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The serotonin-2 receptor modulator, (–)-trans-PAT, decreases voluntary ethanol consumption in rats

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Abstract

Serotonin (5-HT) 5-HT_{2C} receptor agonists have shown promise as novel alcoholism pharmacotherapies, but developing selective agonists has been problematic. Female Sprague Dawley rats were given ethanol in a palatable gel vehicle during operant sessions. 5-HT_{2C} receptor modulators (Ro60-0175, SB242,084, and (–)-trans-PAT) were administered before operant sessions. As a control for the effects of 5-HT_{2C} receptor agonism on caloric intake, drugs were also tested using non-ethanol containing gelatin. Ro60-0175, a 5-HT₂ family receptor agonist, decreased both ethanol and vehicle responding while (–)-trans-PAT, a 5-HT_{2C} receptor agonist with 5-HT_{2A-2B} receptor inverse agonist activity, selectively reduced only ethanol responding. The effect of 5-HT_{2C} receptor agonists on self-administration after reinstatement of ethanol after a three week deprivation was also determined. (–)-trans-PAT eliminated increases in ethanol intake following ethanol deprivation whereas Ro60-0175 had no effect. These results emphasize the need for caloric controls and further support the idea that selective modulation of 5-HT₂ family receptors is a potential pharmacotherapeutic approach in the treatment of alcoholism.

Keywords

Ethanol; serotonin 2C receptor; alcoholism; operant self-administration; alcohol deprivation

1. Introduction

Agonism of serotonin (5-hydroxytryptamine, 5-HT) 5-HT_{2C} receptors has been shown to decrease voluntary ethanol consumption in rats (Tomkins et al., 2002). However, as evidenced by the recent FDA approval of lorcaserin for weight loss, 5-HT_{2C} receptor agonism also has anorexiatic effects (Garfield and Heisler, 2009). The 5-HT_{2C} receptor agonist Ro60-0175 demonstrates only a 2–3-fold dose separation for inhibition of ethanol

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consumption versus food consumption (Higgins and Fletcher, 2003). Additionally, Ro60-0175 has only 30-fold selectivity for activating the 5-HT_{2C} receptors over the structurally related 5-HT_{2A} receptors (Porter et al., 1999), which cause unwanted psychotomimetic effects (Nichols, 2004). Thus it is important to test selective 5-HT_{2C} receptor agonists and assess their inhibitory effects on ethanol self-administration compared to that of other caloric substances.

There are currently no commercially-available agonists specific for activation of 5-HT_{2C} receptors (i.e., without activation of 5-HT_{2A} receptors or 5-HT_{2B} receptors) due, in part, to about 75% identical sequence homology in transmembrane binding regions, and similar second messenger signaling between 5-HT₂ receptor subtypes (Julius et al., 1988; 1990). We recently reported a unique 5-HT₂ receptor modulator, (–)-trans-4-phenyl-2-*N,N*-dimethylaminotetralin ((–)-trans-PAT), that demonstrates agonism at 5-HT_{2C} receptors together with inverse agonism at 5-HT_{2A-2B} receptors (Booth et al., 2009), suggesting its suitability for development as pharmacotherapy for alcoholism.

The “jello shot” model of ethanol reinforcement uses 10% ethanol containing gel (Rowland et al., 2005) and is easily adapted for use in operant chambers (Li et al., 2008; 2010). This model results in highly stable self-administration of about 1.0 g/kg body weight of ethanol within a 30 minute period, without the need for food or water restriction, and produces peak brain ethanol levels between 5–10 mM (Peris et al., 2006). Non-selective effects of possible therapeutic drugs (e.g. inhibition of caloric intake, inhibition of operant behavior, sedation) can be assessed on self-administration of plain gel lacking ethanol (Li et al., 2010). This method is similar to the beer and near beer studies (McGregor and Gallate, 2004) with plain gel acting as near beer. The advantage to this technique is that both plain gel and ethanol gel have similar taste, texture, novelty, and both can serve as strong reinforcers. An ideal pharmacotherapy would decrease only ethanol gel responding without affecting response rates for plain gel.

Besides simply decreasing the reinforcement value of ethanol, an ideal alcoholism pharmacotherapy would also attenuate elevated ethanol consumption caused by increased ethanol craving. During ethanol deprivation, human alcoholics find craving to be a leading cause of relapse (Martin-Fardon and Weiss, 2013). In animals, when ethanol is reintroduced following a period of abstinence, consumption will temporarily increase compared to basal levels (Rodd, et al. 2004).

The aim of this study was to test if 5-HT₂ family receptor agonist Ro60-0175 and the selective 5-HT_{2C} receptor agonist and 5-HT_{2A} receptor antagonist, (–)-trans-PAT decrease voluntary ethanol consumption, motivation for ethanol-reinforced responding, and the severity and duration of post deprivation increases in consumption. Additionally, the selective 5-HT_{2C} receptor antagonist SB242,084 (Kennett et al., 1997) was used to confirm the role that 5-HT_{2C} receptor agonists play in both ethanol and caloric reinforcement.

2. Methods

2.1 Animals and housing

Female Sprague-Dawley rats (90 days old), weighing about 275 g at the start of the studies, were individually housed in plastic cages with food and water available *ad libitum* throughout the study, except for the time spent in the operant chambers. Female rats were used since they maintain more stable body weight over time compared to male rats. Body weight was recorded weekly. Rats were maintained on a 12-h light/dark cycle in an environmentally-controlled room (lights on: 0600, temperature: 23±3 C, humidity: 45±25%) and allowed a 1-week acclimation period to the animal facilities prior to the start of the

studies. All studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2 Behavioral apparatus

Each of the operant chambers and accessories were located inside a sound-attenuating isolation cubicle (Coulbourn Instruments, Allentown, PA). Operant chambers consisted of a grid floor, solid walls, and measured 50.8 cm W × 25.4 cm D × 30.5 cm H. A drinking spout protruded into the chamber and was affixed behind an optical lickometer, which tracked each time a rat licked the drinking spout. Two levers symmetrically flanked the spout on either side of the same wall. The drinking spout was connected to tubing that affixed to a 20 ml syringe filled with 10% ethanol or plain gel on a pump located outside the operant chamber, but inside the sound-attenuating isolation cubicle. Gel-containing syringes were refrigerated between daily operant sessions. Gel delivery and behavioral data collection were controlled by Graphic State 5.2 operating software (Coulbourn Instruments, Allentown, PA).

2.3 Reinforcers

Ethanol gel was composed of 8% Polycose (Abbott Laboratories, Abbott Park, IL), 10% ethanol, and 2.5% gel (Knox brand, Kraft Foods, Northfield, IL), all by weight in water. Plain gel containing Polycose and gel at the above concentrations but without ethanol added was used as a vehicle control. The standard amount of gel delivered per reinforcement was 0.15 g over 5 seconds for fixed ratio and 0.28 g over 10 seconds for progressive ratio experiments.

2.4 Drugs

Ro60-0175 ((S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 C₄H₄O₄) and SB242,084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbonyl]indoline) were purchased from Tocris, Bristol UK, and the novel 5-HT_{2C} agonist/5-HT_{2A-2B} inverse agonist (2*S*, 4*R*)-(-)-*trans*-4-phenyl-2-*N,N*-dimethylaminotetralin ((-)-*trans*-PAT) (Booth et al., 2009) was synthesized in our laboratory (Vincek and Booth, 2009). Ro60-0175 and (-)-*trans*-PAT were dissolved in 0.9% saline. SB242,084 was prepared in a 0.9% saline solution containing 8% w/v hydroxypropyl- β -cyclodextrin and 25 mM citric acid. All drug doses are expressed as that of the salt.

2.5 Statistical analysis

Gel consumption data was expressed as grams of gel per kilogram of body weight while ethanol consumption was converted to grams of ethanol per kilogram of body weight. All data were analyzed by one, two, and three way repeated measures ANOVA or Friedman test (for breakpoint studies) using SPSS software. Main factors included drug treatment, days, and ethanol deprivation (when applicable). Post hoc comparisons were carried out with Bonferroni tests. In all cases, the accepted levels of significance were taken at $P < 0.05$.

2.6 Ethanol consumption experimental procedures

Initially, all rats were given free access to ethanol gel in their home cages. The ethanol gel was presented in a 50 ml glass jar hung from a metal stirrup (see Peris et al., 2006). At first, the ethanol gel was present for 48 hours and, over 7 days, shortened to one hour per day, as described previously (Li et al., 2008).

After consumption of ethanol gel during daily one-hour free access sessions became stabilized (approximately two weeks; less than 15% day to day variability in consumption), rats were placed in the operant chambers with 0.3 ml of ethanol gel placed on the edge of the

active lever and a 30 minute fixed ratio 1 session was started. This “shaping” procedure occurred only on the first day of operant training. Only responses on the active levers resulted in gel delivery (0.15 g) and illumination of the house lights for 5 seconds. The fixed ratio 1 schedule was maintained for 10 days, after which the rats were placed on 30 minute fixed ratio 5 schedules (five active lever presses yielded one gel delivery) for 10 days. All operant sessions occurred during the light period at approximately the same time each day. Responses on both the active and inactive levers were monitored, as well as the number and times of gel deliveries and licking behaviors. Any gel spillage was monitored and the weights of spilled gel (measured to the nearest 0.01 g) were subtracted from the session total. Spillage occurred mostly during fixed ratio 1 responding and was negligible under fixed ratio 5 schedules.

At this point, animals (n = 10) began acclimating to drug administration procedures by receiving once daily s.c. and i.p. vehicle injections 30 and 50 minutes before the operant session. When the response patterns again stabilized, the effects of the different drug treatments on ethanol self-administration behavior were evaluated. Four treatments were implemented: Ro60-0175 at two doses (0.5 and 1 mg/kg s.c., 30-minute pretreatment), SB242,084 (0.5 mg/kg i.p., 50-minute pretreatment) in combination with Ro60-0175 (0.5 mg/kg s.c., 30-minute pretreatment), and SB242,084 (0.5 mg/kg i.p., 50-minute pretreatment) alone. A Latin square design was employed, to reduce total animal use, such that each animal received each dose in a balanced order. Each treatment day was separated from the next by at least 3 days during which the animals received saline injections prior to operant sessions. After all rats received each drug treatment, the reinforcer for daily sessions was switched to plain gel for all rats. When responding for plain gel stabilized (about one week), the Latin square design was repeated to determine the effects of the same four drug treatments on plain gel consumption.

Using the same group of rats, the Latin-Square procedure was then repeated in order to measure the effects of different doses of (–)-trans-PAT (0.5, 1, 5 and 10 mg/kg i.p., 20-minute pretreatment) on ethanol and plain gel self-administration behavior. A new groups of rats (n = 6) were used to test the effects of (–)-trans-PAT (5 mg/kg), SB242,084 (0.5 mg/kg), and the combination of both, again in a Latin-Square design.

Progressive ratio responding was examined using two groups (n = 4–5) responding for 0.28 g of ethanol gel on a progressive ratio 10 schedule. A progressive ratio 10 schedule indicates that within each session, delivery of the first reinforcer required 10 active bar presses, the second reinforcer required 20 presses and so forth. The last completed ratio was taken as the breakpoint. In keeping with methodology used in the fixed ratio schedules, progressive ratio sessions were 30 min in length regardless of the rats’ performances. However, there was very little responding during the last 15 min of any session. Rats were injected with 0.5 or 1.0 mg/kg Ro60-0175 30-minute prior to the operant session. Four days later, these same animals were randomized, divided into two groups, and given 5 or 10 mg/kg (–)-trans-PAT 20-minute before operant responding. This procedure was repeated with the same rats after switching them to plain gel reinforcement, again using a progressive ratio 10 schedule.

2.7 Ethanol deprivation experimental procedures

An ethanol deprivation procedure was used to investigate the therapeutic potential of anti-alcoholism treatments. Rats (n = 24) were trained to respond for ethanol gel using a fixed ratio 5 schedule, as described above. Animals were then divided into four groups (n = 6): the non-deprived saline control group, the non-deprived 5 mg/kg (–)-trans-PAT group, the ethanol-deprived saline control group, and the ethanol-deprived 5 mg/kg (–)-trans-PAT group. Rats were habituated to saline injections 20 minutes before every operant session. The experimental timeline included 10 days of all groups responding for ethanol gel to

establish baseline consumptions for each group. Then the two ethanol-deprived groups responded for plain gel for 3 weeks while the non-deprived animals continued to respond for ethanol gel. After the 3-week deprivation, all animals responded for ethanol gel again and this day was called the reinstatement day. The reinstatement day also marked the day where two groups of animals received (–)-trans-PAT injections 20-minute prior to the start of the operant sessions while the other two groups continued to receive daily saline. All four groups continued responding for ethanol gel for 15 days after reinstatement to observe the duration of any deprivation-induced changes in ethanol consumption.

At this point, the 5-week long experiment was repeated using the same group of rats with Ro60-0175 (0.5 mg/kg, s.c.) instead of (–)-trans-PAT. Animals that were non-deprived in the (–)-trans-PAT experiment remained non-deprived during the Ro60-0175 experiment and likewise for the deprived animals. However, to minimize bias in the Ro60-0175 study due to the animals' drug histories, half of all animals in each group were those that had previously received (–)-trans-PAT administration while the other half had received saline injections.

3. Results

3.1 Effect of Ro60-0175 and SB242,084 on ethanol and plain gel consumption

Average basal consumption before any drug treatment was 8.5 ± 2.1 (average \pm standard error of the mean) grams of ethanol-containing gel per kilogram of body weight (equal to 0.85 g ethanol/kg body weight). Ro60-0175, the 5-HT₂ family receptor agonist, caused a significant decrease in daily voluntary ethanol gel consumption at both 0.5 and 1.0 mg/kg (Fig. 1A). The 5-HT_{2C} receptor antagonist SB242,084 significantly increased ethanol gel consumption to 13 ± 1.9 g/kg (1.3 g ethanol/kg). When both SB242,084 and Ro60-0175 were administered, there was no significant change in ethanol gel consumption compared to saline treatment. ANOVA indicated significant effects of days ($F(3,36) = 6.6, P < 0.01$), drug treatment ($F(3,36) = 6.3, P < 0.01$), and an interaction of days by drug treatment ($F(9,144) = 9.6, P < 0.001$).

When Ro60-0175 and SB242,084 were tested on operant responding for plain gel (basal consumption = 18 ± 2.6 g/kg), Ro60-0175 caused a significant decrease in plain gel consumption at both 0.5 and 1.0 mg/kg (Fig. 1B). The 5-HT_{2C} receptor antagonist, SB242,084, had no effect on plain gel consumption compared to basal, both when given alone and when co-administered with Ro60-0175. ANOVA indicated significant effects of days ($F(3,36) = 5.0, P < 0.01$), drug treatment ($F(3,36) = 7.0, P < 0.01$), and an interaction of days by drug treatment ($F(9,144) = 11.5, P < 0.001$).

3.2 Effect of (–)-trans-PAT and SB242,084 on ethanol and plain gel consumption

The average basal ethanol gel consumption during testing of the (–)-trans-PAT dose response curve was 12 ± 2.2 g/kg. The 5-HT_{2C} receptor agonist/5-HT_{2A-2B} receptor inverse agonist, (–)-trans-PAT, caused a dose-dependent decrease in ethanol gel consumption (Fig. 2A). The lowest dose of (–)-trans-PAT that caused a significant 30 % decrease was 5 mg/kg. ANOVA found a significant effect of days ($F(3,36) = 2.6, P < 0.05$) and an interaction of days by drug treatment ($F(3,144) = 2.6, P < 0.05$), but no significant effect of drug treatment alone ($F(3,36) = 2.6, P = 0.07$). On the other hand, (–)-trans-PAT did not alter plain gel consumption at any dose (Fig. 2B). ANOVA indicated no significant effects of days ($F(3,36) = 1.6, P = 0.22$), drug treatment ($F(3,36) = 1.1, P = 0.36$), or interaction between days by drug treatment ($F(9,144) = 0.38, P = 0.94$).

The average ethanol gel consumption was 14 ± 3.1 g/kg for rats tested using the combination of (–)-trans-PAT and SB242,084. (–)-trans-PAT administered at 5 mg/kg caused a significant decrease in ethanol consumption of about 50% while SB242,084 at 0.5

mg/kg significantly increased ethanol gel consumption by about 30% (Fig. 3). Animals receiving both (–)-trans-PAT and SB242,084 showed no significant change from basal consumption. ANOVA demonstrated significant effects of drug ($F(2,15) = 6.8, P < 0.05$) and a days by drug interaction ($F(6,30) = 9.4, P = 0.001$), but no significant effect of days ($F(1,5) = 0.07, P = 0.80$).

3.3 Effect of Ro60-0175 and (–)-trans-PAT on ethanol and plain gel breakpoints

Average breakpoints for ethanol gel (65.3 ± 11.6) and plain gel (67.1 ± 16.3) were similar and stable on a day to day basis (data not shown). Because of high basal breakpoint variability between individual rats, data are expressed as a percent of each rat's basal performance. At 1.0 mg/kg, Ro-60-0175 decreased both ethanol gel (to $68.3 \pm 11.1\%$ of basal) and plain gel (to $79.2 \pm 4.8\%$ of basal) breakpoints (Fig. 4), though it did not have significant effects at a dose of 0.5 mg/kg. On the other hand, at 10 mg/kg, (–)-trans-PAT decreased breakpoints for ethanol gel (to $70.4 \pm 4.1\%$ of basal) but not plain gel (to $106.3 \pm 7.2\%$ of basal). Repeated measures ANOVA of breakpoints, revealed a significant main effect of days ($F(1,32) = 4.3, P < 0.05$) and significant interactions of days by drug treatment ($F(3,13) = 19.0, P < 0.001$), days by gel type ($F(1,64) = 10.2, P < 0.05$), and days by drug treatment by gel type ($F(3,52) = 3.7, P < 0.05$). Breakpoints for all rats returned to basal the day following drug administration and remained stable for the following 4 days (data not shown).

3.4 Effect of Ro60-0175 on deprivation-induced increases in ethanol consumption

Prior to ethanol deprivation, rats demonstrated a stable baseline of about 1.0 g/kg of ethanol consumption (Fig. 5). When rats underwent a 3-week period of responding for plain gel, the level of ethanol self-administration was higher upon reaccess to ethanol gel (20–40% increase on Post 1–3) compared to non-deprived rats (Fig. 5A and B). Systemic administration of Ro60-0175 on the first day of ethanol gel reaccess produced differing effects on ethanol consumption, depending on ethanol deprivation. In non-deprived rats, Ro60-0175 reduced ethanol consumption by about 30% compared to non-deprived rats that received saline (Fig. 5A) with ethanol self-administration returning to normal levels on subsequent days. In the deprived rats, Ro60-0175 did not significantly alter ethanol consumption compared to saline-injected ethanol-deprived rats on either the experimental day or any day thereafter. Three-way ANOVA showed a significant main effect of ethanol deprivation ($F(1,5) = 10.9, P < 0.05$) but no main effects or interactions involving drug treatment.

3.5 Effect of (–)-trans-PAT on deprivation-induced increases in ethanol consumption

Prior to ethanol deprivation, rats demonstrated a stable baseline of about 1.2 g/kg of ethanol consumption (Fig. 6). In the non-deprived rats, (–)-trans-PAT decreased ethanol consumption by about 40% (Fig. 6A) on the experimental day (1.2 ± 0.37 g/kg to 0.69 ± 0.03 g/kg) with a return to baseline consumption the next day. Rats that experienced a 3-week ethanol deprivation period again showed elevation of ethanol self-administration by 30–60% during the first five days after reaccess to ethanol gel (Fig. 6B). When (–)-trans-PAT was injected on the day of reinstatement, there were decreases in consumption comparable to that caused by (–)-trans-PAT in non-deprived rats (Fig. 6). Ethanol consumption in (–)-trans-PAT-treated ethanol-deprived rats returned to basal levels the days following reaccess and was significantly reduced compared to that in the saline-treated ethanol-deprived rats (Fig. 6B).

Three-way ANOVA indicated that there were significant effects of deprivation ($F(1,11) = 24, P < 0.05$), drug treatment ($F(1,11) = 1800, P < 0.001$), and days ($F(4,25) = 36, P < 0.05$). All interactions were also significant, including deprivation by drug ($F(3,20) = 34, P$

< 0.05), deprivation by days ($F(9,110) = 21, P < 0.05$), drug treatment by days ($F(9,110) = 23, P < 0.05$), and deprivation by drug treatment by days ($F(19,230) = 7.1, P < 0.05$). Follow-up ANOVA of data from just the non-deprived animals found a significant effect of drug treatment ($F(1,5) = 190, P < 0.001$) and a significant interaction between days by drug treatment ($F(9,110) = 9.2, P < 0.05$), but no effect of days alone ($F(4,19) = 3.2, P = 0.054$). Bonferroni post-hoc tests showed that the only significant difference between (–)-trans-PAT- and saline-treated non-deprived animals was on the experimental day. Follow-up ANOVA of data from only the ethanol-deprived rats indicated significant effects of drug treatment ($F(1,5) = 100, P < 0.001$), days ($F(4,19) = 30, P < 0.001$), and an interaction of days by drug treatment ($F(9,110) = 8.0, P < 0.05$).

4. Discussion

4.1 5-HT_{2C} receptor agonists modulate ethanol consumption

These results support the findings of Tomkins et al. (2002) using the nonspecific 5-HT_{2C} receptor agonist Ro60-0175; activation of 5-HT_{2C} receptors decreases voluntary consumption and motivation for ethanol-containing gel. However, Ro60-0175 also decreased plain gel consumption at doses where Ro60-0175 is known to decrease normal chow consumption (Higgins and Fletcher, 2003). Meanwhile, the putatively selective 5-HT_{2C} receptor antagonist SB242,084 increased ethanol gel responding as well as blocked Ro60-0175's decrease of both ethanol and plain gel consumption. Clearly, interpretation of results using Ro60-0175 is confounded due to the overlapping 5-HT₂ receptor activities of Ro60-0175 (and perhaps SB242,084) that may translate to effects on both caloric and ethanol intake. Possibly, a dose of Ro60-0175 lower than 0.5 mg/kg used in the current studies may specifically decrease ethanol consumption relative to plain gel (and, presumably, chow), however, statistical significance would be difficult to establish given the modest 40% reduction of ethanol and plain gel consumption at 0.5 mg/kg observed here.

The 5-HT_{2C} receptor-specific agonist (and 5-HT_{2A-2B} receptor inverse agonist) (–)-trans-PAT produced a dose-dependent specific decrease in ethanol gel consumption on both fixed and progressive ratio schedules of reinforcement. The lack of an effect of (–)-trans-PAT on plain gel responding was surprising since previous studies using mice found that (–)-trans-PAT decreased consumption of “dessert” feeding in a compulsive eating behavioral model at 5 mg/kg (Rowland et al., 2008). It is not clear whether this difference is due to a species effect or to the conditions of the behavioral assay. The composition of the “crunchies” used by Rowland et al., (2008) differs considerably from the composition of the plain gel in terms of having higher sugar content as well as the presence of other carbohydrates. Thus, while activation of 5-HT_{2C} receptors may be able to inhibit all types of food consumption particularly when combined with agonism of 5-HT_{2A-2B} receptors, selective activation of 5-HT_{2C} receptors combined with antagonism or inverse agonism of 5-HT_{2A-2B} receptors results in a more selective effect on consumption of only certain types of food, perhaps those that support more compulsive eating patterns. Thus, the 5-HT_{2A} receptor inverse agonist effects of (–)-trans-PAT may be important in treating addiction (Bubar and Cunningham, 2008) as well as other compulsive behavioral disorders such as binge eating (Simansky, 2005). Activation of 5-HT_{2A} receptors is associated with the reinforcing effects of ethanol in rats (Ding et al., 2009) as well as psychostimulant craving (Bubar and Cunningham, 2008) and it is possible that (–)-trans-PAT's combined blockade of 5-HT_{2A} receptors with agonism of 5-HT_{2C} receptors provides for a more potent action against drug craving. In any event, there was no evidence of a general anorexic effect of (–)-trans-PAT observed here in rats. Furthermore, the ability of (–)-trans-PAT to reduce ethanol gel consumption without affecting plain gel responding indicates the drug effects are not due to drug-induced sedation, cognitive dysfunction or similar neurobehavioral activity that would otherwise render rats unable to perform the bar-pressing procedures regardless of reinforcement type.

Previous studies have seen no effect of (–)-trans-PAT on locomotor activity in mice (Canal et al., 2013). This is important given (–)-trans-PAT's previously studied role as a histamine 1 receptor antagonist (Booth et al., 2002), which might have led to sedation as a side effect.

It is possible that administration of serotonin agents in this study simply shifted the dose-response curve for ethanol intoxication to the left, resulting in less ethanol consumption but equal or greater intoxication. To address this, we tested both Ro60-0175 and (–)-trans-PAT on responding for ethanol and plain gel using a progressive ratio 10 schedule of reinforcement. The breakpoints for ethanol gel and plain gel were similar, with an average breakpoint of 65 bar presses. For ethanol gel, this resulted in total consumption of less than 0.3 g/kg ethanol, far below sedative levels. Both Ro60-0175 and (–)-trans-PAT decreased breakpoints for ethanol gel while only Ro60-0175 decreased breakpoints for plain gel. If either drug were shifting the dose-response curve for ethanol (or food) reinforcement to the left, then breakpoints would have been increased by drug exposure. Thus, (–)-trans-PAT is capable of decreasing motivation for ethanol responding, even at non-intoxicating levels. That (–)-trans-PAT decreases the effort rats are willing to exert in order to receive ethanol implies a decreased reward potency of ethanol (Roane, 2008) but not plain gel reinforcement.

Ethanol deprivation effects were demonstrated using the “jello shot” model of ethanol intake, such that a 3 week period of ethanol deprivation produced a 3 to 5 day long increase in ethanol consumption of about 30–50%. While the shape of the post deprivation consumption is not typical of those more commonly found in the literature (Martin-Fardon and Weiss, 2012; Rodd et al., 2004), the delayed onset and prolonged duration observed in this study is not without precedent (Schroeder et al., 2005). It is unclear why some ethanol consumption models produce different deprivation patterns, but pharmacological intervention that decrease any deprivation-induced increases in ethanol consumption show promise as a pharmacotherapy.

Though 0.5 mg/kg Ro60-0175 was as effective as 5 mg/kg (–)-trans-PAT in decreasing ethanol consumption (Figs. 1 and 2), Ro60-0175 only had a modest and non-significant effect on increases in ethanol consumption seen in ethanol-deprived rats. It was surprising that Ro60-0175 did not produce a significant effect of days given how well Ro60-0175 reduced non-deprived ethanol consumption seen in Fig. 1. Ro60-0175's lack of significant days main effect may be due to the inclusion of both deprived and non-deprived animals in the statistical test, the smaller number of animals used in each treatment group, dose of Ro60-0175, or repeated deprivation model. Based on Ro60-0175's nonselective effect on gel consumption, we did not pursue these experiments.

However, (–)-trans-PAT reduces the increase in ethanol consumption that occurs after 3 weeks of ethanol deprivation. Surprisingly, the effects of one injection of (–)-trans-PAT were capable of eliminating any increases in ethanol consumption for the entire week following reinstatement of ethanol responding. This suggests that functionally selective 5-HT_{2C} receptor agonists, e.g. (–)-trans-PAT, demonstrate a pharmacological profile that may have therapeutic potential that appears to be long-lasting in nature.

4.2 Criticisms of the “jello shot” model of ethanol consumption

It should be noted that the “jello shot” model of ethanol consumption has some disadvantages. Although responding for plain gel can serve as an appropriate control for some confounding variables, it is difficult to completely equate the palatability and caloric values of the ethanol gel with the gel vehicle, so that only the rewarding effects of ethanol are what differentiate these two reinforcers. Palatability concerns can be addressed in future experiments by changing Polydose concentration or by quinine adulteration (Fachin-Scheit

et al., 2006). Additionally, while (–)-trans-PAT alone may not cause sedative or cognitive effects, interactions between ethanol and (–)-trans-PAT may have synergistic actions that only occur when both PAT and ethanol are present. These effects would not be present when measuring the response of (–)-trans-PAT on plain gel. Although this question needs to be addressed more fully, we have not seen evidence of (–)-trans-PAT increasing the sedative properties of ethanol (data not shown) while we show evidence of (–)-trans-PAT inhibiting consumption of non-sedating doses of ethanol.

The “jello shot” model of ethanol consumption can also be criticized for lower consumption levels of ethanol gel compared to plain gel during fixed ratio 5 operant responding. This raises concerns about differing reward values of the two gels. Rat self-titration of ethanol is a common occurrence in a variety of models (Becker, 2013) and it is no surprise that rats self titrate to 1.0 g/kg during a 30 minute operant session in this model. These rats, however, are able to consume much more of the plain gel compared to the ethanol gel because appetitive titration has a much higher threshold than ethanol titration. This makes it difficult to make direct comparisons between changes in ethanol and plain gel consumption, unless the drug treatment specifically alters ethanol gel consumption without changing consumption of plain gel.

Finally, the “jello shot” model of ethanol consumption can only serve as an index of the reinforcement properties of ethanol and in no way serves as a model of alcoholism. Even under deprivation conditions, where the reward value of ethanol appears to be increased, this model still does not approximate the degree of ethanol craving that would be expected of an abstinent alcoholic patient. To further explore the pharmacotherapeutic value of 5-HT_{2C} receptors, a variety of ethanol intake models will need to be tested, such as alcohol preferring animals (Bell et al., 2006) or drinking in the dark (Rhodes et al., 2005).

5. Conclusions

The inhibitory effects of novel drugs on both fixed and progressive ratio schedules of ethanol reinforcement as well as blockade of deprivation-induced increases in ethanol consumption are important for studying the drug’s pharmacotherapeutic value (Lê and Shaham, 2002). (–)-trans-PAT, a 5-HT_{2C} receptor specific agonist with 5-HT_{2A-2B} receptor inverse agonism, selectively decreased ethanol titration and motivation, and prevents the increase in ethanol consumption seen after a period of ethanol deprivation. However, the non-selective 5-HT_{2C} receptor agonist, Ro60-0175 altered consumption and reward value of both ethanol and plain gel but did not prevent the deprivation-induced increases in ethanol consumption. In summary, serotonin 5-HT₂ receptor modulators that combine 5-HT_{2C} receptor agonist activity together with 5-HT_{2A-2B} receptor inverse agonism, such as (–)-trans-PAT, show promise as a novel pharmacotherapy for alcoholism.

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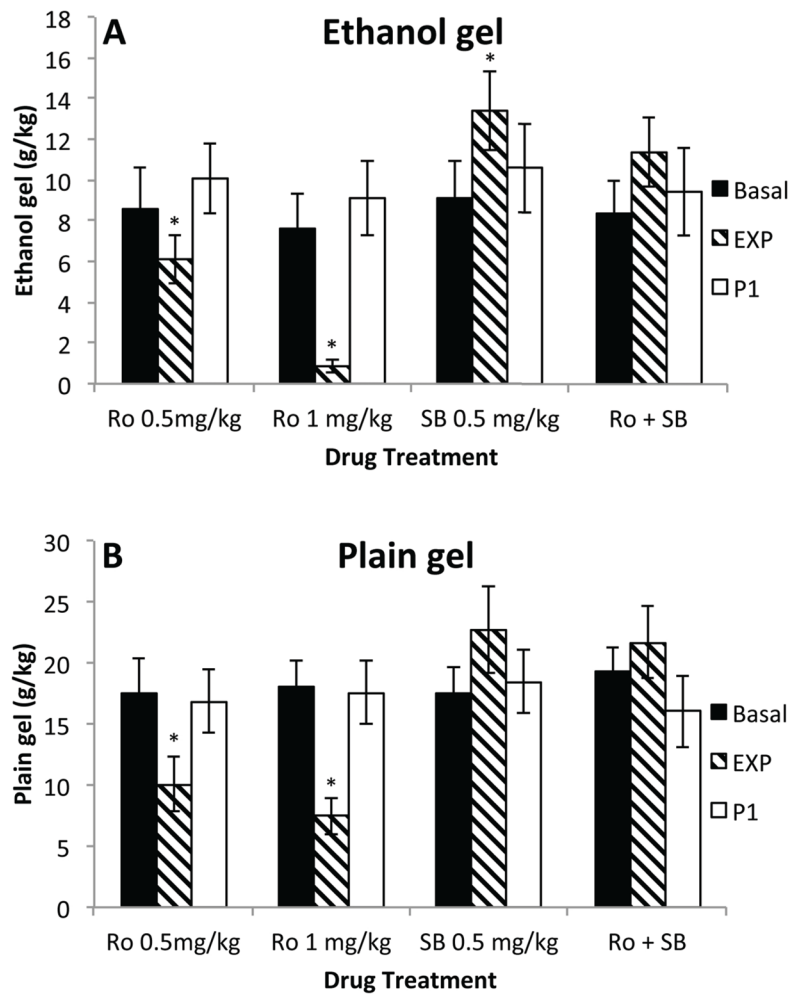


Fig. 1. Ro60-0175 decreases voluntary ethanol and plain gel consumption in rats and this effect is blocked by SB242,084. (A) Consumption of 10% ethanol-containing gel in response to 5-HT_{2C} modulators. (B) Consumption of plain (control) gel in response to the same 5-HT_{2C} modulators as in (A). Black columns represent the average consumption before experimental day, striped represents consumption on the day of drug administration, and white represents consumption the day after drug administration. Shown are mean values \pm S.E.M. for $n = 10$. * indicates $P < 0.05$ compared to basal.

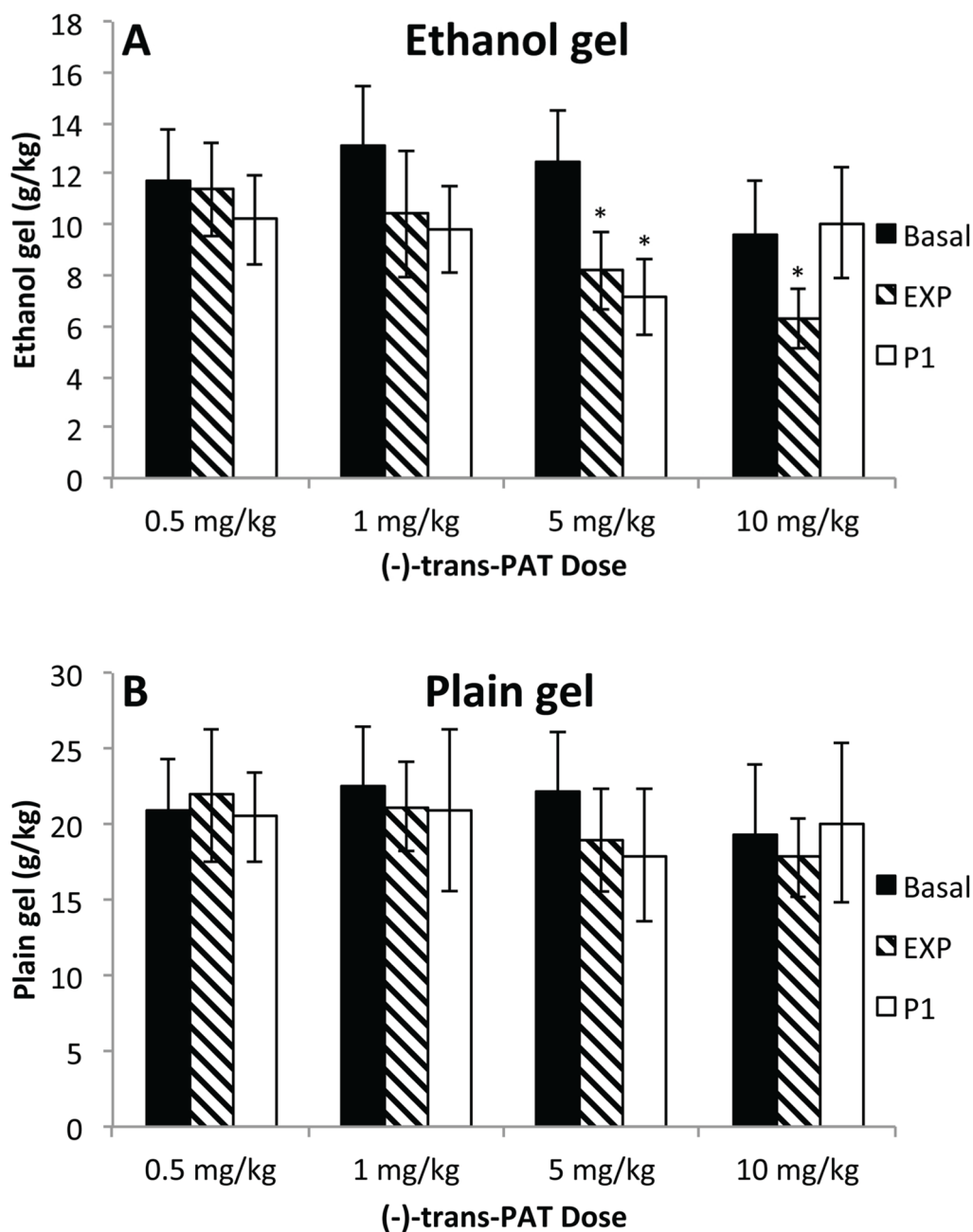


Fig. 2. (-)-trans-PAT decreases voluntary ethanol gel consumption in a dose dependent manner but not plain gel consumption. (A) Consumption of 10% ethanol-containing gel after injection of different doses of (-)-trans-PAT. (B) Consumption of plain (control) gel after injection of the same doses of (-)-trans-PAT as in (A). Black columns represent the average consumption before experimental day, striped represent consumption on the day of drug administration, and white represents consumption the day after drug administration. Shown are mean values \pm S.E.M. for $n = 10$. * indicates $P < 0.05$ compared to basal.

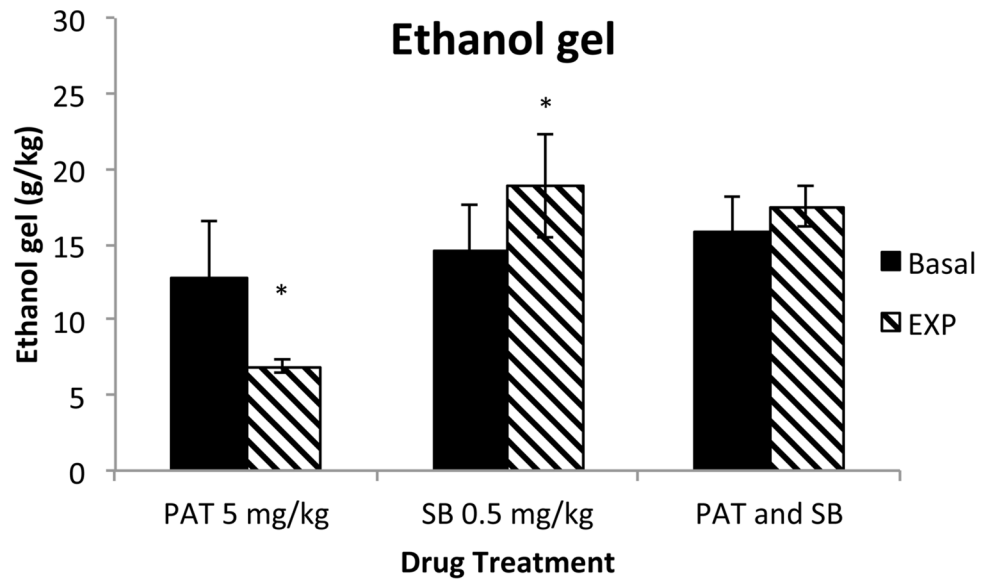
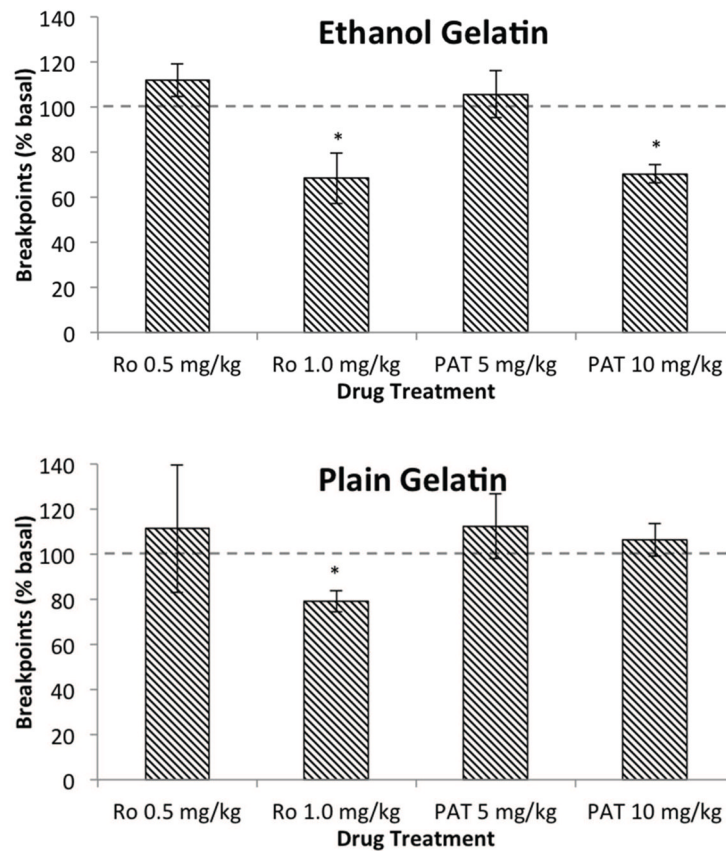


Fig. 3. (–)-trans-PAT decreases, and SB242,084 increases ethanol gel consumption. Black columns represent the average consumption before experimental day, striped represent consumption after drug administration. Shown are mean values \pm S.E.M. for $n = 6$. * indicates $P < 0.05$ compared to basal.

**Fig. 4.**

(-)-trans-PAT specifically decreases ethanol breakpoints and Ro60-0175 decreases both ethanol and plain gel breakpoints during progressive ratio 10. (A) Breakpoints for ethanol gel in the presence of 5-HT_{2C} agonists. (B) Breakpoints for plain gel controls for (A). Data are represented as a percent of basal breakpoints. Striped columns represent change in breakpoints after drug administration expressed as a percent of basal. Shown are mean values \pm S.E.M. for $n = 4-5$. * indicates $P < 0.05$ compared to basal.

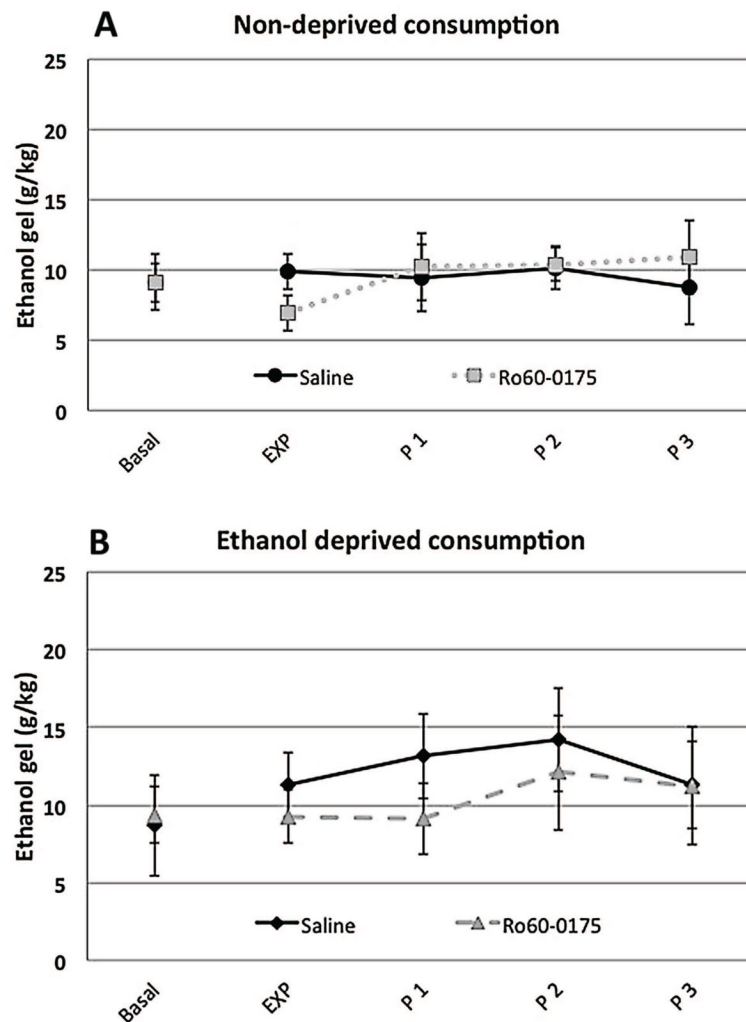


Fig. 5. Ro60-0175 administration causes a temporary non-significant decrease in basal ethanol consumption but has no effect on ADE-induced increases of ethanol consumption. (A) Ethanol consumption of non-deprived rats both with and without 0.5 mg/kg Ro60-0175. (B) Ethanol consumption before and after a 3-week ethanol deprivation with and without 0.5 mg/kg Ro60-0175 administered on the experimental day. Basal consumption values were taken 3 weeks before experimental day with Post 1–3 showing consumption the days immediately following the experimental day. $n = 6$. * indicates a significant difference between groups on that day.

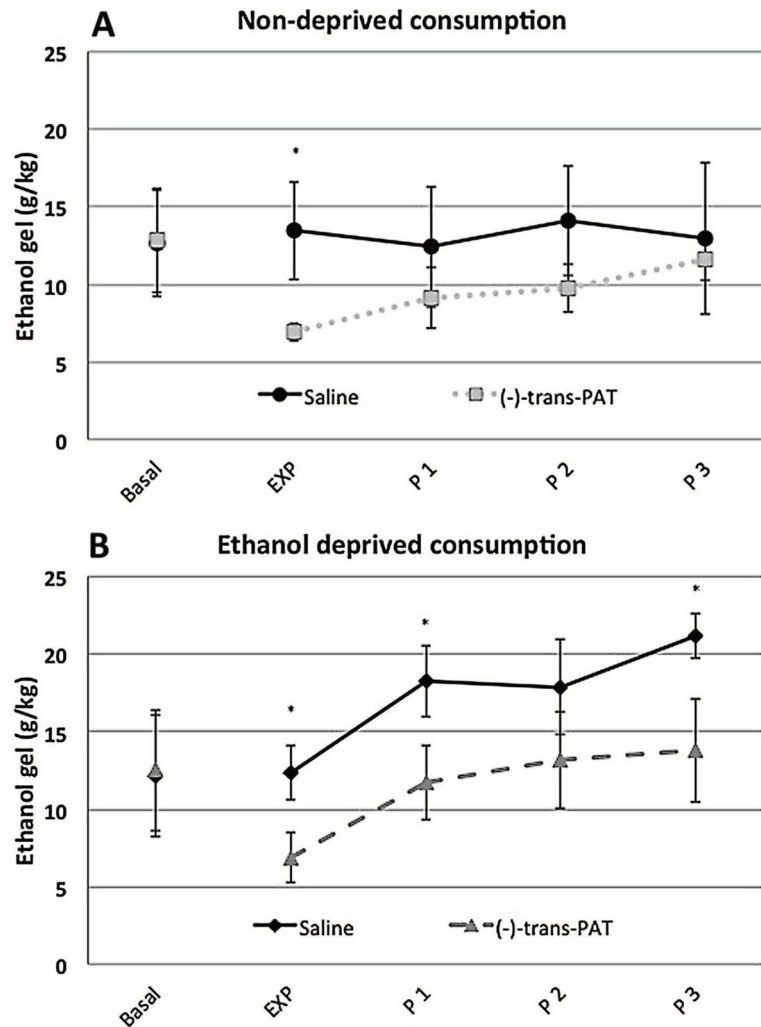


Fig. 6. (-)-trans-PAT administration causes a temporary decrease in baseline ethanol consumption and prevents ADE-induced increases of ethanol consumption. (A) Ethanol consumption of non-deprived rats both with and without 5 mg/kg (-)-trans-PAT. (B) Ethanol consumption before and after a 3-week ethanol deprivation, with and without 5 mg/kg (-)-trans-PAT administered on experimental day. Basal consumption values were taken 3 weeks before experimental day with Post 1–3 showing consumption for the 3 days immediately following the experimental day. $n = 6$. * indicates a significant difference between groups on that day.