Activation of trace amine-associated receptor 1 (TAAR1) reduces alcohol drinking behaviors in mice

Bartosz A. Frycz[1,2] (PhD), Klaudia Nowicka[1] (MSc), Anna Konopka[3] (PhD), Marius C. Hoener[4] (PhD), Ewa Bulska[3] (Prof), Leszek Kaczmarek[1] (Prof), Marzena Stefaniuk[1] (PhD)

[1] Laboratory of Neurobiology, BRAINCITY, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland.

[2] Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland.

[3] Faculty of Chemistry, Biological, and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02-093 Warsaw, Poland.

[4] Neuroscience and Rare Diseases Discovery and Translational Area, pRED, Roche Innovation Center Basel, F. Hoffmann-La Roche, 4070 Basel, Switzerland.

Communicating author:

Bartosz Adam Frycz, PhD

E-mail: b.frycz@nencki.edu.pl

Telephone number: +48 22 5892 316

Address: Nencki Institute of Experimental Biology PAS

Laboratory of Cell Biophysics

3 Pasteur Street, 02-093 Warsaw

Acknowledgments

This work was funded by the Polish National Science Centre (NCN), Sonatina grant: 2018/28/C/NZ4/00453. The MS-based quantification analysis was performed at the Biological and Chemical Research Centre, University of Warsaw, established within the project that was co-financed by the European Union from the European Regional Development Fund under the Operational Programme Innovative Economy 2007–2013.

Conflict of interest

Dr. Marius C. Hoener is employed by F. Hoffmann-La Roche AG where the RO5256390 was developed.

Abstract

Rationale: Trace amine-associated receptor 1 (TAAR1) is an important modulator of dopaminergic neurotransmission and has been recognized as a potential therapeutic target for alcoholism treatment. The interaction of alcohol with the dopamine reward system in the brain may promote the development of alcohol abuse.

Objectives: The aim of the study was to evaluate the role of TAAR1 receptor stimulation on alcohol drinking behaviors in mice.

Methods: We investigated the blood-brain barrier (BBB) penetration of the RO5256390 in mice by mass spectrometry. Then, wild-type (WT) mice were housed in an automatic behavioral system, IntelliCage, and habituated to drinking alcohol. The animals were further administrated either with RO526390 (10 mg/kg, intraperitoneally) or vehicle and studied on alcohol consumption, alcohol consumption after abstinence, and motivation to drink alcohol.

Results: We found that RO5256390 highly penetrated the BBB. R05256390 administration also transiently reduced alcohol consumption and alcohol consumption after abstinence in mice with a high preference for alcohol in comparison to high-drinking mice from the vehicle group. Furthermore, RO5256390 attenuated the motivation for drinking alcohol.

Conclusions: Our data suggest that activation of Taar1 reduces alcohol drinking behavior in mice.

Keywords: TAAR1, alcohol addiction, alcoholism, dopamine, RO5256390

Introduction

Alcohol is one of the most abused drugs (Ritchie and Roser 2018). Chronic use of alcohol can lead to an increased risk of health problems and the development of addiction referred to as alcoholism. The development of alcoholism is associated with physiological changes in the brain's neuronal pathways that control stress and reward behaviors (Cargiulo 2007). Specifically, alcohol interacts with several neurotransmitter pathways including dopaminergic, serotoninergic, γ-aminobutyric acid, and glutamate pathways. These interactions trigger neuroadaptive changes that result in the reinforcement effect of alcohol. In the longer term, disturbances in neurotransmission systems can lead to the development of addiction and alcohol withdrawal syndrome, which are characterized by severe psychological discomfort following discontinuation of alcohol drinking (Banerjee 2014; Morel et al. 2018). One of the most affected by both binge and chronic alcohol consumption is the mesocorticolimbic system, which comprises dopamine neurons projecting from the ventral tegmental area (VTA) to multiple downstream structures, including the nucleus accumbens (NAcc), prefrontal cortex, and the amygdala. Dopamine neurons of the mesocorticolimbic pathways are essential mediators in associative learning, natural reward like food, and reinforcement of drugs of abuse (Lammel et al. 2014). Direct administration of ethanol into the VTA of alcohol-preferring rats promoted its reinforcing effects (Rodd et al. 2004a, b). Furthermore, acute ethanol increased the release of dopamine in the NAcc (Melendez et al. 2002) and the prefrontal cortex (Schier et al. 2013) in these animals. Thus, alcohol-induced neuroadaptation in the mesocorticolimbic system can underlie the reinforcement effect of this drug (Morel et al. 2018; You et al. 2018).

In 2001, two independent research groups identified trace amine-associated receptor 1 (TAAR1) which has been further demonstrated to be involved in a broad range of neuropsychiatric disorders, including drug addiction (Borowsky et al. 2001; Bunzow et al. 2001a; Grandy et al. 2016). TAAR1 is an intracellular G_s-coupled and G_q-coupled G protein-coupled receptor (GPCR) activated by an endogenous group of so-called “trace amines”, particularly by p-tyramine, β-phenylethylamine, octopamine as well as by certain psychoactive drugs, i.e., amphetamine and methamphetamine (Bunzow et al. 2001b). In the mammalian brain, TAAR1 expression was detected in the dopaminergic (VTA, substantia nigra, SN, dorsal and ventral striatum), glutamatergic (frontal cortex, amygdala, subiculum), and serotonergic (dorsal raphe) neurons (Borowsky et al. 2001; Rutigliano et al. 2018). A number of evidence suggest that TAAR1 is an important regulator of monoaminergic neurotransmission (Borowsky et al. 2001; Bunzow et al. 2001a). Furthermore, its activation may inhibit compulsive behaviors of stimulant addiction (Wolinsky et al. 2007; Lindemann et al. 2008; Revel et al. 2012).

The role of the TAAR1 in the regulation of alcohol drinking behaviors is poorly understood. Recent studies have shown that TAAR1 KO mice had a higher preference and consumed more alcohol in comparison to WT mice (Lynch et al. 2013). Furthermore, the selective partial agonist of TAAR1, RO5263397, significantly decreased the expression of ethanol-induced behavioral sensitization both in male and female WT mice (Wu et al. 2020). In the present study, we aimed to find out whether the administration of a full selective agonist of TAAR1, RO5256390, affects alcohol drinking behaviors. First, we investigated the blood-brain barrier penetration of RO5256390 by mass spectrometry. Then, we determined the effect of RO5256390 intraperitoneal (IP) administration on alcohol consumption, alcohol

consumption after the abstinence period, and motivation to drink alcohol in WT female mice habituated to drinking alcohol. We used IntelliCage in our study. IntelliCage is the automatic recording system of the home cage behavior of animals living in a social environment. Preference for alcohol drinking in animals was developed based on the drinking in the dark DiD paradigm (Thiele and Navarro 2014). The DiD promotes high levels of ethanol drinking up to pharmacologically relevant blood concentrations and does not require the inclusion of sucrose to motivate animals to high ethanol intake (Thiele and Navarro 2014).

We showed that RO5256390 highly penetrates the blood-brain barrier in mice. Furthermore, the IP injection of this compound transiently reduced alcohol drinking during daily alcohol exposure and alcohol drinking after abstinence in mice with a high preference for drinking alcohol (high drinkers) in comparison to high drinkers in the vehicle-treated group. We also found that mice treated with RO5256390 got a lower score on the motivation test in comparison to the score obtained in pre-treatment motivational tests. We did not observe the difference comparing pre-and post-treatment scores achieved by mice in the vehicle group.

These results suggest that administration of RO5256390 decreases alcohol consumption in mice, and thus Taar1 can be involved in the regulation of alcohol drinking behaviors.

Methods

Animals

The experiments were carried out on adult 8-week C57Bl/6 female mice (n=43), bred at the animal house of the Medical University of Białystok. The study used female mice to eliminate aggressive and leadership behaviors. Animals were housed in 3 IntelliCage either 15 or 13 mice per cage, in rooms with a controlled temperature of 22°C under a 12/12 h light-dark cycle. Before caging, mice were anesthetized with isoflurane and were implanted with RFID transponders (8.5 mm in length and 1.4 mm in diameter). Mice were weighed three times during the study. After behavioral studies, mice were sacrificed by decapitation under isoflurane anesthesia. All procedures were performed following the Animal Protection Act in Poland, Directive 2010/63/EU, and were approved by the 1st Local Ethics Committee (Permission no. 793/2018).

Drugs

The highly selective TAAR1 full agonist, RO5256390, and Kolliphor EL were purchased in Merck/Millipore Sigma (Burlington, MA, United States). RO5256390 was dissolved in a mixture of ethanol (5% v/v), Kolliphor EL (10% v/v), and saline, just before the start of the experiment. Both RO5256390 and the vehicle were injected intraperitoneally (IP). The dose was expressed as an mg of the compound per kg of body weight (mg/kg). Alcohol consumption was presented as g of consumed alcohol per kg of body weight (g/kg). The dose of 10 mg/kg for RO5256390 was based on previously published reports (Pei et al. 2014; Ferragud et al. 2017a).

Blood-brain barrier (BBB) penetration

Extraction procedure

RO5256390 at the dose of 10 mg/kg was injected IP to 12 mice. The animals were sacrificed at 3, 6, 12, and 24h post-injection, and blood and brain tissue samples were collected. Three animals were sacrificed for every timepoint. The mice were perfused with 0.9% NaCl before the brain collection. Blood was subjected to centrifugation (2000 x g for 10 min) in the presence of sodium citrate to obtain a clear plasma sample. Brain tissues were weighted using an analytical scale and homogenized in 1 ml of deionized water using TissueRuptor II electric homogenizer by Qiagen (Germantown, MA, USA). RO5256390 was extracted using an acetonitrile/water 50%/50% (v/v) (LC/MS grade, JT Baker) as extraction solution. Briefly, 100 µl and 50 µl of brain homogenate and plasma samples were added to 500 µl and 250 µl of extraction buffer, respectively. Samples were incubated for 10 min. in thermomixer (Eppendorf) at room temperature at 800 × rpm. Supernatants were separated from residues by centrifugation for 10 min. at 17 500 × g (Centrifuge 5804/5804R, Eppendorf). Pellets were discarded, whereas 200 µl of supernatants were transferred to LC autosampler vials (Agilent Technologies) and analyzed by UHPLC-ESI-MS/MS. The extraction procedure was carried out in three technical replicates for each examined tissue sample.

UHPLC-ESI-MS/MS analysis

UHPLC-ESI-MS/MS analysis was performed using an Agilent 1290 Infinity LC system (Agilent Technologies) coupled to Agilent 6460 Triple Quadrupole Mass Spectrometer (Agilent Technologies) equipped with an electrospray ionization source (ESI) operating in positive ion mode. ESI conditions were as follows: nebulizing gas temperature – 300 °C, nebulizing gas flow – 10 l/min., sheath gas temperature – 200 °C, sheath gas flow – 11 l/min., positive ionization mode source voltage – 3.75 kV. Nitrogen was used as nebulizing and sheath gas. The mass spectrometer was operated in the multiple reaction monitoring mode (MRM) following optimization of working conditions (fragmentor voltage and collision energy, CE) for RO5256390 using a standard solution at the concentration of 1 mg/L (in acetonitrile/water 50%/50% (v/v) solution) in the direct infusion mode. The optimal fragmentor voltage was found to be 80 V. The following transitions of m/z (precursor ion → product ion) were monitored: 219.1 → 159.1 (CE=8 eV) (as the most intense transition taken for quantitative analysis) and 219.1 → 117.1 (CE=19 eV), 219.1 → 91.1 (CE=41 eV), 219.1 → 61.2 (CE = 12 eV) (confirming transitions for qualitative analysis). Compounds, present in the prepared extracts, were separated on a Zorbax Eclipse Plus C18 Rapid Resolution column (Agilent Technologies) (100 mm x 4.6 mm, 3.5 µm) at 35 °C. Water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used as eluent A and B, respectively. The mobile phase was delivered at 0.5 mL/min in isocratic mode with 50% of eluent B. The injection volume was 10 µl. The total time of chromatographic separation was 10 min. Measurements were carried out in three technical replicates. Quantification of RO5256390 was achieved by the external matrix calibration curve method. Standard solutions were made by adding appropriate amounts of RO5256390 stock solution to extracts of blank brain homogenates and plasma. Blank extracts were obtained from tissues derived from untreated animals following the same extraction procedure. Standard curves for brain aliquots and blood plasma were generated within the concentration range of 0.1 ng/ml – 100 ng/ml (R^2=0.9999) and 0.1 ng/ml – 50 ng/ml (R^2=0.9995), respectively. Considering the dilution factor and the masses of tissues collected, the content of RO5256390 was calculated per g of tissue or ml of plasma for brain tissue and blood plasma samples, respectively.

Behavioral procedures

IntelliCage training

Animals were trained in the IntelliCage system (Kiryk et al. 2020) (Fig 1A and B). Two corners of the cage were equipped with a presence detector and an antenna to detect the signal from the RFIDs. Access to bottles with water and/or alcohol was limited by a door situated behind the entrance of the corner. Activity towards the entrance (i.e., nosepoke) opened the door and allowed the mouse to make a lick from the bottle. The activity of mice in a corner, including visits, nosepoke, and licks was automatically recorded. Drinking preference, amount of alcohol consumed, and motivation to drink alcohol were examined by placing bottles containing water and/or alcohol in appropriate corners and setting the conditions for the bottle access (Fig. 1B).

For the first ten days in the cage, all mice were habituated to using automatic corners of IntelliCage. Then, the behavioral training based on DiD paradigm was used to develop a preference for drinking alcohol in mice (Thiele and Navarro 2014). Briefly, all water bottles were replaced with a bottle containing 20% ethanol [v/v] for 2.5 h, beginning 2 h into the dark cycle. The presence of mice in corners with alcohol was accompanied by the lighting up of green led light. Sessions with DiD lasted 4 weeks, every day, excluding one day in a week when the mice only had access to water. The DiD was followed by the sessions with free alcohol access (FAA) which were conducted similarly to DiD, except that half of the bottles contained water. Thus, the mice could choose whether they preferred to drink water or alcohol. Bottles of alcohol were placed in a corner that was previously visited less frequently by mice to exclude the effect of place preference on alcohol consumption. Based on the average consumption of alcohol in the pre-treatment FAA sessions (2.5 h), the animals were assigned as either 'high drinker' or 'low drinker'. The high drinkers consisted of 30% of all animals with the highest average alcohol consumption of 1.4-1.9 g/kg per FAA session, while all others were classified as 'low drinkers' with average alcohol consumption of 0.0-1.1 g/kg per session (Thiele and Navarro 2014). The following formula was used to calculate the consumption of alcohol: (number of licks × 1.94 × alcohol concentration × 0.79 g/ml)/animal weight. The value of 1.94 µl ± 0.2 µl corresponds to the average lick volume.

RO5256390 administration

The high- (n=5) and low drinker (n=15) mice were assigned to the 'vehicle' and the 'RO5256390' groups. There were 5 high and 10 low drinker mice in each group. Before each of the seven FAA sessions preceding the experiment, the mice were taken out of the cage and the injection was mimicked for habituation. To evaluate the effect of RO5256390 on alcohol drinking, mice were injected IP either RO5256390 or vehicle, depending on the group. Then, the animals had 20 h long, free access to water and alcohol. We decided to extend the FAA session to 20 instead of the 2.5 h due to decreased locomotor activity in the RO5256390 group within the first two h after injection (very low no. of visits to water and alcohol corners for R05256390 in comparison to the vehicle group).

The effect of RO5256390 on alcohol drinking after abstinence was investigated by blocking access to the alcohol corner (only the water corner was active) for 9 days. The experiment was carried out on the same mice as previously (n=30). The 20 h long FAA session was carried out as above. At the end of the session, mice were sacrificed and both cortex and striatum were collected for qPCR analysis.

The study of the effect of RO5256390 on motivation to drink alcohol was investigated in another group of mice (n=13). Like in the previous groups, the mice underwent behavioral training consisting of DiD and FAA sessions. Considering that low drinking animals can display a high motivation for alcohol, we decided to include low drinkers in the analysis (5 animals with avg. alcohol consumption of 0.0-1.2 g/kg per FAA session). Motivation testing included 6 motivation tests conducted at an interval of one week. Assignment to RO5256390 and vehicle groups was based on the mice's total score in the first 3 motivation pre-treatment tests. Groups were established in a way that both groups contained the individuals with the similar motivation to drink alcohol. Then, another 3 motivation tests were performed, however, before these tests, the animals were treated with RO5256390 or vehicle. The motivation tests are based on the predetermined number of nosepoke mice had

to perform to gain the access to alcohol corners. According to the following ratio sequence, the number of nosepoke increased with every visit to the corner: 1, 4, 8, 12, 16, 20, 24, 28, and 32. The measure of motivation was the maximum ratio of nosepoke (reward NP) the mouse was prone to perform to get to the bottle of alcohol. Each test lasted 20 h. The mice were habituated to IP before the tests as previously.

The effect of RO5256390 on long-term alcohol drinking was carried out on high drinkers (avg. alcohol consumption 1.4-3.5 g/kg per FAA session) from a group previously tested for motivation. RO5256390 and vehicle groups consisted of 4 individuals in each. Animals were given either RO5256390 or a vehicle and then the amount of alcohol consumption, nosepoke, and licks in the corners were measured for the following 48 h.

Statistical analysis

All results are presented as mean ± SEM. The appropriate tests were used, considering whether data had normal distribution and equal variation. Differences in alcohol consumption between mice in the RO5256390 and vehicle groups were estimated using the Unpaired t-test or Mann Whitney U test. The effect of the drug administration on motivation for alcohol drinking was evaluated by the Wilcoxon test.

Statistical analysis was conducted using GraphPad Prism, version 9.3.1 (GraphPad Software, Inc., La Jolla, CA). Differences between the experimental groups were considered significant if the type 1 error was less than 5%.

Results

RO5256390 highly penetrates the blood-brain barrier

RO5256390 was detected in both blood plasma and the brain after IP administration (Fig. 2A and C). The highest concentration of RO5256390 in brain and plasma was observed 3 h post-injection (2260 ng/g and 82ng/ml, respectively) (Fig. 2A). At subsequent time points, the amount of RO5256390 in these tissues was decreased (Fig. 2A). Twenty-four hours after injection, the amount of RO5256390 in plasma was at the trace level (<0.1 ng/ml) while in the brain it was 27.6 ng/g (1.2% of the maximum amount reached at 3 h post-injection) (Fig. 2A and B). Notably, the B/P ratios for all studied time points exceeded 1, demonstrating higher brain RO5256390 concentrations than plasma concentrations (Fig. 2B).

Furthermore, we calculated the ratio of areas under the kinetic curve (AUC brain/AUC plasma) of the RO5256390 concentration in blood plasma and brain tissue (Fig. 2C). The ratio was 23.7, which shows the high ability of RO5256390 to penetrate the BBB.

RO5256390 administration decreased alcohol consumption in high drinkers

Mice were assigned to either RO5256390 or the vehicle group and tested for alcohol drinking (Fig. 3A). Each of these groups consisted of 5 high and 10 low drinkers respectively. The groups did not differ in the amount of alcohol consumed during the pre-treatment session (data not shown). However, we found that high drinkers treated with RO5256390 had lower total alcohol consumption ($p=0.034$) (Fig. 3B) and performed a less total number of nosepoke ($p=0.022$) (Fig. 3C) and licks ($p=0.034$) (Fig. 3E) in alcohol corner in comparison to high drinkers treated with vehicle, during 20 h of FAA session. Furthermore, high drinkers treated with RO5256390 performed more water licks than high drinkers treated with vehicle (Fig. 3E); however, the difference was not statistically significant. There were no differences in nosepoke (Fig. 3D), and licks (Fig. 3F) performed in water and alcohol corners comparing low drinkers in the RO5256390 group and low drinkers in the vehicle group.

RO5256390 administration decreased alcohol consumption in high drinkers after the abstinence

The analysis of the effect of RO5256390 administration on alcohol consumption was followed by blocked access to alcohol for 9 days. Then, we studied the effect of RO5256390 administration on alcohol drinking after the abstinence period (Fig. 4A). Similarly, we found that high drinkers treated with RO5256390 had lower total alcohol consumption ($p=0.016$) (Fig. 4B) and performed a less total number of nosepoke ($p=0.024$) (Fig. 4C) and licks ($p=0.016$) (Fig. 4E) in alcohol corner in comparison to high drinkers treated with vehicle, during 20 h of FAA session. There were no differences in nosepoke (Fig. 4D), and licks (Fig. 4F) performed in water and alcohol corners comparing low drinkers in the RO5256390 group and low drinkers in the vehicle group.

RO5256390 administration attenuated motivation to alcohol drinking

We compared the reward nosepoke (reward NP) of the mice obtained in the motivation test before and after administration of RO5256390 or vehicle (Fig. 5A). Although the mean score of both groups on the motivation tests was lower after the administration versus before the administration of the compounds, statistically significant differences were observed only in the RO5256390 group (Fig. 5B). Namely, mice that were administered RO5256390 scored lower on the motivation test in comparison to the pre-treatment score (p=0.0021) (Fig. 5B). There was no difference in the licks of water comparing pre-and post-administration motivation tests for both groups (Fig. 5C). However, we found that R05256390 treated group consumed significantly less alcohol during motivation tests performed after RO5256390 treatment in comparison to motivation tests performed before the drug administration (p=0.016) (Fig. 5D). The difference was not found in the vehicle-treated groups (Fig. 5D).

The effect of RO5256390 on alcohol drinking is short-lasting

We investigated the time of reduced alcohol drinking in mice treated with RO5256390 (Fig. 6A). Similarly, to the previous experiments, high drinkers in the RO5256390 group drank less (p=0.024) (Fig. 6B) and performed fewer licks of alcohol (p=0.017) (Fig. 6C) during the first 24 h of the FAA session in comparison to the high drinkers in the vehicle group. However, during the following 24 h, the level of consumed alcohol end licks performed in the alcohol corner was similar in both groups (Fig. 6B and C). These results are consistent with our previous experiments where the RO5256390 group consumed a similar amount of alcohol in comparison to the vehicle group in subsequent FAA sessions performed without the compound administration (data not shown).

Discussion

Our study reports that administration of the full selective TAAR1 agonist, RO5256390, reduced alcohol drinking in female C57BL/6 mice in comparison to their littermates administered with a vehicle. Mice in the RO5256390 group also consumed less alcohol in comparison to vehicle mice after the abstinence period. Furthermore, we have shown that RO526390 administration can attenuate motivation for alcohol drinking. Similarly, to the recent findings, we confirmed that RO5256390 effectively penetrates the BBB after IP administration. This is the first study showing the effect of RO5256390 on alcohol drinking in mice. Our results support the role of TAAR1 in the development of alcohol abuse and alcoholism.

The agonists of TAAR1 have been mostly studied in psychostimulant addictions (Achat-Mendes et al. 2012; Revel et al. 2012; Jing et al. 2014; Harkness et al. 2015; Sukhanov et al. 2016) due to the structural resemblance of trace amines to amphetamine. On the other hand, little is known about whether they affect the other abused substances like alcohol. Lynch et al. (Lynch et al. 2013) showed that Taar1 KO mice had a greater preference and drank more alcohol than their WT counterparts. Therefore, it has been suggested that TAAR1 may potentially regulate alcohol consumption. Our results support this hypothesis since we observed reduced alcohol consumption in high-drinking mice treated with RO5256390 in comparison to the high drinkers in the vehicle group. A similar effect of this compound was found in the psychostimulant study. Administration of RO5256390 produced a downward shift in the dose-response curve for cocaine self-administration and dose-

dependently prevented the cocaine-induced lowering of intracranial self-stimulation thresholds (Pei et al. 2015). Recent studies have shown that RO5256390 can also regulate compulsive behavior related to food. It was found that the compound dose-dependently blocks binge-like eating of the palatable diet in rats (Ferragud et al. 2017b).

Wu et al. (Wu et al. 2020) showed that a highly selective partial TAAR1 agonist, RO5263397, significantly decreased the expression of ethanol-induced behavioral sensitization both in male and female WT mice. Since there is a remarkable overlap in the neurocircuitry between behavioral sensitization and relapse, TAAR1 activation may affect these phenomena. Consistently, we found that RO5256390 decreased alcohol consumption in high-drinking mice after the abstinence period. Similarly, this compound dose-dependently decreased cocaine seeking in rats, after a 2-week withdrawal from chronic cocaine self-administration (Pei et al. 2014). Furthermore, RO5263397 blocked methamphetamine-primed reinstatement of the drug-seeking which was accompanied by a decrease in the release of dopamine in NAcc (Pei et al. 2017). Therefore, activation of TAAR1 may attenuate relapse-like behaviors in drug abuse.

We also found that RO5256390 administration attenuated the motivation for drinking alcohol. Similarly, RO5263397 decreased the motivation for methamphetamine self-administration under a progressive ratio schedule of reinforcement (Pei et al. 2017). The same compound also significantly decreased nicotine's physical and motivational withdrawal effects in the long-access self-administration rats model (Wu et al. 2022). This indicates the possible involvement of TAAR1 activation in the regulation of motivational behavior associated with drug addiction. Notably, Lynch et al. (Lynch et al. 2013) found no difference in sucrose consumption between WT and Taar1 KO mice, suggesting that TAAR1 does not affect natural reward. It was also shown that deletion of Taar1 did not significantly affect sexual motivation in mice (Zhukov et al. 2022). Although RO5263397 attenuated the expression of behavioral sensitization to methamphetamine, and reduced methamphetamine self-administration it did not alter cue-induced reinstatement of sucrose-seeking behavior (Jing et al. 2014). In another study, RO5256390 did not affect either baseline intake or overeating of the standard chow diet (Ferragud et al. 2017b).

To avoid dominance fights that could disturb the obtained results, we used female mice in our study, and the presence of gender differences in the development of alcohol dependence was also reported (Cortez et al. 2020; Flores-Bonilla and Richardson 2020; Grace et al. 2021). Women develop an addiction to alcohol much more quickly than men (Zilberman et al. 2008). Consistently, female mice had augmented ethanol-induced locomotor activity in comparison to male individuals (Wu et al. 2020). There is also evidence of increased alcohol consumption in female than male rats, occurring after the second and third deprivations (García-Burgos et al. 2010). However, it remains unknown whether the administration of TAAR1 agonists affects differently on males and females (Mckinzie et al. 1998).

RO5256390 has been recognized as a potent full agonist at hTAAR1 and has excellent pharmacokinetic properties in mice and rats (Revel et al. 2013; Galley et al. 2016). We confirmed that the compound highly penetrates the blood-brain barrier in mice. Despite the low to moderate oral bioavailability of RO5256390, Revel et al. (Revel et al. 2013) showed that IP administration of the compound to mice increases its bioavailability. An elimination half-life of RO5256390 was estimated to be several hours with a high brain/plasma concentration ratio (11 times more in the brain) (Revel et al. 2013). In our study, 3 h after

administration, the amount of RO5256390 in the brain was nearly 30 times higher than in plasma and was approx. 30% of the original dose. However, we used a higher dose than Revel et al. (10 mg/kg vs 2.1 mg/kg IP). Interestingly, 24 h after administration, the amount of RO5256390 in both brain and plasma was drastically low compared to the original dose. It is worth noting here that the reduction in alcohol intake in RO5256390 mice in comparison to vehicles resolved after approximately 24 h and remained similar in both groups thereafter.

Finally, on the second day after compound administration, we observed no differences in alcohol consumption between the studied groups. This indicates an effect of RO5256390 on drinking alcohol only while it is present in the brain. It is also important to consider that we used the whole-brain extract in mass spectrometry studies. Thus, we do not know whether RO5256390 does not better penetrate specific brain areas.

In conclusion, our findings indicate that TAAR1 activation may be involved in the regulation of alcohol drinking-related behaviors. Further, TAAR1 agonists such as RO5256390 may have a role in the treatment of alcohol abuse, specifically inhibiting motivation to drink alcohol and drinking alcohol after abstinence. Thus, TAAR1 is a promising pharmacological target for the treatment of alcoholism; however, more research is needed to better understand its involvement in the development of this disease.

Figures caption

Fig. 1. IntelliCage was used to study the effects of RO5256390 administration on alcohol-related behavior

C57Bl/6 mice underwent behavioral training in IntelliCage and then were tested for alcohol drinking behaviors. **(A)** To access water or alcohol, a mouse had to enter the corresponding corner and approach a sensor (nosepoke) placed inside, which opens a door blocking access to the bottle. **(B)** IntelliCage setup during training and behavioral tests. During the adaptation and free access to alcohol (FAA) sessions, mice had unrestricted access to water or water and alcohol, respectively. To promote high alcohol consumption in mice, the FAA sessions were preceded by a "Drinking in the Dark (DiD)" based paradigm. During the motivation tests, the animals had to perform an increasing number of nosepoke at each visit to get access to alcohol. In the abstinence period, access to alcohol was blocked. The presence of mice in a corner with alcohol was always accompanied by the lighting of green led.

Fig. 2. RO5256390 effectively penetrated the blood-brain barrier

(A) RO5256390 was found in both, the brain and plasma after IP administration. **(B)** RO5256390 showed a brain/plasma ratio (B/P) >1 at all studied time points which indicates a high BBB penetration rate of the compound **(C)** High penetration rate of BBB by RO5256390 was additionally demonstrated by are under the curve (AUC) calculations.

Fig. 3. RO5256390 administration reduced nosepoke, licks, and total alcohol consumption in high drinkers

(A) Experimental timeline. **(B)** Total alcohol consumption was lower in high drinkers in the RO5256390 group in comparison to high drinkers in the vehicle group (p=0.034, Unpaired t-test). **(C)** High drinkers in the RO5256390 group performed fewer nosepoke than high drinkers in the vehicle group during 20 h FAA in the corner with alcohol (p=0.022, Unpaired t-test). **(D)** There was no difference in the number of nosepoke performed in the water and alcohol corners between low drinkers from the RO5256390 and vehicle groups. **(E)** High drinkers from the RO5256390 group performed fewer licks of alcohol in comparison to the high drinkers from the vehicle group (p=0.034, Unpaired t-test). **(F)** There was no difference in the number of licks performed in the water and alcohol corners between low drinkers from the RO5256390 and vehicle groups.

Fig. 4. RO5256390 administration reduced nosepoke, licks, and total alcohol consumption in high drinkers after abstinence

(A) Experimental timeline. **(B)** Total alcohol consumption was lower in high drinkers in the RO5256390 group in comparison to high drinkers in the vehicle group (p=0.016, Mann Whitney U test). **(C)** High drinkers in the RO5256390 group performed fewer nosepoke than high drinkers in the vehicle group in the corner with alcohol during 20 h FAA after abstinence (p=0.024, Mann Whitney U test). **(D)** There was no difference in the number of nosepoke performed in the water and alcohol corners between low drinkers from the RO5256390 and vehicle groups. **(E)** High drinkers in the RO5256390 group performed fewer licks than high drinkers in the vehicle group in the corner with alcohol during 20 h FAA after abstinence (p=0.016, Mann Whitney U test). **(F)** There was no difference in the number of licks performed in the water and alcohol corners between low drinkers from the RO5256390 and vehicle groups.

Fig. 5. RO5256390 administration attenuated motivation for alcohol drinking

(A) Experimental timeline. **(B)** RO5256390 mice but not vehicle group mice achieved a statistically significant lower score in the motivation tests after compound administration in comparison to the pre-treatment tests score (p=0.0021, Wilcoxon test). **(C)** There was no difference in licks performed in water corners after RO526390 or vehicle treatment in comparison to licks performed by the groups in the pre-treatment test. **(D)** RO5256390 mice drank less alcohol during motivation tests conducted after compound administration in comparison to the amount of alcohol consumed in pre-treatment tests (p=0.016, Wilcoxon test). There was no difference in alcohol consumption in pre-and post-treatment tests in the vehicle group.

Fig. 6. RO5256390 administration transiently reduced alcohol consumption

(A) Experimental timeline. **(B)** High drinkers in the RO5256390 group consumed less alcohol 24 h after compound administration in comparison to the high drinkers in the vehicle group (p=0.024, Unpaired t-test). The difference was lost during the following 24 h. **(C)** High drinkers in the RO5256390 group performed fewer licks in the corner with alcohol 24 h after

compound administration in comparison to the high drinkers in the vehicle group (p=0.017, Unpaired t-test). The difference was lost during the following 24 h. There were no differences in the number of licks in the water corner between RO5256390 and vehicle groups within 48 h session

References

Achat-Mendes C, Lynch LJ, Sullivan KA, et al (2012) Augmentation of methamphetamine-induced behaviors in transgenic mice lacking the trace amine-associated receptor 1. Pharmacol Biochem Behav 101:201–207. https://doi.org/10.1016/j.jmb.2008.10.054.The

Banerjee N (2014) Neurotransmitters in alcoholism: A review of neurobiological and genetic studies. Indian J Hum Genet 20:20–31. https://doi.org/10.4103/0971-6866.132750

Borowsky B, Adham N, Jones KA, et al (2001) Trace amines: Identification of a family of mammalian G protein-coupled receptors. Proc Natl Acad Sci 98:8966–8971. https://doi.org/10.1073/pnas.151105198

Bunzow JR, Sonders MS, Arttamangkul S, et al (2001a) Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. Mol Pharmacol 60:1181–1188. https://doi.org/10.1124/mol.60.6.1181

Bunzow, Sonders, Arttamangkul, et al (2001b) Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. Mol Pharmacol 60:1181–1188. https://doi.org/10.1124/mol.60.6.1181

Cargiulo T (2007) Understanding the health impact of alcohol dependence. Am J Heal Pharm 1:5–11. https://doi.org/10.2146/ajhp060647

Cortez I, Rodgers SP, Kosten TA, Leasure JL (2020) Sex and Age Effects on Neurobehavioral Toxicity Induced by Binge Alcohol. Brain Plast 6:5–25. https://doi.org/10.3233/bpl-190094

Ferragud A, Howell AD, Moore CF, et al (2017a) The Trace Amine-Associated Receptor 1 Agonist RO5256390 Blocks Compulsive, Binge-like Eating in Rats. Neuropsychopharmacology 42:1458–1470. https://doi.org/10.1038/npp.2016.233

Ferragud A, Howell AD, Moore CF, et al (2017b) The Trace Amine-Associated Receptor 1 Agonist RO5256390 Blocks Compulsive, Binge-like Eating in Rats. Neuropsychopharmacology 42:. https://doi.org/10.1038/npp.2016.233

Flores-Bonilla A, Richardson HN (2020) Sex Differences in the Neurobiology of Alcohol Use Disorder. Alcohol Res Curr Rev 40:. https://doi.org/10.35946/arcr.v40.2.04

Galley G, Beurier A, Décoret G, et al (2016) Discovery and Characterization of 2-Aminooxazolines as Highly Potent, Selective, and Orally Active TAAR1 Agonists. ACS Med Chem Lett 7:192–197. https://doi.org/10.1021/acsmedchemlett.5b00449

García-Burgos D, Manrique Zuluaga T, Gallo Torre M, González Reyes F (2010) [Sex

differences in the alcohol deprivation effect in rats]. Psicothema 22:887–892

Grace S, Rossetti MG, Allen N, et al (2021) Sex differences in the neuroanatomy of alcohol dependence: hippocampus and amygdala subregions in a sample of 966 people from the ENIGMA Addiction Working Group. Transl Psychiatry 11:. https://doi.org/10.1038/s41398-021-01204-1

Grandy DK, Miller GM, Li JX (2016) "TAARgeting Addiction"—The Alamo Bears Witness to Another Revolution: An Overview of the Plenary Symposium of the 2015 Behavior, Biology and Chemistry Conference. Drug Alcohol Depend 159:9–16. https://doi.org/10.1016/J.DRUGALCDEP.2015.11.014

Harkness JH, Shi X, Janowsky A, Phillips TJ (2015) Trace Amine-Associated Receptor 1 Regulation of Methamphetamine Intake and Related Traits. Neuropsychopharmacology 40:2175–2184. https://doi.org/10.1038/npp.2015.61

Jing L, Zhang Y, Li JX (2014) Effects of the trace amine associated receptor 1 agonist RO5263397 on abuse-related behavioral indices of methamphetamine in rats. Int J Neuropsychopharmacol 18:1–7. https://doi.org/10.1093/ijnp/pyu060

Kiryk A, Janusz A, Zglinicki B, et al (2020) IntelliCage as a tool for measuring mouse behavior – 20 years perspective. Behav Brain Res 388:112620. https://doi.org/10.1016/j.bbr.2020.112620

Lammel S, Lim BK, Malenka RC (2014) Reward and aversion in a heterogeneous midbrain dopamine system. Neuropharmacology 76:351–359. https://doi.org/10.1016/J.NEUROPHARM.2013.03.019

Lindemann L, Meyer CA, Jeanneau K, et al (2008) Trace amine-associated receptor 1 modulates dopaminergic activity. J Pharmacol Exp Ther 324:948–956. https://doi.org/10.1124/jpet.107.132647

Lynch LJ, Sullivan KA, Vallender EJ, et al (2013) Trace amine associated receptor 1 modulates behavioral effects of ethanol. Subst Abus Res Treat 7:117–126. https://doi.org/10.4137/SART.S12110

Mckinzie DL, Nowak KL, Yorger L, et al (1998) The Alcohol Deprivation Effect in the Alcohol-Preferring P Rat under Free-Drinking and Operant Access Conditions

Melendez RI, Rodd-Henricks ZA, Engleman EA, et al (2002) Microdialysis of dopamine in the nucleus accumbens of alcohol-preferring (P) rats during anticipation and operant self-administration of ethanol. Alcohol Clin Exp Res 26:318–325

Morel C, Montgomery S, Han M-H (2018) Nicotine and alcohol: the role of midbrain dopaminergic neurons in drug reinforcement. Eur J Neurosci 50:2180–2200. https://doi.org/10.1111/ejn.14160

Pei Y, Asif-Malik A, Hoener M, Canales JJ (2017) A partial trace amine-associated receptor 1 agonist exhibits properties consistent with a methamphetamine substitution treatment. Addict Biol 22:1246–1256. https://doi.org/10.1111/adb.12410

Pei Y, Lee J, Leo D, et al (2014) Activation of the trace amine-associated receptor 1 prevents relapse to cocaine seeking. Neuropsychopharmacology 39:2299–2308. https://doi.org/10.1038/npp.2014.88

Pei Y, Mortas P, Hoener MC, Canales JJ (2015) Selective activation of the trace amine-

associated receptor 1 decreases cocaine's reinforcing efficacy and prevents cocaine-induced changes in brain reward thresholds. Prog Neuro-Psychopharmacology Biol Psychiatry 63:70–75. https://doi.org/10.1016/j.pnpbp.2015.05.014

Revel FG, Meyer CA, Bradaia A, et al (2012) Brain-specific overexpression of trace amine-associated receptor 1 alters monoaminergic neurotransmission and decreases sensitivity to amphetamine. Neuropsychopharmacology 37:2580–2592. https://doi.org/10.1038/npp.2012.109

Revel FG, Moreau JL, Pouzet B, et al (2013) A new perspective for schizophrenia: TAAR1 agonists reveal antipsychotic- and antidepressant-like activity, improve cognition and control body weight. Mol Psychiatry 18:543–556. https://doi.org/10.1038/mp.2012.57

Ritchie H, Roser M (2018) Alcohol Consumption. In: OurWorldInData.org

Rodd ZA, Bell RL, Melendez RI, et al (2004a) Comparison of Intracranial Self-Administration of Ethanol Within the Posterior Ventral Tegmental Area Between Alcohol-Preferring and Wistar Rats. https://doi.org/10.1097/01.ALC.0000134401.30394.7F

Rodd ZA, Melendez RI, Bell RL, et al (2004b) Behavioral/Systems/Cognitive Intracranial Self-Administration of Ethanol within the Ventral Tegmental Area of Male Wistar Rats: Evidence for Involvement of Dopamine Neurons. https://doi.org/10.1523/JNEUROSCI.1319-03.2004

Rutigliano G, Accorroni A, Zucchi R (2018) The case for TAAR1 as a modulator of central nervous system function. Front Pharmacol 8:1–18. https://doi.org/10.3389/fphar.2017.00987

Schier CJ, Dilly GA, Gonzales RA (2013) Intravenous Ethanol Increases Extracellular Dopamine in the Medial Prefrontal Cortex of the Long-Evans Rat. https://doi.org/10.1111/acer.12042

Sukhanov I, Caffino L, Efimova E V., et al (2016) Increased context-dependent conditioning to amphetamine in mice lacking TAAR1. Pharmacol Res 103:206–214. https://doi.org/10.1016/j.phrs.2015.11.002

Thiele TE, Navarro M (2014) "Drinking in the dark" (DID) procedures: A model of binge-like ethanol drinking in non-dependent mice. Alcohol 48:235–241. https://doi.org/10.1016/j.alcohol.2013.08.005

Wolinsky TD, Swanson CJ, Smith KE, et al (2007) The Trace Amine 1 receptor knockout mouse: An animal model with relevance to schizophrenia. Genes, Brain Behav 6:628–639. https://doi.org/10.1111/j.1601-183X.2006.00292.x

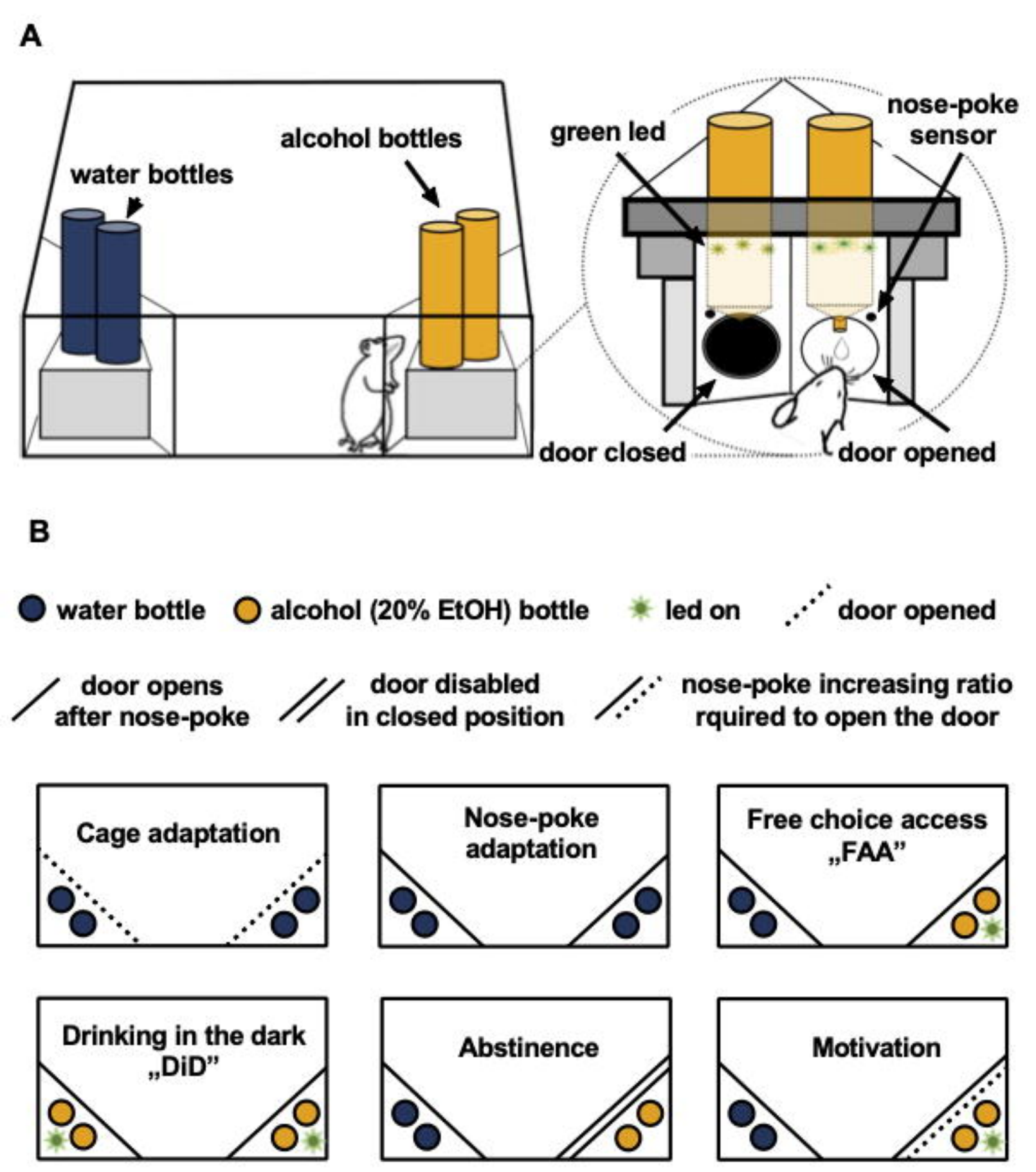
Wu R, Liu J, Johnson B, et al (2022) Activation of trace amine-associated receptor 1 attenuates nicotine withdrawal-related effects. Addict Biol 27:. https://doi.org/10.1111/ADB.13075

Wu R, Liu J, Wang K, et al (2020) Effects of a trace amine-associated receptor 1 agonist RO 5263397 on ethanol-induced behavioral sensitization. Behav Brain Res 390:112641. https://doi.org/10.1016/j.bbr.2020.112641

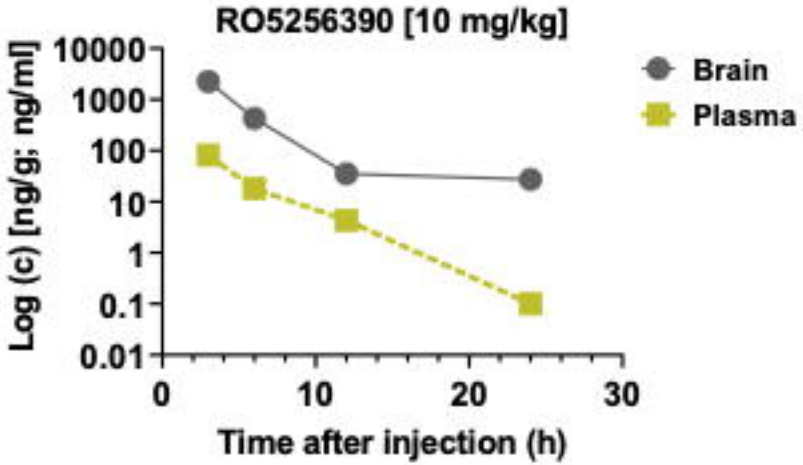
You C, Vandegrift B, Brodie MS (2018) Ethanol actions on the ventral tegmental area: novel potential targets on reward pathway neurons. Psychopharmacology (Berl) 235:1711–1726. https://doi.org/10.1007/s00213-018-4875-y

Zhukov IS, Ptukha MA, Zolotoverkhaja EA, et al (2022) Evaluation of Approach to a Conspecific and Blood Biochemical Parameters in TAAR1 Knockout Mice. 12:614. https://doi.org/10.3390/brainsci12050614

Zilberman M, Tavares H, El-Guebaly N (2008) Journal of Addictive Diseases Gender Similarities and Differences The Prevalence and Course of Alcohol and Other Substance-Related Disorders. https://doi.org/10.1300/J069v22n04_06


A
water bottles
alcohol bottles
green led
nose-poke sensor
door closed
door opened
B
water bottle
alcohol (20% EtOH) bottle
led on
door opened
door opens after nose-poke
door disabled in closed position
nose-poke increasing ratio rquired to open the door
Cage adaptation
Nose-poke adaptation
Free choice access „FAA"
Drinking in the dark „DiD"
Abstinence
Motivation

A

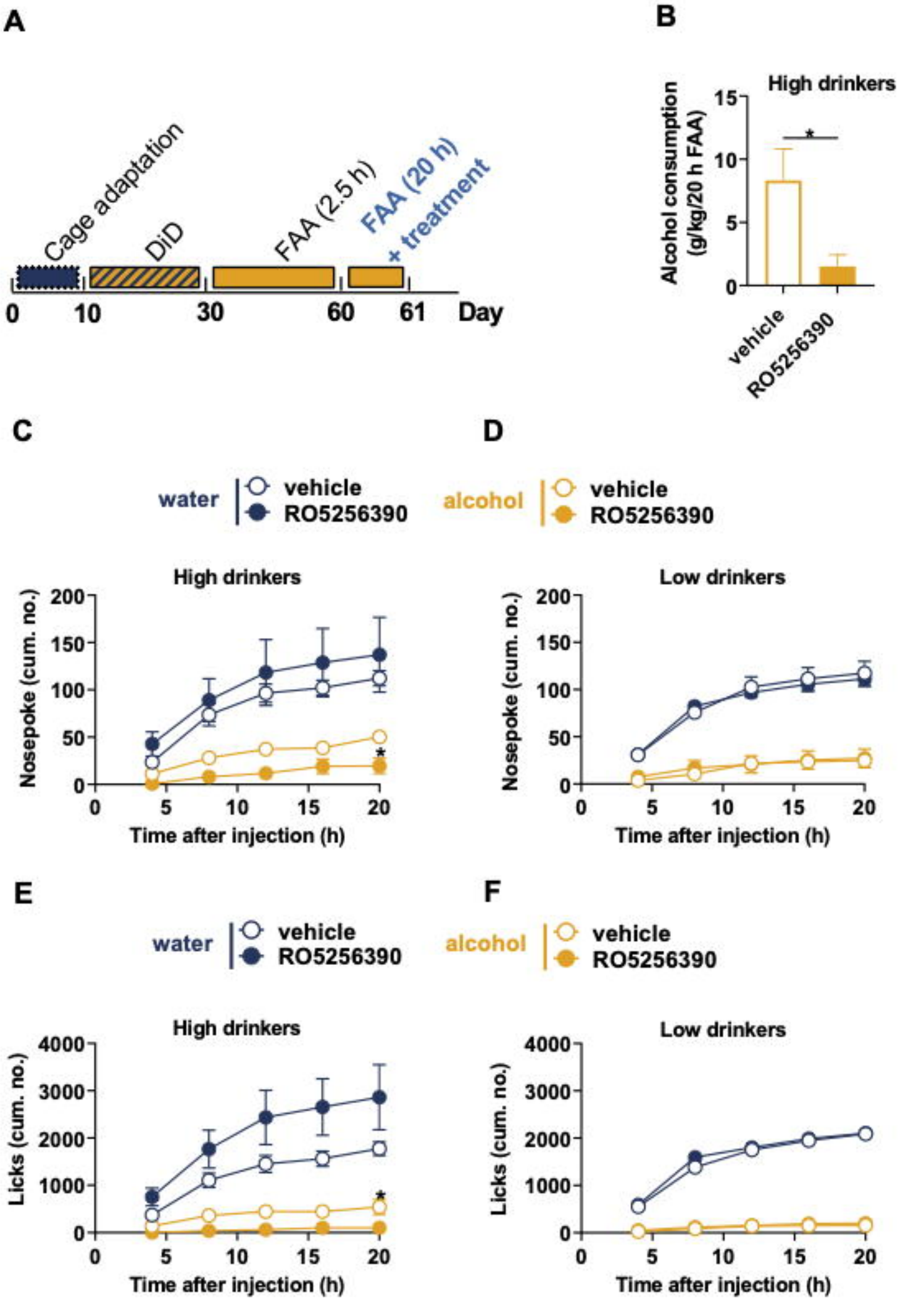


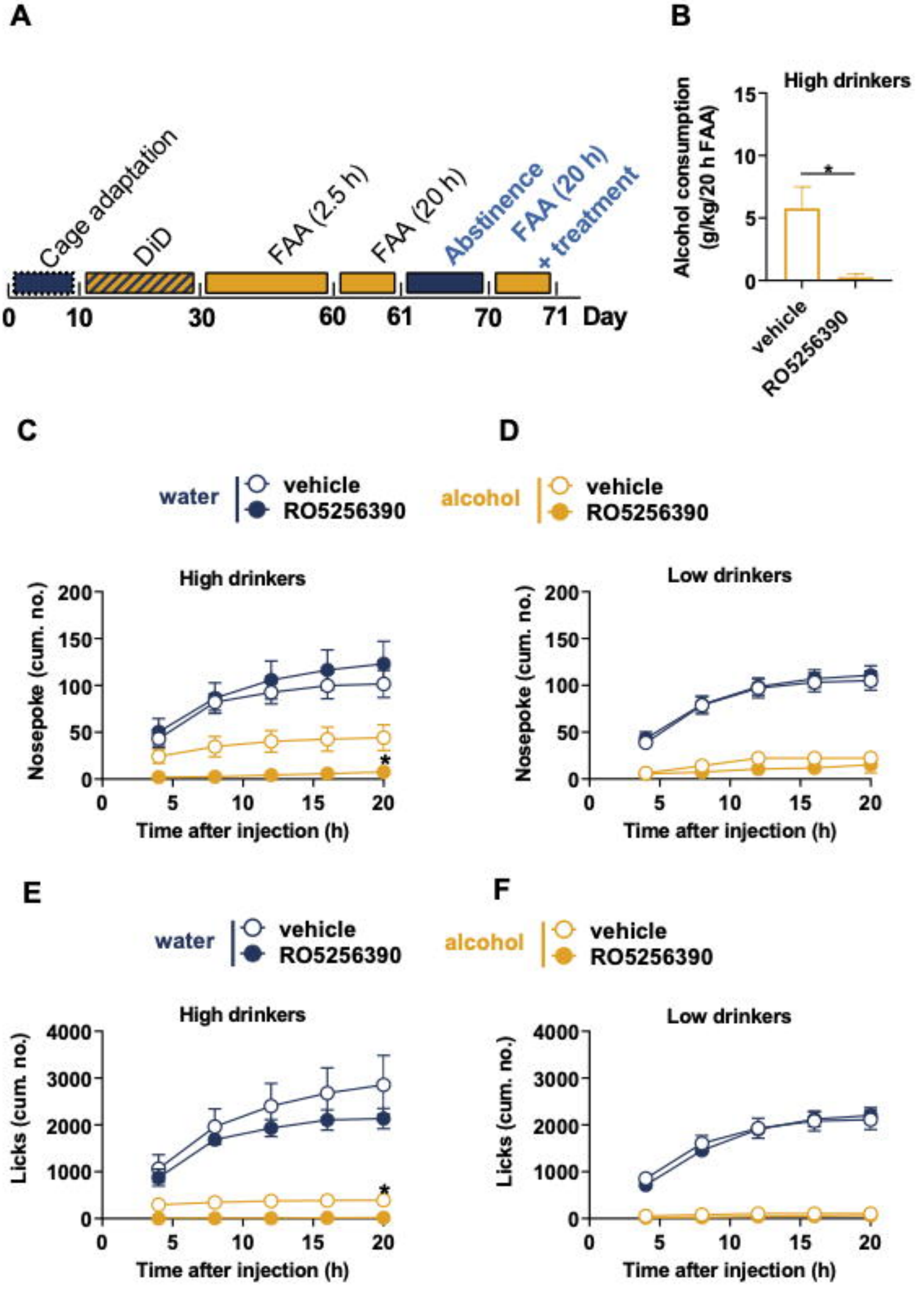
B

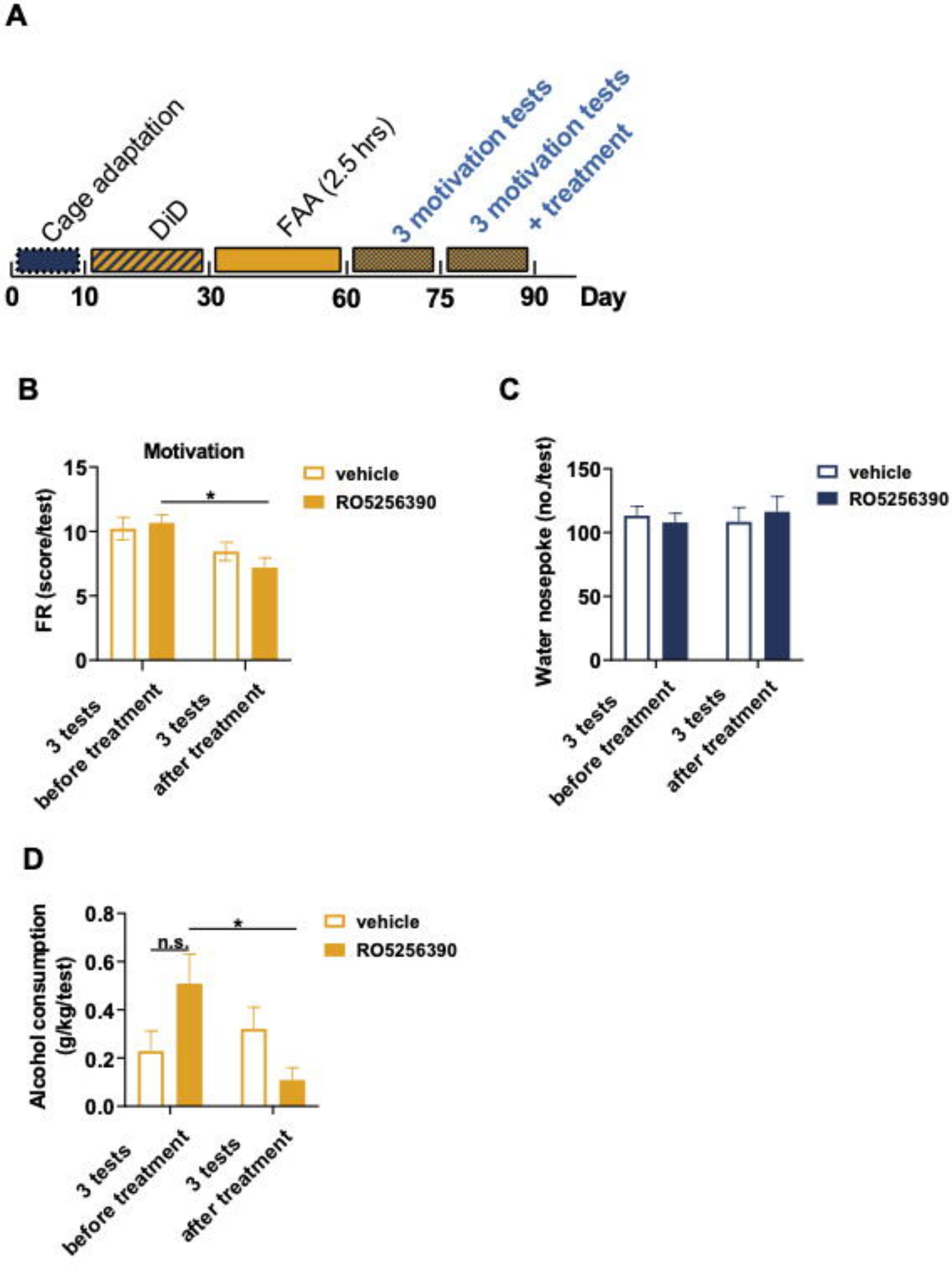
Time (h)	B/P ratio
3	27.5
6	23.1
12	7.5
24	275.8

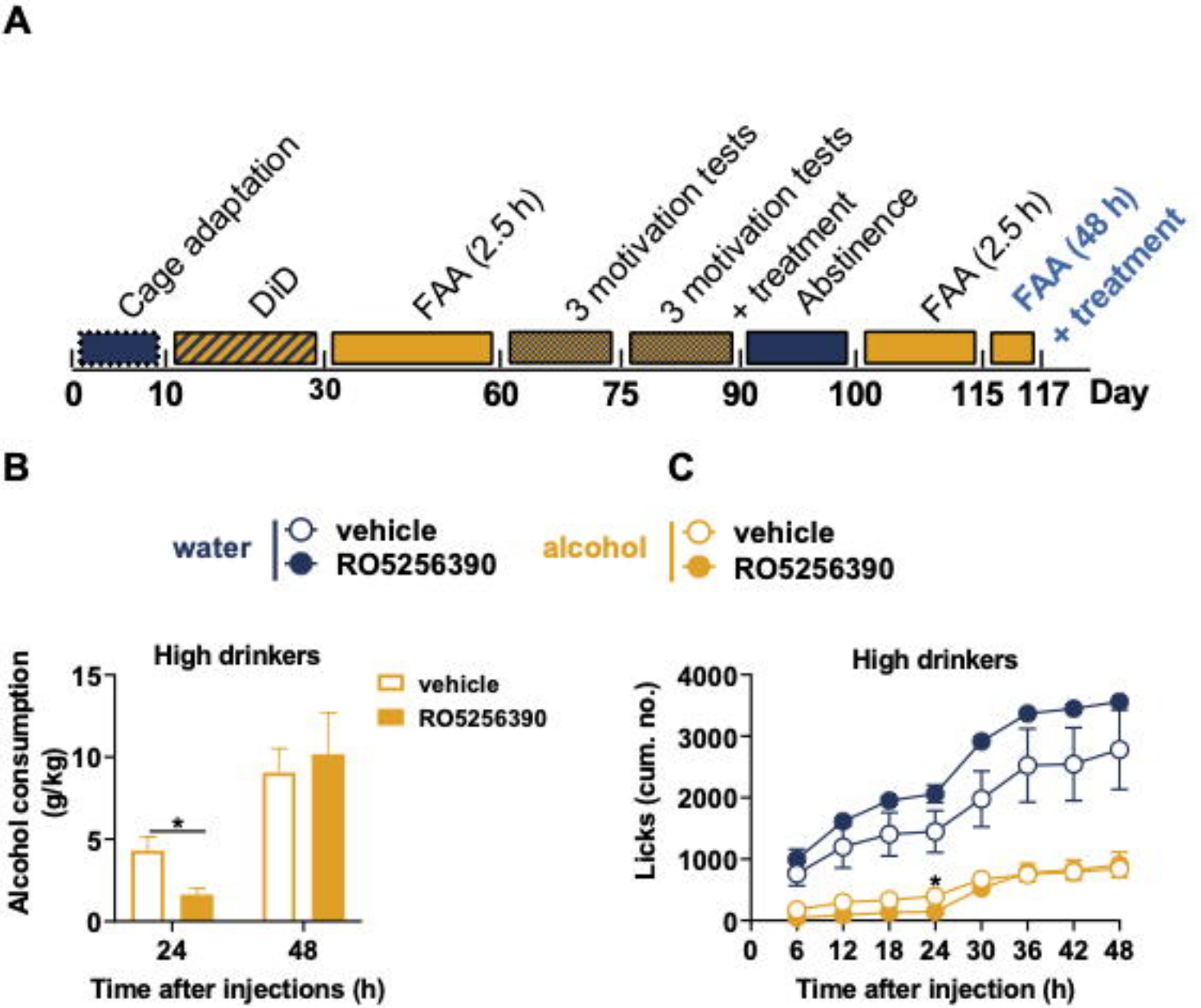
C

	AUC (are under curve)	AUC brain/AUC plasma
Blood plasma [ng min/ml]	245.34±36.5	23.7
Brain [ng min/ml]	5812.73±1090.4	


A
Cage adaptation
DiD
FAA (2.5 h)
FAA (20 h)
+ treatment
0
10
30
60
61
Day
B
High drinkers
Alcohol consumption
(g/kg/20 h FAA)
15
10
5
0
*
vehicle
RO5256390
C
water
vehicle
RO5256390
High drinkers
Nosepoke (cum. no.)
200
150
100
50
0
*
0
5
10
15
20
Time after injection (h)
D
alcohol
vehicle
RO5256390
Low drinkers
Nosepoke (cum. no.)
200
150
100
50
0
0
5
10
15
20
Time after injection (h)
E
water
vehicle
RO5256390
High drinkers
Licks (cum. no.)
4000
3000
2000
1000
0
*
0
5
10
15
20
Time after injection (h)
F
alcohol
vehicle
RO5256390
Low drinkers
Licks (cum. no.)
4000
3000
2000
1000
0
0
5
10
15
20
Time after injection (h)


A
Cage adaptation
DiD
FAA (2.5 h)
FAA (20 h)
Abstinence
FAA (20 h)
+ treatment
0
10
30
60
61
70
71
Day
B
High drinkers
Alcohol consumption (g/kg/20 h FAA)
15
10
5
0
*
vehicle
RO5256390
C
water
vehicle
RO5256390
High drinkers
Nosepoke (cum. no.)
200
150
100
50
0
*
0
5
10
15
20
Time after injection (h)
D
alcohol
vehicle
RO5256390
Low drinkers
Nosepoke (cum. no.)
200
150
100
50
0
0
5
10
15
20
Time after injection (h)
E
water
vehicle
RO5256390
High drinkers
Licks (cum. no.)
4000
3000
2000
1000
0
*
0
5
10
15
20
Time after injection (h)
F
alcohol
vehicle
RO5256390
Low drinkers
Licks (cum. no.)
4000
3000
2000
1000
0
0
5
10
15
20
Time after injection (h)


A
Cage adaptation
DiD
FAA (2.5 hrs)
3 motivation tests
3 motivation tests
+ treatment
0
10
30
60
75
90
Day
B
Motivation
FR (score/test)
15
10
5
0
*
vehicle
RO5256390
3 tests
before treatment
3 tests
after treatment
C
Water nosepoke (no./test)
150
100
50
0
vehicle
RO5256390
3 tests
before treatment
3 tests
after treatment
D
Alcohol consumption
(g/kg/test)
0.8
0.6
0.4
0.2
0.0
n.s.
*
vehicle
RO5256390
3 tests
before treatment
3 tests
after treatment


A
Cage adaptation
DiD
FAA (2.5 h)
3 motivation tests
3 motivation tests
+ treatment
Abstinence
FAA (2.5 h)
FAA (48 h)
+ treatment
0
10
30
60
75
90
100
115
117
Day
B
water
vehicle
RO5256390
High drinkers
Alcohol consumption (g/kg)
15
10
5
0
vehicle
RO5256390
*
24
48
Time after injections (h)
C
alcohol
vehicle
RO5256390
High drinkers
Licks (cum. no.)
4000
3000
2000
1000
0
*
0
6
12
18
24
30
36
42
48
Time after injection (h)