A Novel Allosteric Modulator of the Cannabinoid CB_1 Receptor Ameliorates Hyperdopaminergia Endophenotypes in Rodent Models

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ABSTRACT

The endocannabinoid system (eCBs) encompasses the endocannabinoids, their synthetic and degradative enzymes, and cannabinoid (CB) receptors. The eCBs mediates inhibition of neurotransmitter release and acts as a major homeostatic system. Many aspects of the eCBs are altered in a number of psychiatric disorders including schizophrenia, which is characterized by dysregulation of dopaminergic signaling. The GluN1-Knockdown (GluN1KD) and Dopamine Transporter Knockout (DATKO) mice are models of hyperdopaminergia, which display abnormal psychosis-related behaviors, including hyperlocomotion and changes in pre-pulse inhibition (PPI). Here we investigate the ability of a novel CB_1 receptor (CB_1R) allosteric modulator, ABM300, to ameliorate these dysregulated behaviors.

ABM300 was characterized *in vitro* (receptor binding, β-arrestin2 recruitment, ERK1/2 phosphorylation, cAMP inhibition) and *in vivo* (anxiety-like behaviors, cannabimimetic effects, novel environment exploratory behavior, pre-pulse inhibition, conditioned avoidance response) to assess the effects of the compound in dysregulated behaviors within the transgenic models. *In vitro,* ABM300 increased CB_1R agonist binding but acted as an inhibitor of CB_1R agonist induced signaling, including β-arrestin2 translocation, ERK phosphorylation and cAMP inhibition. *In vivo*, ABM300 did not elicit anxiogenic-like or cannabimimetic effects, but it decreased novelty-induced hyperactivity, exaggerated stereotypy, and vertical exploration in both transgenic models of hyperdopaminergia, as well as normalizing pre-pulse inhibition (PPI) in DATKO mice.

The data demonstrate for the first time that a CB_1R allosteric modulator ameliorates the behavioral deficits in two model of increased dopamine, warranting further investigation as a potential therapeutic target in psychiatry.

INTRODUCTION

Dysregulation of dopaminergic and glutamatergic signaling are thought to underpin the development of psychosis and schizophrenia (1). Pharmacological treatment of schizophrenia and psychosis includes the use of antipsychotics, which act as orthosteric receptor antagonists / partial agonists of various GPCR targets including dopamine receptor D_2 and serotonin receptor $5HT_{1A}$. However, antipsychotics are associated with extrapyramidal side effects, sedation, metabolic syndrome and weight gain (2, 3).

The endocannabinoids, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), are orthosteric agonists of the cannabinoid CB_1 receptor (CB_1R). CB_1R are expressed presynaptically on various neuronal types, including GABAergic, glutamatergic and serotonergic neurons, where they mediate an inhibition of transmitter release. While not directly expressed on dopaminergic neurons, the endocannabinoid system acts as a crucial filter that integrates both inhibitory and excitatory signaling that modulates dopamine neuron signaling (4). Furthermore, studies have shown that the endocannabinoid system is a negative modulator of both D_1 and D_2 receptor-mediated behaviours, implicating them in basal ganglia disorders (5). As such, in combination with the complex dysregulation and circuit-based mechanisms for brain region-dependent alterations in dopaminergic signaling in psychiatry, this suggests that the CB_1R may be a more attractive, alternative therapeutic target to the classical D_2 receptor antagonism approaches of antipsychotics (4).

Furthermore, there is strong evidence from humans that both endocannabinoid levels and CB_1R are dysregulated in schizophrenia (6–9). Serum and CSF levels of AEA are higher in patients with schizophrenia at all stages of the illness and are normalized after treatment with antipsychotics. CB_1R expression and binding is higher in post-mortem brain tissues of patients with schizophrenia (6, 9). While the endocannabinoid system seems to be an important potential therapeutic target in psychiatry, targeting CB_1R at the orthosteric site has not yielded beneficial clinical outcomes. The CB_1R orthosteric inverse agonist, rimonabant, was effective treating obesity and metabolic syndrome, but caused suicidal ideation and was withdrawn from the

market (10–12). Here we propose to investigate a novel pharmacological approach of targeting the CB_1R.

In 2005, we discovered the CB_1R allosteric site and the original, prototype allosteric modulator, Org275. This compound has served as a tool compound to characterize the allosteric site but is not a drug candidate. Org275, and related compounds, display an atypical, complex allosteric profile at CB_1R (13, 14). Org275 *increases* the B_{max} of [^{3}H] CB_1R agonist binding but functionally acts as an *inhibitor* of CB_1R agonist mediated signaling (13, 15). Importantly, in October 2019, Shao *et al.* elucidated the ternary crystal structure of CB_1R in complex with agonist and Org275 (16). The structure shows that Org275 binds to a cholesterol-binding site on the CB_1R, suggesting that the compound works by partitioning into the bilayer and competing with endogenous cholesterol for this surface. Previous studies have demonstrated that cholesterol may act as an endogenous modulator of CB_1R (17). There is growing evidence that instead of targeting the orthosteric site of CB_1R, the allosteric site may have key advantages (15, 18, 19). By modulating the effects of the endogenous ligand, normal physiological tone (spatial and temporal effects of ligand binding to the receptor) are maintained, as opposed to the non-physiological binding and distribution seen with exogenous direct ligands such as orthosteric agonists or antagonists.

Since discovering the CB_1R allosteric site in 2005, and identification of Org275 as the first CB_1R negative allosteric modulator (13), we, and others, have worked to develop both CB_1R negative and positive allosteric modulators. The positive allosteric modulators developed by us, and others, have shown efficacy in the treatment of neuropathic pain (20) and other therapeutic indications (21). Because Org275, and related compounds, have insufficient metabolic stability, in order to further investigate the potential of this unique class of CB_1R allosteric modulator, we embarked on a chemistry campaign, with the goal of generating new molecules with improved drug-like characteristics that are more suitable for *in vivo* testing and clinical development. Our working hypothesis is that the unique pharmacological profile of CB_1R allosterics provides a distinctive pharmacological approach for modulation of the endocannabinoid system in

123 complex disorders, and offers an alternative to CB_1R orthosteric antagonists (22). The *in vivo*
124 outcomes of this complex mechanism are yet to be elucidated, particularly in models in which
125 the endocannabinoid system is dysregulated.
126
127 Here, we present data on the effects of a novel CB_1R allosteric modulator, ABM300, in two
128 distinct transgenic mouse models, both of which present with a state of hyperdopaminergia.
129 Both the GluN1-Knockdown (GluN1KD) and Dopamine Transporter Knockout (DATKO) mice
130 have increased synaptic dopamine in subcortical regions (23–25) which is implicated in their
131 phenotypic behavioral changes (24, 26–28), as well as disrupted sensorimotor gating (29–31).

METHODS & MATERIALS

Animal Ethics

Animal housing and experimentation were carried out in accordance with the Canadian Council in Animal Care (CCAC) guidelines for the care and use of animals and following protocols approved by the Faculty of Medicine and Pharmacy Animal Care Committee at the University of Toronto and the University of Guelph Animal Care Committee, respectively.

Compound Synthesis

See Supplementary Information for details.

Pharmacokinetic Analyses

Microsomal stability assays were conducted by Cyprotex Ltd (Macclesfield, UK). The *in vitro* metabolic stability of ABM300 was measured in the presence of human or rat liver microsomes by determination of the rate of compound disappearance. Single dose *in vivo* PK studies were conducted by Sai Life Ltd (Pune, India) to investigate the plasma pharmacokinetics and brain distribution of ABM300 in male C57Bl/6 mice following a single intraperitoneal (i.p.) administration of a 10 mg/kg dose.

Predictive Model of ABM300 bound to CB_1R

The crystal structure of the CB_1R-CP55940-ORG27569 complex (PDB code 6kqi) (16) was loaded into ICM (Molsoft, San Diego, CA), hydrogens were added, and rotameric states of hydroxy groups, histidine, asparagine and glutamine side-chains optimized. ICM's ligand editor was used to strip ORG27569 to the indole core scaffold shared with ABM300, and to incrementally grow the scaffold into ABM300, with a Monte Carlo-based energy minimization in the internal coordinate space at each step (32).

Equilibrium Binding Assays

Binding assays in hCB_1R CHO cells were performed by Eurofins Cerep with the CB_1R agonist, [^{3}H]CP55,940 (0.5nM, K_d of 3.5nM). Non-specific binding was defined in the presence of 10μM WIN55,212-2.

PathHunter® β-Arrestin Assay

The PathHunter® β-Arrestin assay was conducted by Eurofins Pharma Discovery Services (further details can be found at https://www.eurofinsdiscoveryservices.com).

ERK1/2 Phosphorylation Assay

CB_1R-mediated ERK1/2 phosphorylation was quantified using an AlphaLISA® Surefire® Ultra™ pERK1/2 Assay (PerkinElmer, Woodbridge, ON) according to the manufacturer's protocol, in hCB_1R CHO cells plated at a density of 40,000 cells/well (96 well). ABM300 IC_{50} values were determined in the presence of increasing concentrations of ABM300 at the EC_{80} for CP55,940 (40 nM). Results are presented as the percent stimulation of ERK1/2 phosphorylation by CP55,940 alone.

Cyclic AMP (cAMP) Assays

A DiscoverX HitHunter® cAMP Assay for Small Molecules (DiscoverX, Fremont, CA) was used to quantify cAMP levels as per the manufacturer's instructions. hCB_1R CHO cells were seeded at a density of 40,000 cells/well (white 96 well), and after 24h were serum-starved in the presence of 0.1% BSA for 60 min prior to pretreatment for 30 min with increasing concentrations of ABM300 (10^{-10}-10^{-5} M) and a final 30 min incubation with 40 nM CP55,940 and 5 μM forskolin. The cAMP BRET experiments were performed as previously described (36, 37). Briefly, hCB_1R HEK293 cells were transfected with 5μg/10cm dish of the CAMYEL biosensor using polyethylenimine. After 24h, the cells were plated into white 96 well plates (PerkinElmer) at a density of 60,000 cells/well.

Drug Administration

The following drugs were administered in a volume of 10 ml/kg via i.p. injections in a vehicle consisting of 95% ethanol, Tween80, and 0.9% NaCl in a 1:1:18 ratio, 30 min before behavioral testing, unless otherwise stated: ABM300 (10mg/kg), rimonabant (RIM; 10mg/kg, Cayman Chemical, Cat.# 9000484, Ann Arbor, MI), olanzapine (OLA; 1 mg/kg, Millipore Sigma, Cat.# O1141, Toronto, ON, CA), and Δ^9-tetrahydrocannabinol (THC; 10mg/kg, gift from MedReleaf, Markham, ON, CA).

Cannabinoid-Induced Tetrad Behaviors

Male C57Bl/6J mice (PD>70) were tested on the cannabinoid-induced tetrad, as previously described (35).

Behavioral Testing in Murine Models of Hyperdopaminergia

The effect of ABM300 was compared to OLA. GluN1KD (F1 on C57Bl/6J x 129/SvlmJ background) (26) and DATKO (C57Bl/6J background) (24) mice were used as murine models of hyperdopaminergia. All mice were tested as follows: Day 1 – open field test and Day 3 – pre-pulse inhibition, as previously described (28, 30, 36, 37).

Quantification and Statistical Analysis

For *in vitro* assays, results were analyzed by non-linear regression analysis of sigmoidal dose-response curves. For *in vivo* assays, statistical parameters, the definition of measures and statistical significance are reported in the figures and the figure legends. Data are represented as mean ± SEM. Studies and data analysis were not blinded. Differences in means were considered statistically significant at $p<0.05$. All data analyses were performed using GraphPad Prism 6.0 or 8.0 software (San Diego, CA) and/or IBM SPSS 23.0 Software (Armonk, NY).

See Supplementary Materials and Methods for more detail.

RESULTS

Pharmacokinetics of ABM300

ABM300 (5-(5-chloro-3-ethyl-1H-indol-2-yl)-*N*-phenyl-1,3,4-oxadiazol-2-amine) showed promising *in vitro* metabolic stability in human and rat liver microsomal preparations with half-life values of 109 and 110 min (compared to a typical developmental target for progression of >45 to 60 minutes), respectively (CL_{int} = 12.7 ± 3.4 and 12.6 ± 2.0 µL/min/mg protein). Subsequent *in vivo* pharmacokinetic studies in male C57Bl/6 mice (8 timepoints, N = 3 per timepoint) confirmed acceptable metabolic stability for our studies ($T_{1/2}$ approximately 2 h ~~1.8h~~) and CNS exposure, with a brain to plasma ratio of 0.77 from a dose 10 mg/kg, i.p., ($AUC_{8h\ brain}/AUC_{8h\ plasma}$) and brain concentrations of 374 ± 39 ng/mL at 30 min (Figure S1, Table S1).

Docking model of ABM300 bound to CB_1R

A model of ABM300 bound to CB_1R was derived from the CB_1-CP55940-ORG27569 ternary complex (16) (Figure 1). ABM300 recapitulates hydrophobic interactions observed with ORG27569 in the crystal structure. Due to its increased rigidity, ABM300 needs to shift by 2Å towards the wall formed by I141 to accommodate the phenyl ring that abuts I245. The docked conformation optimally occupies the pocket, the oxadiazole ring is stacked against the indole of W241, and the secondary amine bridging the phenyl and oxadiazole rings is engaged in a hydrogen-bond with C238.

In vitro pharmacology: ABM300 increases agonist binding but inhibits CB_1R orthosteric agonist signaling

In line with our previous studies using a related compound (ORG275) (13), we find that ABM300 causes a significant and concentration-dependent increase in the specific binding of [^{3}H]CP55,940 to hCB_1R CHO (Figure 2A) with an E_{max} value of 328 ± 47% and a EC_{50} value of 132 nM (pEC_{50} 6.90 ± 0.09) and an α value of 4.33 ± 0.79 (logα 0.622 ± 0.08) (Table S2).

In the PathHunter® β-arrestin CB_1R assay, CP55,940 stimulated β-arrestin recruitment with an EC_{50} value of 5.37nM (pIC_{50} 8.28 ± 0.06) and an E_{max} of 104.3 ± 0.90%. In the presence of the

243 EC_{80} of CP55,940 (10nM), ABM300 produced a concentration-related reduction in β-arrestin
244 recruitment with an IC_{50} value of 49.7nM (pIC_{50} 7.31 ± 0.02) (Figure 2B).
245
246 Using an AlphaScreen® SureFire® ERK 1/2 phosphorylation assay kit, we measured the effect of
247 ABM300 on activation of ERK1/2 phosphorylation by CP55,940 in hCB_1R CHO cells. In the
248 presence of vehicle, CP55,940 induced ERK1/2 phosphorylation with an EC_{50} of 12.1 nM (pEC_{50}
249 8.14 ± 0.23) (Figure 2C). At concentrations of 100 or 1000 nM, ABM300 significantly decreased
250 CP55,940 E_{max} (efficacy), to 40.3 ± 5.57% and 14.7 ± 5.22% respectively (Figure 2C). ABM300
251 alone did not affect ERK1/2 phosphorylation at concentrations up to 10 µM (Figure 2C).
252 Additionally, 100 or 1000 nM ABM300 significantly decreased the E_{max} (efficacy) of AEA from
253 100.3 ± 0.20% to 80.23 ± 14.4% and 31.88 ± 6.09% respectively (Figure 2D). In the presence of
254 the EC_{80} (40nM) of CP55,940, ABM300 produced a concentration-related reduction in ERK1/2
255 phosphorylation with an IC_{50} value of 47.0nM (pIC_{50} 7.38 ± 0.12) (Figure 2E). CP55,940 inhibited
256 forskolin-stimulated cAMP accumulation in hCB_1R CHO cells (data not shown). At CP55,940 EC_{80}
257 (40nM), ABM300 blocked this inhibition with an IC_{50} value of 379nM (pIC_{50} 6.45 ± 0.08) (Figure
258 2F).
259
260 We further characterized the real time kinetic effect of ABM300 using a cAMP BRET sensor
261 assay in HEK293 cells. Similar to previous observations with Org275 (38), ABM300 produced a
262 complex, concentration and time-dependent modulation of agonist-mediated regulation of
263 cAMP levels (Figure 2G,H). Levels of cAMP were measured over time with a high concentration
264 of CP55,940 (1 µM) in the presence of varying concentrations of ABM300. Consistent with
265 Org275 observations, ABM300 did not affect the initial inhibition of cAMP by CP55,940 but,
266 following a concentration-dependent "lag" in drug onset, inhibition of the agonist effect
267 became apparent. At high concentrations, the ABM300 inhibitory effect overcame the
268 CP55,940 effect and further enhanced cAMP levels above those produced by forskolin alone –
269 reflecting inverse agonism.
270

ABM300 does not bind to the CB_2R and does not have off target effects in a Safety Screen® 44 panel.

Off-target effects of ABM300 were assessed using the SafetyScreen44, conducted at Eurofins Discovery Services. The screen assesses the selectivity of the compound on a diverse panel of targets that includes GPCRs, drug transporters, ion channels, nuclear receptors, kinases, and other non-kinase enzymes. The screen employs radioligand binding or enzyme assays for these 44 targets. At 1 µM, ABM300 did not display any significant binding to these targets, including; receptors CB_2, Dopamine D_1 and D_2, NMDA, $5HT_{1A}$, $5HT_{1B}$, $5HT_{2A}$, $5HT_{2B}$, and $5HT_3$ (Figure S2).

ABM300 has no effect in the cannabinoid-induced tetrad alone and does not display anxiogenic-like effects.

We confirmed that ABM300 (10 mg/kg) did not have agonist activity via the cannabinoid-induced tetrad, compared to THC (10 mg/kg). In all four tetrad measures (Figure 3), THC, but not ABM300, produced effects ($p<0.0001$ for all outputs). Previous reports demonstrated adverse effects observed with CB_1R orthosteric agonists/inverse agonists (39, 40). Possible anxiogenic effects of ABM300 (10 mg/kg) were investigated using the EPM and compared to rimonabant (10 mg/kg) (Figure S3). ABM300 did not affect time spent in the open arms ($p=0.3335$), whereas rimonabant significantly reduced open arm time compared to vehicle ($p=0.0059$).

ABM300 decreases novelty-induced hyperactivity, exaggerated stereotypy, and vertical exploration in GluN1KD mice.

GluN1KD mice display hyperactivity, increased stereotypy and vertical exploration patterns, along with impairment in sensorimotor gating (26–29). GluN1KD mice do not display a difference in *Cnr1* mRNA expression in key brain regions mediating these behaviours (Figure S4). ABM300 decreased the number of dysregulated behaviours in the GluN1KD model of hyperdopaminergia (Figure 4). Hyperactivity (Figure 4A,B) was affected by genotype ($F[1,94]=100.7$, $p<0.0001$), and GluN1KD mice responded to ABM300 ($p<0.0001$) when compared to vehicle. Significant effects of genotype were observed for stereotypy and vertical

exploration (Figure 4C,D) ($F[1,94]=223.6$, $p<0.0001$; $F[1,94]=70.87$, $p<0.0001$, respectively). ABM300 significantly reduced exaggerated stereotypic activity (Figure 4C), as well as phenotypic increased rearing behavior (Figure 4D) ($p<0.0001$). Sensorimotor gating deficits, along with acoustic startle response, did not respond to ABM300 or the atypical antipsychotic olanzapine (*4dB*: $F[2,95]=1.068$, $p=0.3476$; *8dB*: $F[2,95]=0.4576$, $p=0.6342$; *16dB*: $F[2,95]=0.4790$, $p=0.6209$; *ASR:* $F[2,95]=3.016$, $p=0.0537$) (Figure S5).

ABM300 decreases novelty-induced hyperactivity, exaggerated stereotypy, vertical exploration and normalizes PPI in DATKO mice.

The DATKO mouse, another model of hyperdopaminergia, displays hyperactivity, increased stereotypy and vertical exploration, with an impairment in sensorimotor gating (24, 31). Similar to the GluN1KD model, DATKO mice showed no change in *Cnr1* mRNA expression (Figure S6). ABM300 normalized dysregulated hyperactivity, stereotypic movements, vertical exploration and sensorimotor gating in the DATKO mice (Figure 5), a pattern of findings similar to what was seen in the GluN1KD model (Figure 4). For the hyperactivity measure (Figure 5A), a significant interaction was found between ABM300 and genotype ($F[1,46]=9.38$, $p=0.004$), indicating that ABM300 has genotype-specific effects on the exacerbated hyperactivity endophenotype in DATKO. There was an effect of genotype on exaggerated stereotypic movements and mania-like rearing behaviour ($F[1,46]=40.18$, $p<0.001$; $F[1,46]=26.50$, $p<0.001$, respectively), which ABM300 had a beneficial effect in decreasing ($F[1,46]=14.22$, $p<0.001$; $F[1,46]=7.38$, $p=0.009$, respectively) (Figure 5B,C). Furthermore, the actions of ABM300 extended to sensorimotor gating deficits present in DATKO (Figure 5D), with a rescue of the PPI deficit at the 16dB pre-pulse interval ($p=0.018$). Neither genotype, nor treatment with ABM300, had an effect on the acoustic startle response in the DATKO model (data not shown, genotype: $p=0.214$, ABM300: $p=0.516$).

ABM300 has no effect on the conditioned avoidance response (CAR).

The CAR task has been used as a test to infer antipsychotic efficacy via the selective suppression of the avoidance response (41, 42). Administration of olanzapine (1 mg/kg), but not ABM300

(10 mg/kg), attenuated avoidance behaviour in CAR testing (Figure S7). A main effect of drug was observed ($p=0.007$). Furthermore, olanzapine, but not ABM300, enhanced escape responding during CAR (Figure S7B), with a main effect of drug treatment ($p=0.011$) and an interaction between drug treatment and testing day ($p=0.034$). Lastly, neither ABM300 nor olanzapine affected escape failures (Figure S7C). ABM300 did not induce catalepsy, or changes in body temperature (Figure S7D,E).

DISCUSSION

There is growing interest in the possible therapeutic potential of CB_1R allosteric molecules (21). Here, we show for the first time that a novel CB_1R allosteric modulator ameliorates select disrupted behaviors in two distinct models of hyperdopaminergia. The rescue of these phenotypes by ABM300 occurred without adverse anxiogenic-like or cannabimimetic effects traditionally observed with orthosteric CB_1R inverse agonists. Thus, these findings represent the first study demonstrating the potential for the use of a CB_1R allosteric modulator as a therapeutic strategy for the treatment of hyperdopaminergic states, such as psychosis and mania.

Our molecular docking data indicate that ABM300 binds to the recently elucidated binding site for the original allosteric modulator ORG275 (16). The ORG275 binding site on CB_1R overlaps with a cholesterol binding site which is an extrahelical site within the inner leaflet of the membrane. The model is broadly in agreement with literature demonstrating that ORG275 apparently stabilizes a high affinity, agonist bound CB_1R (43), which may impede activation of selected downstream signaling pathways. The structure proposes a mechanism by which Org275, binding to the cholesterol site, captures an intermediate conformation that binds the orthosteric agonist and also inhibits G protein coupling. The model accommodates the complex effects of Org275 on CB_1 orthosteric ligand binding including the increase in B_{max} of agonists. ABM300 displays a similar *in vitro* pharmacological profile to ORG275 (13, 15), whereby it increases CB_1R agonist binding with an α value > 1 (4.33 ± 0.79)(44), but acts as a functional inhibitor of CB_1R agonist-mediated β-arrestin recruitment (IC_{50}, 50nM), ERK phosphorylation (IC_{50}, 47nM), and cAMP inhibition (IC_{50}, 380nM).

As previously described (38), the unique time-dependent mechanism of action of ABM300 (Figure 2G) posits that at moderate concentrations (high nM/low µM), early agonist signaling may remain unaffected; with inhibition initiating after a lag. These observations indicate that the molecular mechanism of action of ABM300 is related to previously characterized CB_1R allosteric modulators such as Org275 and PSNCBAM-1 (38). This may introduce potential for a

unique kinetic profile of modulation of endocannabinoid signaling. The consequences of such effects at a network level and in a disease state are highly complex but may underlie the beneficial effects observed with ABM300 in the genetic models of hyperdopaminergia. Furthermore, expression levels, affinity, and pre-coupling of CB_1Rs can significantly differ in various neuronal cell types; CB_1R on GABAergic interneurons have significantly higher agonist affinity than those found on glutamatergic terminals, but the coupling efficacy of glutamatergic CB_1R is significantly higher (45, 46). This expression and affinity profile leads to the complex nature in the function of the eCBs, which explains the diverse effects of certain cannabinoid drugs, and the opposing effects in different illnesses (46). We hypothesize that, in the genetic murine models of hyperdopaminergia, ABM300 acts as a modulator of endogenous CB_1R signaling *in vivo* and, potentially, selectively modulates the endocannabinoid system in specific neurotransmitter system pathways to ameliorate the dysregulated behaviors observed in these mice.

Studies have demonstrated that administration of compounds that increase endocannabinoid levels modulate a number of schizophrenia-like responses in mice (47). Studies in cultured cells have shown that the related compound, ORG27569, causes migration of CB_1R to the soma, while cholesterol, which binds to the same site as ABM300 and acts as a positive modulator, allows for the enrichment of CB_1R at the axon (48). Thus, in addition to complex effects on endocannabinoid affinity and signaling, there is the potential for the modulation of topological CB_1R membrane localization by CB_1R allosterics. The consequences of this in a complex neuronal network, and pathological states, are yet to be fully elucidated.

To assess psychosis-like behaviours, and therapeutic efficacy of ABM300, we focused on two main behavioural categories: exploratory behaviour (hyperactivity) and sensorimotor gating (disruption in PPI). The literature related to psychosis-like behaviors in genetically modified mouse models has classically focused on the same two categories (49). Although these behaviors do not directly translate to human symptoms present in the disease, they do mimic the neurotransmitter changes that are involved in psychotic symptoms. It has been accepted

that subcortical hyperdopaminergia is implicated in psychosis, confirmed further by the neuropharmacological action of antipsychotic drugs currently available; all licensed pharmacological treatments of psychosis (antipsychotics) require interactions with the dopamine D_2 receptor (50). Therefore, by assessing locomotor behavior within this study, we can infer that, driven by subcortical dopamine levels, an increase in dopamine leads to enhanced motor activity (either horizonal, rearing, and/or stereotypy) (49). Meanwhile, disruption in PPI allows for a more straightforward phenotypic analysis of psychosis, as it has been reported in a number of psychiatric diseases, particularly schizophrenia and psychosis (51).

Here, we show that ABM300 restores dysregulated dopamine-mediated exploratory activity in both genetic models: decreasing exaggerated hyperactivity, stereotypy and rearing. Furthermore, ABM300 rescues PPI deficits in the DATKO model. Since psychosis symptomology is never present alone in a disease state such as schizophrenia, it would be intriguing to investigate the effects of ABM300 in other symptomatic domains, such as cognition. CB_1R antagonism and loss of function may enhance some forms of learning and memory, and it is possible that CB_1R negative allosteric modulators may be pro-cognitive in preclinical schizophrenic models (52, 53).

We saw no effect of the compound in CAR in rats~~, which is one of the most well established~~ even though olanzapine produced significant suppression of CAR as has been shown in previous studies (54). CAR is an extensively validated pre-clinical test used to predict therapeutic efficacy of antipsychotics that directly target the dopaminergic and serotoninergic receptor systems (42, 55). This experiment further supports our assertion that ABM300's antipsychotic effects are not mediated via dopamine D2 receptors, as considerable occupancy of striatal D2 receptors is required to see suppression of CAR (65-80% for typical antipsychotics, ~50% for atypical antipsychotics like clozapine) (42).

It is important to note that we do not have direct evidence for the involvement of the CB_1R in the *in vivo* effect of ABM300 in genetic models. Directly implicating CB_1R is challenging. One approach would be to create a double knockout of CB_1R-/- and DAT-/- or GluN1-/-. However, given the crucial role of the CB_1R in dopaminergic and glutamatergic signaling, there is a strong likelihood that the double knockouts could have a novel phenotype (such as seizures) or that previous phenotypes would be exacerbated (56, 57). Furthermore, genetic manipulation of the CB_1R has been shown to have effects on dopamine signaling (58, 59). Another approach would be to investigate whether a CB_1R orthosteric antagonist would block the effects of ABM300, thereby implicating CB_1R. However, there is also doubt as to whether an orthosteric CB_1R antagonist would block the effects of a negative allosteric inhibitor (antagonist blocking inhibitor); it is conceivable that a synergistic effect might be observed. Taken together, these limitations highlight the complexity of directly implicating CB_1R in the mechanism of action of ABM300 *in vivo* in these genetic models. We plan to make this the subject of future investigations involving a variety of *in vivo* and *ex vivo* approaches. The pharmacological profiling and assessment of the potential for off-target interactions of ABM300 in binding screens suggest that the compound is CB_1R-selective. Taken together, the potent inhibitory effect of the compound on CB_1R signaling at nM concentrations *in vitro* and the favorable PK and brain penetration data, there is substantial support for the hypothesis that the CB_1R mediates the effects of ABM300 *in vivo* in the genetic mouse models of hyperdopaminergia. It is also notable that the present study only included acute administration of one dose of ABM300. Future experiments will focus on various dose regimens, chronic dosing, development of tolerance and the effects of ABM300 in combination with an anti-psychotic.

The data presented here offer the first evidence that acute administration of a CB_1R allosteric modulator, with a unique pharmacological profile, effectively ameliorates certain behavioral deficits in two distinct models of increased dopamine. These data highlight that the allosteric binding pocket on the CB_1R warrants further investigation as a potentially important therapeutic target in psychiatry.

FUNDING AND DISCLOSURES

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AUTHORSHIP

All authors included in this manuscript have contributed to at least one of the following ICMJE guidelines:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work
2. Drafting the work or revising it critically for important intellectual content
3. Final approval of the version to be published
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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FIGURES

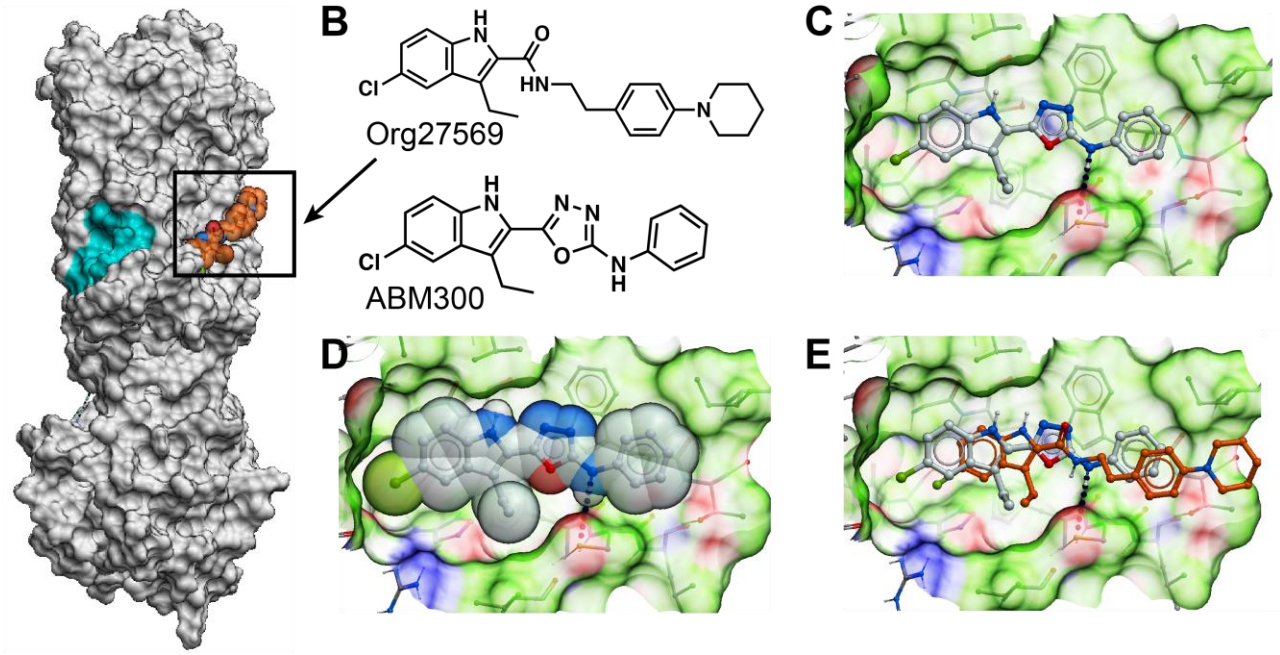
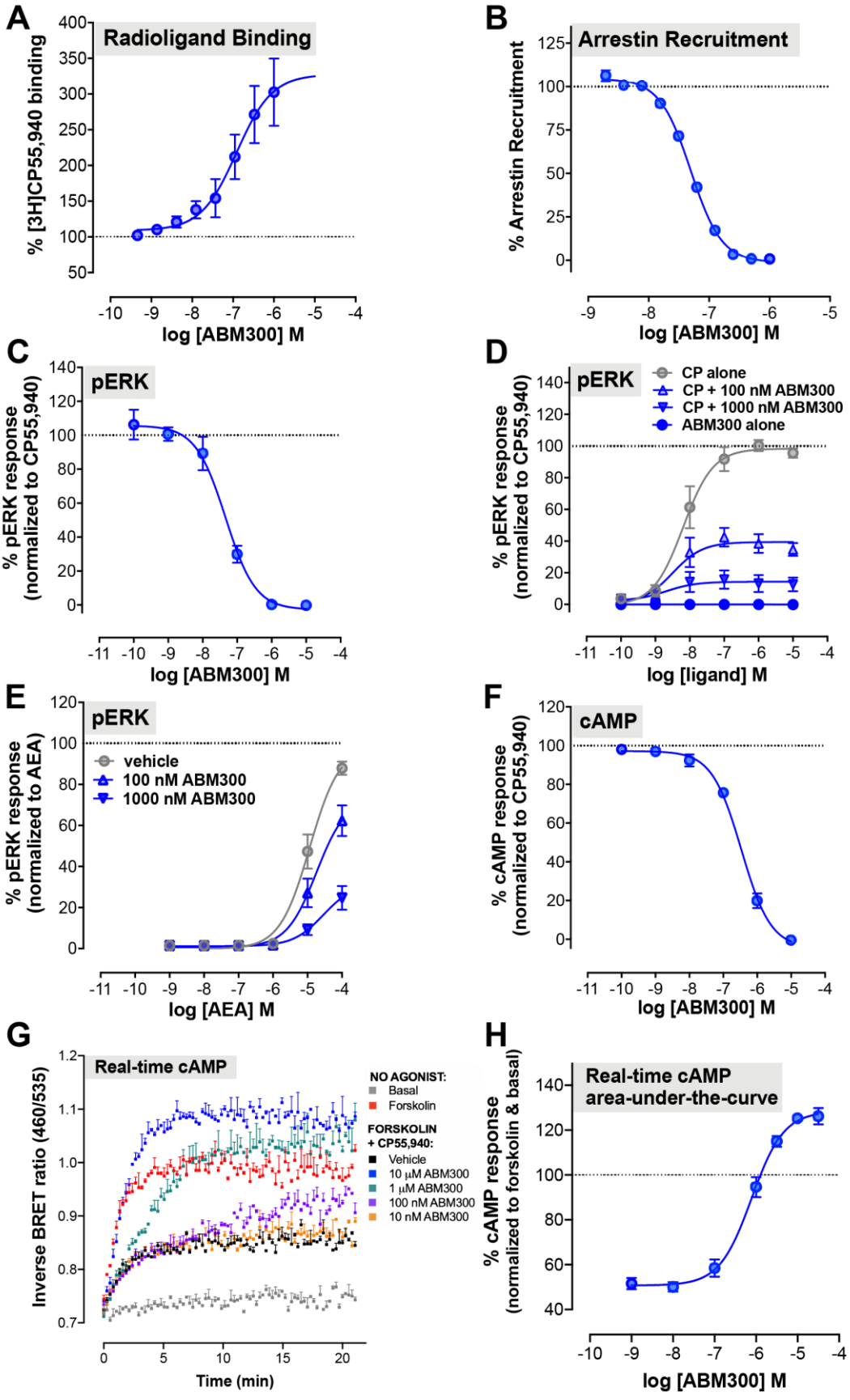


Figure 1: Molecular structure and docking model of ABM300 bound to CB_1R. (A) Overall structure of CB_1R (PDB code: 6kqi) with bound ORG27569 (orange). The pregnenolone allosteric binding site is highlighted in cyan (60), (B) Molecular structures of ABM300 and ORG27569. (C) Overall model showing ABM300 and surrounding side-chains. (D) Space filling representation showing that ABM300 occupies optimally the negative allosteric modulator binding pocket. (E) ABM300 superimposed with the crystal structure of ORG27569 (orange) bound to CB_1R (PDB code: 6kqi). The molecular surface of CB_1R is color-coded based on binding properties. Green: hydrophobic. Red: hydrogen-bond acceptor. Blue: hydrogen-bond donor.


A
Radioligand Binding
% [3H]CP55,940 binding
log [ABM300] M
B
Arrestin Recruitment
% Arrestin Recruitment
log [ABM300] M
C
pERK
% pERK response (normalized to CP55,940)
log [ABM300] M
D
pERK
CP alone
CP + 100 nM ABM300
CP + 1000 nM ABM300
ABM300 alone
% pERK response (normalized to CP55,940)
log [ligand] M
E
pERK
vehicle
100 nM ABM300
1000 nM ABM300
% pERK response (normalized to AEA)
log [AEA] M
F
cAMP
% cAMP response (normalized to CP55,940)
log [ABM300] M
G
Real-time cAMP
NO AGONIST:
Basal
Forskolin
FORSKOLIN + CP55,940:
Vehicle
10 μM ABM300
1 μM ABM300
100 nM ABM300
10 nM ABM300
Inverse BRET ratio (460/535)
Time (min)
H
Real-time cAMP area-under-the-curve
% cAMP response (normalized to forskolin & basal)
log [ABM300] M

646

Figure 2. ABM300 increases agonist binding but inhibits CB_1R orthosteric agonist signaling through arrestin recruitment, ERK phosphorylation and cAMP signalling. (A) ABM300 increases [^{3}H]CP55,940 binding to hCB_1R CHO cell membranes. (B) ABM300 concentration-dependently decreases CP55,940 (10 nM) mediated arrestin recruitment, with the PathHunter® β-arrestin assay. (C) ABM300 concentration-dependently decreases ERK phosphorylation at the EC_{80} concentration of CP55,940 (40 nM), using the AlphaScreen® SureFire® ERK1/2 phosphorylation kit in hCB_1R CHO cells. (D) ABM300 has no effect alone, but decreases the E_{max} for CP55,940-stimulated ERK phosphorylation in a concentration-dependent manner in hCB_1R CHO cells. (E) ABM300 decreases the E_{max} of AEA-stimulated ERK1/2 phosphorylation in a concentration-dependent manner in hCB_1R CHO cells. (F) ABM300 concentration-dependently inhibits CP55,940 (EC_{80} of 40 nM) mediated inhibition of forskolin-stimulated cAMP signaling in hCB_1R CHO cells. Data shown as mean ± SEM from 3-5 independent experiments conducted in triplicate. (G) BRET CAMYEL real-time cAMP signaling data in hCB_1R HEK cells, showing ABM300 concentration-dependently inhibiting the reduction in cAMP level induced by 5µM forskolin and 1µM CP55,940; the time-dependent activity of ABM300 is particularly apparent at moderate concentrations (1µM and 100nM), in which the onset of the ABM300 effect is delayed (representative experiment). (H) Area-under-the-curve analysis of (G), showing that ABM300 concentration-dependently inhibits CP55,940-mediated cAMP reductions in HEK cells. At high concentrations of ABM300, cAMP levels are increased above forskolin alone (100%).

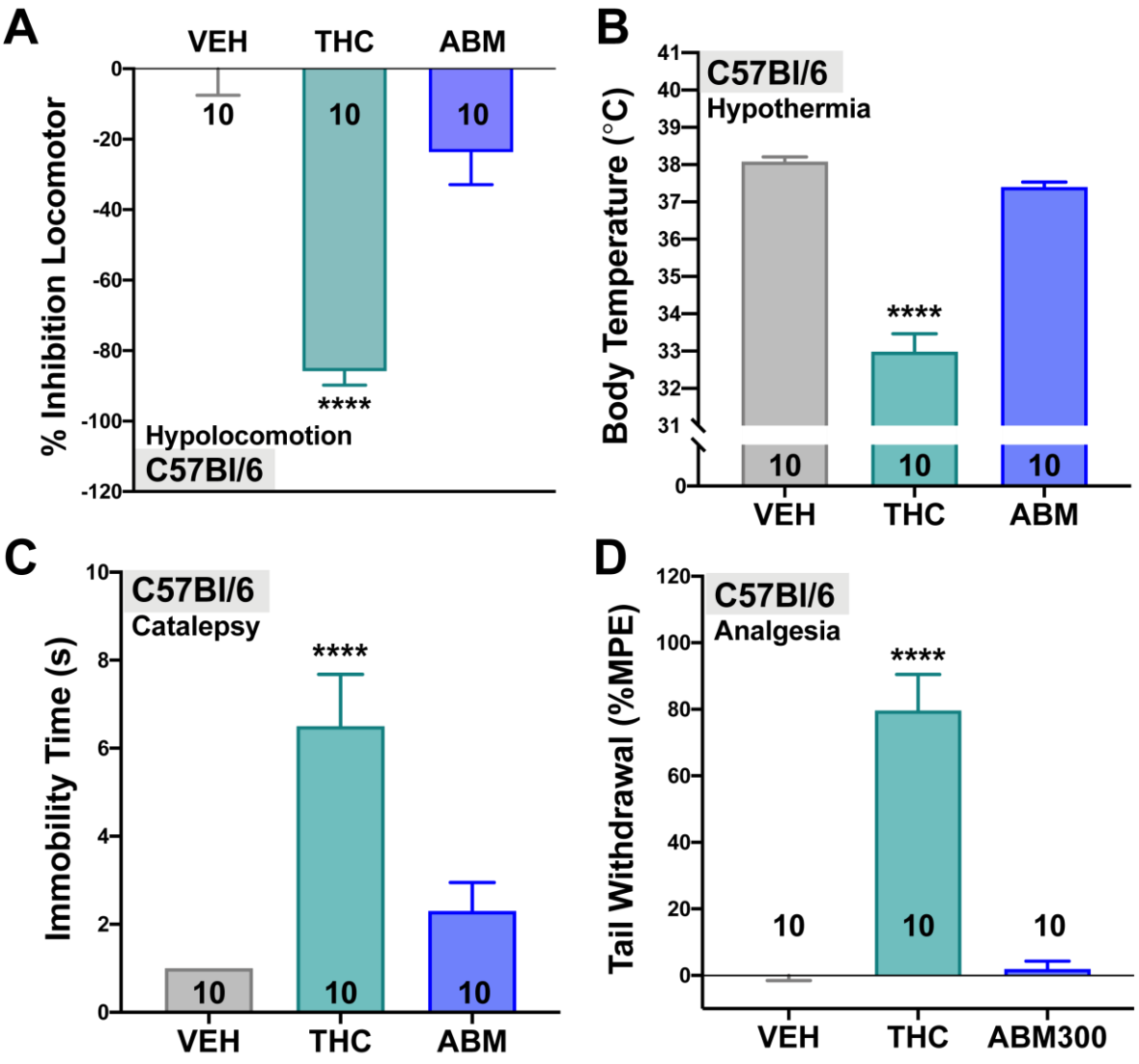


Figure 3. ABM300 (10 mg/kg) has no effect in the cannabinoid-induced tetrad, when compared to THC (10 mg/kg). Cannabinoid-induced tetrad measuring (A) percent inhibition of locomotor activity (15 min), (B) rectal temperature (°C), (C) catalepsy-induced immobility time (s), and (D) tail withdrawal (% MPE). ABM300 (10 mg/kg) alone has no effect on all outputs, when compared to vehicle and THC. All tests performed in male mice, ABM300 or THC were administered 30 min prior to behavioural testing via i.p. injection. Data shown as mean ± SEM, *p≤0.05 compared to vehicle, ****p<0.0001, one-way ANOVA, multiple comparisons, post-hoc Sidak's test. Effect of treatment (A) $F[2,27]=37.47$, $p<0.0001$, (B) $F[2,27]=85.58$, $p<0.0001$, (C) $F[2,27]=13.72$, $p<0.0001$, and (D) $F[2,26]=46.67$, $p<0.0001$.

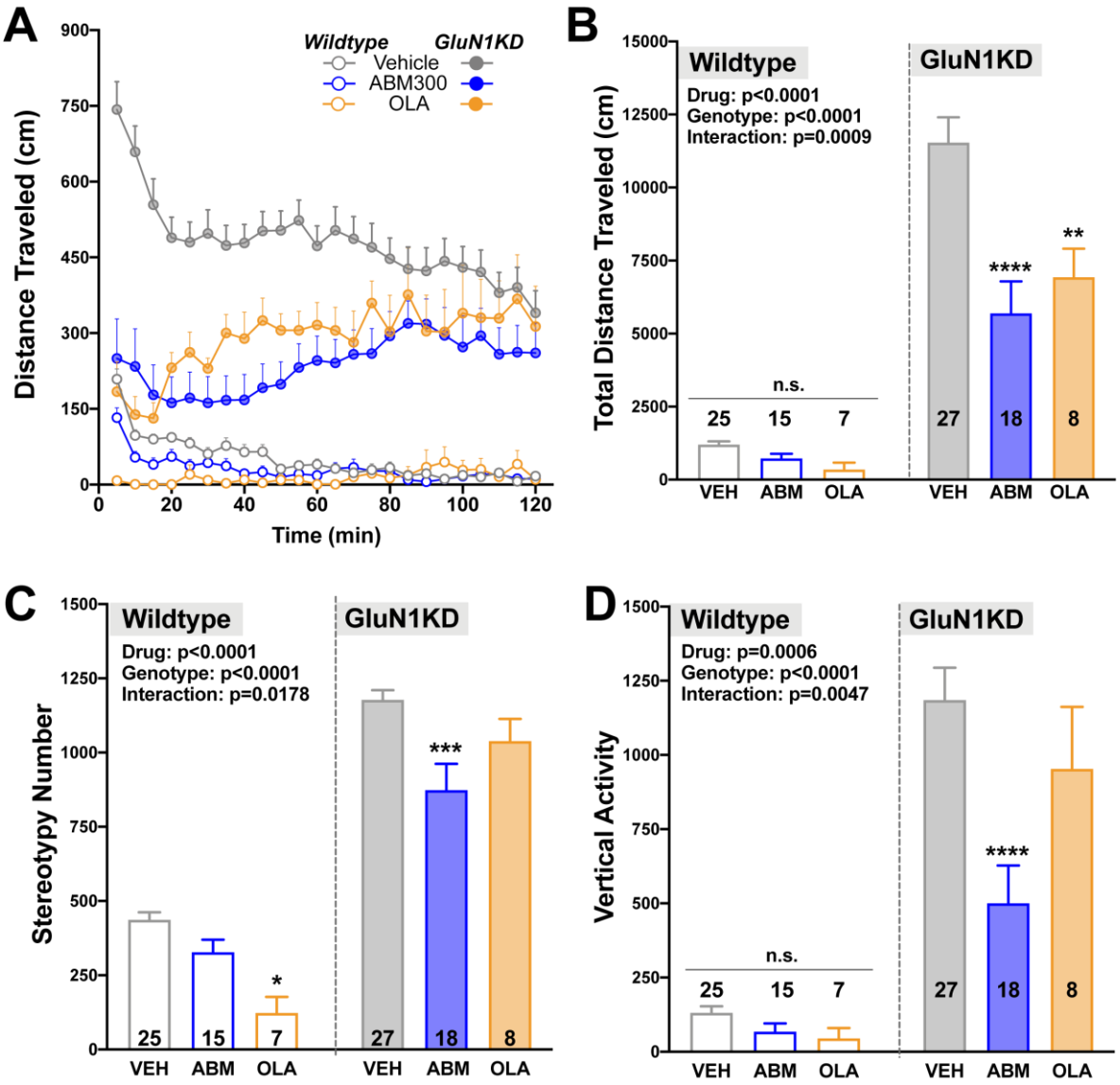


Figure 4. ABM300 corrects hyperactivity, aberrant stereotypic movements and rearing behaviour, resulting from hyperdopaminergia in the GluN1KD mouse model. ABM300 (ABM; 10 mg/kg) decreases novelty-induced hyperactivity (time-course of distance traveled – A, total distance traveled – B) aberrant stereotypic movements (C), and mania-like rearing behaviour (D), in the open field test. Effects of ABM300 are similar to those seen with olanzapine (OLA – 1 mg/kg). All tests balanced for sex, drugs administered 30 min before test via i.p. injection. Data shown as mean ± SEM, *p≤0.05 compared to vehicle (within genotype), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-way ANOVA, multiple comparisons, post-hoc Sidak's test. (A,B) Effect of genotype F[1,94]=100.7, p<0.0001, effect of drug F[2,94]=11.23, p<0.0001, interaction

of genotype x drug $F[2,94]=7.554$, $p=0.0009$. (C) Effect of genotype $F[1,94]=223.6$, $p<0.0001$, effect of drug $F[2,94]=12.13$, $p<0.0001$, interaction of genotype x drug $F[2,94]=4.206$, $p=0.0178$. (D) Effect of genotype $F[1,94]=70.87$, $p<0.0001$, effect of drug $F[2,94]=8.137$, $p=0.0006$, interaction of genotype x drug $F[2,94]=5.679$, $p=0.0047$.

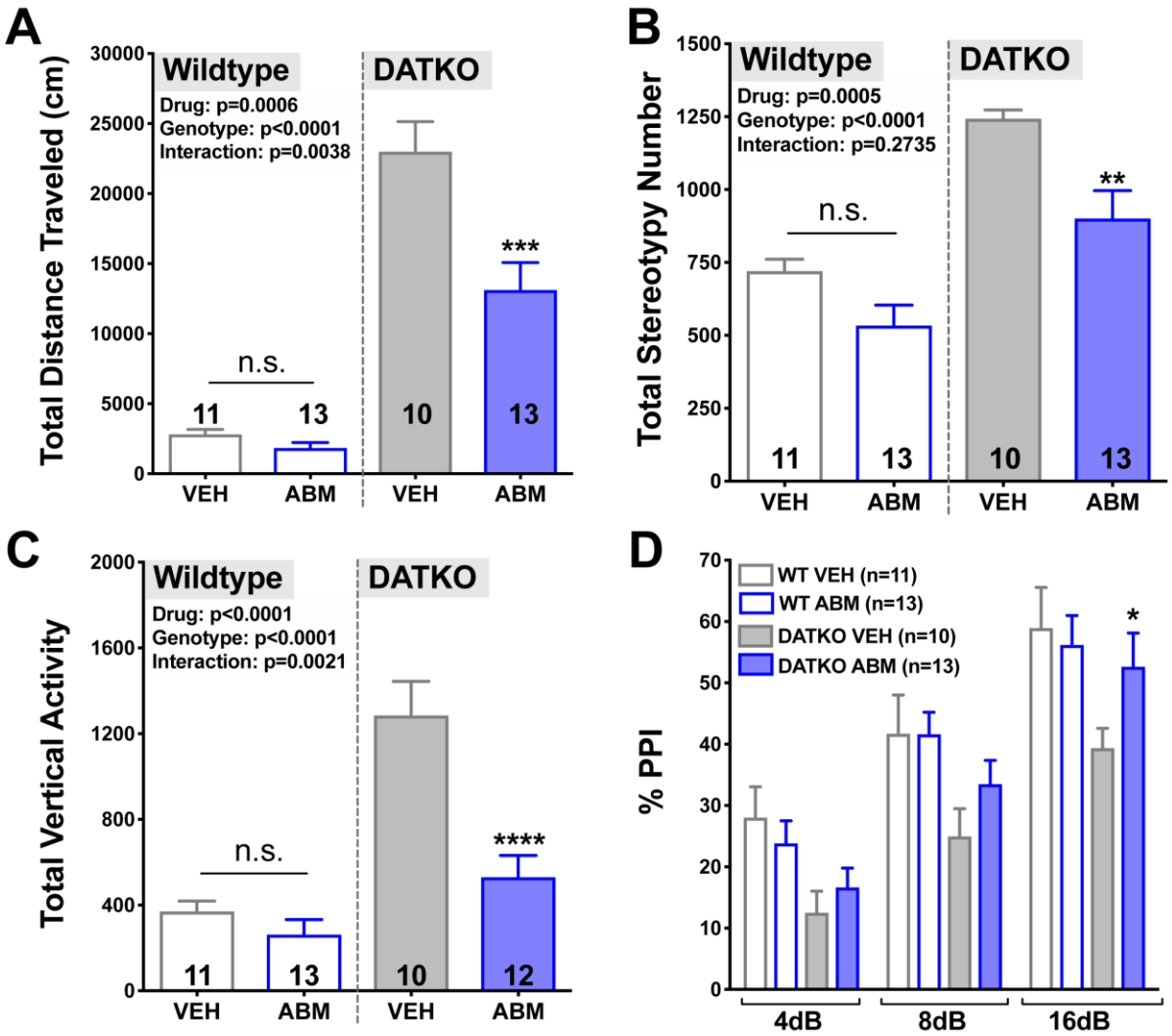


Figure 5. The effectiveness of ABM300 is recapitulated in a second, distinct, mouse model of hyperdopaminergia, the DATKO model, with additional restoration of sensorimotor deficits. ABM300 (10 mg/kg) decreases novelty-induced hyperactivity (total distance traveled; cm) (A), aberrant stereotypic movements (B), and mania-like vertical exploration (C) in the open field test. ABM300 ameliorates sensorimotor gating deficits (D), rescuing the PPI deficit at 16dB pre-pulse. All tests balanced for sex, drugs administered 30 min before test via i.p. Data shown as mean ± SEM, *p≤0.05 compared to vehicle (within genotype), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, two-way ANOVA, multiple comparisons, post-hoc Sidak's test. (A) Effect of genotype F[1,43]=116.9, p<0.0001, effect of drug F[1,43]=13.89, p=0.0006, interaction of genotype x drug F[1,43]=9.384, p=0.0038. (B) Effect of genotype F[1,43]=40.18, p<0.0001 and effect of drug F[1,43]=14.22, p=0.0005, (C) Effect of genotype F[1,42]=36.03, p<0.0001, effect of

drug $F[1,42]=19.16$, $p<0.0001$, interaction of genotype x drug $F[1,42]=10.80$, $p=0.0021$, (D) (4dB) effect of genotype $F[1,43]=8.509$, $p=0.0056$, (8dB) effect of genotype $F[1,43]=7.303$, $p=0.0098$, (16dB) effect of genotype $F[1,43]=4.713$, $p=0.0355$.