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Galanin-3 Receptor Antagonism by SNAP 37889 Reduces Motivation to Self-administer Alcohol and Attenuates Cue-Induced Reinstatement of Alcohol-Seeking in iP Rats

Belinda L. Ash[1], Tim Quach[2], Spencer J. Williams[2], Andrew J. Lawrence[3,4], and Elvan Djouma[1,*]

[1]*Department of Human Biosciences, Faculty of Health Sciences, La Trobe University, Bundoora, Victoria 3086, Australia*
[2]*Bio21 Molecular Science and Biotechnology Institute,* [3]*Florey Institute of Neuroscience and Mental Health,*
[4]*Melbourne Brain Centre, University of Melbourne, Parkville, Victoria 3010, Australia*

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Abstract. The neuropeptide galanin has a role in promoting alcohol consumption and general feeding behavior. The galanin-3 receptor (GALR3) subtype is implicated in modulating the consumption of alcohol and has therefore been identified as a potential target for new pharmacotherapies to treat alcohol use disorders. We have previously shown that the selective GALR3 antagonist SNAP 37889 reduced voluntary alcohol consumption in iP (alcohol-preferring) rats. The present study firstly aimed to investigate the effect of GALR3 antagonism on the motivational properties of alcohol. Secondly, the potential of GALR3 as a therapeutic target in the prevention of relapse was investigated in response to alcohol-conditioned cues. Administration of SNAP 37889 (30 mg/kg, i.p.) significantly reduced the breakpoint for ethanol under a progressive-ratio operant responding schedule of reinforcement. SNAP 37889 also significantly reduced reinstatement of alcohol-seeking in response to re-exposure to conditioned cues that were previously associated with the availability of alcohol. Collectively, results from the current study provide new evidence of GALR3 involvement in cue-induced relapse and provide further evidence that GALR3 antagonism reduces the motivational drive to consume alcohol. These findings validate further research in to the potential use of SNAP 37889 and other GALR3 antagonists to treat alcohol abuse disorders in humans.

Keywords: alcohol, galanin, galanin-3 receptor (GALR3), operant self-administration, cue-induced reinstatement

Introduction

Alcohol abuse is a contributing factor to almost 4% of worldwide deaths and 4.5% of the global burden of disease (1). In recent years, the consumption of alcohol has increased, particularly in developing countries (1), prompting research in to the creation of new medications to effectively treat alcohol use disorders. Accumulating evidence has emerged to indicate that galanin, a neuropeptide, plays a role in promoting alcohol intake (2 – 4) and may therefore be a suitable target for new pharmacotherapies in the treatment of alcoholism (reviewed by 5).

*Corresponding author. e.djouma@latrobe.edu.au
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Galanin is synthesised at high levels in the locus coeruleus and dorsal raphe nucleus (6), where galaninergic neurons project to regions of the forebrain to mediate a variety of physiological functions via binding to three identified receptor-subtypes (GALR1, GALR2, and GALR3) (7). Numerous experiments with rodents have confirmed a role for galanin in learning and memory (8), alcohol consumption, and general feeding behavior (9) as well as affective disorders (for reviews see 10, 11). The exact receptor subtype(s) mediating these behaviors is yet to be elucidated, but the literature to date suggests GALR3 to be the most relevant in the context of alcohol consumption.

GALR3 are anatomically located in brain regions known to regulate feeding and addictive behaviors. Expression of GALR3 is reported to be particularly high in the hypothalamus (12, 13), with moderate levels found

in other brain regions including the hippocampus (13), nucleus accumbens (NAc), amygdala (AMG) (14), bed nucleus of the stria terminalis (BNST), and the dorsal raphe nucleus (12).

Many reviews have highlighted a possible functional role for GALR3 in addiction and drug-seeking behavior, but to date, experimental evidence to support these suggestions is somewhat limited. Our laboratory was the first to report a direct association between GALR3 and altered alcohol-seeking behavior. We reported that SNAP 37889 (30 mg/kg, i.p.), reduced self-administration of ethanol by alcohol-preferring (iP) rats, independent of a sedative effect (15). In our previous study, operant self-administration was assessed under a fixed-ratio (FR) response schedule, which did not provide a quantitative indication of the level to which SNAP 37889 reduced the motivation to work for ethanol. The first experiment described in the current study therefore aimed to investigate the effect of GALR3 antagonism with SNAP 37889 (30 mg/kg; i.p.) on the reinforcing efficacy of alcohol using a progressive-ratio (PR) response schedule.

A major hurdle in overcoming alcohol addiction is the need to treat ongoing cravings for alcohol and vulnerability to relapse, as well as short-term symptoms associated with alcohol withdrawal. A second experiment in this study therefore aimed to investigate the potential of SNAP 37889 to reduce the rate of relapse in response to exposure to conditioned cues.

Materials and Methods

Animals

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986, under the guidelines of the National Health and Medical Research Council code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. Male iP rats were obtained from a breeding colony at the Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia. Rats were maintained on a 12-h light-dark cycle with ad libitum access to food and water.

Drugs

SNAP 37889 was synthesised as described (16). Dimethyl sulfoxide (DMSO) and hydroxypropyl-methyl cellulose (HMC) were purchased from Sigma Pharmaceuticals (Castle Hill, NSW, Australia). SNAP 37889 was administered at a dose of 30 mg/kg via intraperitoneally (i.p.), in accordance with data previously obtained from a pilot study indicating 30 mg/kg to be the most effective in altering alcohol-seeking in this species (17). SNAP 37889 (30 mg/kg) and vehicle (5% DMSO and 1% HMC in saline, 1 ml/kg) were administered via i.p. injection.

Progressive-ratio responding for alcohol

Adult age matched iP rats were trained to administer 10% (v/v) ethanol in sound attenuated operant chambers (Med Associates, Fairfax, VT, USA). Chambers were fitted with two levers calibrated to dispense 100 μl of fluid as a reward when a required number of lever presses was completed by the rat. One lever was paired with the delivery of 10% (v/v) alcohol, while the other lever was paired with the delivery of water. Lever pressing was conditioned in the presence of an olfactory cue (a single drop of vanilla essence placed below the ethanol lever) and a light stimulus cue (adjacent to the lever), which would illuminate upon completion of the required response. Chambers were linked to a computer with Med-PC IV software which recorded lever pressing activity during each session.

The initial training of the rats consisted of a single overnight session, followed by a standard "sucrose fade" protocol conducted over a period of nine days as previously described (15). Following the training, rewards were dispensed on an FR3 schedule (fixed ratio of 3 lever presses for 1 reward) for both the ethanol solution and the water. Twenty-minute sessions were conducted 5 days a week for 5 weeks until a stable baseline response was achieved across FR3 sessions.

Three 90-min PR sessions were then conducted on non-consecutive days (Mon, Wed, and Fri) to assess the viability of repeated sessions and also to determine a baseline figure for the breakpoint. Breakpoint was defined as the last successfully completed ratio within the session. The PR response requirement was increased on a linear scale by adding one additional lever press for each subsequent reward, as previously described (18). FR3 sessions were conducted in between PR sessions on alternate days (Tue, Thu). Treatment commenced the following week and to account for a possible alcohol deprivation effect over the weekend, the PR treatment session was conducted on the Wednesday. Rats were divided into 2 treatment groups (n = 9), using a counterbalanced design according to the average number of FR3 rewards recorded from the previous 10 sessions. One group was treated with vehicle (1 ml/kg, i.p.) and the other, with SNAP 37889 (30 mg/kg, i.p.). Rats were then returned to their home cage for 60 min prior to being placed in the operant chambers for the 90-min session.

Cue-induced alcohol reinstatement

Following the PR treatment sessions, the same cohorts of rats were used to investigate the effect of SNAP 37889 administration on cue-induced alcohol reinstatement. Rats

were subjected to another 2 weeks of FR3 responding to re-establish a base level of responding, after which their lever pressing behavior was extinguished. During extinction, the conditioned cues (light and vanilla essence), which had previously been associated with the delivery of ethanol were removed from the chamber, along with the 10% (v/v) ethanol solution and water so that lever pressing activity resulted in no reward. Extinction sessions were conducted daily (Mon – Fri) for 20 min and the number of lever presses was recorded for each session. Following extinction, rats were divided in to 3 treatment groups: extinction only (n = 5), vehicle treatment (n = 6), and SNAP 37889 treatment (n = 7). The non-treatment group were culled following their final extinction session and their brains were retained for use in a future study. Rats were treated with vehicle (1 ml/kg, i.p.) or SNAP 37889 (30 mg/kg, i.p.) 60 min prior to being placed in to the operant chamber for the reinstatement session. Reinstatement was triggered by replacing the olfactory cue under the "active" lever and reprogramming the software such that the stimulus light was activated (1 s) after every FR3 equivalent, although there was no delivery of ethanol into the receptacle (19, 20).

Statistical analyses

Baseline PR response was determined by calculating the average response across the 3 pre-treatment sessions. The variance across pre-treatment sessions was compared with a one-way analysis of variance (ANOVA) with repeated measures (RM). A one way ANOVA with RM was used to analyze the breakpoint, total number of responses, and the latency to first response on both the ethanol and water levers. A two-way ANOVA with RM was used to analyze lever pressing subtotals at time intervals within the session. Bonferroni's post-hoc test was applied to identify significant interactions, with planned comparisons conducted between vehicle and SNAP 37889–treatment groups and also for water and ethanol lever pressing activity. Ethanol intake (g/kg) was calculated from the body weight (g) of the animal and the number of ethanol reinforcers delivered during the session. Graph Pad Prism 5.00 was used to perform all analyses and a result of $P \leq 0.05$ was considered to be of statistical significance.

Results

Effect of SNAP 37889 on progressive-ratio responding for alcohol

Rats responded on a FR3 schedule (3 lever presses = 1 reward) for a period of 29 days until a stable number of responses was achieved across sessions. The average number of rewards made on the ethanol lever was 32 ± 2 and 2 ± 0.2 on the water lever per 20 minute session, equating to an average ethanol consumption of 1.32 ± 0.07 g/kg per session.

Following 29 days of FR3 responding, 3 PR sessions were conducted to establish a breakpoint baseline of 16.7 ± 1.2, with a one-way ANOVA confirming that variation across all 3 sessions was not significant ($P = 0.94$, data not shown). Treatment with SNAP 37889 60 min prior to the PR session significantly reduced the breakpoint for ethanol, when compared with both the vehicle-treated group and the average breakpoint of pre-treatment sessions [treatment, $F(35,2) = 5.943$, $P < 0.01$, Fig. 1: A and B]. Vehicle treatment did not alter the breakpoint for ethanol and there was no statistical difference in responses for water between the treatment groups (Fig. 1: A and B).

Analyses of the cumulative lever presses for ethanol at each 10-min time point revealed that responses made by the SNAP 37889–treated group were significantly reduced from 20 min through to the end of the session [treatment × time, $F(8,128) = 2.733$, $P < 0.05$ (20 – 60 min); $P < 0.01$ (70 – 90 min); Fig. 1C]. At the 10-min, mark lever pressing activity by the SNAP 37889–treated group was significantly lower than that in the vehicle-treated group [treatment × time, $F(8,128) = 5.375$, $P < 0.001$, Fig. 1D]. When ethanol lever pressing activity was examined across the time course of the session, the majority of lever presses were made within the first 10 min, with little activity after 20 min, indicating that breakpoint was achieved very early in the 90-min session. This was apparent in both the vehicle and SNAP 37889–treated groups, indicating that regardless of treatment type, the breakpoint was quickly reached (Fig. 1D).

Effect of SNAP 37889 on cue-induced alcohol reinstatement

At extinction, rats made an average of 2.4 ± 0.3 presses on the lever, which had previously delivered water and an average of 4.9 ± 0.7 presses on the lever, which had previously delivered alcohol. Re-presentation of cues paired with alcohol availability resulted in reinstatement of lever pressing activity directed towards the "active" lever that had previously delivered alcohol. Active lever presses made by the SNAP 37889–treated group were significantly lower [treatment, $F(2,30) = 101.3$, $P < 0.0001$, Fig. 2A] than those in the vehicle-treated rats, indicating that SNAP 37889 successfully attenuated cue-induced reinstatement. Analyses of the "inactive" lever, which had previously delivered water, revealed no significant differences between the 3 groups (Fig. 2A). The latency to the first right lever press was compared between the vehicle- and drug-treated groups, but no significant difference was found ($P = 0.48$, data not shown). Analyses of the lever pressing sub-totals at

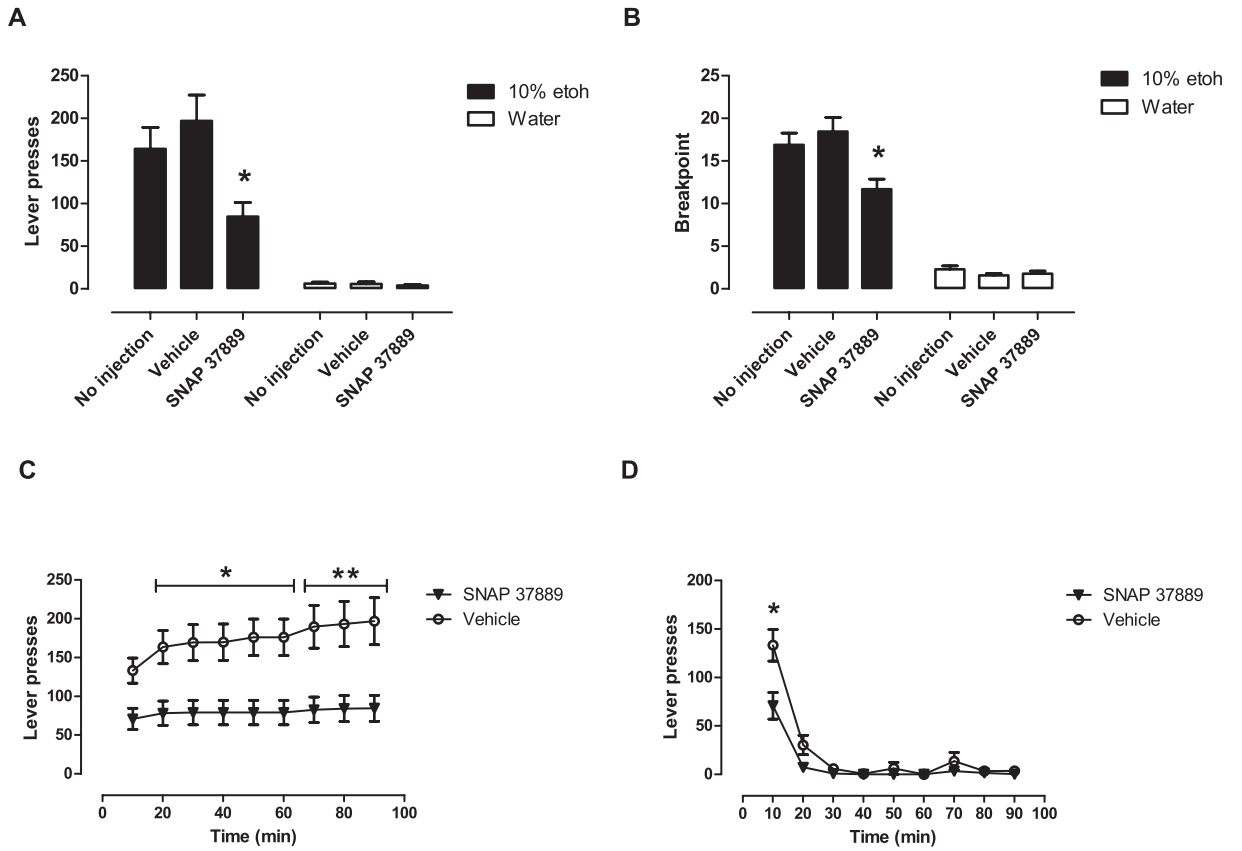

Fig. 1. Effect of SNAP 37889 treatment on PR responding and breakpoint for 10% (v/v) ethanol. SNAP 37889 caused a significant reduction in lever presses (*$P < 0.01$, A) and breakpoint (*$P < 0.01$, B) for ethanol but not water on a PR reinforcement schedule. The cumulative responses made for ethanol were significantly reduced by SNAP 37889 (*$P < 0.05$, **$P < 0.01$; D) in comparison to vehicle treatment from 20 – 90 min, while the most significant reduction in responding for ethanol occurred within the first 10 min of the session (*$P < 0.001$, D).

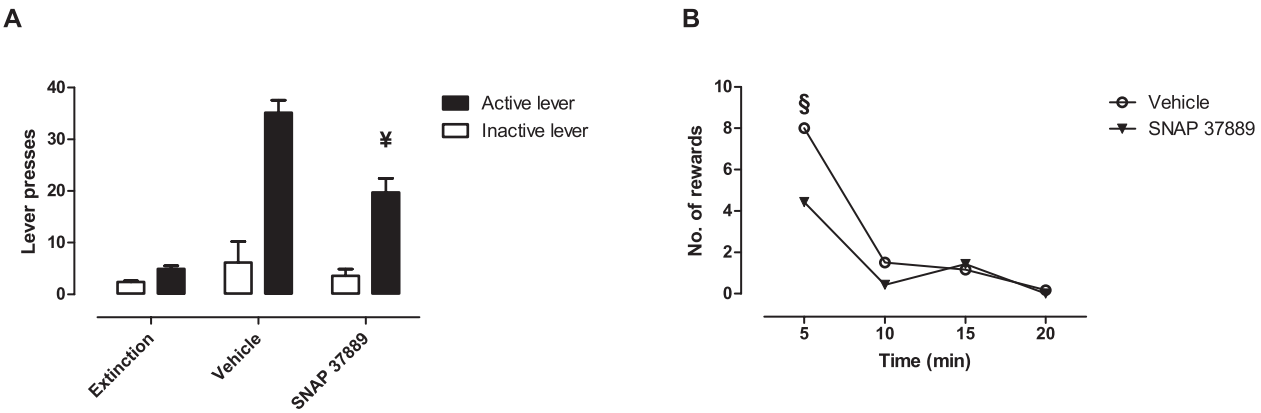

Fig. 2. Effect of SNAP 37889 treatment on cue-induced alcohol reinstatement. SNAP 37889, when compared with vehicle treatment significantly reduced the total responses made on the ethanol lever (with cues present but no ethanol delivery) and without a change in responses made on the water lever ($^{¥}P < 0.0001$, A). Responses on the ethanol lever were significantly reduced by SNAP 37889 5 min into the 20-min session ($^{§}P < 0.001$, B).

each 5-min time interval within the session revealed that 5 min into the 20-min session, SNAP 37889 treatment had significantly reduced the number of active lever presses [treatment × time, $F(3,33) = 4.226$, $P < 0.001$, Fig. 2B].

Discussion

We have previously demonstrated that administration of the GALR3 antagonist SNAP 37889 reduced operant self-administration of ethanol on a FR3 schedule (15). The FR operant model used in our previous study provided valuable preliminary evidence to indicate involvement of GALR3 in self-administration of rewarding substances, but FR paradigms are limited in terms of detecting whether pre-treatment with a pharmacological agent causes an alteration in the reinforcing efficacy of a certain drug (21). Data reported in the present study indicate that SNAP 37889 (30 mg/kg, i.p.) decreased the motivational value of alcohol as a reward under a PR schedule, as well as attenuating cue-induced reinstatement of alcohol-seeking after extinction. Together, these results provide new evidence of the involvement of GALR3 in motivational and reward-seeking processes.

iP rats were obtained from the same breeding colony as our previous study and 30 mg/kg was selected as the dose for administration to maintain consistency with our previous work (15). The breakpoint established prior to treatment was compared with that of another recent study that also used a PR schedule of responding for 10% (v/v) ethanol in iP rats and was found to be equivalent (18). More recently, a different study in our laboratory has confirmed 30 mg/kg (i.p.) to be the most effective dose of SNAP 37889 in reducing-alcohol consumption by C57BL/6J mice as part of a binge-drinking model (Scheller, et al. 2014; unpublished data). SNAP 37889 binds with high selectively to GALR3 ($Ki > 17.44 \pm 0.01$ nM), over the GALR1 and GALR2 subtypes ($Ki > 10{,}000$ nM) (22). Behavioral data reported in the current study are therefore almost certainly a result of GALR3 involvement with negligible influence from GALR1 or GALR2 subtypes.

The half-life of SNAP 37889 following systemic administration appears to be less than 5 hours following i.p. (9 mg/kg) and oral (8.61 mg/kg) administration in rats (23). The present data confirm a pharmacological effect of SNAP 37889 in iP rats which lasted for at least 150 min, as indicated by an increasing difference between treatment groups in the cumulative number of ethanol lever presses over the PR time-course. The usefulness of SNAP 37889 for longer term treatment may be limited by a relatively short duration of action. Further studies may investigate the use of frequent repeated dosing or continuous administration to determine its effectiveness in reducing drug-seeking behavior in the long-term.

Mapping of GALR3 via in situ hybridization indicates the highest levels of GALR3 mRNA within the rodent hypothalamus (12 – 14) and hippocampus (13) and moderate levels within the NAc, ventral tegmental area (14), BNST (12), and AMG (12 – 14). From the GALR3 mRNA distribution pattern, it can be inferred that GALR3 may be anatomically well-situated to contribute to functions such as feeding, memory, emotion, and addiction. Identifying the precise anatomical location of GALR3 is challenged by the lack of receptor-selective radioligands and antibodies that are for use in rodent species (24, 25).

Multiple studies have reported increased c-Fos expression within sub-regions of the AMG of rats, following cue-induced reinstatement for alcohol (19, 26 – 28). Galanin is co-localised with the neurotransmitter gamma-aminobutyric acid (GABA) in the AMG (29), where it is well established that GABA plays a role in alcohol consumption at the level of the CeA (reviewed by ref. 30). A functional role for galanin in the CeA has already been identified as shown by increased feeding behavior when galanin is injected directly into the CeA (31, 32). Furthermore, the non-selective galanin-receptor antagonists C7 and M40 reversed galanin-induced feeding when microinjected intraventricularly in Sprague-Dawley rats (33). Experimental data gathered in vitro indicates that GALR3 antagonists inhibit the likelihood of firing of a sub-population of CeA neurons (34), which supports the likelihood of GALR3 as a likely therapeutic target in treating alcoholism. Given that galanin is known to promote alcohol consumption as well as feeding behavior, it is postulated that microinjection of GALR3 antagonists at the level of the CeA may dampen the usual stimulatory effect of GABA on ethanol self-administration (34). Future studies will undoubtedly seek to identify the anatomic loci where GALR3 antagonism acts to attenuate cue-induced alcohol-seeking.

In summary, the present study has demonstrated SNAP 37889 effectively reduced the motivation to work for alcohol as a reinforcer, as well as reducing reinstatement in response to conditioned cues. Evidently, it is important to determine the action of GALR3 antagonism at a circuit and neurochemical level to gain insight into the pathways that are interrupted following SNAP 37889 administration. Collectively, these findings validate further research into the use of SNAP 37889 and other GALR3 antagonists as a possible means to treat alcohol abuse disorders in humans.

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