the rotarod

Motor coordination and learning were assessed using an accelerating rotarod (4–40 rpm for a cutoff time of 300 sec) as previously described.[31,32] In this paradigm, mice were tested for seven testing trails (with 20 min intervals) each day for two consecutive days so that the learning on motor skills will be evaluated. All mice were naive on the first testing day. Motor learning was analyzed over a total of 14 trials of the latency (sec) to fall off the rotarod.

### Amphetamine-stimulated locomotion measure in an open field

Mice were first habituated by placing them in the open-field area for 2 days (90 min on each day), which included a saline injection. On day 3, mice were placed in the open field, monitored for 90 min, injected with amphetamine (2 mg/kg i.p.), and monitored for an additional 90 min. Mice underwent the same procedure on day 10 to study AS.

### Statistical analyses

Animals were randomly assigned to experimental groups and experimenters were blinded to experimental conditions. ANOVA for repeated measures was used to determine the effects of time course, including PhenoTyper and AS. Two-way ANOVA was used to determine the effects of drug treatment and genotype on acute or AS. The Sphericity-Assumed correction was applied to all repeated factors; degrees of freedom for significant interactions are reported as uncorrected values. One-way ANOVA was used to identify the source of significant interactions and comparisons between genotype or drug treatment, followed by Bonferroni *post hoc* tests or two-tailed *t*-tests, as appropriate. No gender differences were detected in all the behavior tests ($p > 0.15$ for all comparisons), and therefore results from both genders were pooled for statistical analyses. Statistical analyses were performed using IBM-SPSS Statistics version 25.0 (SPSS Inc., Illinois). $p$-Value $< 0.05$ was considered significant.

## Results

### Targeted disruption of *Abhd6* and loss in ABHD6 expression and activity

The *Abhd6* gene is on chromosome 3p14.3 and its sequence encodes for a 38 kDa integral membrane enzyme that has its active domain facing the intracellular space and includes a canonical catalytic triad (S148-D278-H306) necessary for 2-AG hydrolysis.[25,28,33] Mice lacking *Abhd6* were generated in collaboration with the TSRI Mouse Genetics Core by using a targeting construct to delete exon 5 of the *Abhd6* gene that encodes S148. LoxP sites were introduced to flox exon 5 to enable tissue- and cell-specific deletion of *Abhd6* exon 5 with Cre/LoxP recombination technology (Fig. 1A). This construct was used to generate targeted clone #8 in C57Bl/6J-derived murine embryonic stem cells as confirmed by Southern blot analysis (Fig. 1B), and the resulting chimera mouse had germline transmission of the targeted mutation as confirmed by Southern blotting analysis (Fig. 1C) and PCR genotyping (data not shown).

To generate mice that lack *Abhd6* expression in all tissues, we bred mice carrying the floxed *Abhd6* allele with Rose26 Cre transgenic mice (Cre deleter; Taconic Farms). The resulting ABHD6 knockout (KO) mice were viable and born with the expected Mendelian frequency and lacked whole brain ABHD6 activity as determined by gel-based activity-based protein profiling (ABPP) using fluorophosphonate-rhodamine (Fig. 1D). Of note, ABHD6 KO mice were largely indistinguishable from wild-type (WT; $Abhd6^{+/+}$) and heterozygous ($Abdh6^{+/-}$) littermates throughout adulthood and exhibited overall normal behaviors when observed in their home cage, and gain weight similar to WT mice. These results show that genetic deletion of *Abhd6* does not result in either overt developmental defects or overall abnormal behavior in adult mice.

Locomotor behaviors and their modulation by psychostimulants involve changes in neuronal activity in the cerebral cortex and basal ganglia.[34,35] Reported measures of mouse brain ABHD6 activity by gel-based ABPP suggest similar ABHD6 activity in cerebral cortex and striatum.[36] In this study, we sought to extend these results by measuring ABHD6 activity using gel-based ABPP with the $\beta$-lactone (MB064) probe that reliably measures ABHD6 activity, as confirmed by the absence in the corresponding tissue harvested from ABHD6 KO mice (Fig. 1E). We found comparable levels of ABHD6 activity in the membrane proteome from mice cortical

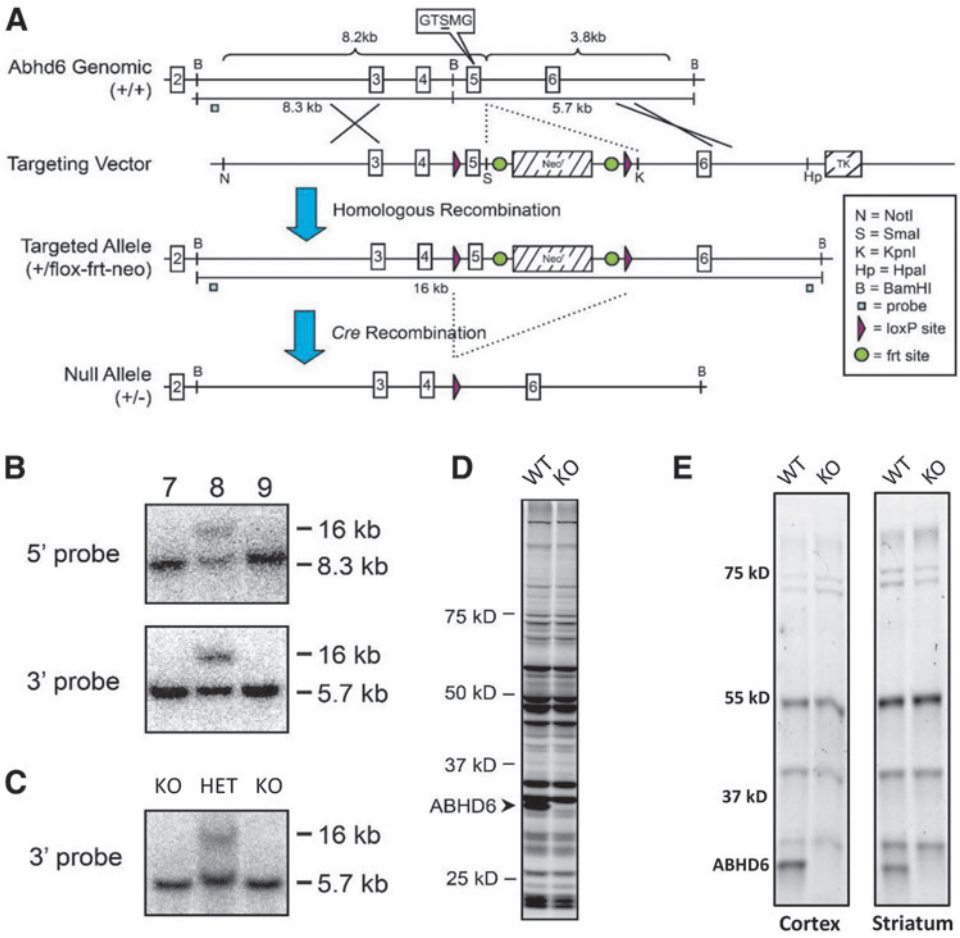


**FIG. 1.** Generation and validation of mouse line that lacks *Abhd6* expression. Mice lacking *Abhd6* were generated using a targeting construct to LoxP-delete exon 5 of the *Abhd6* gene that encodes S148, generating a targeted clone (#8) of C57Bl/6J-derived murine embryonic stem cells and a chimeric progeny with germline transmission and absence of ABHD6 activity. **(A)** Schematic of *Abhd6* targeting strategy. **(B)** Positive ES clone #8 was identified by Southern analysis with 5′ and 3′ probes. **(C)** Germline transmission of the targeted mutation was confirmed in genomic tail DNA by Southern analysis with a 3′ probe. **(D)** Activity-based protein profiling analysis with the FP-rhodamine probe confirmed the loss of ABHD6 activity in whole brain tissue from ABHD6 KO mice compared with WT mice (8 weeks of age). **(E)** Activity-based protein profiling analysis of ABHD6 activity with the MB064 probe shows similar ABHD6 activity in WT mouse cerebral cortex and striatal tissues and loss of activity in corresponding ABHD6 KO tissues (8 weeks of age). ABHD6, $\alpha/\beta$-hydrolase domain containing 6; KO, knockout; WT, wild type. Color images are available online.

and striatal tissues (15 weeks of age; $0.14 \pm 0.01$ and $0.12 \pm 0.01$, arbitrary units, $n = 3$ per sample, Student's $t$-test $= 0.12$; Supplementary Fig. S1). Thus, comparable ABHD6 activities are present in mouse cortex and striatum, brain areas involved in locomotor behaviors and their modulation by amphetamine.

## Mild impairment of spontaneous locomotion in ABHD6 KO mice

To determine if the genetic deletion of *Abhd6* affects spontaneous locomotion, we used the Noldus PhenoTyper® system, an instrumented observation cage that tracks mice over multiple days.[37,31] This monitoring

device detects subtle differences in daily patterns of spontaneous locomotion and time spent in defined areas of the chamber (e.g., close to the edges, in the center and in hidden areas, and in drinking and eating areas).[29,30] ABHD6 KO mice and WT littermates (7–10 weeks of age) were transferred from their home cage to individual Noldus PhenoTyper cages at 8 AM (i.e., start of the 12-h light phase), and their spontaneous locomotion monitored for 48 h. We found no significant difference in the overall distance travelled by ABHD6 KO and WT littermates when analyzing the entire cage during 3 time periods: the initial 2 h of being placed into the chamber (an index of novel environment exploration, $p = 0.57$), the dark phase (when mice are more active, $p = 0.78$), and the light phase (when mice are less active, $p = 0.13$)[38] (Fig. 2A). Analysis of the time spent in defined areas of the chamber during these three periods also showed no significant difference between ABHD6 KO and WT littermate mice, including the time spent close to the edges of the chamber (thigmotaxis, $p > 0.32$), in the shelter (hidden area, $p > 0.13$), and in the drinking ($p > 0.07$) and eating ($p > 0.27$) areas (Supplementary Fig. S2; Supplementary Table S1). An analysis of spontaneous locomotion in 2 h bins revealed small, but significant decrease in spontaneous locomotion of ABHD6 KO mice compared to WT littermate mice at select time points ($F_{23,529} = 3.09$, $p < 0.001$; Fig. 2B), in particular, during periods of transitions from light to dark phase (e.g., from less to more active; $p < 0.05$ and $p < 0.01$ for the first and second transitions, respectively; Fig. 2C). Thus, ABHD6 KO mice do not exhibit overt changes in spontaneous locomotion and exhibit only mild impairments of spontaneous locomotion during transition periods from low activity to higher activity (light to dark phase transition).

### Mild impairment of motor coordination in ABHD6 KO mice

Motor coordination and the acquisition of such motor skills can be studied in mice using a Rotarod apparatus by comparing their latency to fall off the Rotarod over seven trials per day for multiple days.[27,33,39] While ABHD6 KO and WT littermate mice performed similarly on the Rotarod when averaging their performance during the 1st and 2nd day ($F_{1,19} = 1.15$, $p = 0.30$; Fig. 3A), an analysis of individual Rotarod trials during each day indicated a significant reduction in performance by ABHD6 KO mice compared to WT

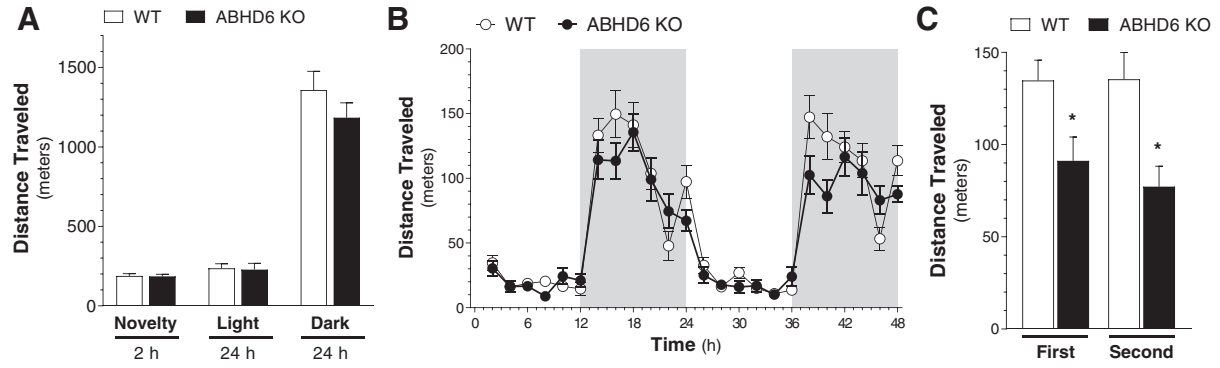


**FIG. 2.** Mild impairment of spontaneous locomotion in ABHD6 KO mice. Spontaneous locomotion of WT and ABHD6 KO mice was measured over 2 days in an instrumented observation home cage (12-h light and dark cycles). **(A)** ABHD6 KO and WT mice exhibited similar overall spontaneous locomotion during the initial 2 h of being placed in the instrumented observation home cage (novel environmental exploration) and during light and dark phases (total 24 h each). **(B)** Analyses of the daily patterns of locomotor activity over 48 h in 2-h bins suggested the reduction of spontaneous locomotion of ABHD6 KO mice during the transition period from light to dark phases. **(C)** Significant reduction in the spontaneous locomotion of ABHD6 KO mice compared to WT mice measured during the transition from light to dark phases (i.e., distance travelled during the last 4 h of the light cycle vs. the first 4 h of the dark cycle). Data are expressed as mean ± S.E.M. ($n = 12$–13 mice per group). *$p < 0.05$ versus WT, repeated measures ANOVA followed by Bonferroni *post hoc* test. ANOVA, analysis of variance; S.E.M., standard error of mean.

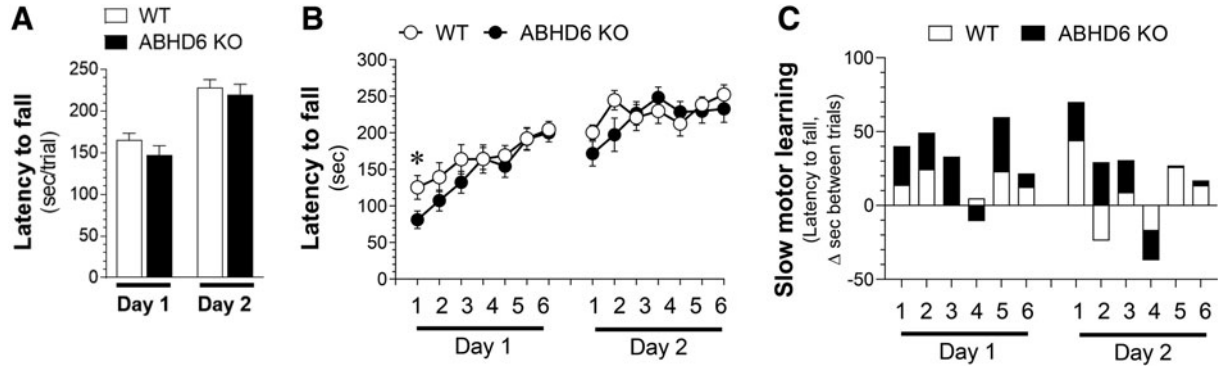


**FIG. 3.** Mild initial impairment of motor coordination and enhanced motor learning in ABHD6 KO mice. Motor coordination and the acquisition of such motor skills were studied in WT and ABHD6 KO mice by measuring their latency to fall off a Rotarod using a 2-day paradigm (each day, 7 trials of 300 sec). **(A)** ABHD6-KO mice and WT littermates do not exhibit significant difference in their rotarod performance when analyzing the average latency to fall off the rotarod across all trials on days 1 and 2. **(B)** ABHD6-KO mice exhibit a significant reduction in the latency to fall off the Rotarod on the first trial of the 1st day of testing (*$p < 0.05$ vs. WT, ANOVA followed by Tuckey). **(C)** ABDH6 KO mice (total = 152 sec) enhanced slow motor learning compared to WT mice (total = 120 sec) as determined by the improvement in performance between trials across all trials (Δ sec between trials). Data are expressed as mean ± S.E.M. ($n = 10$–11 mice per group).

littermates during the first trial of the 1st day of testing (analysis of variance [ANOVA] followed by Tuckey, $p < 0.05$; Fig. 3B). These results suggest that genetic deletion of *Abhd6* has no overt effect on motor coordination, but might influence motor learning.

To determine if ABHD6 KO mice exhibit impaired motor learning, we adopted a method developed by Costa et al.,[39] in which *fast* motor learning is determined by measuring improvement in motor performance on the rotarod within the first training sessions (average % improvement between initial four trials of each day) and *slow* motor learning is determined by measuring improvement across all trials of each day. Fast motor learning was slightly lower in ABHD6 KO mice (8%) compared to WT littermates (20%). By contrast, slow motor learning was slightly greater in ABHD6 KO mice compared to WT littermates (i.e., totaling 152 sec in ABHD6 KO mice compared to 127 sec in WT mice; Fig. 3C). Together, these results suggest a mild impairment of motor coordination in ABHD6 KO mice compared to WT littermates, which is more pronounced during the initial Rotarod trials. These results also suggest enhanced learning of new motor coordination skills ABHD6 KO mice compared to WT littermates, a conclusion that agrees with the fact that ABHD6 KO mice perform similar to WT littermates by the 2nd day of Rotarod testing.

### Blocking ABHD6 enhances amphetamine-stimulated hyperlocomotion without affecting AS: $CB_1R$ dependence

We first determined whether genetic deletion of *Abhd6* affects amphetamine-stimulated hyperlocomotion by treating ABHD6 KO and WT littermate mice with amphetamine (2 mg/kg) and recording their locomotion in an open-field chamber before and after treatment. Figure 4A shows that amphetamine-stimulated hyperlocomotion was two-fold greater in ABHD6 KO mice compared to WT littermates ($F_{17,357} = 3.437$, $p < 0.001$). Enhanced amphetamine-stimulated hyperlocomotion was also evident in ABHD6 heterozygote (HET) mice (2.4-fold) and when treating WT mice with the brain-penetrant ABHD6 inhibitor KT-182 (2 mg/kg, intraperitoneal [i.p.]; 2.2-fold; Fig. 4B). Together, our genetic and pharmacological results strongly suggest that blocking ABHD6 increases amphetamine-stimulated hyperlocomotion.

As previously shown, amphetamine-stimulated hyperlocomotion in WT mice was not affected by the $CB_1R$ antagonist SR141617 (2 mg/kg; Fig. 4C).[22–24] By sharp contrast, the enhanced amphetamine-stimulated hyperlocomotion measured in ABHD6 KO mice was reduced by SR141617 to a WT response ($F_{1,44} = 8.966$, $p < 0.01$; Fig. 4C). This result suggests that reduced ABHD6

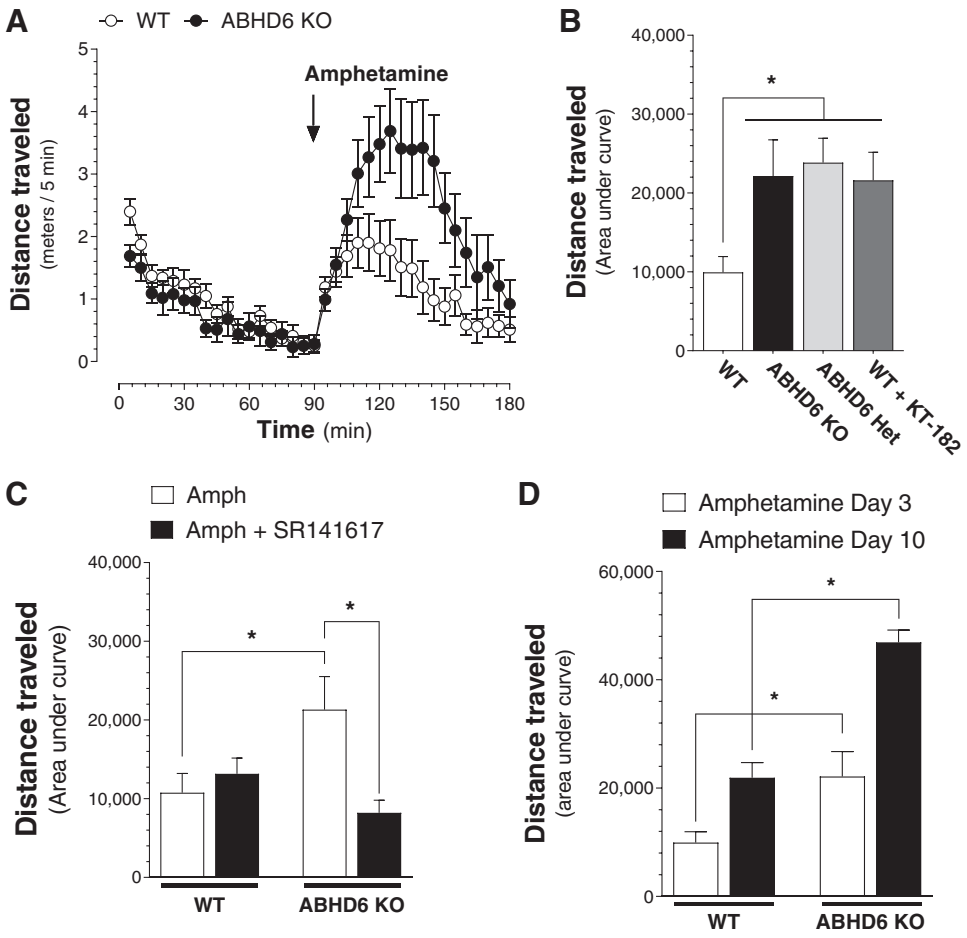


**FIG. 4.** $CB_1R$-dependent increases in amphetamine triggered hyperlocomotion and maintained amphetamine sensitization in ABHD6 KO mice. Amphetamine-stimulated hyperlocomotion and AS was measured by treating ABHD6 KO and WT littermate mice with amphetamine (2 mg/kg) and recording their locomotion in an open-field chamber before and after treatment. **(A)** Amphetamine-stimulated hyperactivity (distance travelled per 5 min) was greater in ABHD6 KO compared to WT mice. **(B)** Amphetamine-stimulated hyperactivity (distance travelled during 90 min following treatment, AUC) was enhanced by 2- and 2.2-fold in ABHD6 KO and HET mice, respectively, and by 2.4-fold in WT mice treated with KT-182 (2 mg/kg). $*p < 0.05$ versus WT group, ANOVA followed by Dunnett's *post hoc* test. **(C)** The $CB_1R$ antagonist SR141617 (2 mg/kg, subcutaneous) does not affect amphetamine-stimulated hyperlocomotion in WT mice and reduced the amphetamine-stimulated hyperlocomotion in ABHD6 KO mice to levels comparable to the WT response. **(D)** ABHD6 KO and WT littermates express AS. $*p < 0.05$ versus WT vehicle group, $*p < 0.05$ vs. ABHD6 KO vehicle group, repeated measures ANOVA followed by Tuckey *post hoc* test. $*p < 0.05$ versus WT vehicle group, $*p < 0.05$ versus ABHD6 KO vehicle group, repeated measures ANOVA followed by Tuckey *post hoc* test. Data are expressed as mean ± S.E.M. ($n = 10$–11 mice per group). AS, amphetamine sensitization; AUC, area under the curve; $CB_1R$, cannabinoid 1 receptor; HET, heterozygote.

activity causes a mechanistic switch from a $CB_1R$-independent amphetamine-stimulated hyperlocomotion to an enhanced, $CB_1R$-dependant, amphetamine-stimulated hyperlocomotion.

AS is expressed as a progressive enhancement of amphetamine-stimulated hyperlocomotion with repeated amphetamine injections, and this behavioral sensitization is known to involve $CB_1Rs$.[5,6,15,24,40] To determine if ABHD6 is involved in AS, we treated mice twice with amphetamine (2 mg/kg, i.p., first on day 3 and then on day 10), and measured locomotion as above. Remarkably, ABHD6 KO and WT littermate mice exhibited a similar AS (2.2-fold in WT and 2.1-fold in ABHD6-KO; $F_{1,27} = 24.308$, $p < 0.001$; Fig. 4D). Importantly, pretreatment with SR141516 blocked the enhanced AS measured in ABHD6-KO mice showing that AS remained $CB_1R$ dependent (data not shown) Thus, while ABHD6 controls acute amphetamine-stimulated hyperlocomotion, it does not control the establishment of AS.

## Discussion

We report a new genetically modified mouse line, ABHD6 KO, which appears to undergo normal development into adulthood and only exhibits a mild reduction in spontaneous locomotion and motor coordination, indicating that adult mice lacking ABHD6 from birth do not exhibit an overt abnormal phenotype. Our results extend a previous report showing that ABHD6 KO mice generated using a different genetic approach do not exhibit an overt abnormal phenotype in adulthood.[41]

Well-balanced spontaneous locomotion and exploration are controlled by $CB_1Rs$, represent essential behaviors for survival, and are often dysfunctional in psychiatric disorders.[42–44] We report a mild reduction in spontaneous locomotion in ABHD6 KO mice compared to WT littermates, which was apparent during the transition between periods of low activity to higher activity (light to dark phase transition), suggesting the involvement of ABHD6 in arousal-dependent changes in locomotion. Furthermore, ABHD6 does not appear to play a role in exploratory behavior as indicated by the similar spontaneous locomotion of ABHD6 KO mice and WT littermate mice measured during the initial period in PhenoTyper cage. These results contrast with the pronounced impairment of spontaneous locomotion measured when blocking MAGL activity in mice.[18,45] Thus, compared to MAGL inhibition, ABHD6 inhibition only mildly affects spontaneous locomotion.

Blockade of MAGL activity in mice does not influence their average performance on the Rotarod.[46] We show that ABHD6 KO mice exhibit a mild impairment of motor coordination detected during the first trial of Rotarod testing and yet perform similar to WT littermate mice during the following Rotarod trials. Considering that motor coordination and learning of motor skills involve corticostriatal neuronal circuits,[47] our results suggest that ABHD6 expressed by these neuronal circuits might contribute to the molecular mechanism involved in improving motor coordination skills.

Amphetamine increases neurotransmission in limbic brain areas involved in locomotion and motivation by several mechanisms that increase dopamine signaling.[1,2] Amphetamine enhances dopamine signaling in the nucleus accumbens (NAc) in a $CB_1R$-dependent manner.[8] We show that loss of ABHD6 activity enhances amphetamine-stimulated hyperlocomotion through a mechanistic switch to a $CB_1R$-dependent amphetamine response. The enhanced amphetamine-stimulated hyperlocomotion was measured in both WT mice treated with an ABHD6 inhibitor and in ABHD6 HET mice, suggesting that the enhanced amphetamine response is not due to neurodevelopmental compensation occurring in ABHD6 KO mice, and that ABHD6 activity represents an enzymatic limiting factor in this response, respectively. There are several brain areas and neuronal circuits where a mechanistic interaction between ABHD6, $CB_1R$, and dopamine signaling might occur. $CB_1Rs$ are expressed by prefrontal cortex-NAc projection terminals that modulate cholinergic interneurons controlling dopamine release in the NAc.[48] Thus, ABHD6 inhibition could enhance $CB_1R$ activation on PFC-NAc terminals and decrease glutamate release onto cholinergic interneurons that control dopamine release in the NAc. Another mechanistic interaction could occur in the ventral tegmental area (VTA) where $CB_1Rs$ are expressed on afferent GABAergic terminals that control the activation of dopamine neurons.[49,50] Thus, ABHD6 inhibition could enhance $CB_1R$ activation on GABAergic neurons in the VTA and increase dopamine release in the striatum. Our results provide an initial mechanistic framework for studying the molecular mechanism by which blockade of ABHD6 might enhance amphetamine-stimulated hyperlocomotion and render it dependent of $CB_1Rs$.

Blocking ABHD6 activity does not affect AS, a result that is in sharp contrast with the known involvement of $CB_1R$, MAGL, and FAAH in this behavioral

sensitization.[4] AS has been proposed to model the development of aberrant dopamine signaling and dysregulation of incentive motivational processes that are involved in the development of addiction, stimulant-induced psychosis, and a number of psychiatric illnesses.[51–54] Accordingly, $CB_1R$ signaling plays a role in reinstatement and relapse of amphetamine-type stimulant use disorders, addiction, and ensuing psychiatric symptoms.[54,55] Our results suggest that ABHD6 does not play a significant role in the expression of AS.

What is the signaling mechanism that links blockade of ABHD6 to activation of $CB_1R$ and enhancement of amphetamine-stimulated hyperlocomotion? This literature suggests that the most likely candidate is 2-AG. Biochemical measures of ABHD6 activity in tissue homogenates show that ABHD6 preferentially hydrolyses 2-AG over other monoacylglycerol lipids and hydrolyses various lysophospholipids and diacylglycerol (DAG) substrates.[28,56,57] However, the involvement of lysophospholipids and DAGs in our response is unlikely since these lipids do not activate $CB_1R$, whereas 2-AG activates $CB_1R$ as a full agonist.[58–60] Our understanding of 2-AG dynamics *in vivo* has been limited by the low spatiotemporal resolution of common analytical chemistry approaches (rapid freezing of tissue follow by liquid chromatography mass spectrometry quantification). While well suited for measuring changes in 2-AG tone, quantification of bulk brain 2-AG does not resolve 2-AG fluctuations occurring in seconds to minutes triggered by transient increase in brain activity. For example, AS reduces 2-AG levels in the ventral striatum, but not in the dorsal striatum, responses that could be linked to changes in overall 2-AG tone rather than rapid and transient changes in localized 2-AG levels.[23] To overcome this limitation, novel genetically encoded eCB sensors, for example, GRABeCB2.0, are paving the way to study localized changes in low micromolar eCB concentrations within seconds.[61,62] Of note, ABHD6 has been shown to control the number of AMPA receptors trafficking to the plasma membrane; however, this molecular mechanism of ABHD6 is not involved in the ABHD6-dependent enhanced amphetamine-stimulated hyperlocomotion because (1) ABHD6-AMPA receptor interactions do not involve the catalytic triad[63] and (2) we report the enhancing response also occurs when treating mice with KT-182, a carbamate-based inhibitor that blocks the 2-AG hydrolyzing activity by ABHD6 by covalent modification of the catalytic Ser148.[25,64–66] Thus, the most likely signaling mechanism that links blockade of ABHD6 to activation of $CB_1R$ and enhancement of amphetamine-stimulated hyperlocomotion is enhancement of 2-AG levels.

In conclusion, we provide the first evidence for a role of ABHD6 in the modulation of amphetamine-stimulated hyperlocomotion and suggest a molecular interaction between ABHD6 and $CB_1R$ signaling in highly active neuronal circuits that control locomotor behaviors influenced by psychostimulants. The ABHD6-dependent mechanistic switch to a $CB_1R$ dependence of amphetamine responses adds to our understanding of the molecular mechanisms involved in the behavioral effects of psychostimulants.

## Author Disclosure Statement

L.D., K.V., S.S., and B.C. reported no financial interests or potential conflicts of interest. N.S. is employed by Stella Consulting LLC. The terms of this arrangement have been reviewed and approved by the University of Washington in accordance with its policies governing outside work and financial conflicts of interest in research.

## Funding Information

This work was supported by the National Institutes of Health (Grant Nos. DA026430 and DA047626).

## Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Table S1

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**Cite this article as:** Deng L, Viray K, Singh S, Cravatt B, Stella N (2022) ABHD6 controls amphetamine-stimulated hyperlocomotion: involvement of $CB_1$ receptors, *Cannabis and Cannabinoid Research* 7:2, 188–198, DOI: 10.1089/can.2021.0066.

## Abbreviations Used

2-AG = 2-arachidonoylglycerol
ABHD6 = $\alpha/\beta$-hydrolase domain containing 6
ABPP = activity-based protein profiling
AEA = arachidonoylethanolamine
ANOVA = analysis of variance
AS = amphetamine sensitization
AUC = area under the curve
$CB_1R$ = cannabinoid 1 receptor
DAG = diacylglycerol
eCB = endocannabinoid
FAAH = fatty acid amide hydrolase
GABA = gamma-aminobutyric acid
HET = heterozygote
i.p. = intraperitoneal
KO = knockout
MAGL = monoacylglycerol lipase
MB064 = $\beta$-lactone
NAc = nucleus accumbens
SR141716 = N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride
VTA = ventral tegmental area
WT = wild type