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The Fyn kinase inhibitor, AZD0530, suppresses mouse alcohol self-administration and seeking

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Abstract

Fyn is a member of the Src family of protein tyrosine kinases (PTKs) that plays an important role not only in normal synaptic functions but also in brain pathologies including alcohol use disorder. We previously reported that repeated cycles of binge drinking and withdrawal activate Fyn in the dorsomedial striatum (DMS) of rodents, and that Fyn signaling in the DMS contributes to rat alcohol intake and relapse. Here, we used AZD0530, a CNS penetrable inhibitor of Src PTKs developed for the treatment of Alzheimer disease and cancer and tested its efficacy to suppress alcohol-dependent molecular and behavioral effects. We show that systemic administration of AZD0530 prevents alcohol-induced Fyn activation and GluN2B phosphorylation in the DMS of mice. We further report that a single dose of AZD0530 reduces alcohol operant self-administration and promotes extinction of alcohol self-administration without altering basal and dopamine D1 receptor-dependent locomotion. Together, our findings suggest that AZD0530, through its inhibitory actions on Fyn kinase, dampens alcohol seeking and drinking.

Introduction

Fyn is a member of the Src family of protein tyrosine kinases (PTKs).1 Fyn is highly expressed throughout the developing and adult brain2, 3 and plays an important role in excitatory and inhibitory synaptic transmission,4–6 synaptic plasticity, and learning and memory.7–9

Fyn also plays a role in Alzheimer pathology through Tau and amyloid β phosphorylation and signaling.10–12 Fyn also contributes to mechanisms underlying pain.5 In addition, studies in humans suggest that Fyn participates in mechanisms that drive and/or maintain phenotypes associated with alcohol use disorder (AUD). For instance, studies in humans identified single nucleotide polymorphisms (SNPs) within the FYN gene as AUD risk factors.13, 14 In addition, the Fyn gene is localized within a gene network associated with alcohol dependence in African Americans and Americans of European decent. 15

Animal studies support the human findings and show that the kinase contributes to biochemical neuroadaptations and behavioral effects that drive phenotypes associated with

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AUD.16 Specifically, ex vivo or in vivo noncontingent administration of alcohol activates Fyn in the cerebellum and hippocampus resulting in the phosphorylation of the Fyn substrate, the GluN2B subunit of the N-methyl D-aspartate receptor (NMDAR).17, 18 In the hippocampus, Fyn-dependent phosphorylation of GluN2B produces rebound potentiation and contributes to the development of acute tolerance to the inhibitory actions of alcohol on the activity of the NMDAR.18 Further studies utilizing a global gene deletion strategy have implicated the Fyn/GluN2B pathway in mechanisms that underlie the acute hypnotic actions of alcohol.17, 19

In addition, over the past decade, we have generated data to suggest that Fyn in the dorsal striatum20 and specifically in the dorsomedial striatum (DMS)21 plays a crucial role in alcohol-dependent synaptic adaptations that in turn drive alcohol drinking behaviors. Specifically, we found that long-term consumption of high amounts of alcohol results in a long-lasting activation of Fyn in the DMS of mice and rats that was maintained even after 16 to 24 hours of withdrawal21–23 and was not observed in the other striatal regions: the nucleus accumbens (NAc) and the dorsolateral striatum (DLS).21–23 We further showed that infusion of the Src PTK inhibitor, PP2, in the dorsal striatum,20 and specifically into the DMS21 of rats attenuated operant self-administration of alcohol. Intra-DMS administration of PP2 did not alter sucrose self-administration or locomotion.20, 21 Furthermore, PP2 administration into the NAc or DLS had no effect on alcohol self-administration suggesting that Fyn's actions are specific for alcohol and are localized to the DMS. Finally, Fyn inhibition reduced rat reinstatement of alcohol seeking,21 suggesting that Fyn may also play a key role in relapse to alcohol use.

The aim of the study was to examine the effects of AZD0530 on alcohol drinking behaviors. AZD0530 (also known as saracatinib) is a Src PTK inhibitor that was originally developed and tested clinically as an orally available cancer therapy drug.24 More recently, AZD0530 has been developed as a specific Fyn inhibitor for the treatment of Alzheimer disease.25 We show herein that a systemic administration of AZD0530 inhibits Fyn activation and GluN2B phosphorylation in the mouse DMS. We further report that the drug attenuates alcohol self-administration and promotes extinction without altering locomotion, thus implicating Fyn in the mechanisms underlying these alcohol-dependent behavioral phenotypes. Our results lay out the foundation for further studies elucidation the potential use of AZD0530 for the treatment of AUD.

2 METHODS

2.1 Subjects

Male C57/Bl6 mice (Jackson Laboratory) were 7 weeks old upon arrival and were single-housed in a temperature- and humidity-controlled room (22 ± 2°C, relative humidity: 50-60%) under a reversed 12-hour dark/light cycle (lights off at 10:00 AM) with food and water available ad libitum. Mice were given 2 weeks of habituation to the housing conditions before the beginning of the experiments. Mice were handled and habituated to intraperitoneum (ip) injections once a day for three consecutive days prior the beginning of drug testing. Mice were weighed every other week and prior to receiving drug administration. All animal procedures were approved by the University of California San

Francisco Institutional Animal Care and Use Committee (IACUC) and were conducted in agreement with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). A total of 60 mice were used for the study.

2.2 Reagents

Anti-phosphoTyr418/420[Src/Fyn] antibodies (#2101; 1:500) and anti-phosphoTyr1472[GluN2B] (Y1472, #4208, 1:500) antibodies were purchased from Cell Signaling Technology (Danvers, MA). Anti-Fyn antibodies (#SC16; 1:500), anti-Actin (#SC1616, 1:500), and anti-GluN2B (#SC1469, 1:500) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Nitrocellulose membranes were purchased from EMD Millipore (Billerica, MA). Donkey anti-rabbit horseradish peroxidase (HRP) was purchased from Jackson ImmunoResearch (West Grove, PA). EDTA-free complete mini protease inhibitor cocktail was purchased from Roche (Indianapolis, IN). Phosphatase Inhibitor Cocktails 2 and 3 were from Sigma-Aldrich (St. Louis, MO). NuPAGE Bis-Tris precast gels were purchased from Life Technologies (Grand Island, NY). Pierce bicinchoninic acid (BCA) protein assay kit was obtained from Thermo Scientific (Rockford, IL), and BioMax MR Film was purchased from Kodak (Rochester, NY). Ethyl alcohol (190 proof) was purchased from VWR (Radnor, PA), 2-hydroxypropyl-beta-cyclodextrin (HPBCD) was purchased from Sigma-Aldrich, and saline (0.9% NaCl) was purchased from Hospira Inc. (Lake Forest, IL). AZD0530 was synthetized by AstraZeneca (Waltham, MA). SKF81297 was purchased from R&D Systems (Minneapolis MN).

2.3 Preparation of solutions

Alcohol was diluted to 20% (v/v) in 0.9% saline for systemic administration and in tap water for alcohol drinking experiments. AZD0530 (10 mg/kg) was dissolved in 20% HPBCD diluted in water and injected in a volume of 10 mL/kg. The dose of AZD0530 was chosen based on previous studies. 11, 26 SKF81297 (5 mg/kg) was dissolved in 2% DMSO and was injected in a volume of 5 mL/kg.27

2.4 Western blot analysis

Naïve mice received a systemic administration of vehicle (20% HPBCD) or AZD0530. One hour later, mice were randomly treated with saline or alcohol (2 g/kg). Thirty minutes later, mice were killed by cervical dislocation. Brains were removed and dissected on ice. The DMS was isolated from a 1-mm-thick coronal section located +1.70 to +0.70 mm anterior to bregma. Tissues were homogenized in ice-cold radio immunoprecipitation assay (RIPA) buffer (in mM: 50 Tris-CI, 5 EDTA, 120 NaCI, 1% NP-40, 0.1% deoxycholate, 0.5% SDS, protease and phosphatase inhibitors), using a sonic dismembrator. Protein content was determined using a BCA kit. Tissue homogenates (30 μg per sample) were resolved on NuPAGE 10% Bis-Tris gels at 100 V for 2 hours and transferred onto nitrocellulose membranes at 30 V for 2 hours. Membranes were blocked with 5% milk-phosphate-buffered saline with 0.1% tween-20 at room temperature and then probed with primary antibodies overnight at 4°C. Membranes were washed and incubated with HRP-conjugated secondary antibodies for 2 hours at room temperature. Bands were visualized using ECL. Band intensities were quantified by ImageJ (National Institutes of Health, MD, USA).

2.5 Alcohol self-administration

First, mice underwent intermittent access to 20% alcohol in a two-bottle choice procedure (IA2BC) for 7 weeks as described previously.28 Briefly, mice had access to a bottle of 20% alcohol and a bottle of water on Monday, Wednesday, and Friday of each week and two bottles of water on Tuesday, Thursday, Saturday, and Sunday. Mice were not food restricted at any point of the experiment. Mice that drank more than 14 g/kg per 24 hours on the last week of IA-2BC were selected for alcohol operant self-administration training. Operant training was conducted during the dark phase of the reversed dark/light cycle in chambers (length: 22 cm, width: 20 cm, and height: 14 cm) equipped with two levers (1.5 cm in length, 11 cm apart, and 2.5 cm from the grid floor) mounted at the opposite ends of the same wall (Med-Associates; Georgia, VT). The operant chambers included a reward port centered between the levers (0.5 cm from the grid floor) with photo-beams to allow monitoring of reward magazine visits, a light centered above the reward magazine and a tone-delivering tweeter situated on the opposite wall of the levers. Each chamber was housed within a sound-attenuating box with a fan providing background noise and ventilation. Each chamber was connected to a computer to control and record program events. Mice received five daily self-administration sessions per week (Monday-Friday). Sessions started with the presentation of only one lever. Lever press resulted in the delivery of a 20% alcohol delivery via a motorized dipper that held 10 μL of liquid in the magazine. Reward delivery was paired with a 3 seconds tone (2900 Hz), and the illumination of the cue-light above the magazine. Alcohol solution was delivered after three port entries have been made (ensuring the consumption of the previously delivered alcohol solution). The third reward port entry turned off the cue-light. The number and timing of the active lever presses and reward port entries, as well as the number of reward deliveries, were recorded during each session. Sessions ended with the retraction of the lever and the light were turned off. Operant self-administration training was initiated under a fixed ratio (FR) 1, ie, one lever press results in the delivery of one reward, for three 6-hour sessions (10 AM to 4 PM) followed by three 4-hour sessions (11 AM to 3 PM). Afterwards, the sessions lasted for 2 hours (1-3 PM). Two sessions under the FR1 schedule were conducted. Then, a variable ratio (VR) schedule was used during which each reward was delivered following a variable number of lever presses pseudorandomly determined by the computer program. Five sessions under VR2 (ie, one reward delivery following on average two lever presses with number of presses ranging from 1 to 3) followed by five VR3 (number of presses ranged from 2 to 4) and 10 VR4 sessions (number of presses ranged from 3 to 5) were conducted prior to the beginning of drug testing. Only mice that displayed a discrimination ratio between the active and inactive lever (number of active lever presses/total active + inactive × 100) above 60%, which is an index of instrumental learning, were included in the study. The high ratio (greater than 75%) of discrimination between the active and inactive lever reveals that the mice effectively learnt the operant task (data not shown). Blood alcohol concentration (BAC) was measured at the end of the 2-hour self-administration session as previously described.29 Consumption of 1.75 ± 0.2 g/kg corresponded to BAC of 42.82 ± 17.7 mg/dL. AZD0530 was administered following stable VR4 responding. Mice maintained a baseline level of alcohol responding for at least three operant self-administration sessions between each tested condition. To test AZD0530's effect on alcohol self-administration and seeking, mice were systemically administered with vehicle or AZD0530 (10 mg/kg), 1 hour prior to the beginning of an

operant self-administration session or before a single 2-hour extinction session (ie, lever presses were not paired with alcohol deliveries), respectively. All drug testing was performed using a "within-subject" design in which mice received both treatments in counterbalanced order, with at least two standard self-administration sessions conducted between tests. Two mice were excluded from the study.

2.6 Response-outcome contingency degradation

To test for sensitivity to changes in the response-outcome association, a contingency degradation was used as described previously.30 The procedure was conducted across 2 days, termed nondegraded and degraded days. On the degraded day, the lever was extended but response to the manipulation produced no consequences. Rather, noncontingent alcohol deliveries occurred at a rate that was determined based on each animal reinforcement rate on the week preceding to the contingency degradation testing. On nondegraded day lever presses were paired with alcohol delivery. The degradation test occurred in a counterbalanced manner, removing the possibility that extraneous factors could have influenced the reduction in lever pressing.

2.7 Locomotion test

To test whether the observed behavioral effects were due to locomotor deficits, a separate cohort of mice was habituated in an open field apparatus (Med-Associates; Georgia, VT; length: 20 cm, widths: 20 cm, and height: 20 cm) for 2 days. For the experiment evaluating basal locomotion, mice were treated with vehicle or AZD0530 (10 mg/kg) 1 hour prior to an hour locomotion test. For the experiment evaluating the possible interaction between AZD0530 and the D1/D5R agonist SKF81297, mice locomotion was tested for 2 hours. Mice received a systemic administration of vehicle or AZD0530 (10 mg/kg) 30 minutes into the test, and a second injection of vehicle or SKF81297 (5 mg/kg) 60 minutes into the test. Subsequent locomotor activity depicted as distance traveled in cm was recorded. Three mice were excluded from the study.

2.8 Data analysis

Data were analyzed using the appropriate statistical test, including one- or two-tailed paired t tests and analysis of variance (ANOVA) with or without repeated measures, as detailed in the results section. Significant main effects and interactions of ANOVAs were further investigated with the LSD post hoc test. Statistical significance was set at $P < 0.05$ unless indicated otherwise.

3 RESULTS

3.1 AZD0530 prevents alcohol-induced Fyn activation in the mouse DMS

We previously showed that a single administration of a nonhypnotic dose of alcohol activates Fyn in the dorsal striatum.20 Therefore, we first tested whether systemic administration of alcohol activates Fyn in the DMS and if so, whether systemic administration of AZD0530 inhibits alcohol-dependent Fyn activation in this brain region. To do so, mice received a systemic administration of vehicle or AZD0530 (10 mg/kg), 1 hour later, mice received a systemic administration of saline or alcohol (2 g/kg), and the

DMS was harvested 30 minutes later (Figure 1A). Fyn activation was measured using anti-phosphoTyr418/420[Src/Fyn] antibodies, which recognize the autophosphorylated, and thus activated, kinase.31 As shown in Figure 1B,C, acute systemic administration of 2 g/kg of alcohol activates Fyn in the DMS of vehicle-treated mice (Figure 1C; main effect of alcohol, two-way ANOVA: $F_{1,12} = 22.9$, $P = 0.0004$). Anti-phosphoTyr418/420[Src/Fyn] antibodies recognize the active form of both Src and Fyn31; however, we previously showed that Src is not activated by alcohol in the dorsal striatum.20 Once active, Fyn phosphorylates residues within the cytoplasmic tail of GluN2B subunit,32, 33 and we previously showed that the consequence of Fyn activation by alcohol is the phosphorylation of GluN2B.16 As shown in Figure 1B,D, GluN2B phosphorylation was robustly increased in the DMS in response to a single dose of alcohol in vehicle-treated mice (Figure 1D; main effect of alcohol, two-way ANOVA: $F_{1,12} = 14.08$, $P = 0.0028$). AZD0530 pretreatment reduced the basal levels of Fyn activation (Figure 1C; main effect of drug, two-way ANOVA: $F_{1,12} = 30.05$, $P = 0.0001$) and GluN2B phosphorylation (Figure 1D; main effect of drug, two-way ANOVA: $F_{1,12} = 15.54$, $P = 0.002$), and importantly, Fyn activation and GluN2B phosphorylation were significantly attenuated in mice that were pretreated with AZD0530 prior to the administration of alcohol (Figure 1C; p-Tyr418/420[Src/Fyn]: $P = 0.0001$; Figure 1D; p-Tyr1472[GluN2B]: $P = 0.036$). Together, these data demonstrate that acute alcohol exposure activates the Fyn/GluN2B pathway in the DMS and show that AZD0530 is a potent, CNS penetrable Fyn inhibitor.

3.2 Alcohol self-administration is goal-directed

Next, we determined whether systemic administration of AZD0530 alters alcohol self-administration and seeking. As the DMS is part of the circuity that drives goal-directed behavior,34–36 and since impairments in goal-directed behaviors are an integral part of the addiction cycle,37 we hypothesized that inhibition of Fyn kinase will attenuate goal-directed alcohol seeking and drinking. To test this hypothesis, mice had intermittent access to 20% alcohol for 7 weeks and were then trained to press on an active lever to obtain 20% alcohol under a VR schedule, which is known to promote goal-directed actions.36 Goal-directed actions are sensitive to changes in contingency between response (ie, lever press) and outcome (ie, alcohol delivery). 38, 39 Therefore, we used the contingency degradation procedure to confirm that the alcohol self-administration schedule we used models goal-directed alcohol seeking (Figure 2A,B). As shown in Figure 2C, alcohol operant self-administration is sensitive to manipulations of response-outcome contingency as lever presses were reduced on the degraded day ($t_9 = 3.083$, $P = 0.013$), although the amount of alcohol consumed was similar on both days (Figure 2D) indicating that the alcohol self-administration schedule we use does indeed model goal-directed alcohol seeking.

3.3 AZD0530 reduces alcohol self-administration

Next, we determined whether AZD0530 alters alcohol self-administration. Alcohol self-administration was conducted as described above, and after stable responding, vehicle or AZD0530 (10 mg/kg) were systemically administered 1 hour prior to the beginning of an operant self-administration session (Figure 3A). Treatment of mice with AZD0530 produced a significant reduction in alcohol self-administration as evidenced by the reduction in cumulative (Figure 3B; treatment X time interaction effect, two-way ANOVA: $F_{11,242} =$

3.46, P = 0.0002), and total number of lever presses (Figure 3C; t11 = 2.93, P = 0.014). The reduction in the number of lever presses by AZD0530 corresponded with the frequency (Figure 3D; t11 = 2.93, P = 0.014) of lever presses. We also observed that AZD0530 administration led to a trend in reduction of cumulative number (Figure 3E; treatment X time interaction effect, two-way ANOVA: F11,242 = 1.66, P = 0.08) and frequency (Figure 3F; t11 = 1.92, P = 0.08) of port entries. Finally, AZD0530-treated mice displayed a robust reduction in the amount of alcohol consumed (Figure 3G; t11 = 2.77, P = 0.018) resulting from a decreased frequency of alcohol delivery (Figure 3H; t11 = 2.95, P = 0.013). Together, these data suggest that AZD0530 attenuates goal-directed alcohol self-administration.

3.4 AZD0530 promotes extinction of alcohol self-administration

To test whether AZD0530 reduces alcohol self-administration by reducing alcohol seeking and/or by promoting extinction, mice trained to self-administer alcohol received a systemic administration of vehicle or AZD0530 (10 mg/kg) 1 hour prior to the beginning of an acute extinction session (Figure 4A; ie, lever presses were not rewarded). Treatment of mice with AZD0530 produced a significant decrease in the cumulative number (Figure 4B; treatment X time interaction effect, two-way ANOVA: F11,242 = 4.00, P < 0.0001) and frequency (Figure 4C; t11 = 2.24, P = 0.047) of lever presses, revealing a greater degree of extinction of alcohol responding compared with controls. AZD0530 treatment did not affect the cumulative number (Figure 4D; treatment effect, two-way ANOVA: F11,242 = 0.47, P = 0.92) and frequency (Figure 4E; t11 = 0.23, P = 0.83) of port entries. However, this may be due to a possible floor effect, as the frequency of port entries in vehicle-treated mice is lower during extinction than during self-administration testing (Figures 3D,E vs 4D,E). Nevertheless, our results indicate that AZD0530 enhances extinction of alcohol responding.

3.5 AZD0530 does not alter locomotion

To exclude the possibility that the inhibition of alcohol self-administration in response to AZD0530 treatment is due to alterations in locomotion, we measured whether systemic administration of the drug alters locomotor activity in an open field test. We found that AZD0530 and vehicle-treated mice traveled a similar distance in an open field (Figure 5A; drug effect, two-way ANOVA: F1,16 = 0.177, P = 0.680; Figure 5B; t16 = 0.421, P = 0.680). We further determined whether AZD0530 alters drug-induced locomotion by measuring the level of locomotion induced in response to the systemic administration of the dopamine D1/D5 receptor agonist, SKF81297 (5 mg/kg), with and without AZD0530 (10 mg/kg). As shown in Figure 5C,D, AZD0530 did not alter SKF81297-dependent hyper-locomotion (Figure 5C; treatment effect, two-way ANOVA: F3,312 = 112.1, P < 0.0001, vehicle/SKF81297 vs AZD0530/SKF81297: P = 0.9251; Figure 5D; effect of SKF81297: F1,13 = 184.2, P < 0.0001, effect of AZD0530: F1,13 = 0.1466, P = 0.708, vehicle/vehicle vs vehicle/SKF81297: P < 0.0001, AZD0530/SKF81297 vs AZD0530/vehicle: P = 0.0001, vehicle/SKF81297 vs AZD0530/SKF81297: P = 0.7903). Together, these data reveal that AZD0530 at a dose that produces a reduction in alcohol intake does not affect locomotion.

4 DISCUSSION

We show that acute systemic administration of a nonhypnotic dose of alcohol produces a robust increase in Fyn activation and GluN2B phosphorylation in the DMS, which were abolished by the systemic administration of AZD0530. Previous work indicates that dorsal striatal GluN2B is critical for choice learning.40 Thus, it is plausible that the inhibition of alcohol-dependent GluN2B phosphorylation via AZD0530 in the DMS is part of the mechanism by which the Fyn inhibitor attenuates alcohol seeking and drinking. However, other Fyn substrates may also play a role in mechanisms underlying alcohol drinking behaviors. For example, Fyn was reported to phosphorylate the metabotropic glutamate receptor 1 (mGluR1s) in cell preparations.41 Jin et al further showed that Fyn phosphorylation of mGluR1 increases the membrane expression of the receptor.41 As mGluR1 has been implicated in mechanisms underlying addiction,42 it would be of interest to determine whether Fyn contributes to alcohol seeking in part via the phosphorylation and forward trafficking of mGluR1. Further studies are therefore required to determine whether other Fyn substrates are required for the development and maintenance of alcohol-dependent behaviors.

We show that mice undergoing the protocol described in Figure 2A are sensitive to contingency degradation. Specifically, lever pressing was reduced during trials in which noncontingent alcohol was delivered (degraded days) as compared with nondegraded days in which lever presses were paired with alcohol deliveries. Using this protocol, we found that a single dose of AZD0530 attenuates alcohol self-administration. Putting this finding together with the observation that Fyn is activated by voluntary intake of alcohol specifically in the DMS,21 a central brain region for goal-directed behavior,34, 36 our data implies that Fyn contributes to goal-directed alcohol seeking. However, one caveat of our study is the fact that we used a single dose of AZD0530, and a future dose-response study may reveal additional information. It would also be of interest to determine if AZD0530 alters self-administration of other abused drugs as well as natural rewarding substance.

We also found that AZD0530 promotes extinction of alcohol seeking. Interestingly, we previously showed that intra-DMS administration of the Src PTKs inhibitor PP2 inhibited reinstatement of alcohol self-administration of rats; however, PP2 did not alter alcohol seeking as measured in an extinction session.21 How can the differential effect of systemic administration of AZD0530 and intra-DMS infusion of PP2 on alcohol seeking be explained? First, it is plausible that the differences in the results reflects species differences and/or drug kinetics. It is also plausible that AZD0530 prevents alcohol-induced Fyn activation in brain regions other than the DMS, which could contribute to alcohol seeking. For example, the hippocampus has been implicated in extinction of fear learning,43 and PP2 infusion in the dorsal hippocampus (DH) reduces lever pressing during extinction in rats trained to self-administer cocaine,44 revealing that Src family of protein kinases in the DH contributes to drug seeking. Interestingly, bath application of alcohol or systemic administration of alcohol increases Fyn activation in the hippocampus.18 Future studies are needed to determine whether excessive alcohol drinking promotes Fyn activation in the DH and whether hippocampal Fyn signaling plays a role in alcohol seeking.

Alcohol addiction is defined by the inability to suppress alcohol taking and seeking despite negative consequences.45 As a result, the development of the disease has been hypothesized to follow a shift from goal-directed to habitual alcohol use.46 This theory generated a growing interest for the study of goal-directed actions vs habits in the alcohol addiction field.47–49 However, harmful heavy consumption could still be goal-directed and not necessarily entirely due to the development of habit as addicts exhibit classical goal-directed behaviors when seeking to obtain drugs.50 Interestingly, using a new goal-directed self-administration procedure in which rats had to solve a puzzle prior to cocaine administration, Singer et al reported that habitual responding is not necessary for the development of addiction-like behavioral phenotypes.51 Thus, elucidating molecular substrates of goal-directed behavior such as this study has significant merit both for basic science and for the development of new medications to treat the AUD.

AZD0530 has numerous desirable properties. For example, AZD0530 inhibits Fyn activity in the low nM range26 has a half-life of 16 hours in the mouse and is highly brain penetrable. 11 Phases I and II clinical trials for cancer and Alzheimer disease indications show that oral administration of AZD0530 is safe and well tolerated in patients. 11, 25, 52, 53 Here, we provide preclinical behavioral data to suggest that AZD0530, through its actions on Fyn kinase, could be a potential therapeutic for the treatment of AUD. However, extensive animal studies are still needed to evaluate the potential use of AZD0530 in AUD.

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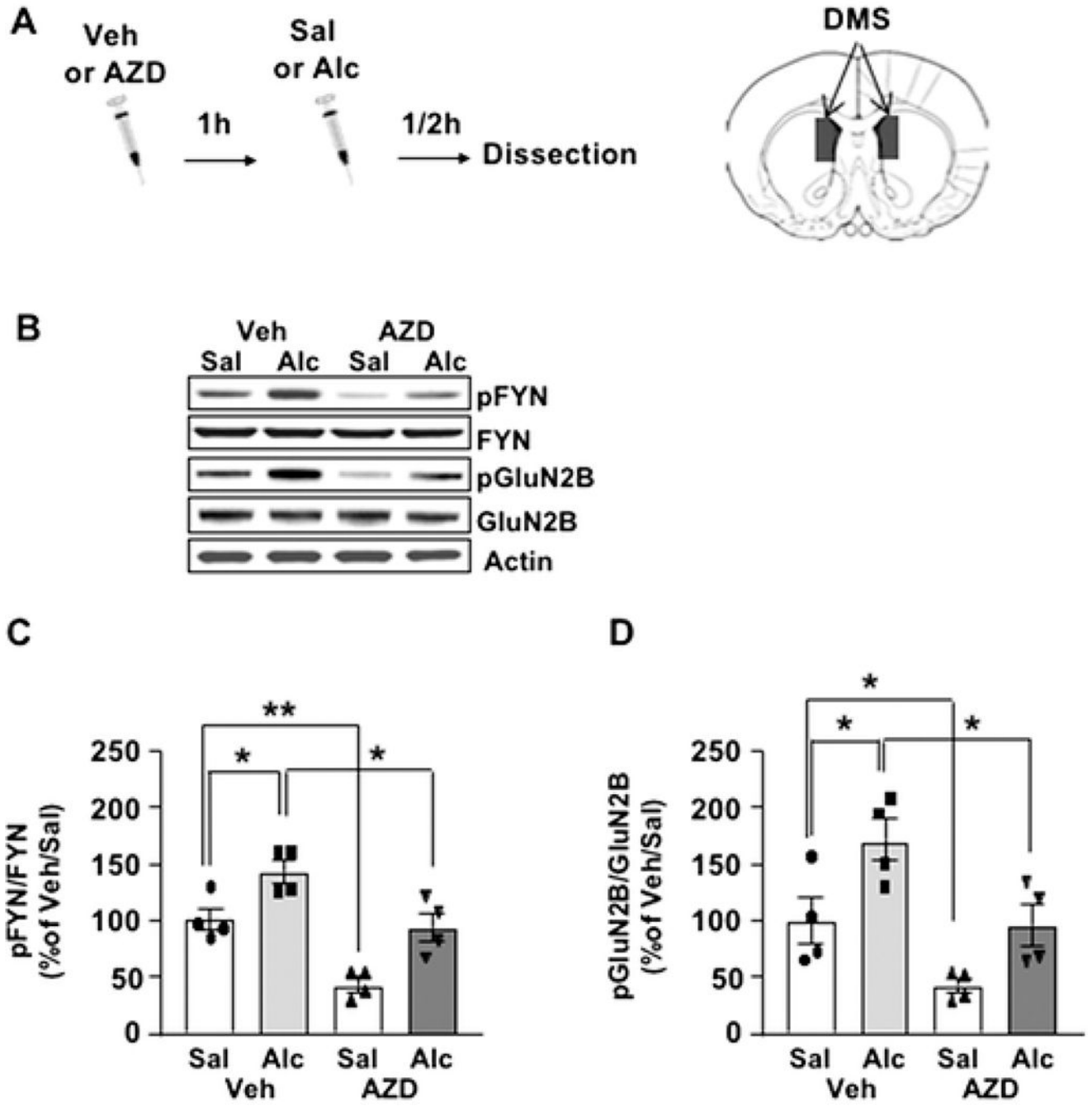

Figure 1.

AZD0530 prevents alcohol-induced Fyn activation and GluN2B phosphorylation in the mouse DMS. A, Alcohol-naïve mice received vehicle (Veh) or AZD0530 (AZD, 10 mg/kg ip) 1 h prior to the administration of saline (Sal) or alcohol (Alc, 20% v/v, 2 g/kg ip), and half an hour later, the DMS of the mice was dissected. B, Schematic drawing of a coronal section of the mouse brain showing the sectioned DMS at bregma +1.10/+0.70. C, The phosphorylation level of Tyr 418/420[Src/Fyn] and Tyr1472[GluN2B] as well as the total amount of the proteins in DMS homogenates were determined by western blot analysis.

Actin immunostaining was used as a loading control. ImageJ was used for optical density quantification. Data are expressed as the mean ratio ± SEM of phosphoprotein to total protein and are expressed as percentage of the control vehicle/saline group; n = 4 per group. $*P < 0.05$ and $**P < 0.01$

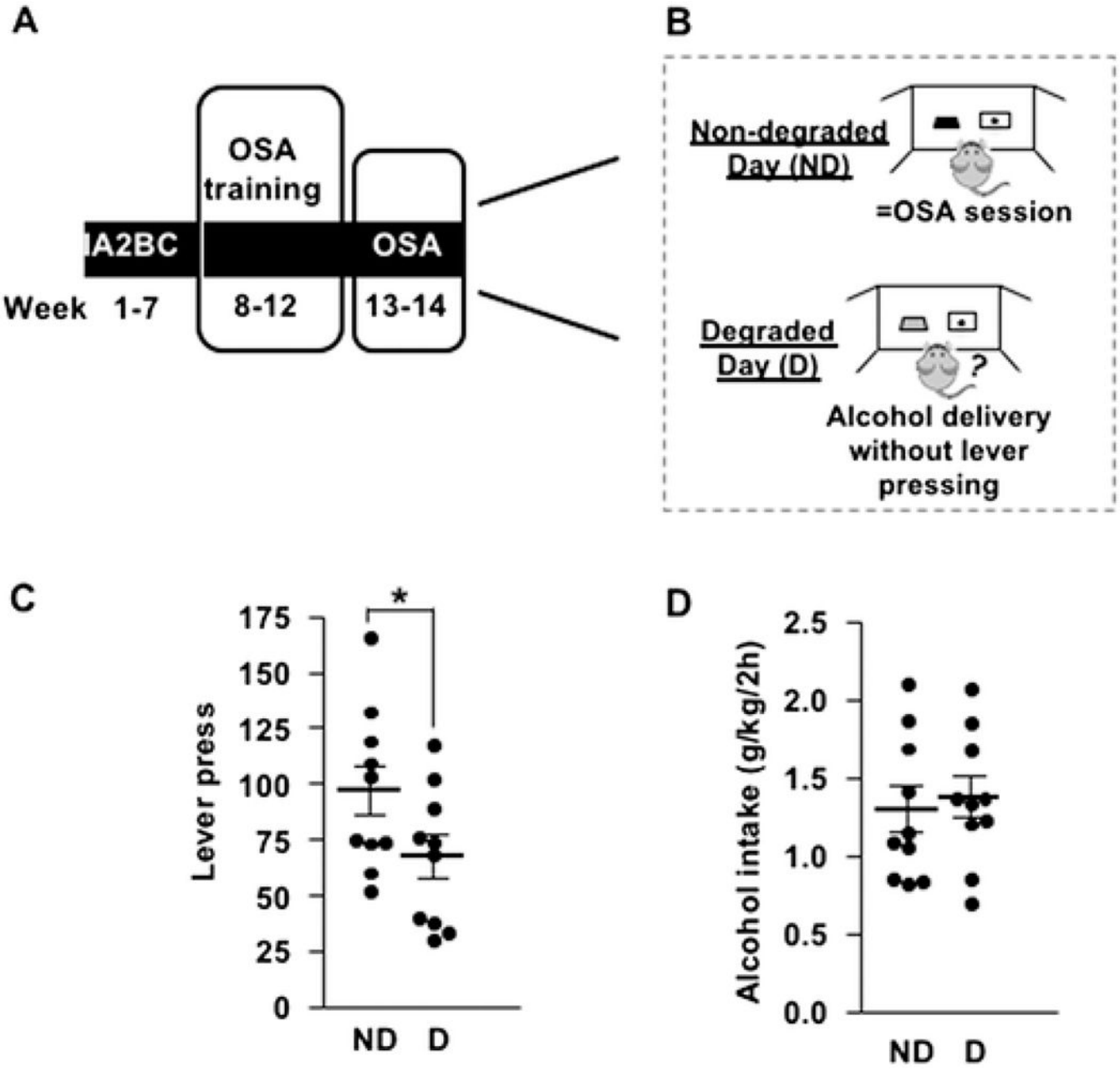

Figure 2.

Contingency degradation of alcohol self-administration. A,B, Experimental design. Mice underwent an intermittent access to 20% two-bottle choice paradigm for 7 wk followed by operant self-administration of 20% alcohol training period of 4 wk and were the tested for response-outcome contingency degradation. Number of lever presses (C) and alcohol intake (g/kg per 2 h) (D) were recorded. Data are presented as individual data points and mean ± SEM; n = 12. *$P < 0.05$

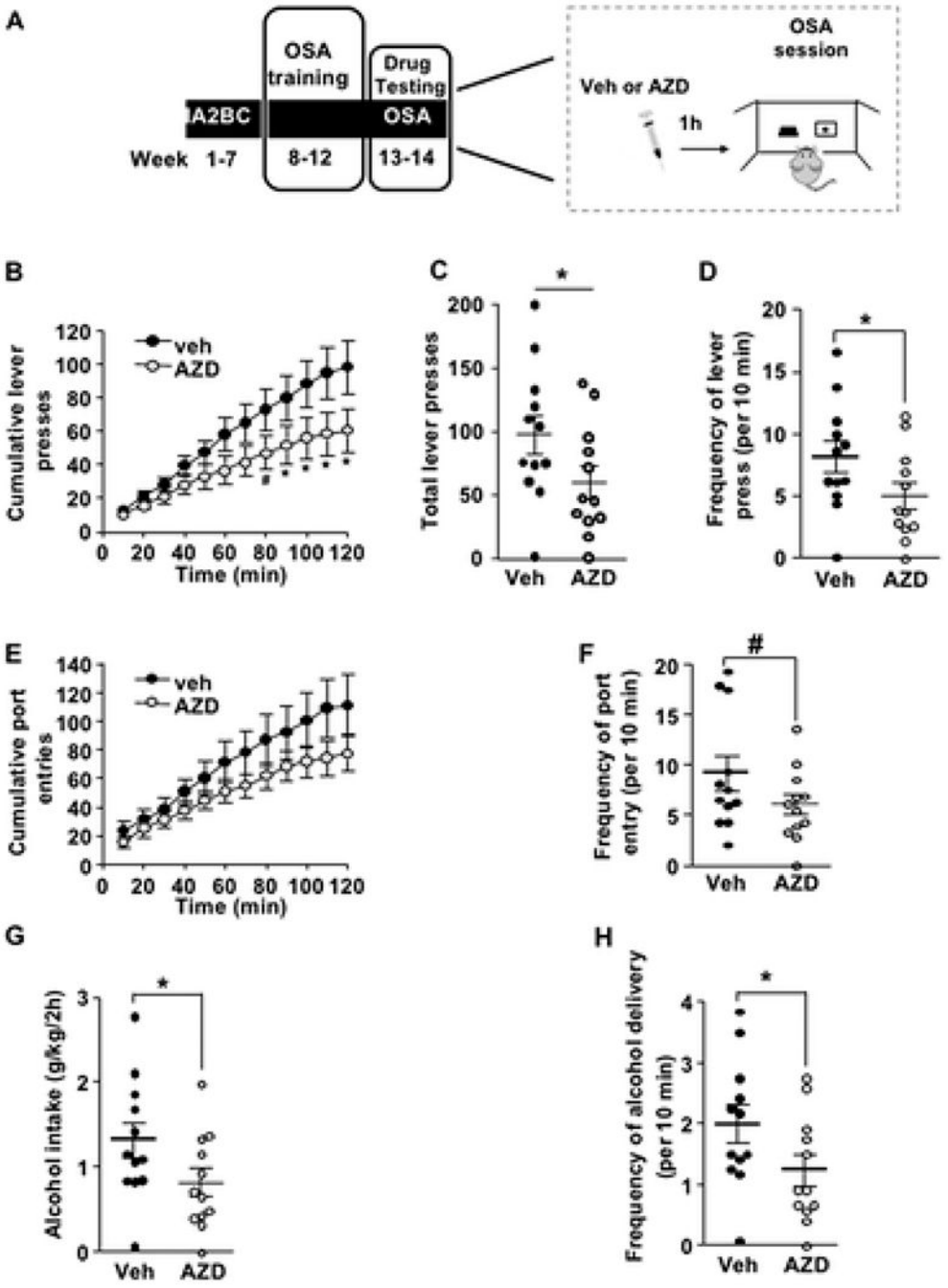

Figure 3.

AZD0530 reduces alcohol self-administration and seeking. A, Experimental plan. On weeks 13-14, mice received vehicle (Veh) or AZD0530 (AZD, 10 mg/kg ip) 1 h prior to the beginning of an alcohol self-administration session. The cumulative number of lever presses (B) as well as total number of lever presses (C) and frequency (D) were recorded. Also measured were the cumulative number of port entries (E), frequency of port entries (F), alcohol intake (G) and frequency of alcohol delivery (H). Data are displayed as individual data points and as mean ± SEM; n = 12. $^{*}P < 0.05$ and $^{\#}P = 0.08$

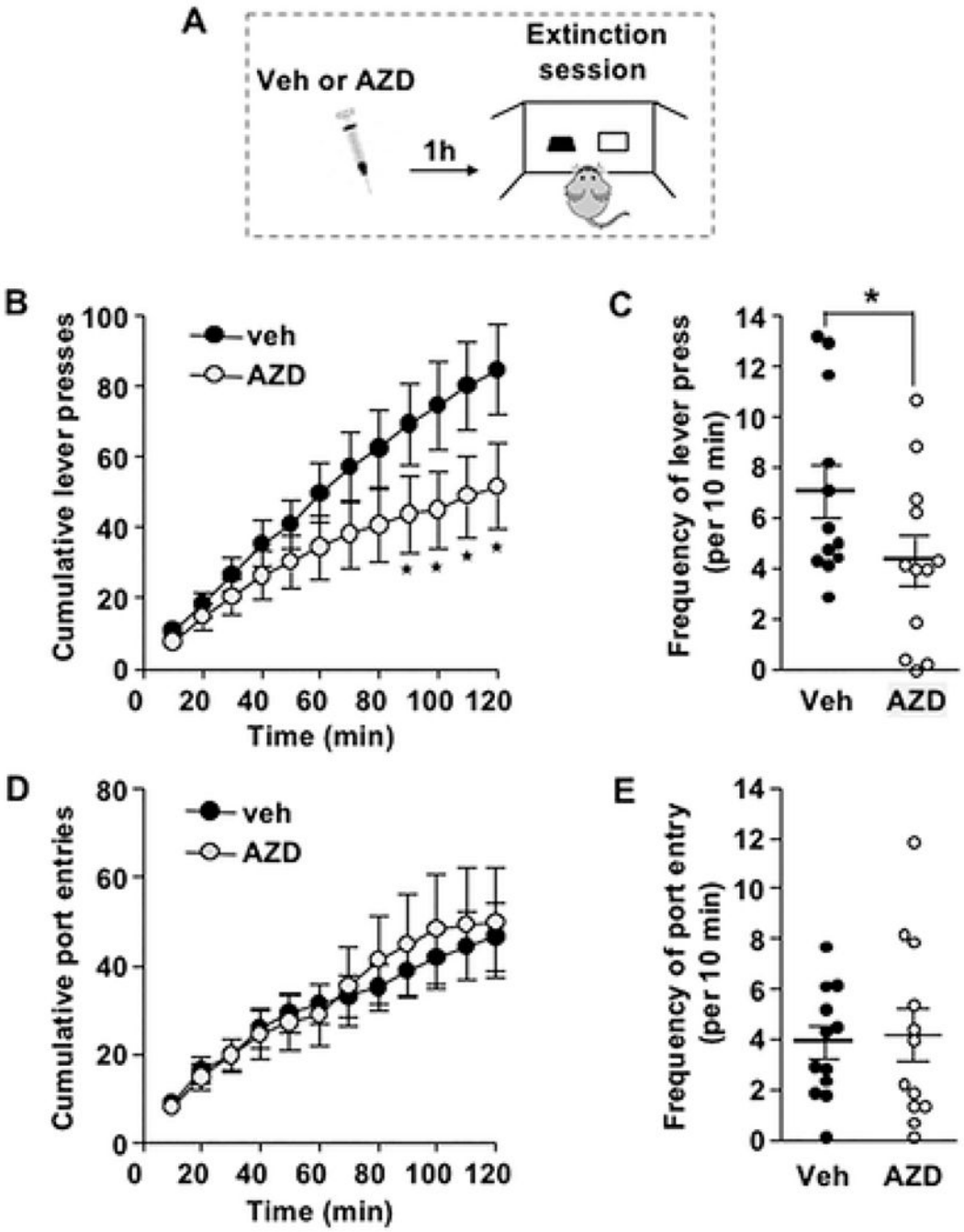

Figure 4.

AZD0530 promotes extinction of alcohol self-administration. A, Mice underwent the experimental design described in Figure 3A. On weeks 15-16, mice received vehicle (vehicle) or AZD0530 (AZD0530, 10 mg/kg ip) 1 h prior to the beginning of a single extinction session. The cumulative number (B and D) and frequency (C and E) of lever presses (B and C) and port entries (D and E) were recorded. Data are represented as mean ± SEM. Individual data points are also displayed in (C) and (E); n = 12. **P* < 0.05

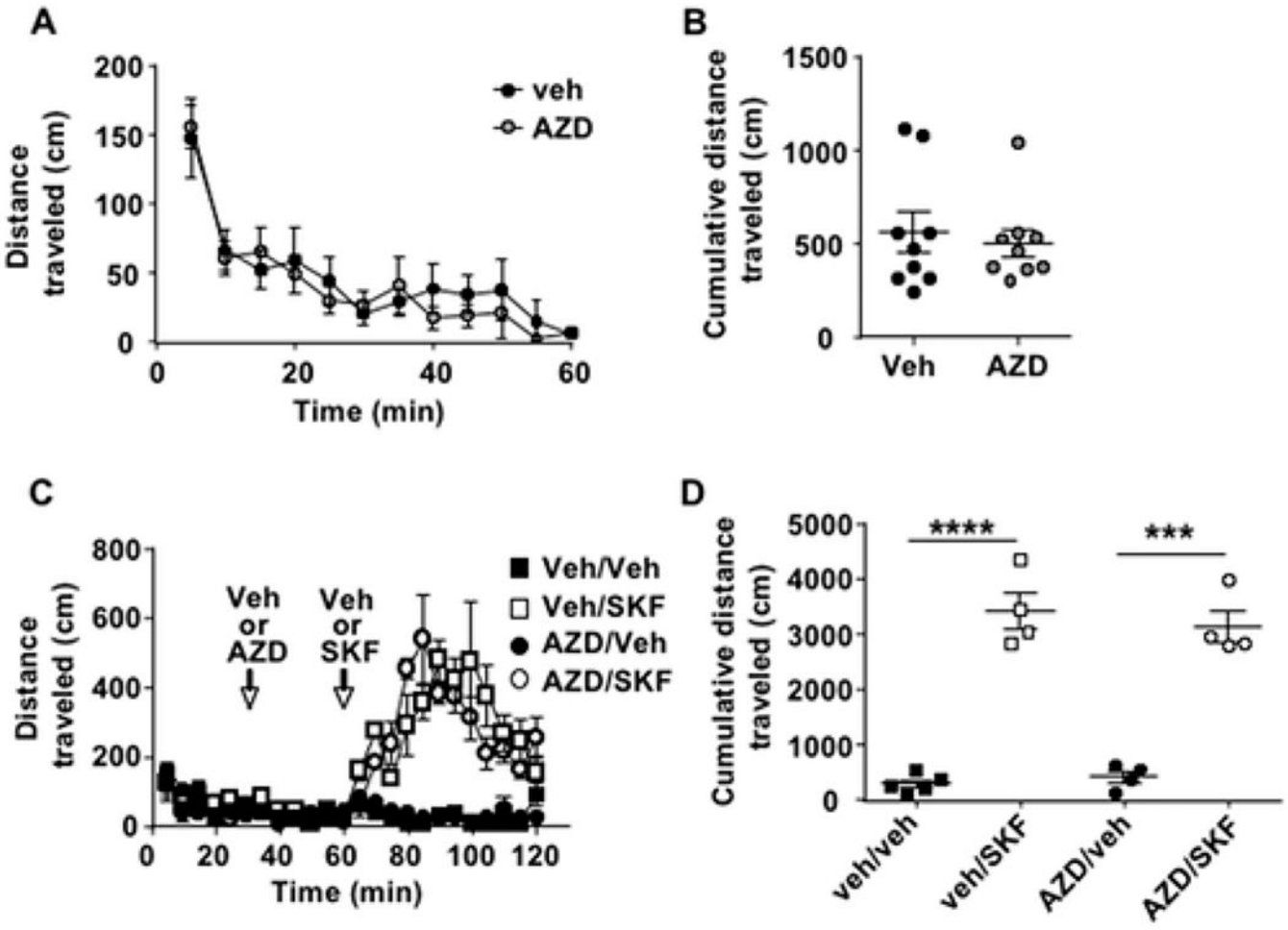

Figure 5.

AZD0530 does not alter basal or SKF81297-induced locomotion. A and B, Mice received vehicle (Veh) or AZD0530 (AZD, 10 mg/kg ip) 1 h prior to a 1-h locomotor test, and subsequent distance traveled across time (A) and cumulative distance traveled (B) were recorded. C and D, Another cohort of mice was tested in a 2-h locomotor test and received an injection of vehicle or AZD0530 (10 mg/kg ip) 30 min into the test, and a second injection of Veh or the D1/D5R agonist SKF81297 (SKF, 5 mg/kg ip) 60 min into the experiment, and subsequent distance traveled across time (C) and cumulative distance traveled (D) were recorded. Data are presented as mean ± SEM. Individual data points are also displayed in (B) and (D); n = 4-9. ***$P = 0.0001$ and ****$P < 0.0001$