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Where and how does d-amphetamine act to reveal antipsychotic-induced dopamine supersensitivity in rats?

Running title (50 characters max): Antipsychotics and dopamine supersensitivity

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CONFLICT OF INTEREST

ANS was a scientific consultant for H. Lundbeck A/S as this research was being carried out. This has had no influence on the work.

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ABSTRACT

Antipsychotic treatment can produce a dopamine supersensitive state. In both schizophrenia patients and rodents, this is linked to antipsychotic treatment failure. In rodents, dopamine supersensitivity is often confirmed by an exaggerated behavioural response to the indirect monoamine agonist, d-amphetamine, after discontinuation of antipsychotic treatment. Here we investigated where and how d-amphetamine acts to trigger behavioural expression of dopamine supersensitivity, as this could uncover pathophysiological mechanisms underlying this supersensitivity. First, we examined the contributions of a central increase in dopamine/monoamine activity. Haloperidol-treated rats showed a potentiated psychomotor response to systemic d-amphetamine, confirming dopamine supersensitivity. However, they showed a normal psychomotor response to an increase in ventral midbrain dopamine impulse flow or to intracerebroventricular injection of d-amphetamine. This suggests that d-amphetamine's peripheral effects are required for a supersensitive response. Second, we determined the specific contributions of dopamine neurotransmission. The D2 agonist quinpirole, but not the D1 agonist SKF38393 or the dopamine reuptake blocker GBR12783 produced a supersensitive psychomotor response in haloperidol-treated rats. In these rats, the D1 antagonist SCH39166 decreased d-amphetamine-induced psychomotor activity, whereas the D2 antagonist sulpiride enhanced it. Thus, when d-amphetamine triggers a supersensitive response, this involves both D1- and D2-mediated transmission. Finally, we measured d-amphetamine-induced changes in D1- and D2-mediated intracellular signalling pathways in the striatum. In haloperidol-treated rats, a supersensitive response to d-amphetamine was linked to enhanced GSK3β activity and suppressed ERK1/2 activity in the nucleus accumbens, suggesting increased D2-mediated signalling. These findings provide new insights into the neurobiology of antipsychotic-evoked dopamine supersensitivity.

Keywords: Schizophrenia, Rat, D1 receptor, D2 receptor, Dopamine transporter, Mesocorticolimbic system

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INTRODUCTION

Antipsychotic drugs attenuate schizophrenia symptoms by blunting dopamine D2 receptor activity. However, long-term antipsychotic treatment can produce neuroadaptations that lead to supersensitivity to dopamine receptor stimulation. Antipsychotic-induced dopamine supersensitivity is linked to antipsychotic treatment failure and to an exacerbation of psychosis symptoms [1-7]. In animals, a widely-used index of antipsychotic-induced dopamine supersensitivity is an exaggerated locomotor response to d-amphetamine [8-18]. In this context, d-amphetamine serves as a pharmacological tool to probe the functional consequences of an acute increase in striatal dopamine release, as seen during psychosis [19]. D-amphetamine is an indirect monoamine agonist [20] with multiple sites of action and neurochemical effects. The anatomical location and nature of the neurochemical effects through which d-amphetamine produces a supersensitive behavioural response in antipsychotic-treated rats are largely unknown. We investigated these effects here, as the answers could reveal underlying biological mechanisms and eventual therapeutic targets to suppress antipsychotic-evoked dopamine supersensitivity.

A first question concerns where d-amphetamine acts to trigger the expression of antipsychotic-evoked dopamine supersensitivity. In dopamine-supersensitive rats, infusing d-amphetamine into the striatum does not trigger expression of established supersensitivity, suggesting that d-amphetamine actions in extra-striatal sites are also required [13]. Hence, we determined if increasing ventral tegmental area (VTA) dopamine impulse flow is sufficient to trigger a supersensitive psychomotor response. In other models where rats also show exaggerated d-amphetamine-induced psychomotor activity, this requires d-amphetamine actions in the periphery [21, 22]. Thus, we also determined whether limiting d-amphetamine's effects to the brain triggers a supersensitive psychomotor response in antipsychotic-treated rats.

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A second question concerns the role of dopamine-mediated neurotransmission. D-amphetamine stimulates dopamine, but also noradrenaline and serotonin transmission [20, 23]. Noradrenaline and serotonin also modulate the expression of antipsychotic-evoked dopamine supersensitivity [24, 25]. Thus, we determined whether selective dopamine reuptake inhibition is sufficient to evoke a supersensitive response in antipsychotic-treated rats. Dopamine signals through dopamine D1-type and D2-type receptors. Selective D2 receptor stimulation evokes a supersensitive behavioural response in antipsychotic-treated rats [25, 26], but whether D1 stimulation does the same is unknown. We also addressed this here. Furthermore, we determined whether D1 or D2 receptor antagonists suppress the exaggerated response to d-amphetamine in antipsychotic-treated rats. Lastly, we assessed the effects of d-amphetamine on D1- and D2-mediated signalling in the striatum by quantifying protein activity in the AKT/GSK3β- and cAMP/PKA-dependent pathways [27-30]. This is because although injecting d-amphetamine into the striatum is not sufficient to produce an enhanced psychomotor response in dopamine-supersensitive rats [13], d-amphetamine-induced signalling in the striatum might still be necessary.

METHODS

See Supplement for further information on rats, drugs, intra-cranial manipulations, measurement of psychomotor activity and Western Blots. Experimental procedures were approved by the Université de Montréal's ethics committee and followed the guidelines of the Canadian Council on Animal Care.

Antipsychotic treatment

Adult male Sprague-Dawley rats received haloperidol via osmotic minipumps (Alzet model 2ML2; Durect Corporation, Cupertino, CA) to achieve steady-state brain concentrations of the drug [9, 31], as produced by standard antipsychotic treatment regimens in the clinic [32-34]. We used 0.5

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mg/kg/day haloperidol. This achieves 73% ± 14 SD striatal D2 receptor occupancy (unpublished observations, see [9, 31]), and this is within the occupancy range that is therapeutically-efficacious in patients [35-37]. Under isoflurane anaesthesia, minipumps were implanted subcutaneously (s.c.) for haloperidol-treated rats, and controls were sham-operated [9]. Seventeen days later, minipumps were removed, and controls were sham-operated again.

Psychomotor Activity

Psychomotor activity was assessed using 2 measures: 1) photocell counts to measure horizontal locomotor activity and 2) observer ratings based on a 1-to-9 scale [38]. Ratings between 1 and 4 indicate no-to-normal locomotor activity, 5 indicates hyperlocomotion without stereotypy, and ratings between 6 and 9 indicate stereotypy [38].

Experiments

Fig. 1 illustrates experimental timelines. Locomotion tests started at least 3 days after haloperidol discontinuation and were given every 48 hours, 1 test/day. In each experiment below, we confirmed antipsychotic-induced dopamine supersensitivity by measuring the psychomotor response to s.c. d-amphetamine (0 or 1.5 mg/kg).

Exp. 1: Increasing VTA dopamine impulse flow. We determined if enhancing VTA dopamine impulse flow produces a supersensitive psychomotor response in haloperidol-treated rats. We evaluated the locomotor response to bilateral infusions of vehicle, neurotensin (1 nmol/hemisphere) or DAMGO (0.3 nmol/hemisphere) into the VTA, at concentrations that increase dopamine release in terminal regions [39, 40]. Neurotensin increases dopamine impulse flow by producing an inward current on dopamine neurons [41], reducing D2 autoreceptor-mediated inhibition [42-44], and enhancing glutamatergic inputs to dopamine neurons [45, 46]. DAMGO, a μ-opioid receptor agonist

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[47], inhibits GABA release thereby disinhibiting dopamine neuron activity [39, 48]. All rats received all treatments, counterbalanced.

Exp. 2: Intracerebroventricular d-amphetamine. We determined if limiting d-amphetamine's effects to the brain still triggers a supersensitive response in haloperidol-treated rats. We infused d-amphetamine into the lateral ventricles (0, 50 or 150 µg/hemisphere [49], counterbalanced, one d-amphetamine dose/rat), and measured psychomotor activity.

Exp. 3: Selective dopamine reuptake inhibition. We assessed whether selectively blocking dopamine reuptake with GBR12783 [50] (0, 5 or 10 mg/kg [51]) produces a supersensitive psychomotor response in haloperidol-treated rats. For comparison, we also assessed effects of the monoamine reuptake blocker cocaine [52] (0, 2.5 or 10 mg/kg [53]) and the monoamine receptor agonist apomorphine [23] (0, 0.25 or 0.5 mg/kg [54, 55]). Each rat received 3 agonist injections, counterbalanced.

Exp. 4: D1 and D2 receptor stimulation. We determined whether selective D1 or D2 stimulation produces an enhanced psychomotor response in haloperidol-treated rats. Locomotion was recorded for 30 min before administration of a D1 (SKF38393 [56, 57]; 0, 1 or 10 mg/kg [58, 59]) or D2 agonist (quinpirole [23]; 0, 0.15 or 0.5 mg/kg [26, 60]) and for 2 hours thereafter. Each rat received 2 agonist injections, counterbalanced.

Exp. 5: Systemic d-amphetamine with D1 or D2 receptor blockade. We assessed whether D1 and/or D2 transmission is necessary for the expression of dopamine supersensitivity. Rats received the D2 antagonist sulpiride [61, 62] (0, 25 or 80 mg/kg [63, 64]) or the D1 antagonist SCH39166 [65] (0, 0.03 or 0.1 mg/kg [66, 67]), and 30 min later, they received d-amphetamine (0 or 1.5 mg/kg). Each rat received 2 d-amphetamine and 2 antagonist injections, counterbalanced.

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Exp. 6: D-amphetamine effects on D1- and D2-mediated signalling. We measured d-amphetamine-induced protein activity in the AKT/GSK3β- and cAMP/PKA-dependent pathways. Locomotion was recorded for 30 minutes, then control and haloperidol-treated rats received s.c. saline or d-amphetamine. One hour later, brains were extracted, samples were taken from the nucleus accumbens and the dorsal, ventrolateral and centromedial caudate-putamen. We quantified total and phosphorylated protein levels of DARPP-32, ERK1, ERK2, AKT and GSK3β using Western Blot procedures.

Statistical analysis

Locomotor activity and protein levels were expressed as the percent change relative to vehicle-injected controls. Repeated measures or mixed-model ANOVA were used to analyse the influence of Dose or Injection or Group on locomotion, psychomotor activity ratings or protein level (Group × Dose or Injection × Time, 'Injection' as a within-subjects variable in Exp. 1 and 'Dose' and 'Injection' as between-subjects variables in Exps. 2-6). When interaction and/or main effects were significant ($p < 0.05$), effects were analysed further using Bonferroni's multiple comparisons' tests. Values in figures are mean ± SEM.

RESULTS

Across experiments, all haloperidol-treated groups developed dopamine supersensitivity, as indicated by enhanced d-amphetamine-induced locomotion relative to controls (Fig. 2; Group × Injection interaction; 2A, $F_{1,11}$ = 25.95; 2C, $F_{1,51}$ = 19.71; 2D, $F_{1,30}$ = 8.1; 2E, $F_{1,61}$ = 5.66; 2F, $F_{1,20}$ = 18.76; Group effect; 2A, $F_{1,11}$ = 5.31; 2B, $F_{1,8}$ = 5.51; 2C, $F_{1,51}$ = 9.05; 2D, $F_{1,30}$ = 5.57; 2E, $F_{1,61}$ = 7.88; 2F, $F_{1,20}$ = 12.67; Injection effect; 2A, $F_{1,11}$ = 92.63; 2B, $F_{1,8}$ = 31.77; 2C, $F_{1,51}$ = 497.5; 2D, $F_{1,30}$ = 117.0; 2E, $F_{1,61}$ = 260.4; 2F, $F_{1,20}$ = 129; 2-A-C-D-E-F; d-amph > veh in all groups; after d-amph, haloperidol >

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controls; all *P*'s < 0.05). Haloperidol and control rats generally showed similar D-amphetamine-induced psychomotor activity ratings (Fig. S1).

Exp. 1: Increasing VTA dopamine impulse flow

Across groups, intra-VTA neurotensin and DAMGO increased locomotion and psychomotor activity ratings compared to vehicle, without significant group differences (Fig. 3; Injection × Time interaction on minutes 10-120; 3B versus 3C, $F_{11,132}$ = 4.32; 3B versus 3D, $F_{11,132}$ = 7.75; Injection effect; 3B versus 3C, $F_{1,12}$ = 14.41; 3B versus 3D, $F_{1,12}$ = 17.04. Fig. 3E; Injection effect; vehicle versus neurotensin, $F_{1,12}$ = 6.92; vehicle versus DAMGO, $F_{1,12}$ = 13.91; all *P*'s < 0.05).

Hence, in rats with established antipsychotic-evoked dopamine supersensitivity, increasing VTA dopamine impulse flow does not evoke an enhanced psychomotor response.

Exp. 2: Intracerebroventricular d-amphetamine

Across groups, intracerebroventricular d-amphetamine dose-dependently increased locomotion and psychomotor activity ratings compared to vehicle, without group differences (Figs. 3F-H; minutes 10-120; Dose × Time interaction, $F_{22,726}$ = 6.38; Dose effect, $F_{2,66}$ = 29.49; Fig. 3I; Dose effect, $F_{2,66}$ = 50.43; all *P*'s < 0.0001).

Hence, circumscribing d-amphetamine's effects to the brain does not evoke the expression of established dopamine supersensitivity.

Exp. 3: Selective dopamine reuptake inhibition

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GBR12783 increased locomotion and psychomotor activity ratings above vehicle, without group differences (Fig. 4; vehicle not shown; 4A-B versus vehicle; minutes 70-180; Dose × Time interaction, $F_{22,1133}$ = 5.87; Dose effect, $F_{2,103}$ = 29.72; 4C versus vehicle; Dose effect, $F_{2,91}$ = 41.4; all *P*'s < 0.0001). Both cocaine and apomorphine increased locomotion and ratings above vehicle, with a mildly enhanced response in haloperidol rats (minutes 70-180; 4D-E versus vehicle; Dose × Time interaction, $F_{22,957}$ = 5.98; Dose effect, $F_{2,87}$ = 47.45; Group effect, $F_{1,87}$ = 4.35; 4F versus vehicle; Dose effect, $F_{2,75}$ = 53.45; 4G-H versus vehicle; minutes 70-180; Dose × Time interaction, $F_{22,1023}$ = 6.4; Dose effect, $F_{2,93}$ = 10.3; Group × Time interaction, $F_{11,1023}$ = 3.05; Group effect, $F_{1,93}$ = 8.05; 4I versus vehicle; Group × Dose interaction, $F_{2,81}$ = 5.43; Dose effect, $F_{2,81}$ = 189.8; Group effect, $F_{1,81}$ = 28.48; Fig. 4I; haloperidol > controls at both doses; all *P*'s < 0.05).

Thus, in dopamine-supersensitive rats, monoamine agonists (cocaine and apomorphine) but not a selective dopamine reuptake inhibitor (GBR12783) produce a mildly enhanced psychomotor response.

Exp. 4: D1 and D2 receptor stimulation

The D1 agonist SKF38393 evokes stereotypy but little hyperlocomotion [58, 68, 69]. Accordingly, SKF38393 increased psychomotor activity ratings, and did so similarly across groups (Fig. 5; vehicle not shown; 5A versus vehicle; Dose effect, $F_{2,58}$ = 15.89, p < 0.0001) without increasing locomotion (5B-C versus vehicle).

The D2 agonist quinpirole dose-dependently increased locomotion and ratings relative to vehicle, and this effect was greatest in haloperidol rats (5D versus vehicle; Dose effect, $F_{2,58}$ = 17.85; Group effect, $F_{1,58}$ = 3.89; 5E-F versus vehicle; minutes 40-210; Dose × Time interaction, $F_{34,\ 986}$ = 16.55; Group × Time interaction, $F_{17,986}$ = 4.57; Dose effect, $F_{2,58}$ = 32.11; Group effect, $F_{1,58}$ = 6.8; all *P*'s ≤ 0.05).

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Hence, rats with antipsychotic-induced supersensitivity show an augmented behavioural response to D2, but not D1 receptor stimulation.

Exp. 5: Systemic d-amphetamine with D1 or D2 receptor blockade

Haloperidol-treated rats showed greater d-amphetamine-induced ratings and locomotion than controls did (Fig. 6A; Group effect, $F_{1,88}$ = 14.48; Fig. 6B-C; minutes 40-150; Group × Time interaction, $F_{11,968}$ = 3.48; Group effect, $F_{1,88}$ = 7.3; all *P*'s < 0.01), confirming dopamine supersensitivity. Across groups, the D1 antagonist SCH39166 decreased both vehicle- (Figs. S2A-C) and d-amphetamine-induced locomotion and ratings (Fig. 6A; Dose effect, $F_{2,88}$ = 39.11; Figs. 6B-C; minutes 40-150; Dose × Time interaction, $F_{22,968}$ = 6.33; Dose effect, $F_{2,88}$ = 28.69; all *P*'s < 0.0001). Notably, in haloperidol-treated rats, 0.03 mg/kg SCH39166 restored d-amphetamine-induced locomotion to control levels (compare light purple curve in Fig. 6B to white curve in Fig. 6C).

Across groups, the D2 antagonist sulpiride did not influence vehicle-induced ratings or locomotion (Fig. S2D-F). Sulpiride *suppressed* d-amphetamine-induced psychomotor activity ratings in controls but, surprisingly, it *enhanced* this response in haloperidol-treated rats (Fig. 6D; Group × Dose interaction, $F_{2,89}$ = 8.47; Group effect, $F_{1,89}$ = 38.25; haloperidol > controls at both sulpiride doses; haloperidol rats, 0 < 25 and 80 mg/kg; control rats, 0 > 80 mg/kg; all *P*'s < 0.05). Sulpiride also influenced d-amphetamine-induced locomotion (Figs. 6E-F; minutes 40-150; Dose × Time interaction, $F_{22,979}$ = 2.51; Dose effect, $F_{2,89}$ = 3.09; all *P*'s ≤ 0.05), with group differences in this effect. Specifically, sulpiride decreased d-amphetamine-induced hyperlocomotion in controls but not in haloperidol rats (Figs. 6E-F; Group × Time interaction, $F_{11,979}$ = 3.14, Group effect, $F_{1,89}$ = 14.38; all *P*'s ≤ 0.05).

Thus, in dopamine-supersensitive rats, D1- but not D2-mediated activity is required for the expression of an enhanced psychomotor response to d-amphetamine. In parallel, D2 receptor

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blockade *potentiated* d-amphetamine-induced psychomotor activity in dopamine-supersensitive rats, suggesting that D2 receptor activity normally tempers the expression of dopamine supersensitivity.

Exp. 6: D-amphetamine effects on D1- and D2-mediated signalling

Caudate-putamen. D-amphetamine produced similar effects in haloperidol-treated and control rats. In the dorsal, ventrolateral and centromedial caudate-putamen, d-amphetamine had mixed effects on total proteins levels, but it either decreased or had no effect on phosphorylated/total protein ratios (Figs. S3-5). Thus, d-amphetamine did not increase protein phosphorylation in AKT/GSK3β- or cAMP/PKA-dependent pathways in the caudate-putamen. Some of our results differ from work showing that in otherwise naïve rats, d-amphetamine increases DARPP-32, ERK1/ERK2 and GSK3β activity, and decreases AKT activity in the striatum [29, 30, 70, 71]. However, these previous studies analysed the caudate-putamen as a whole or with nucleus accumbens included [29, 30, 70, 71].

Nucleus accumbens. Relative to saline, d-amphetamine increased total GSK3β levels only in haloperidol-treated rats (Fig. 7C; Group × Injection interaction, $F_{1,20}$ = 4.23; Injection effect, $F_{1,20}$ = 14.61; haloperidol rats, d-amph > saline; all *P*'s ≤ 0.05). This reflects higher levels of non-phosphorylated (active) versus phosphorylated (inactive) GSK3β [72], because d-amphetamine decreased pGSK3β/total GSK3β ratios across groups (Fig. 7D; Injection effect, $F_{1,20}$ = 7.57, p = 0.012). D-amphetamine decreased total AKT levels and increased pAKT/total AKT ratios, with no group differences (Injection effect; Fig. 7E; $F_{1,15}$ = 13.01; Fig. 7F; $F_{1,15}$ = 6.611; all *P*'s < 0.05). Hence, in the nucleus accumbens, d-amphetamine influences AKT similarly in dopamine-supersensitive and control rats, but d-amphetamine enhances GSK3β activity to a greater extent in dopamine-supersensitive rats.

In haloperidol-treated rats, total DARPP-32 levels were increased at baseline and decreased after d-amphetamine (Fig. 7G; Group × Injection interaction, $F_{1,19}$ = 9.97; Injection effect, $F_{1,19}$ = 5.47;

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after saline, haloperidol rats > controls; Haloperidol rats, saline > d-amph; all *P*'s < 0.05). At baseline, total levels of both ERK1 and ERK2 were highest in haloperidol-treated rats (Fig. 7I; Group × Injection interaction, $F_{1,20}$ = 4.13; Group effect, $F_{1,20}$ = 6.41; haloperidol rats > controls after saline injection; Fig. 7K; Group effect, $F_{1,20}$ = 4.56; all *P*'s ≤ 0.05). D-amphetamine decreased total ERK2 levels similarly across groups (Fig. 7K; Injection effect, $F_{1,20}$ = 17.79; all *P*'s < 0.05). Hence, d-amphetamine-induced expression of dopamine supersensitivity potentially involves decreased total DARPP-32 levels in the accumbens, without distinct effects on total ERK1/ERK2 levels.

D-amphetamine enhanced the proportion of phosphorylated (active) versus total ERK1 and ERK2 levels in controls (see also [29, 70, 71]), but not in haloperidol-treated rats (Fig. 7J; Group × Injection interaction, $F_{1,20}$ = 9.73; Injection effect, $F_{1,20}$ = 15.62; Fig. 7L; Group × Injection interaction, $F_{1,20}$ = 8.41; Injection effect, $F_{1,20}$ = 11.82; Figs. 7J-L; controls, d-amph > saline; after d-amph, controls > haloperidol rats; all *P*'s ≤ 0.05). D-amphetamine did not change the proportion of phosphorylated DARPP-32 in either group (Fig. 7H; p > 0.05). Thus, in the accumbens, the expression of dopamine supersensitivity is potentially linked to suppressed phosphorylation of ERK1 and ERK2.

In summary, in dopamine-supersensitive rats, enhanced d-amphetamine-induced psychomotor activity is accompanied by enhanced GSK3β activity and decreased ERK activity in the nucleus accumbens.

DISCUSSION

In rats given a clinically-relevant antipsychotic treatment regimen, we examined where and how d-amphetamine acts to reveal the expression of dopamine supersensitivity. We report four main findings. First, systemic d-amphetamine reliably triggered the expression of established dopamine supersensitivity, whereas intracerebroventricular d-amphetamine infusion or an increase in VTA dopamine impulse flow did not. Second, dopamine-supersensitive rats showed an enhanced

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psychomotor response to selective D2, but not to D1 receptor stimulation or selective dopamine reuptake inhibition. Third, in dopamine-supersensitive rats, blocking D2 receptors *enhanced* the psychomotor response to d-amphetamine, whereas blocking D1 receptors *suppressed* d-amphetamine-induced responding. Fourth, in dopamine-supersensitive rats, d-amphetamine increased GSK3β levels in the nucleus accumbens, but d-amphetamine failed to increase ERK1/2 phosphorylation as it did in controls. These results give new insights into the mechanisms underlying the behavioural expression of antipsychotic-evoked dopamine supersensitivity.

Central processes

Across experiments, rats withdrawn from haloperidol treatment showed an enhanced psychomotor response to systemic d-amphetamine, indicating dopamine supersensitivity [9, 14, 15]. In these rats, increasing VTA dopamine impulse flow or restricting d-amphetamine's effects to the brain did not trigger the expression of established dopamine supersensitivity. This concords with findings that intra-striatal d-amphetamine infusions also fail to trigger this response [13]. Still, our findings contrast with studies showing that antipsychotic-treated rats show a sensitized locomotor response to intra-striatal dopamine infusions [73, 74]. However, these previous studies used high and clinically unrepresentative antipsychotic doses [31]. Using a clinically representative antipsychotic treatment regimen [31, 32, 34, 37], our results suggest that once antipsychotic-evoked dopamine supersensitivity has developed, its behavioural expression requires peripheral activity. This could involve adrenal glucocorticoids, as these are required for the expression of behavioural supersensitivity to d-amphetamine in other contexts (*e.g.*, after repeated exposure to d-amphetamine [21] or stress [22]).

Dopamine reuptake

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Dopamine-supersensitive rats showed a normal psychomotor response to the dopamine reuptake blocker GBR12783, and only a marginally enhanced response to the monoamine reuptake blocker, cocaine. In contrast, these same rats showed markedly augmented d-amphetamine-induced psychomotor activity. GBR12783, cocaine and d-amphetamine all act at the dopamine transporter (DAT). D-amphetamine could trigger a more robust supersensitive response through more potent effects at the DAT (*e.g.* by both blocking dopamine uptake and enhancing dopamine release [52]) and/or through DAT-independent effects. For instance, in the caudate-putamen, d-amphetamine—but not cocaine—depletes dopamine-containing vesicles and enhances tonic dopamine release [75]. The effects of antipsychotic treatment on these processes are not yet known, but antipsychotic-treated rats have potentially enhanced striatal DAT function [11].

D2 and D1 receptors

Our results suggest that dopamine-supersensitive rats have enhanced D2-mediated activity, extending prior observations of increased striatal D2 receptor density and function [9, 10, 76-78]. First, our dopamine-supersensitive rats showed an enhanced psychomotor response to a D2 receptor agonist. Second, acute D2 receptor blockade suppressed the psychomotor response to d-amphetamine in controls, but it *potentiated* d-amphetamine responding in dopamine-supersensitive rats. This potentiation could involve blockade of D2 autoreceptors, which would disinhibit dopamine synthesis/release and thus promote psychomotor activity. Indeed, chronic antipsychotic treatment can enhance presynaptic D2 receptor activity in the caudate-putamen [79] (but not in the nucleus accumbens [80]). Third, dopamine-supersensitive rats showed changes in nucleus accumbens cAMP/PKA- and GSK3β/AKT-dependent activity consistent with enhanced D2-mediated signalling. D2 receptor stimulation disinhibits GSK3β activity and inhibits both cAMP/PKA-dependent activity and ERK1/2 phosphorylation [27, 81, 82]. Our dopamine-supersensitive rats showed enhanced d-amphetamine-induced increases in GSK3β activity, but diminished d-amphetamine-induced ERK1/2 phosphorylation. Our biochemical and behavioural findings remain correlational. Moreover, we have

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shown previously that injecting d-amphetamine into the accumbens does not trigger the expression of established dopamine supersensitivity [13]. However, future work can determine whether dopamine-mediated signalling in the accumbens is *necessary* for the expression of dopamine supersensitivity.

Our findings also suggest that D1 receptor activity could be required for the full expression of antipsychotic-induced supersensitivity. Dopamine-supersensitive rats showed a normal psychomotor response to a D1 agonist, and d-amphetamine failed to increase ERK1/2 activity in the accumbens in these rats, suggesting that D1 transmission is not potentiated. However, blocking D1 receptors normalized d-amphetamine-induced locomotion in dopamine-supersensitive rats. This extends findings that chronic stimulation of D1 (but not D2) receptors reverses the expression of antipsychotic-evoked dopamine supersensitivity [83]. However, a caveat is that D1 blockade also supressed basal locomotion in our rats, raising the possibility of non-specific motor effects. This requires further investigation. Nonetheless, our results show that dopamine-supersensitive rats remain responsive to the anti-dopaminergic effects of D1, but not D2 receptor blockade. Indeed, antipsychotic-evoked dopamine supersensitivity produces tolerance to the anti-dopaminergic effects of D2 receptor antagonists in both rodents [9, 10, 84] and schizophrenia patients [3, 85]. Hence, D1 but not D2 receptors represent potential targets to temper the behavioural manifestations of antipsychotic-evoked dopamine supersensitivity.

Conclusions

Effective treatments to prevent the expression of antipsychotic-evoked dopamine supersensitivity depend on a better understanding of the biological mechanisms through which this supersensitivity is expressed. In this context, our findings both extend existing knowledge on the role of D2 receptors in antipsychotic-evoked dopamine supersensitivity and suggest two new underlying mechanisms. First, the expression of antipsychotic-evoked dopamine supersensitivity requires D1-

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mediated transmission. Second, beyond central processes, the expression of this supersensitivity likely involves peripheral mechanisms.

ACKNOWLEDGMENTS

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FIGURES

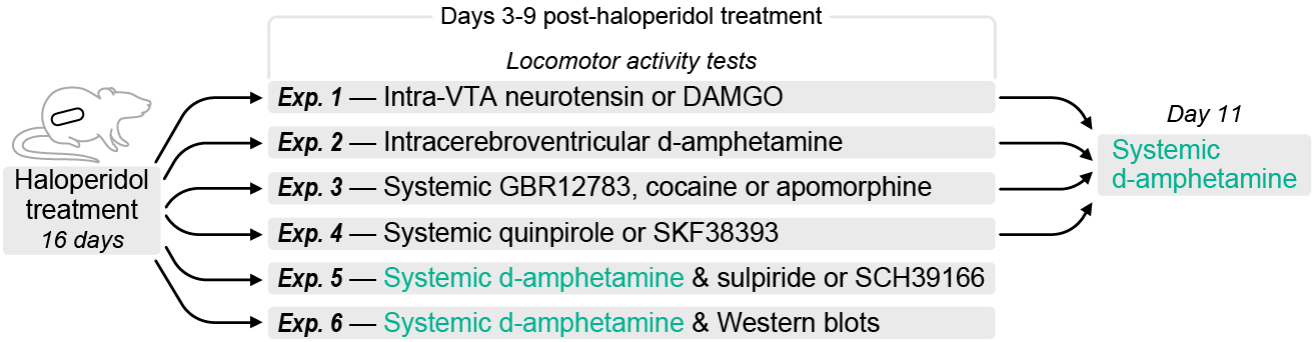

FIG. 1 ─ Experimental timelines.

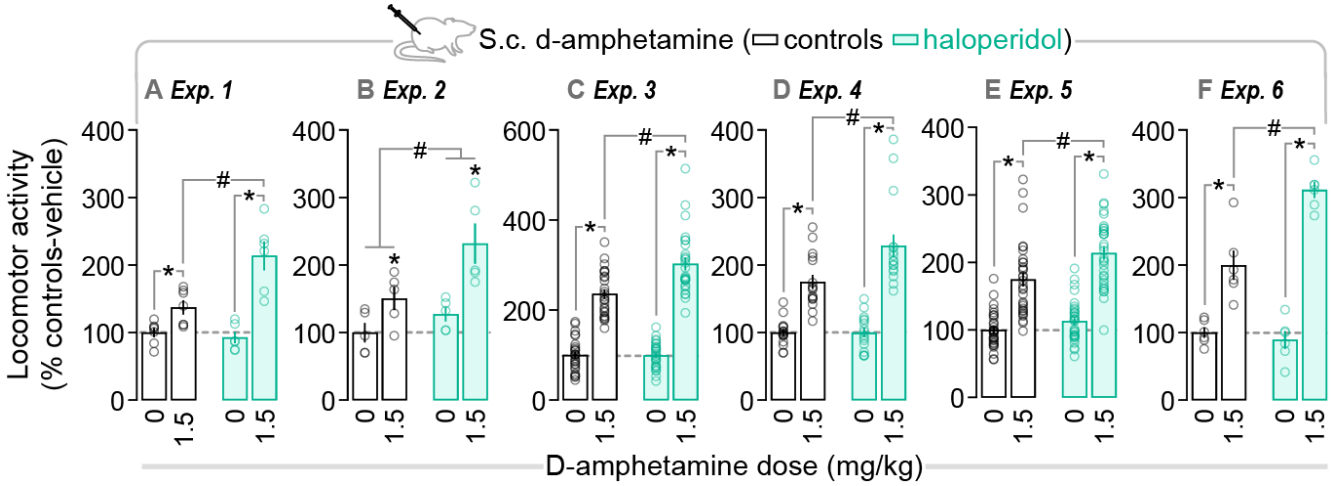

FIG. 2 ─ Chronic haloperidol treatment produced dopamine supersensitivity, as indicated by an exaggerated psychomotor response to d-amphetamine. (**A**-**F**) Locomotor response to subcutaneous (s.c.) d-amphetamine (0 or 1.5 mg/kg) in each experiment. Dotted lines indicate locomotion of vehicle-injected controls. *n*'s = 5-32/condition. **p* < 0.05; in (**B**), Injection effect. # *p* < 0.05; in (**B**), Group effect.

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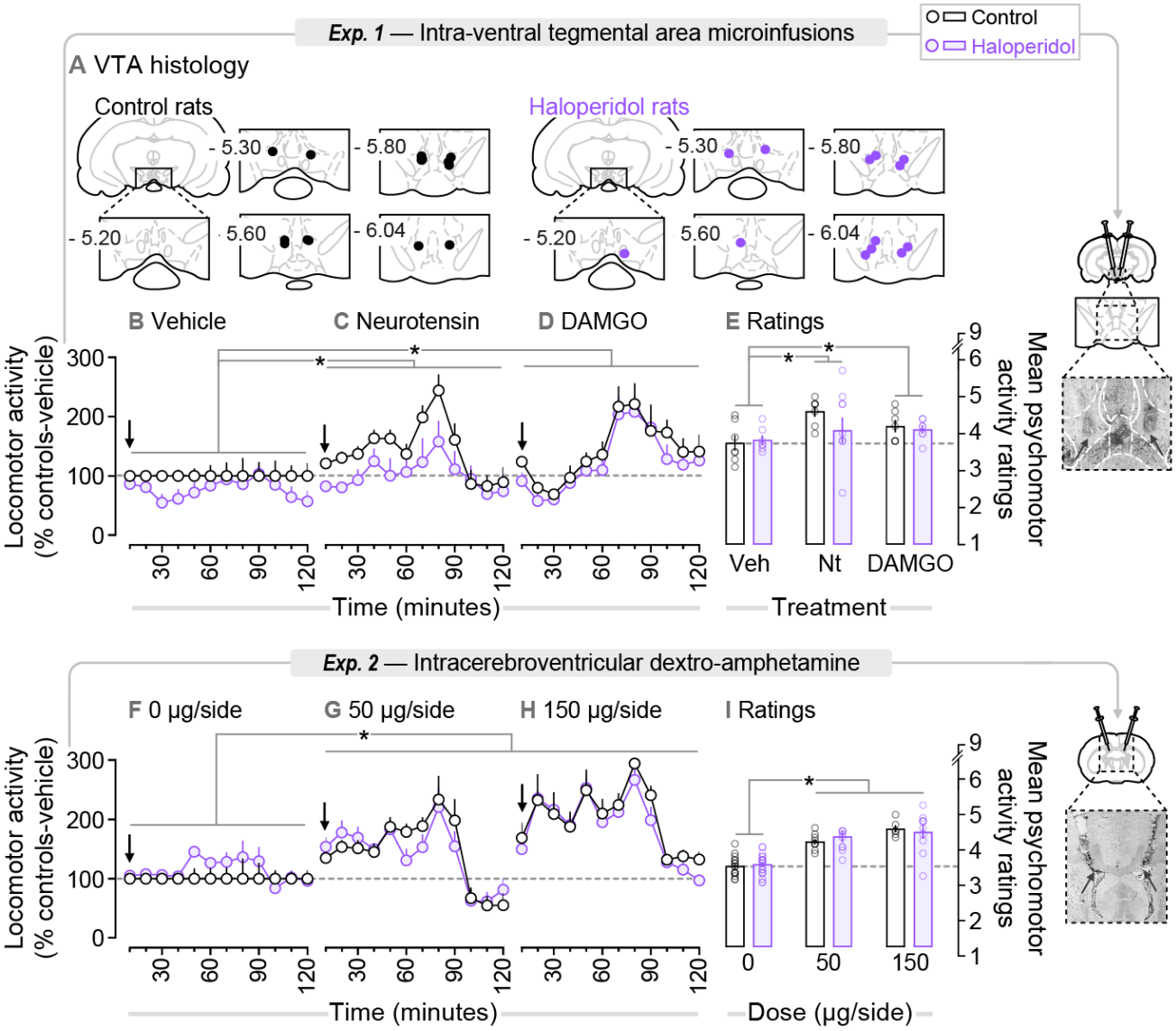

FIG. 3 — Neither increasing ventral tegmental area (VTA) dopamine impulse flow nor injecting d-amphetamine into the brain triggers the expression of established dopamine supersensitivity. (**A**) VTA histology. (**B**-**E**) Psychomotor response to intra-VTA vehicle, neurotensin or DAMGO. (**F**-**I**) Psychomotor response to intracerebroventricular d-amphetamine. On the right, representative injector placements (arrows indicate injectors). Dotted lines indicate response of vehicle-injected controls. *n*'s = 7-21/condition. *$p < 0.05$.

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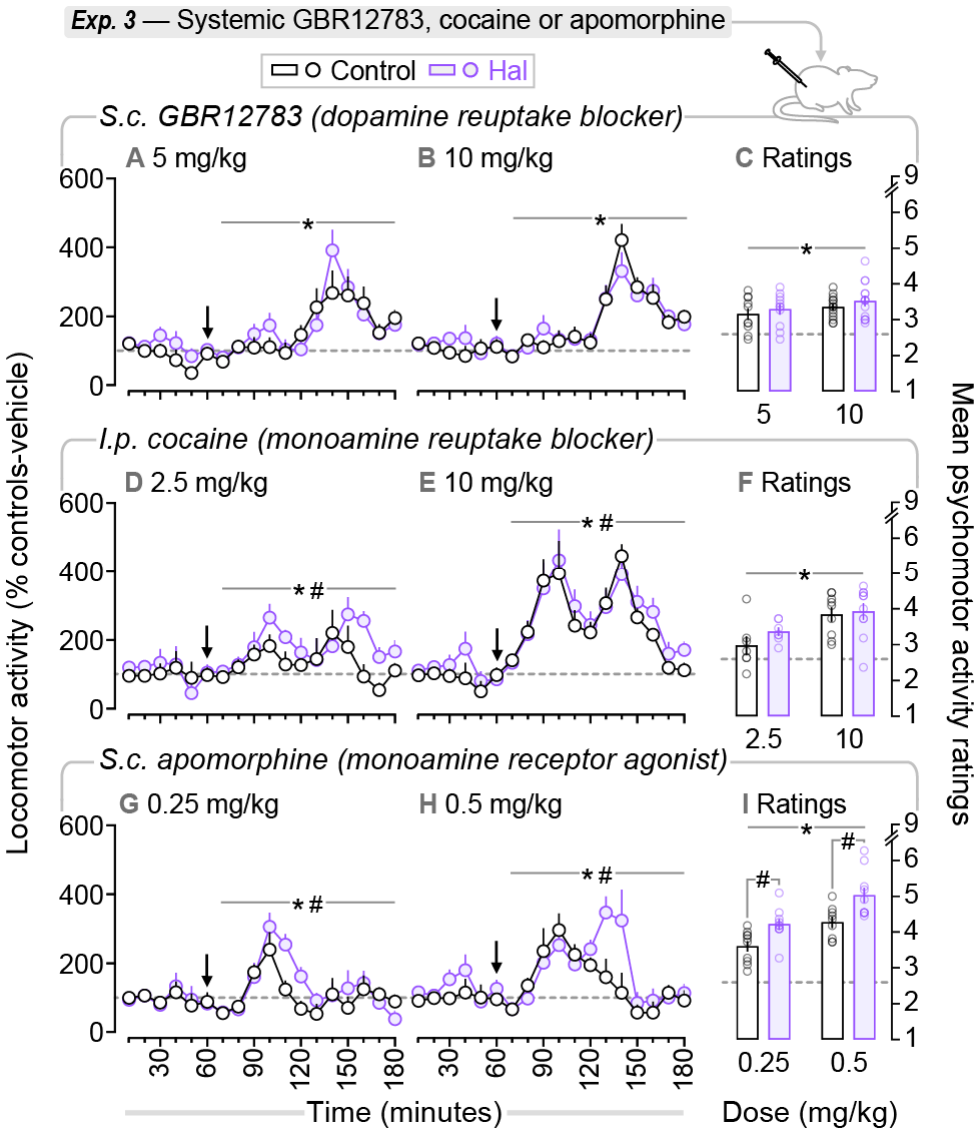

FIG. 4 — Psychomotor effects of GBR12783, cocaine and apomorphine in haloperidol-treated rats versus controls. Psychomotor response to (**A**-**C**) subcutaneous (s.c.) GBR12783, (**D**-**F**) intraperitoneal (i.p.) cocaine or (**G**-**I**) s.c. apomorphine. Dotted lines indicate response of vehicle-injected controls. *n*'s = 7-31/condition. #, *$p < 0.05$. In (**A**-**B**); *Dose × Time interaction and Dose effect. In (**C**); *Dose effect. In (**D**-**E**); *Dose × Time interaction and Dose effect, # Group effect. In (**F**); *Dose effect. In (**G**-**H**); *Dose × Time interaction and Dose effect, # Group × Time interaction and Group effect. In (**I**); *Dose effect.

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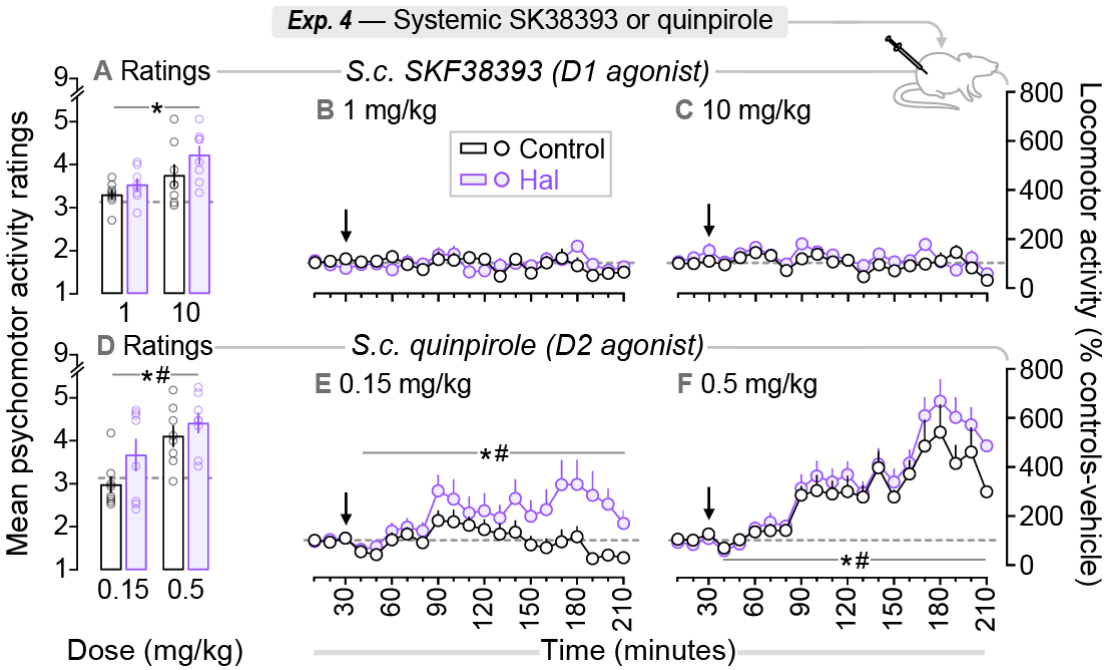

FIG. 5 — Stimulation of D2 but not D1 transmission is sufficient to reveal the expression of antipsychotic-evoked dopamine supersensitivity. (**A**-**F**) Psychomotor response subcutaneous (s.c.) quinpirole or SKF38393. Dotted lines indicate response of vehicle-injected controls. *n*'s = 7-32/condition. #, *$p < 0.05$. In (**A**); *Dose effect. In (**D**); *Dose effect, # Group effect. In (**E**-F), *Dose × Time interaction and Dose effect, # Group × Time interaction and Group effect.

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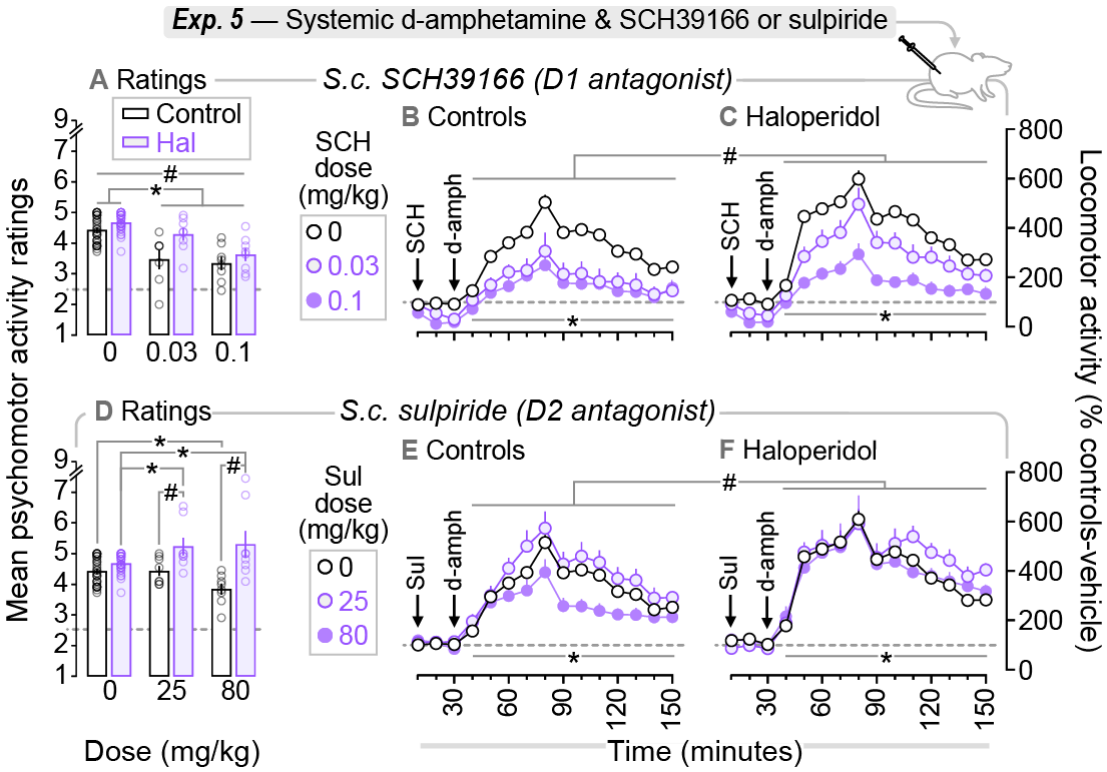

FIG. 6 — D1- but not D2-mediating signalling is necessary to the expression of antipsychotic-evoked dopamine supersensitivity. (**A**-**F**) Psychomotor response to s.c. SCH39166 or sulpiride and s.c. d-amphetamine. Dotted lines indicate response of vehicle-injected controls. *n*'s = 7-32/condition. #, *$p < 0.05$. In (**A**); *Dose effect, # Group effect. In (**B**-**C**, **E**-**F**); *Dose × Time interaction and Dose effect, # Group × Time interaction and Group effect.

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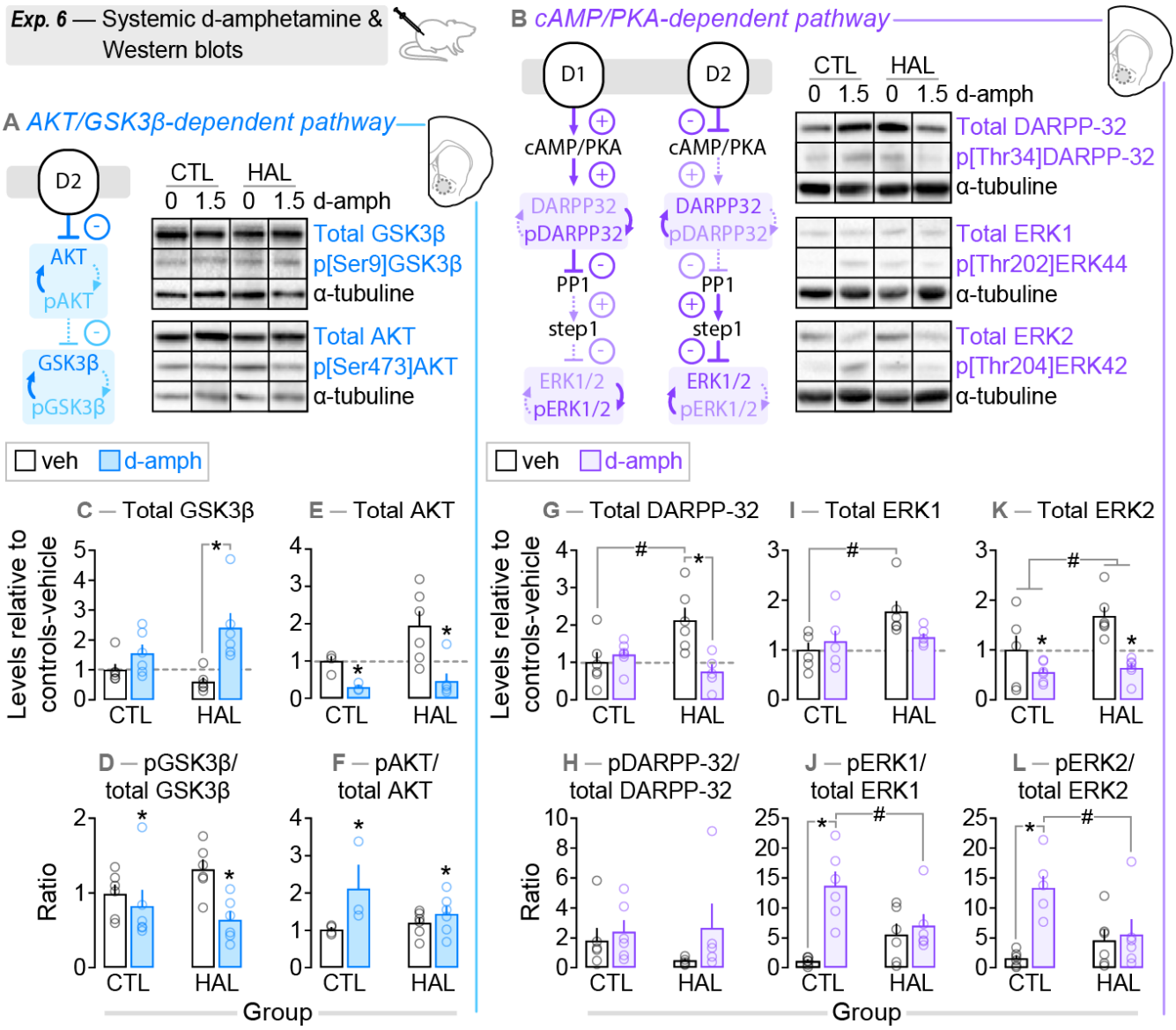

FIG. 7 — Dopamine-supersensitive rats have enhanced d-amphetamine-induced GSK3β activity and suppressed d-amphetamine-induced ERK1/2 activity in the nucleus accumbens. (**A**-**B**) AKT/GSK3β- and cAMP/PKA-dependent pathways and Western blots in accumbens tissue. Total protein levels and phosphorylated/total protein ratios within the (**C**-**F**) AKT/GSK3β- and (**G**-**L**) cAMP/PKA-dependent pathways. Dotted lines indicate mean protein level of vehicle-injected controls. *n*'s = 3-6/condition. # $p < 0.05$; in (**K**), Group effect. *$p < 0.05$; in (**D**-**F**, **K**), Injection effect.