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The monoamine stabilizer (−)-OSU6162 counteracts downregulated dopamine output in the nucleus accumbens of long-term drinking Wistar rats

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ABSTRACT

We recently established that the monoamine stabilizer (−)-OSU6162 (OSU6162) decreased voluntary alcohol-mediated behaviors, including alcohol intake and cue/priming-induced reinstatement, in long-term drinking rats, while blunting alcohol-induced dopamine output in the nucleus accumbens (NAc) of alcohol-naïve rats. Therefore, we hypothesized that OSU6162 attenuates alcohol-mediated behaviors by blunting alcohol's rewarding effects. Here, we evaluated the effects of long-term drinking and OSU6162 treatment (30 mg/kg, sc) on basal and alcohol-induced (2.5 g/kg, ip) NAc dopamine outputs in Wistar rats after 10 months of intermittent access to 20% alcohol. The results showed that basal and alcohol-induced NAc dopamine outputs were significantly lower in long-term drinking rats, compared with alcohol-naïve rats. In the long-term drinking rats, OSU6162 slowly increased and maintained the dopamine output significantly elevated compared with baseline for at least 4 hours. Furthermore, OSU6162 pre-treatment did not blunt the alcohol-induced output in the long-term drinking rats, a finding that contrasted with our previous results in alcohol-naïve rats. Finally, OSU6162 did not induce conditioned place preference (CPP) in either long-term drinking or alcohol-naïve rats, indicating that OSU6162 has no reinforcing properties. To verify that the CPP results were not due to memory acquisition impairment, we demonstrated that OSU6162 did not affect novel object recognition. In conclusion, these results indicate that OSU6162 attenuates alcohol-mediated behaviors by counteracting NAc dopamine deficits in long-term drinking rats and that OSU6162 is not rewarding on its own. Together with OSU6162's beneficial side-effect profile, the present study merits evaluation of OSU6162's clinical efficacy to attenuate alcohol use in alcohol-dependent patients.

Keywords Alcohol dependence, condition place preference, ethanol, medication development, microdialysis.

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INTRODUCTION

Alcohol use disorder (AUD) is a chronic, relapsing disorder significantly contributing to the global burden of disease (Rehm *et al.* 2009). The limited clinical efficacy of the few available medications (Anton *et al.* 2006) emphasizes a crucial need for better treatments.

The mesolimbic dopamine system is one possible treatment target. Alcohol increases dopamine release in the nucleus accumbens (NAc) of alcohol-naïve rats (Di Chiara and Imperato 1988) and healthy subjects (Boileau *et al.* 2003) and thereby induces reinforcing effects (Tupala and Tiihonen 2004). In the striatum of individuals with AUD, however, both dopamine D2 receptor density (Hietala *et al.* 1994; Volkow *et al.* 1996) and central stimulant-induced dopamine release are reduced (Martinez *et al.* 2005; Volkow *et al.* 2007). These adaptations have been suggested to drive alcohol craving, compulsive drinking and increase the risk of relapse (Diana 2011). In fact, craving severity has been suggested to be correlated with a rapid dopamine turnover (Kumakura *et al.* 2013) and a reduced D2 receptor

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density in the striatum of detoxified alcohol-dependent patients (Heinz *et al.* 2004). In outbred rodents, however, the effects on the mesolimbic dopamine system following chronic alcohol treatment are inconsistent (Tupala and Tiihonen 2004). One possible explanation for these discrepancies may be that most preclinical studies to date have used forced alcohol administration, which introduces an element of stress and artifice into the experiment, casting doubt on the applicability to our understanding of human AUD. Recently, we reintroduced the preclinical intermittent-access 20% ethanol two-bottle-choice procedure (IA20E) (Wise 1973; Simms *et al.* 2008), which shows clear face validity and several hallmarks of an AUD profile (Carnicella *et al.* 2014) including the following: (1) escalation of voluntary drinking; (2) cycles of excessive drinking and abstinence; (3) pharmacologically relevant blood alcohol concentrations (Simms *et al.* 2008); and (4) compulsive drinking (Hopf *et al.*, 2010). Moreover, the IA20E model has shown predictive validity, at least for the case of current AUD medications (Simms *et al.* 2008) and varenicline (Steensland *et al.* 2007; Litten *et al.* 2013). The IA20E model also induces neuroadaptations relevant for the understanding of AUD, including a dysregulated glutamatergic neurotransmission in the medial prefrontal cortex (Fredriksson *et al.* 2015). Furthermore, 7 weeks of IA20E decreases NAc dopamine output (Barak *et al.* 2011), supporting the hypothesis that chronic alcohol consumption induces a hypo-functioning dopamine state in AUD (Diana 2011; Becker and Mulholland 2014). However, dopamine receptor gene expression levels have previously been shown to be lower, compared with age-matched alcohol-naïve rats, after 2 and 4 months, but not 10 months, of voluntary alcohol consumption (Jonsson *et al.* 2014). Thus, the present microdialysis study investigates the effects of ten months of IA20E on basal and alcohol-induced NAc dopamine outputs to further understand the influence of long-term voluntary alcohol consumption on the dopamine system.

Despite the importance of the dopamine system in the development and maintenance of AUD (Tupala and Tiihonen, 2004), studies with traditional dopamine D2 antagonists and agonists in AUD patients have been discouraging (Swift 2010). However, recent studies with modafinil (a dopamine transporter modulator) and aripiprazole (a partial D2 agonist) show promising results in alcohol-dependent patients (Brunetti *et al.* 2012; Joos *et al.* 2013). Thus, optimal treatment may not rely on blockade or activation, but rather a modulation or 'stabilization' of the dopaminergic system.

(−)-OSU6162 (OSU6162) belongs to a class of compounds that when developed was named 'dopamine stabilizers' based on its ability to inhibit or stimulate dopamine-related behaviors depending on the prevailing dopaminergic tone (Sonesson *et al.* 1994; Rung *et al.* 2008). OSU6162 is clinically safe and has been evaluated in several neuropsychiatric disorders such as Huntington's disease and mental fatigue following stroke and brain trauma (Johansson *et al.* 2012; Kloberg *et al.* 2014). *In vitro* studies indicate that OSU6162, like aripiprazole, acts as a partial agonist with low intrinsic activity at D2 receptors (Seeman and Guan 2007; Kara *et al.* 2010); however, behavioral studies have failed to demonstrate any intrinsic activity of OSU6162 (Sonesson *et al.* 1994; Natesan *et al.* 2006), indicating a different mechanism than that of a classical partial agonist. Instead, it has been hypothesized that OSU6162 predominantly acts as an antagonist at presynaptic D2 receptors (Carlsson *et al.* 2004). However, recent observations demonstrate that OSU6162 also acts as a partial agonist on serotonergic, notably 5-hydroxytryptamine 2A receptors, which may well contribute to its stabilizing properties (Carlsson *et al.* 2011) and indicates that 'monoamine stabilizers' might be a more appropriate name for this class of compounds. Nevertheless, a human brain-imaging study (Tolboom *et al.* 2015) showed that OSU6162 occupied D2/D3 receptors concentration-dependently in the striatum, including the ventral striatum (NAc), highlighting OSU6162's potential to target the dopamine system and brain regions connected to AUD. Indeed, we recently identified OSU6162 as a potential AUD medication by showing that it reduced voluntary alcohol intake, alcohol seeking, cue/priming-induced reinstatement and withdrawal-like symptoms in long-term drinking rats (Steensland *et al.* 2012). We further found that OSU6162 blunted the dopamine output induced by a systemic alcohol challenge in alcohol-naïve rats (Steensland *et al.* 2012), highlighting OSU6162's ability to counteract an acute dopamine surge. In addition, OSU6162 by itself increased NAc dopamine output during basal conditions in the alcohol-naïve rats (Steensland *et al.* 2012), which could indicate a potential for abuse liability. The present study further evaluates the mechanism behind OSU6162's potential as an AUD medication. First, the effects of OSU6162, alone and in combination with an alcohol challenge, on NAc dopamine output were investigated using microdialysis in awake rats after 10 months of IA20E. Second, OSU6162's rewarding properties were evaluated using conditioned place preference (CPP). Finally, because memory acquisition contributes to establishing CPP, the effects of OSU6162 on novel object recognition (NOR) were evaluated.

MATERIAL AND METHODS

Detailed descriptions of animals [male Rcc Wistar Han Rats (Harlan, the Netherlands)], housing conditions, the IA20E-drinking paradigm (Wise 1973; Simms *et al.* 2008)

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and drugs and chemicals are available in the Supporting Information (SI). All experiments were carried out in strict accordance with recommendations in the Swedish Animal Welfare Act and were approved by the Swedish Ethical Committee on Animal Research in Stockholm (Dnr: N475/12 and N64/12).

In vivo microdialysis

Microdialysis was performed in the NAc of rats after 10 months of voluntary alcohol consumption (IA20E; $n = 44$) and in age-matched alcohol-naïve rats ($n = 8$) to measure the basal and alcohol-induced (2.5 g/kg, ip) outputs of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The group of long-term drinking rats was further divided into four treatment groups to evaluate the effects of OSU6162 (30 mg/kg, sc), alone and in combination with alcohol (2.5 g ethanol/kg, ip), on NAc dopamine, DOPAC and HVA output levels. The OSU6162 dose was based on previous results showing effectiveness in attenuating alcohol-mediated behaviors (Steensland *et al.* 2012), and the 60-minute interval between injections was chosen to allow the OSU6162-induced dopamine output to reach a maximum before the second injection (Steensland *et al.* 2012). The alcohol dose was based on previous studies applying microdialysis in the NAc of awake rats (Lido *et al.* 2009; Steensland *et al.* 2012).

Surgery was performed as described previously (Schilström *et al.* 1998; Steensland *et al.* 2012); however, isoflurane anesthesia was used. Concentric dialysis probes were constructed as described previously (Malmlöf *et al.* 2015) with a 2.25-mm active membrane (Filtral 10 AN69, Hospal, Meyzieu, France) and implanted in the right NAc at AP +1.6, ML −1.1 and DV −8.2 mm relative to the bregma and dura mater (Atlas of Paxinos and Watson 2007). Two hours after the rats had awoken from anesthesia, they were given access to one cycle of IA20E (i.e. 24 hours of voluntary alcohol intake, followed by free access to water).

Dialysis was initiated approximately 21 hours after the end of the last alcohol-drinking session (i.e. approximately 48 hours after surgery), by perfusion (2.5 µl/min) with physiological perfusion solution (mM: 147 NaCl, 3.0 KCl, 1.3 $CaCl_2$, 1 MgCl, 1.0 $NaHPO_4$; pH = 7.4). After approximately 2 hours, dialysate samples were collected at 15-minute intervals into tubes containing 5-µl 0.5-M perchloric acid/1.76-µM ethylenediaminetetraacetic acid (EDTA). Baseline samples were collected during 45 minutes before OSU6162 (30 mg/kg, sc) or vehicle (saline) was administered. Sixty minutes later, rats were given an injection of alcohol (2.5 g/kg, ip) or vehicle (saline), and samples were collected for an additional 180 minutes. Dialysis samples were immediately cooled down to 4°C, transferred every hour to the −20°C freezer and stored at −80°C until analyzed using high-performance liquid chromatography (HPLC) as described previously but with manual sample injection (Steensland *et al.* 2012).

During HPLC, the mobile phase (55 mM acetate, 10–12% methanol, 0.1 mM octanesulfonic acid, 0.01 mM Na_2EDTA; pH = 4.1) was pumped (0.8 ml/min) through a C-18 column with dense core particles (Kinetex 150 × 4.6 mm, 2.6 µm, Phenomenex, Torrance, CA, USA). Following separation on the column, dopamine, DOPAC and HVA were electrochemically detected using an ESA analytical cell (Thermo Scientific, Waltham, MA, USA) at an oxidizing potential of 400 mV and a reducing potential of −200 mV, and concentrations were quantified by comparing peak areas with external standards (TotalChrom software, PerkinElmer, Waltham, MA, USA).

Probe placement was verified using light microscopy of 50-µm sections, stained with neutral red. In total, 16 of the 44 long-term drinking rats were excluded because of probe displacement ($n = 5$) or technical difficulties during surgery ($n = 2$), dialysis ($n = 6$) and HPLC analysis ($n = 3$). One rat was excluded from the comparison of basal output (fmol), because absolute dopamine concentration could not be assessed. However, relative changes could be determined and therefore this rat was included when the data were presented as percent of baseline changes. In the alcohol-naïve group ($n = 8$), one rat was excluded because of probe displacement. Two rats were only included in the comparison of basal output as technical difficulties with the microdialysis procedure occurred after baseline sampling.

Conditioned place preference

Conditioned place preference was carried out using a biased design (i.e. test compounds were paired with the least preferred compartment and vehicle with the preferred compartment) as described previously (Jerlhag *et al.* 2009) in four identical CPP boxes (TSE Systems, Bad Homburg, Germany), each consisting of two compartments (45 × 22 × 40 cm) separated by a guillotine door and distinguishable by wall and floor patterns as well as floor texture. Time spent in each compartment was automatically recorded by 32 infrared sensors located 2 cm above the compartment floor (ActiMot, TSE Systems).

The CPP experiment consisted of three phases: pre-conditioning (day 1; 15 minutes), conditioning (day 2–5; 60 minutes/session), and post-conditioning test (day 6; 15 minutes). The initial side preference (i.e. the side where the rat spent more than 50% of the time) was determined during the pre-conditioning phase. To comply with the biased design, rats spending less than 51 and

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more than 75% of their time on the preferred side were excluded. During the conditioning phase (guillotine door closed), each rat received two injections with 6 hours apart per day and were randomly assigned to conditioning with either test compound in the morning and vehicle in the afternoon or vice versa. Four conditioning sessions were chosen as three to four sessions are generally used to obtain robust CPP to rewarding substances in rodents (Cunningham *et al.* 2006; Tzschentke 2007). Control rats were paired with vehicle on both sides. During the post-conditioning test, rats were again given free access to both compartments, and time spent in each compartment was recorded. CPP expression was evaluated by comparing time spent in the drug-paired compartment during post-conditioning, with time spent in the same compartment during pre-conditioning. Robust CPP expression has repeatedly been shown following morphine conditioning (Karami and Zarrindast 2008); the ability of the environmental context in our CPP boxes to induce CPP was therefore confirmed using morphine (10 mg/kg, sc, $n = 10$) or saline ($n = 10$) conditioning as described earlier with the exception that the conditioning phase lasted for 30 minutes.

OSU6162's effect on CPP expression was evaluated as described above in both alcohol-naïve rats ($n = 20$) and rats given IA20E for approximately 3 months before the experiment ($n = 20$). During the conditioning phase, OSU6162 (30 mg/kg, sc) or vehicle injections were given 30 minutes before the rats were confined to one compartment. The length of the conditioning session (60 minutes) and the pre-treatment time (30 minutes) was based on our previous studies showing that dopamine output in the NAc reached a maximum at 45 minutes after a systemic OSU6162 (30 mg/kg) injection and was thereafter maintained for more than 60 minutes in alcohol-naïve rats (Steensland *et al.* 2012).

Novel object recognition

Details about the NOR chamber and objects can be found in SI. During the first NOR session, each rat was free to explore the chamber without any objects present during 8 minutes (habituation session). After 12 minutes in the home cage, the rat was placed in the chamber again and allowed to explore two identical objects attached to opposite walls of the chamber for 2 minutes (training session). After an intersession interval of 2 or 24 hours, respectively, the rat was again placed in the chamber and allowed to explore a copy of one familiar object from the training session together with one novel object during five minutes (test session). Object positions and combinations were counter-balanced between each rat. Both training and test sessions were video-recorded and the time rats spent exploring each object was scored manually by a blinded researcher (XNote Stopwatch). During the test session, the discrimination ratio (i.e. total time exploring the novel object divided by the total time exploring both objects) was calculated and because of the natural preference of rats to explore novel objects, predominant exploration of the novel object was regarded as remembrance of the familiar object. Exploration of an object was defined as sniffing, biting or licking the object. Sitting, climbing or leaning on the object without any sign of active exploration was not scored as exploration.

First, we confirmed that a 2-hour intersession interval would allow the rats to remember the familiar object introduced during the training session, during the test session, by comparing the discrimination ratio after a 2-hour interval ($n = 16$) with that after a 24-hour interval ($n = 16$), when natural forgetting occurs (Nilsson and Carlsson 2013). Thereafter, the 2-hour intersession interval was used to test the effects of OSU6162 on memory acquisition. OSU6162 (30 mg/kg, sc, $n = 8$) or vehicle ($n = 8$) was given 60 minutes before the training session, and the discrimination ratio during the test session was compared between the two treatment groups.

Statistical analysis

In the microdialysis experiment, basal dopamine, DOPAC and HVA outputs (fmol) were compared between long-term drinking ($n = 27$) and alcohol-naïve ($n = 7$) rats using the Mann–Whitney *U*-test because assumptions for the *t*-test [equal standard deviations (SD)] were not met.

To analyze the effect of a systemic alcohol challenge on the dopamine output in alcohol-drinking ($n = 7$) versus alcohol-naïve rats ($n = 5$), a two-way repeated-measures ANOVA with condition as the between-subject factor and time as the within-subject factor was used. The absolute values (fmol) of dopamine output were used in the analysis due to significantly different basal dopamine levels in the two conditions (alcohol drinking or alcohol naïve). Following a significant condition and interaction effect, the area under the curve (AUC) (timepoint 0–45 minutes) relative to the value at timepoint 0 (ΔAUC) was calculated and analyzed using unpaired Student's *t*-test to compare the alcohol-induced peak between long-term drinking and alcohol-naïve rats. The basal DOPAC and HVA outputs on the other hand were independent of condition and were thus normalized to percent of respective baseline values (mean of timepoints −90 to −60 minutes) and thereafter analyzed using two-way repeated-measures ANOVA as described for dopamine earlier.

In the alcohol-drinking rats, there were no significant differences in the basal dopamine, DOPAC and HVA (fmol) outputs (mean of timepoints −90 to −60 minutes)

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between the different treatment groups: vehicle–vehicle, OSU6162–vehicle, vehicle–alcohol and OSU6162–alcohol (one-way ANOVA; $n = 6–8$ per group). Thus, data on output of dopamine and metabolites were normalized to percent of baseline and analyzed separately for each treatment group using repeated-measures one-way ANOVA with time as the within-subject factor followed by Fisher's least significant difference *post hoc* test to compare significant changes toward baseline.

To evaluate the effects of OSU6162, compared with vehicle pre-treatment on the alcohol-induced dopamine output, the percent change in dopamine output after the alcohol injection was calculated using the sample immediately before the alcohol injection as reference (timepoint 0 minutes). Data were analyzed with repeated-measures two-way ANOVA with treatment as the between-subject factor and time as the within-subject factor.

Statistical analysis of the CPP data was performed using paired Student's *t*-test within each treatment group as determined a priori. Analysis of the NOR data was performed using unpaired Student's *t*-test within each session.

Average alcohol intake (g/kg per 24 hours) was presented as median and interquartile range (IQR). All other values were presented as mean and standard error of the mean (SEM). The significance level was set at $\alpha = 0.05$. Statistical analysis was performed using the software SPSS version 22 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 (La Jolla, CA, USA).

RESULTS

Ten months of voluntary alcohol drinking induced a hypo-dopaminergic state in the nucleus accumbens

Using microdialysis, basal and alcohol-induced (2.5 g/kg, ip) NAc outputs of dopamine and its metabolites were compared between rats that voluntary had been drinking alcohol for 10 months [4.2 (2.4–4.7) g/kg per 24 hours; median and IQR of their life-time alcohol intake] and age-matched alcohol-naïve rats. The basal dopamine output (expressed as median and IQR in fmol/min) was significantly decreased ($U = 41.5$; $P < 0.05$) in long-term drinking [1.8 (1.2–2.7); $n = 27$] compared with alcohol-naïve rats [2.5 (2.3–3.3); $n = 7$]. In contrast, there was no significant difference between the groups in basal DOPAC [alcohol: 638 (504–734), alcohol-naïve: 691 (582–734); $U = 85.5$; non-significant (ns)] or HVA [alcohol: 243 (199–294), alcohol-naïve: 251 (192–263); $U = 90.5$; ns] output.

Analysis of dopamine output following a systemic alcohol challenge (Fig. 1a) showed an overall main effect of time ($F_{12, 120} = 51.1$; $P < 0.001$), condition [alcohol-drinking ($n = 7$) or alcohol-naïve rats ($n = 5$)] ($F_{1, 10} = 7.3$; $P < 0.05$) and a time*condition interaction ($F_{12, 120} = 2.7$; $P < 0.01$). *Post hoc* analysis showed that the alcohol-induced dopamine peak (ΔAUC, timepoint 0–45 minutes) was significantly lower in alcohol-drinking compared with alcohol-naïve rats (Fig. 1b, $t_{10} = 2.8$; $P < 0.05$). Analysis of DOPAC and HVA outputs following the alcohol challenge revealed no significant differences between

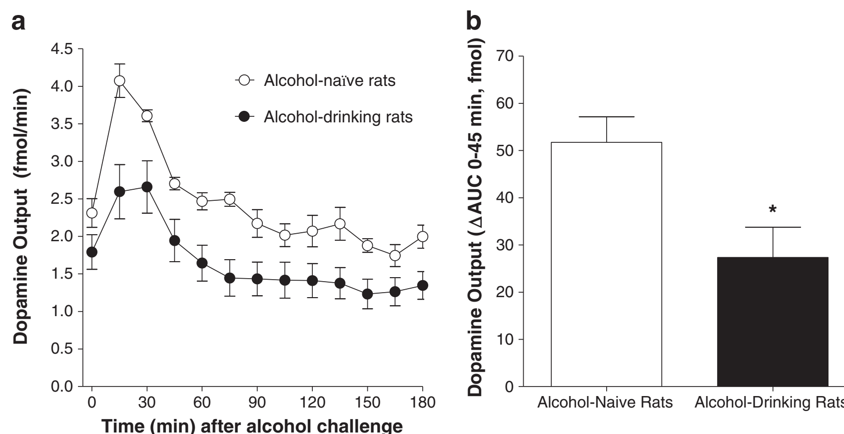

Figure 1 The dopamine output in the nucleus accumbens following a systemic alcohol challenge was blunted in long-term drinking rats. To evaluate the effects of an acute alcohol challenge (2.5 g/kg, ip) on the dopamine output in the nucleus accumbens following long-term voluntary alcohol consumption, microdialysis was applied in awake Wistar rats after 10 months of intermittent access to 20% ethanol in a two-bottle-choice paradigm ($n = 7$) and in age-matched alcohol-naïve rats ($n = 5$). (a) The alcohol challenge affected dopamine output (mean ± SEM, fmol/min) differently in long-term drinking and alcohol-naïve rats as shown by a significant overall condition and a condition * time interaction effect (two-way repeated-measures ANOVA with condition as the between-subject factor and time as the within-subject factor). (b) The alcohol-induced dopamine peak was significantly blunted in the alcohol-drinking compared with alcohol-naïve rats as revealed by comparison of the area under the curve (ΔAUC) between timepoints 0 and 45 minutes in relation to timepoint 0 within each group (Student's *t*-test, *$P < 0.05$ compared with alcohol-naïve rats)

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alcohol-drinking and alcohol-naïve rats (Supporting Information Fig. S1; see statistical details in SI).

(−)-OSU6162 counteracted the hypo-dopaminergic state in the nucleus accumbens induced by long-term voluntary alcohol drinking

The group of long-term drinking rats (with reduced basal dopamine output compared with alcohol-naïve rats, see results earlier) was divided into four treatment groups: vehicle–vehicle ($n = 6$), vehicle–alcohol ($n = 7$), OSU6162–vehicle ($n = 8$) and OSU6162–alcohol ($n = 7$) to evaluate the effects of OSU6162, alone or in combination with alcohol, on dopamine, DOPAC and HVA outputs in the NAc. There was no significant difference between the treatment groups in basal dopamine (1.97 ± 0.22 fmol/min; $F_{3, 23} = 0.2$), DOPAC (649 ± 46 fmol/min; $F_{3, 23} = 0.3$) or HVA (242 ± 22 fmol/min; $F_{3, 23} = 1.2$) output. Thus, the data were normalized and presented as percent of individual baseline. In the vehicle–vehicle group (Fig. S2), there was no overall main effect on dopamine ($F_{16, 80} = 0.9$; ns) or HVA ($F_{16, 80} = 1.1$; ns) outputs. There was, however, an overall main effect on the DOPAC output ($F_{16, 80} = 3.2$; $P < 0.001$), and *post hoc* analysis revealed a significant decrease toward baseline at timepoints 150 and 180 minutes.

In the alcohol–vehicle group (Fig. 2a), there was a significant effect on dopamine ($F_{16, 96} = 18.0$; $P < 0.001$), DOPAC ($F_{16, 96} = 8.6$; $P < 0.001$) and HVA ($F_{16, 96} = 9.3$; $P < 0.001$) outputs. *Post hoc* analysis revealed that the dopamine output was significantly increased at the 15- and 30-minute timepoints compared with baseline, whereas it was significantly decreased compared with baseline from the 90- to 165-minute timepoints. Furthermore, DOPAC output was significantly increased compared with baseline at the 30-minute timepoint, whereas HVA output was significantly elevated from the 30- to 135-minute timepoints compared with baseline.

In the OSU6162–vehicle group (Fig. 2b), there was a significant overall main effect on dopamine ($F_{16, 112} = 4.0$; $P < 0.001$), DOPAC ($F_{16, 112} = 19.6$; $P < 0.001$) and HVA ($F_{16, 112} = 53.6$; $P < 0.001$) outputs. *Post hoc* analysis revealed that dopamine output was significantly elevated compared with baseline immediately after injection (timepoint −45 minutes), reached a maximum 75 minutes after injection (timepoint 15 minutes) and remained significantly elevated throughout the dialysis session (timepoint 180 minutes). DOPAC and HVA were significantly increased compared with baseline from the −30- to 180-minute timepoints.

In the OSU6162–alcohol group (Fig. 2c), there was an overall main effect on the dopamine ($F_{16, 96} = 7.3$; $P < 0.001$), DOPAC ($F_{16, 96} = 17.5$; $P < 0.001$) and HVA ($F_{16, 96} = 29.9$; $P < 0.001$) outputs. *Post hoc* analysis revealed that dopamine output was significantly elevated compared with baseline from the −30- to 60-minute timepoints. Outputs of DOPAC and HVA were significantly elevated compared with baseline at all timepoints after the OSU6162 injection.

To further evaluate the effect of OSU6162 on alcohol-induced dopamine output in the NAc, the percent difference in dopamine output from the timepoint immediately before the alcohol injection (timepoint 0 minute) was compared between the vehicle–alcohol and OSU6162–alcohol treatment groups. There was a significant overall main effect of time ($F_{3.1, 36.7} = 20.1$; $P < 0.001$), but not of treatment ($F_{1, 12} = 0.46$; ns), and no time * treatment interaction ($F_{3.1, 36.7} = 2.6$; $P = 0.067$) on the alcohol-induced dopamine output (Fig. 3). Thus, no *post hoc* analysis was conducted.

(−)-OSU6162 did not induce conditioned place preference in long-term drinking or alcohol-naïve rats

The ability to facilitate a CPP in our CPP boxes was confirmed by showing that morphine-conditioned rats ($n = 8$) spent significantly more time on the morphine-paired side during the post-conditioning session, compared with the same side during pre-conditioning (Fig. 4a, right panel), whereas no such effect was seen in the vehicle-conditioned rats ($n = 9$; Fig. 4a, left panel).

To evaluate possible reinforcing properties of OSU6162, a CPP experiment was conducted in rats that had been drinking alcohol [IA20E; 2.6 (2.1–3.2) g/kg/day] for 3 months as well as in alcohol-naïve rats. The results showed that neither alcohol-naïve nor alcohol-drinking rats (Fig. 4b and c, respectively), conditioned with either OSU6162 or vehicle ($n = 8$–9 per treatment group), showed significant difference in the amount of time spent on the OSU6162-paired or vehicle-paired side, respectively, during post-conditioning compared with the same side during pre-conditioning.

(−)-OSU6162 did not affect memory acquisition in the novel object recognition test

In the NOR test, rats had a significantly higher discrimination ratio following a 2-hour intersession interval, compared with a 24-hour intersession interval (Fig. 5a), confirming that the rats, during the test session, remembered the object introduced during the training session conducted 2 hours earlier. Hence, a 2-hour intersession interval was used to test OSU6162's ability to affect memory acquisition in long-term drinking rats. The results showed no significant difference in total time spent exploring the two objects during the training session between the OSU6162-treated or vehicle-treated rats (Fig. 5b). Both OSU6162-treated and vehicle-treated rats had a discrimination ratio above 0.5 during the test session (with

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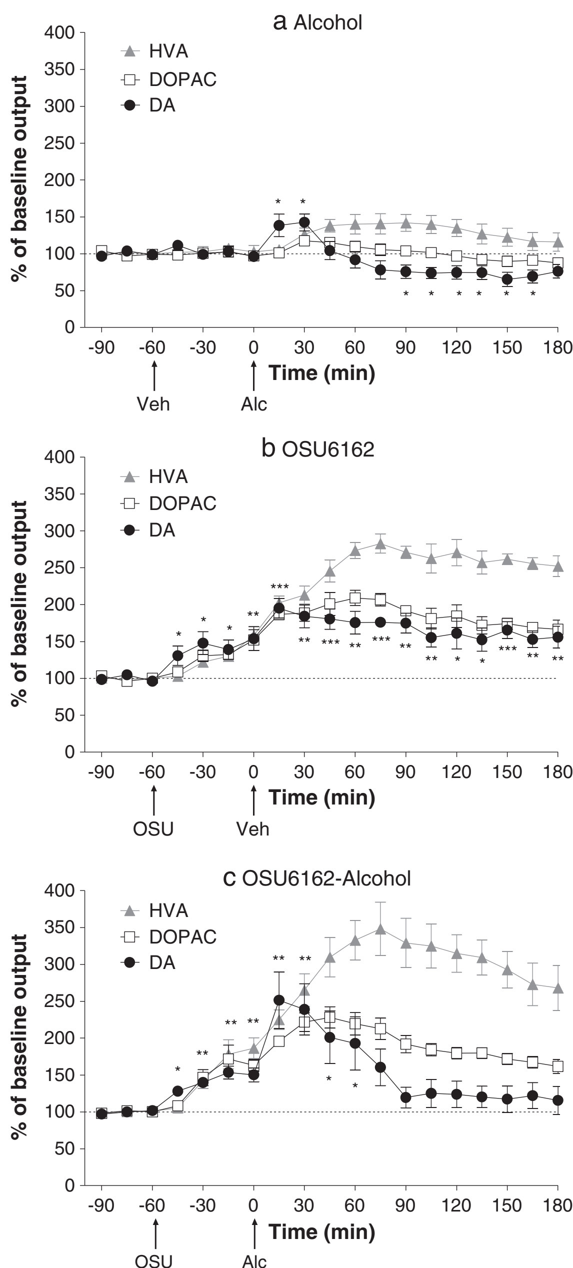

Figure 2 (−)-OSU6162 (OSU6162) counteracts the hypo-dopaminergic state in the nucleus accumbens induced by long-term voluntary alcohol drinking. Microdialysis was used to measure nucleus accumbens output of dopamine and the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) following treatment with vehicle–alcohol (2.5 g/kg, ip) (a), OSU6162 (30 mg/kg, sc)–vehicle (b) and OSU6162–alcohol (c) ($n = 7$–8 per treatment) in awake Wistar rats, which had lower basal dopamine output after 10 months of drinking in the intermittent access to 20% ethanol two-bottle-choice paradigm (during 24-hour withdrawal) than alcohol-naïve age-matched controls. (a) The acute alcohol challenge significantly increased and decreased dopamine output compared with baseline between 15 and 30 minutes and between 90 and 165 minutes after injection, respectively. DOPAC and HVA outputs were significantly increased compared with baseline at 30 and 30–135 minutes following the alcohol challenge, respectively. (b) OSU6162 administration significantly increased and maintained dopamine (from timepoint −45 minutes), DOPAC and HVA (from timepoint −30 minutes) outputs elevated compared with baseline throughout the experiment (i.e. at least 4 hours after the OSU6162 injection). (c) When OSU6162 was administered 60 minutes before the alcohol challenge, dopamine was significantly elevated compared with baseline from −45 to 60 minutes, and DOPAC and HVA outputs were significantly increased compared with baseline at all measured timepoints. Values are presented as percent of respective individual baseline (mean ± SEM), because basal values (fmol/min) did not differ between treatment groups (one-way ANOVA). Data were analyzed using repeated-measures one-way ANOVA within each separate treatment group with time as the within-subject factor followed by Fisher's least significant difference *post hoc* test. Arrows indicate time of injections. Asterisks indicate timepoints when dopamine output was significantly different from baseline (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). To avoid clutter, asterisks were omitted for DOPAC and HVA. OSU, OSU6162; Alc, alcohol; Veh, vehicle

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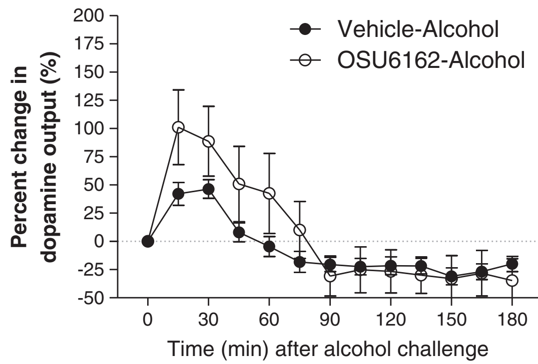

Figure 3 Pre-treatment with (−)-OSU6162 (OSU6162) had no significant effect on alcohol-induced dopamine output in the nucleus accumbens of long-term drinking rats. To evaluate the effects of OSU6162 specifically on the alcohol-induced dopamine output in the nucleus accumbens in the long-term drinking rats, the percent change in dopamine output in relation to the output at the timepoint immediately prior to the alcohol injection (timepoint 0 minutes) was compared between the rats treated with vehicle–alcohol (2.5 g/kg, ip) or OSU6162 (30 mg/kg, sc)–alcohol. There was no significant difference in the OSU6162-pretreated compared with vehicle-pretreated groups (repeated-measures two-way ANOVA with treatment as the between-subject factor and time as the within-subject factor). Values are presented as mean ± SEM ($n = 7$ per treatment)

no significant differences between the treatments; Fig. 5c), indicating a functional memory acquisition.

DISCUSSION

In the present study, we showed for the first time that the monoamine stabilizer OSU6162 counteracts dopamine deficits in the NAc during withdrawal in long-term drinking Wistar rats. Furthermore, we showed that OSU6162 was not rewarding on its own as measured by CPP, and therefore most likely does not possess any abuse liability. Collectively, these findings support our previous study establishing that OSU6162 (at the same dose as evaluated in the present study) attenuates several alcohol-mediated behaviors in long-term drinking rats (Steensland *et al*. 2012) and thus has potential as a novel AUD medication.

The present study indicates that 10 months of voluntary alcohol intake (IA20E) induces a hypo-dopaminergic state in the NAc compared with age-matched alcohol-naïve rats. First, the basal dopamine output (fmol values) was significantly decreased after 24 hours of withdrawal, supporting another microdialysis study showing similar results in Wistar rats after 7 weeks of IA20E (Barak *et al*. 2011). These findings further indicate that the effects of alcohol drinking on the dopamine system are long-lasting, which is in contrast to a previous study showing a decreased dopamine receptor gene expression after 4 months, but not 10 months, of alcohol drinking (Jonsson *et al*. 2014). However, it should be noted that a rather low alcohol intake and the continuous-access schedule might account for the lack of sustained long-lasting effects in the latter study. Second, there was a blunted dopamine response following a systemic alcohol challenge in the long-term drinking compared with alcohol-naïve rats, supporting brain-imaging studies showing reduced central stimulant-induced dopamine release in detoxified alcohol-dependent patients (Martinez *et al*. 2005; Volkow *et al*. 2007). Finally, dopamine output declined below baseline

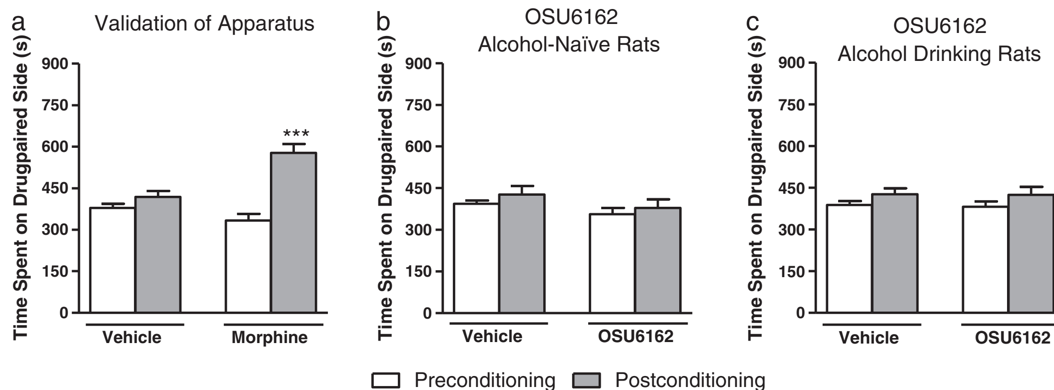

Figure 4 (−)-OSU6162 (OSU6162) did not induce conditioned place preference (CPP) in either long-term drinking or alcohol-naïve rats. To evaluate the reinforcing effects of OSU6162, alcohol-naïve rats and rats that had been drinking alcohol (intermittent access to 20% ethanol) for 3 months prior to the experiment were subjected to the CPP paradigm. (a) First, the CPP boxes were validated by showing that morphine conditioning (10 mg/kg, sc) induced CPP as shown by a significant increased time spent on the morphine-paired side during post-conditioning compared with pre-conditioning. In contrast, there was no expression of CPP in the vehicle-treated rats. OSU6162 (30 mg/kg, sc) and vehicle treatment did not induce CPP in either (b) alcohol-naïve or (c) alcohol-drinking rats. All values are presented as mean ± SEM ($n = 8$–9 per treatment). Data were analyzed using paired Student's *t*-test within each treatment group (comparing time spent on the drug-paired side during post-conditioning with the same side during pre-conditioning); ***$P < 0.001$ compared with corresponding pre-conditioning

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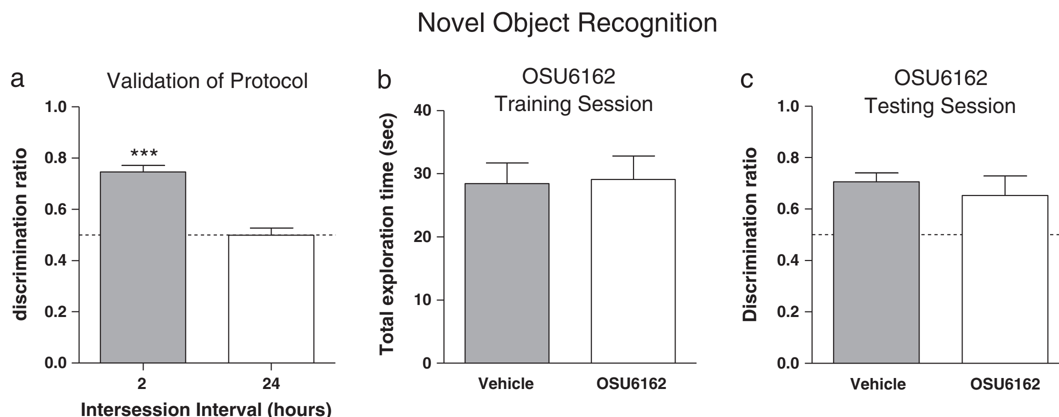

Figure 5 (−)-OSU6162 (OSU6162) did not impair object memory 2 hours after object presentation. Rats were allowed to explore two identical objects for 2 minutes during a training session, and following a 2- or 24-hour intersession interval, rats were presented with one familiar object from the training session and one novel object during the 5-minute testing session. A discrimination ratio (mean ± SEM, exploration of novel object divided by exploration of both objects) above 0.50 during the testing phase indicates remembrance of the familiar object. (a) Rats remembered the familiar object after a 2-hour, but not after a 24-hour, intersession interval ($n = 16$ per condition). (b) Total time spent to explore both objects during the training session was not significantly different between OSU6162-treated (30 mg/kg, sc; $n = 8$, 60 minutes before S1) and vehicle-treated rats ($n = 8$). (c) Using a 2-hour intersession interval, there was no significant difference on discrimination ratio between OSU6162-treated and vehicle-treated rats during the testing session. All values are expressed as mean ± SEM. Data were analyzed using unpaired Student's *t*-test within each session;***$P < 0.001$ compared with the 24-hour intersession interval

values following the cessation of the alcohol-induced dopamine peak. Collectively, the present microdialysis results, showing a hypo-functioning dopamine system following 10 months of voluntary intermittent intake of high amounts of alcohol, provide support for the hypothesis that chronic alcohol consumption contributes to a downregulated dopamine system in alcohol-dependent patients—a state that has been suggested to be associated with dysphoria, which in turn may trigger alcohol craving leading to relapse in AUD patients (Weiss *et al.* 1996; Grace 2000; Diana 2011; Becker and Mulholland 2014).

We have previously established that the monoamine stabilizer OSU6162 attenuates voluntary alcohol drinking, alcohol seeking, cue/priming-induced reinstatement and withdrawal-like symptoms in long-term drinking rats (Steensland *et al.* 2012). In addition, our previous study showed that OSU6162 blunted an alcohol-induced dopamine peak in the NAc of alcohol-naïve rats (Steensland *et al.* 2012). However, the present microdialysis study conducted in long-term drinking rats showed that OSU6162, compared with vehicle pre-treatment, had no significant effect on the alcohol-induced dopamine peak. The contrasting microdialysis results in alcohol-drinking versus alcohol-naïve rats led us to reject our previous hypothesis that OSU6162 attenuates alcohol-mediated behaviors by blunting alcohol's rewarding properties (Steensland *et al.* 2012). Instead, the present results indicate that OSU6162 has the ability to counteract the hypo-dopaminergic state induced by long-term drinking. This hypothesis is supported by the present findings that OSU6162 alone induced a long-lasting increase in NAc dopamine output and that OSU6162 pre-treatment prevented the dopamine output from declining below baseline values following the cessation of the alcohol-induced NAc dopamine peak in the long-term drinking rats. This suggestion is further supported by a previous study showing that rats undergoing alcohol withdrawal self-administer just enough alcohol to return dopamine output back to baseline (Weiss *et al.* 1996) and the hypothesis that a hypo-dopaminergic state during AUD drives alcohol craving, compulsive drinking and increase the risk of relapse (Diana 2011). Thus, it is possible that OSU6162, through its ability to counteract an alcohol-induced hypo-dopaminergic state, attenuates voluntary alcohol-mediated behaviors by diminishing the urge to drink alcohol.

The exact mechanism behind OSU6162's ability to counteract the NAc hypo-dopaminergic state in the long-term drinking rats remains unknown. Nevertheless, OSU6162 has been suggested to enhance dopamine activity by acting as follows: (1) an antagonist at D2 autoreceptors, thereby disinhibiting the negative feedback of dopamine release (Carlsson *et al.* 2004), or (2) an allosteric modulator at the postsynaptic D2 receptors, thereby enhancing the effects of dopamine (Kara *et al.* 2010). A rise in dopamine levels as recorded by microdialysis, following OSU6162 treatment in the present study, indicates either an increased dopamine release or a decreased reuptake of dopamine. The present results showed that OSU6162 slowly increased and maintained both the dopamine and DOPAC output elevated above baseline to a similar extent (an indication of an increase

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of dopamine release instead of a decreased dopamine reuptake), and thus indicate that antagonism at the presynaptic autoreceptor is the most likely mechanism. Moreover, the OSU6162-induced dopamine elevation was more long-lasting in the alcohol-drinking rats in the present study compared with that seen previously in alcohol-naïve rats (Steensland *et al.* 2012), possibly because of an increased D2 autoreceptor sensitivity, which has been shown previously in monkeys following 18 months of voluntary alcohol consumption (Budygin *et al.* 2003) and in mice using the chronic intermittent ethanol vapor exposure paradigm (Karkhanis *et al.* 2015). It should be noted, however, that the alcohol-naïve rat in our previous study (Steensland *et al.* 2012) were a few months younger than the long-term drinking rats in the present study. Thus, the possibility exists that the observed differential effects of OSU6162 in alcohol-naïve versus alcohol-drinking rats may result from age-related differences in the dopamine system. Finally, a recent study using optogenetics showed that tonic (low, but long-lasting), but not phasic (large, but short-lived), dopamine release in the NAc reduced voluntary alcohol intake in rats that had undergone the IA20E procedure for 7 weeks (Bass *et al.* 2013). These results support our overall interpretation that OSU6162's capacity to induce stable, elevated dopamine levels might be the key feature in the compound's ability to attenuate alcohol-mediated behaviors in rats.

With regard to OSU6162's potential therapeutic applicability in a patient population with AUD, it should be noted that the present and previous findings showing that OSU6162 increases the NAc dopamine output in long-term drinking, as well as alcohol-naïve rats, could possibly indicate abuse liability. However, in contrast to the rapid peak typically associated with the reinforcing effects of alcohol and other drugs of abuse (Di Chiara and Imperato 1988; Volkow and Swanson 2003), the OSU6162-induced dopamine rise was slow and long-lasting in both alcohol-drinking and alcohol-naïve rats. In addition, OSU6162 did not induce a CPP in either long-term drinking or alcohol-naïve rats. The possibility that an impaired ability to learn the association between the potential reinforcing effects and the paired chamber was the reason behind the lack of an OSU6162-induced CPP was ruled out by the finding that OSU6162-treated rats readily discriminated between a novel and familiar object in the NOR paradigm. Furthermore, a recent study showed that OSU6162-treatment in fact enhanced object location memory (involving association to spatial cues) (Nilsson and Carlsson 2013), indicating that OSU6162 treatment enhances cognitive properties in a natural forgetting paradigm. Collectively, these findings indicate that OSU6162 most likely does not possess any reinforcing properties on its own and argue against a potential abuse liability.

In conclusion, the present study showing a decreased basal dopamine output in the NAc of long-term drinking Wistar rats strengthens the hypothesis that chronic alcohol consumption induces a hypo-dopaminergic state in AUD patients. Furthermore, we show for the first time that the monoamine stabilizer OSU6162 has the ability to counteract these allostatic changes within the mesolimbic dopamine system induced by cycles of long-term voluntary alcohol consumption and periods of abstinence. Finally, the present results indicate that OSU6162 has no abuse liability. These findings together with our recent 'proof-of-concept' human laboratory study showing that OSU6162 attenuates priming-induced craving and liking of the consumed alcohol in alcohol-dependent patients (unpublished findings from our research group) and the compound's favorable side-effect profile (Johansson *et al.* 2012; Kloberg *et al.* 2014) merit further evaluation of OSU6162's clinical efficacy to attenuate alcohol use in AUD patients.

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Disclosure/Conflict of Interest

None of the authors declare any biomedical financial interests or conflicts of interests.

Authors Contribution

Authors KF, BS and PS designed, analyzed and interpreted the data from the microdualysis experiment. KF and PS designed, analyzed and interpreted the data from the NOR experiment. KF performed the microdialysis and the NOR experiments. IF and PS designed analyzed and interpreted the data the CPP experiments. IF and MW performed the CPP experiments. KF, IF and PS

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wrote the first draft of the manuscript. All authors contributed to and approved the final version of the manuscript.

References

Anton RF, O'Malley SS, Ciraulo DA, Cisler RA, Couper D, Donovan DM, Gastfriend DR, Hosking JD, Johnson BA, LoCastro JS, Longabaugh R, Mason BJ, Mattson ME, Miller WR, Pettinati HM, Randall CL, Swift R, Weiss RD, Williams LD, Zweben A, Group CSR (2006) Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. JAMA 295:2003–2017.

Barak S, Carnicella S, Yowell QV, Ron D (2011) Glial cell line-derived neurotrophic factor reverses alcohol-induced allostasis of the mesolimbic dopaminergic system: implications for alcohol reward and seeking. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 31:9885–9894.

Bass CE, Grinevich VP, Gioia D, Day-Brown JD, Bonin KD, Stuber GD, Weiner JL, Budygin EA (2013) Optogenetic stimulation of VTA dopamine neurons reveals that tonic but not phasic patterns of dopamine transmission reduce ethanol self-administration. Frontiers in Behavioral Neuroscience 7:173.

Becker HC, Mulholland PJ (2014) Neurochemical mechanisms of alcohol withdrawal. Handb Clin Neurol 125:133–156.

Boileau I, Assaad JM, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay RE, Dagher A (2003) Alcohol promotes dopamine release in the human nucleus accumbens. Synapse 49:226–231.

Brunetti M, Di Tizio L, Dezi S, Pozzi G, Grandinetti P, Martinotti G (2012) Aripiprazole, alcohol and substance abuse: a review. Eur Rev Med Pharmacol Sci 16:1346–1354.

Budygin EA, John CE, Mateo Y, Daunais JB, Friedman DP, Grant KA, Jones SR (2003) Chronic ethanol exposure alters presynaptic dopamine function in the striatum of monkeys: a preliminary study. Synapse 50:266–268.

Carlsson ML, Carlsson A, Nilsson M (2004) Schizophrenia: from dopamine to glutamate and back. Curr Med Chem 11:267–277.

Carlsson ML, Burstein ES, Kloberg A, Hansson S, Schedwin A, Nilsson M, Rung JP, Carlsson A (2011) I. In vivo evidence for partial agonist effects of (−)-OSU6162 and (+)-OSU6162 on 5-HT2A serotonin receptors. J Neural Transm 118: 1511–1522.

Carnicella S, Ron D, Barak S (2014) Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. Alcohol 48:243–252.

Cunningham CL, Gremel CM, Groblewski PA (2006) Drug-induced conditioned place preference and aversion in mice. Nat Protoc 1:1662–1670.

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274–5278.

Diana M (2011) The dopamine hypothesis of drug addiction and its potential therapeutic value. Front Psychiatr 2:64.

Fredriksson I, Jayaram-Lindstrom N, Wirf M, Nylander E, Nystrom E, Jardemark K, Steensland P (2015) Evaluation of guanfacine as a potential medication for alcohol use disorder in long-term drinking rats: behavioral and electrophysiological findings. Neuropsychopharmacology 40:1130–1140.

Grace AA (2000) The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. Addiction 95:S119–128.

Heinz A, Siessmeier T, Wrase J, Hermann D, Klein S, Grusser SM, Flor H, Braus DF, Buchholz HG, Grunder G, Schreckenberger M, Smolka MN, Rosch F, Mann K, Bartenstein P (2004) Correlation between dopamine D(2) receptors in the ventral striatum and central processing of alcohol cues and craving. Am J Psychiatry 161:1783–1789.

Hietala J, West C, Syvalahti E, Nagren K, Lehikoinen P, Sonninen P, Ruotsalainen U (1994) Striatal D2 dopamine receptor binding characteristics *in vivo* in patients with alcohol dependence. Psychopharmacology (Berl) 116:285–290.

Hopf FW, Chang SJ, Sparta DR, Bowers MS, Bonci A (2010) Motivation for alcohol becomes resistant to quinine adulteration after 3 to 4 months of intermittent alcohol self-administration. Alcohol Clin Exp Res 34:1565–1573.

Jerlhag E, Egecioglu E, Landgren S, Salome N, Heilig M, Moechars D, Datta R, Perrissoud D, Dickson SL, Engel JA (2009) Requirement of central ghrelin signaling for alcohol reward. Proc Natl Acad Sci U S A 106:11318–11323.

Johansson B, Carlsson A, Carlsson ML, Karlsson M, Nilsson MKL, Nordquist-Brandt E, Rönnbäck L (2012) Placebo-controlled cross-over study of the monoaminergic stabiliser (−)-OSU6162 in mental fatigue following stroke or traumatic brain injury. Acta Neuropsychiatrica 24:266–274.

Jonsson S, Ericson M, Soderpalm B (2014) Modest long-term ethanol consumption affects expression of neurotransmitter receptor genes in the rat nucleus accumbens. Alcohol Clin Exp Res 38:722–729.

Joos L, Goudriaan AE, Schmaal L, Fransen E, van den Brink W, Sabbe BG, Dom G (2013) Effect of modafinil on impulsivity and relapse in alcohol dependent patients: a randomized, placebo-controlled trial. European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology 23:948–955.

Kara E, Lin H, Svensson K, Johansson AM, Strange PG (2010) Analysis of the actions of the novel dopamine receptor-directed compounds (*S*)-OSU6162 and ACR16 at the D2 dopamine receptor. Br J Pharmacol 161:1343–1350.

Karami M, Zarrindast MR (2008) Morphine sex-dependently induced place conditioning in adult Wistar rats. Eur J Pharmacol 582:78–87.

Karkhanis AN, Rose JH, Huggins KN, Konstantopoulos JK, Jones SR (2015) Chronic intermittent ethanol exposure reduces presynaptic dopamine neurotransmission in the mouse nucleus accumbens. Drug Alcohol Depend 150:24–30.

Kloberg A, Constantinescu R, Nilsson MK, Carlsson ML, Carlsson A, Wahlstrom J, Haghighi S (2014) Tolerability and efficacy of the monoaminergic stabilizer (−)-OSU6162 (PNU-96391A) in Huntington's disease: a double-blind cross-over study. Acta Neuropsychiatrica. Officieel Wetenschappelijk Orgaan van Het IGBP (Interdisciplinair Genootschap voor Biologische Psychiatrie) 26:298–306.

Kumakura Y, Gjedde A, Caprioli D, Kienast T, Beck A, Plotkin M, Schlagenhauf F, Vernaleken I, Grunder G, Bartenstein P, Heinz A, Cumming P (2013) Increased turnover of dopamine in caudate nucleus of detoxified alcoholic patients. PLoS One 8e73903:.

Lido HH, Stomberg R, Fagerberg A, Ericson M, Soderpalm B (2009) The glycine reuptake inhibitor org 25935 interacts with basal and ethanol-induced dopamine release in rat nucleus accumbens. Alcohol Clin Exp Res 33:1151–1157.

Litten RZ, Ryan ML, Fertig JB, Falk DE, Johnson B, Dunn KE, Green AI, Pettinati HM, Ciraulo DA, Sarid-Segal O, Kampman

© 2015 The Authors.

K, Brunette MF, Strain EC, Tiouririne NA, Ransom J, Scott C, Stout R, Group NS (2013) A double-blind, placebo-controlled trial assessing the efficacy of varenicline tartrate for alcohol dependence. J Addiction Med 7:277–286.

Malmlöf T, Feltmann K, Konradsson-Geuken A, Schneider F, Alken RG, Svensson TH, Schilstrom B (2015) Deuterium-substituted L-DOPA displays increased behavioral potency and dopamine output in an animal model of Parkinson's disease: comparison with the effects produced by L-DOPA and an MAO-B inhibitor. J Neural Transm 122:259–272.

Martinez D, Gil R, Slifstein M, Hwang DR, Huang Y, Perez A, Kegeles L, Talbot P, Evans S, Krystal J, Laruelle M, Abi-Dargham A (2005) Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. Biol Psychiatry 58:779–786.

Natesan S, Svensson KA, Reckless GE, Nobrega JN, Barlow KB, Johansson AM, Kapur S (2006) The dopamine stabilizers (*S*)-(−)-(3-methanesulfonyl-phenyl)-1-propyl-piperidine [(−)-OSU6162] and 4-(3-methanesulfonylphenyl)-1-propyl-piperidine (ACR16) show high *in vivo* D2 receptor occupancy, antipsychotic-like efficacy, and low potential for motor side effects in the rat. J Pharmacol Exp Ther 318: 810–818.

Nilsson MK, Carlsson ML (2013) The monoaminergic stabilizer (−)-OSU6162 reverses delay-dependent natural forgetting and improves memory impairment induced by scopolamine in mice. Neuropharmacology 75:399–406.

Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J (2009) Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. Lancet 373:2223–2233.

Rung JP, Rung E, Helgeson L, Johansson AM, Svensson K, Carlsson A, Carlsson ML (2008) Effects of (−)-OSU6162 and ACR16 on motor activity in rats, indicating a unique mechanism of dopaminergic stabilization. J Neural Transm 115:899–908.

Schilström B, Nomikos GG, Nisell M, Hertel P, Svensson TH (1998) *N*-methyl-D-aspartate receptor antagonism in the ventral tegmental area diminishes the systemic nicotine-induced dopamine release in the nucleus accumbens. Neuroscience 82:781–789.

Seeman P, Guan HC (2007) Dopamine partial agonist action of (−)OSU6162 is consistent with dopamine hyperactivity in psychosis. Eur J Pharmacol 557:151–153.

Simms JA, Steensland P, Medina B, Abernathy KE, Chandler LJ, Wise R, Bartlett SE (2008) Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. Alcohol Clin Exp Res 32:1816–1823.

Sonesson C, Lin CH, Hansson L, Waters N, Svensson K, Carlsson A, Smith MW, Wikstrom H (1994) Substituted (S)-phenylpiperidines and rigid congeners as preferential dopamine autoreceptor antagonists: synthesis and structure–activity relationships. J Med Chem 37:2735–2753.

Steensland P, Simms JA, Holgate J, Richards JK, Bartlett SE (2007) Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. Proc Natl Acad Sci U S A 104: 12518–12523.

Steensland P, Fredriksson I, Holst S, Feltmann K, Franck J, Schilstrom B, Carlsson A (2012) The monoamine stabilizer (−)-OSU6162 attenuates voluntary ethanol intake and ethanol-induced dopamine output in nucleus accumbens. Biol Psychiatry 72:823–831.

Swift R (2010) Medications acting on the dopaminergic system in the treatment of alcoholic patients. Curr Pharm Des 16:2136–2140.

Tolboom N, Berendse HW, Leysen JE, Yaqub M, van Berckel BN, Schuit RC, Ponsen MM, Bakker E, Hoetjes NJ, Windhorst AD, Carlsson ML, Lammertsma AA, Carlsson A (2015) The dopamine stabilizer (−)-OSU6162 occupies a subpopulation of striatal dopamine D2/D3 receptors: an [C]raclopride PET study in healthy human subjects. Neuropsychopharmacology 40:472–479.

Tupala E, Tiihonen J (2004) Dopamine and alcoholism: neurobiological basis of ethanol abuse. Prog Neuropsychopharmacol Biol Psychiatry 28:1221–1247.

Tzschentke TM (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 12:227–462.

Volkow ND, Swanson JM (2003) Variables that affect the clinical use and abuse of methylphenidate in the treatment of ADHD. Am J Psychiatry 160:1909–1918.

Volkow ND, Wang GJ, Fowler JS, Logan J, Hitzemann R, Ding YS, Pappas N, Shea C, Piscani K (1996) Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcohol Clin Exp Res 20:1594–1598.

Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Jayne M, Ma Y, Pradhan K, Wong C (2007) Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 27:12700–12706.

Weiss F, Parsons LH, Schulteis G, Hyytia P, Lorang MT, Bloom FE, Koob GF (1996) Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:3474–3485.

Wise RA (1973) Voluntary ethanol intake in rats following exposure to ethanol on various schedules. Psychopharmacologia 29:203–210.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Alcohol-injection increases dopamine metabolites in the nucleus accumbens equally in alcohol-drinking and alcohol-naïve rat.

Figure S2 Effects of vehicle injections on output of dopamine and dopamine metabolites in the nucleus accumbens.

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