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Sigma-1 Receptor Mediates Acquisition of Alcohol Drinking and Seeking behavior in Alcohol-Preferring Rats

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Abstract

Sigma-1 receptor (Sig-1R) has been proposed as a novel therapeutic target for drug and alcohol addiction. We have shown previously that Sig-1R agonists facilitate the reinforcing effects of ethanol and induce binge-like drinking, while Sig-1R antagonists block excessive drinking in both genetic and environmental models of alcoholism, without affecting intake in outbred non-dependent rats. Even though significant progress has been made in understanding the function of Sig-1Rs in alcohol reinforcement, its role in the early and late stage of alcohol addiction remains unclear.

Administration of the selective Sig-1R antagonist BD-1063 dramatically reduced the acquisition of alcohol drinking behavior as well as the preference for alcohol in genetically selected TSRI Sardinian alcohol preferring (Scr:sP) rats; the treatment had no effect on total fluid intake, food intake or body weight gain, proving selectivity of action. Furthermore, BD-1063 dose-dependently decreased alcohol-seeking behavior in rats trained under a second-order schedule of

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Conflicts of Interest

The authors declare no conflict of interest.

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reinforcement, in which responding is maintained by contingent presentation of a conditioned reinforcer. Finally, an innate elevation in Sig-1R protein levels was found in the nucleus accumbens of alcohol-preferring Scr:sP rats, compared to outbred Wistar rats, alteration which was normalized by chronic, voluntary alcohol drinking.

Taken together these findings demonstrate that Sig-1R blockade reduces the propensity to both acquire alcohol drinking and to seek alcohol, and point to the nucleus accumbens as a potential key region for the effects observed. Our data suggest that Sig-1R antagonists may have therapeutic potential in multiple stages of alcohol addiction.

Keywords

Ethanol; Addiction; Intake; Rat; Reinforcement; Reward

1. Introduction

Sigma-1 receptor (Sig-1R) has been object of pharmacological studies for decades. Originally categorized as an opioid receptor [1] and then mistaken for the phencyclidine (PCP) binding site on NMDA receptors [2], Sig-1R was finally recognized as a non-opioid, non-PCP unique receptor [3, 4] which was then cloned in 1996 [5–7]. More recently, Sig-1R was identified as a ligand-operated molecular chaperone located post-synaptically in the endoplasmic reticulum and on the plasma membrane through a dynamic translocation [8]. Once activated the Sig-1R receptor can modulate glutamatergic, cholinergic, noradrenergic, and dopaminergic neurotransmissions [9–13], as well as the release of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line derived neurotrophic factor (GDNF) [14–16].

Given its wide distribution in the limbic system and the brainstem, Sig-1R has been shown to play a key role in motivation, learning and memory [17–20]. Furthermore, Sig-1R is considered among the most promising targets in addiction. Several studies have shown that Sig-1R is involved in the motivational effect of psychostimulants such as cocaine and methamphetamine. Antagonists exert a plethora of actions, including the blockade of psychostimulant-induced behavioral sensitization, conditioned place preference, and cue-induced reinstatement of seeking behavior [21–25]. Interestingly, Sig-1R also mediates the motivational effect of ethanol: Sig-1R antagonists are able to block ethanol-induced hyper-locomotion and conditioned place preference [26], and our laboratory has shown that Sig-1R antagonists reduce excessive drinking in ethanol-dependent and alcohol-preferring rats, [27–29], while agonists induce binge-like drinking [27].

While the effects of Sig-1R antagonism on the reinforcing effects of alcohol have been investigated, the role of Sig-1Rs on the acquisition of alcohol drinking as well as alcohol-seeking behavior has yet to be elucidated.

The first aim of this study was to test the effects of the Sig-1R antagonist BD-1063 on the home-cage acquisition of alcohol-drinking behavior in TSRI Sardinians alcohol-preferring rats (Scr:sP). Our line descends from the original line which was selectively bred from a Wistar stock by Prof. G.L. Gessa (University of Cagliari, Italy). This line provides a model

with significant face and predictive validity for alcoholism [30–32] as these rats voluntarily drink high quantities of ethanol, respond to pharmacological treatments which are proven to work in humans, and have a heritable component similar to human ethanol dependence [33–36].

The second aim of this study was to investigate the effect of BD-1063 rats on a later stage alcohol–seeking behavior using a second order schedule of reinforcement, in which seeking behavior is maintained by a response-contingent exposure to a drug-associated stimulus [37–41].

Finally, the third aim was to evaluate innate levels of Sig-1R in the nucleus accumbens (NAcc) and the central nucleus of the amygdala (CeA) of ethanol-naïve Scr:sP and Wistar rats, two brain areas highly involved in the rewarding properties of alcohol.

2. Material and Methods

2.1. Subjects

Subjects of this study were adult male Wistar rats (Charles River, Wilmington, MA) and rats derived from the Scripps Research Institute subline of Sardinian alcohol-preferring rats (Scr:sP, 29–30th generation, http://rgd.mcw.edu/rgdweb/report/strain/main.html?id=2302666) which were then maintained for 12 generations at Boston University without further selective breeding. Scr:sP rats were generated from intra-line breeding at The Scripps Research Institute from sP rats generously provided after 32 generations of selective breeding by Prof. G.L. Gessa (University of Cagliari, Italy). Scr:sP rats were housed in a humidity- and temperature-controlled vivarium on a 12-h light–dark cycle (lights off at 9:00 am), with water and regular rodent chow available *ad libitum*, except otherwise specified. Experiments were conducted during the rats' dark cycle. Different sets of rats were used for each experiment. Procedures adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and *the Principles of Laboratory Animal Care*, and were approved by Boston University Medical Campus Institutional Animal Care and Use Committee.

2.2. Drugs

Ethanol solutions for the home-cage alcohol drinking acquisition procedure (10% *v/v*) and for the operant alcohol seeking procedure (10% *w/v*) were prepared using 95% ethyl-alcohol and tap water. BD-1063 × 2 HBr salt (1-[2-(3,4-Dichlorophenyl)ethyl]-4-methylpiperazine dihydrobromide]) was synthesized according to the previously reported procedure [42]. BD-1063 was solubilized in sterile, isotonic saline and administered subcutaneously (*s.c.* 1 ml/kg).

2.3. Home cage alcohol drinking acquisition

Scr:sP rats (n=18) were singly-housed and habituated to two water bottles for 3 days. Rats were then divided into two groups, matched for body weight and water intake over the last three days. Rats were treated daily with BD-1063 or vehicle 15 min before the dark cycle onset, using a between-subject design. Pre-weighed ethanol and water solutions and food,

withdrawn during the 15 min between drug administration and the onset of the dark cycle, were provided at the beginning of the dark cycle. Ethanol, water, food intake and body weight gain were measured 24 h from the onset of the dark cycle.

2.4. Apparatus for operant second order schedule of reinforcement

The test chambers used for operant second order schedule of reinforcement (Med Associates, Inc., St. Albans, VT) were located in sound-attenuating, ventilated environmental cubicles. Syringe pumps (Med Associates, St. Albans, VT) dispensed ethanol in one of the two stainless steel drinking cups mounted 2 cm above the grid floor in the middle of one side panel. Two retractable levers were located 3.2 cm to either side of the drinking cups. Ethanol delivery and recording of operant responses were controlled by microcomputers.

2.5. Alcohol-seeking behavior procedure

The procedure was adopted and modified from [43]. Scr:sP rats were first allowed continuous (24 h/day) two-bottle choice access to ethanol (10% *w/v*) and water in their home cages for 1 week. Rats were then allowed one or two overnight (16 h) operant sessions to ethanol with chow available *ad libitum*. Subjects were trained to press a lever to acquire 0.05 ml of 10% *w/v* ethanol solution under a fixed ratio 1 (FR1) schedule of reinforcement. Responses on the inactive lever resulted in no consequences, but were recorded as an index of motor activity. Lever presses on the active lever resulted in ethanol solution delivery concomitantly with illumination of a conditioned stimulus (CS) light above the active lever for 20 s (time out, TO) followed by lever retraction. The session ended when subjects reached 30 rewards or after 2 h, whichever occurred first. Subjects were then introduced to the next step of the training, a fixed interval (FI) schedule of reinforcement. The FI schedule increased daily from FI1 min to FI2, FI4, FI8, and FI10 min before stabilizing at FI15 min. After the FI schedule, a press on the active lever resulted in ethanol delivery; the volume of ethanol delivered after the FI progressively increased from 0.05 to 0.12 ml according to the following schedule: FI2 0.05 ml, FI4 0.08 ml FI 8 0.10 ml and FI10 and FI15 0.12 ml. After three consecutive completed session of FI15, subjects were moved to a second-order schedule of reinforcement. The second order schedule was divided in two trials. During the FI15 min interval, every 10th active lever press resulted in a brief CS presentation for 1 s above the active lever (FI15(FR10:S)); after the FI15 min, ten active lever presses resulted in a delivery of 0.12 ml of ethanol solution and CS presentation for 20 s. The session ended when subjects completed two trials or after 40 min, whichever occurred first. Sessions were conducted daily during the dark cycle and, at the end of the session, subjects were returned to the animal facility. When stable responding over three consecutive days was achieved (<20%), rats were administered BD-1063 15 min prior to the alcohol seeking session in a latin square design (0, 3, 10 and 30 mg/kg), allowing at least 2–3 intervening treatment-free days.

2.6. Sigma-1 receptor western blotting

For the analysis of Sig-1R protein levels, Scr:sP rats (n=14) and Wistar rats (n=7) were habituated to two water bottles. Half of the Scr:sP rats (n=7) were then allowed to drink a

10%(*v/v*) ethanol solution and a water solution 24 h/day for 4 consecutive weeks. Rats were anesthetized using isoflurane and rapidly decapitated 0 to 2 h before dark cycle onset. Brains were sectioned coronally (2 mm slices) in a rat brain matrix and the brain regions of interested (NAcc and CeA) were punched using stainless steel needles (Fine Science Tools, Inc., Foster City, CA) guided by a brain atlas [44]. Samples were stored at −80°C until processed for western blotting. Tissue was homogenized with a microsonicator in sodium dodecyl sulfate lysis buffer without β-mercaptoethanol. After protein determination with a BCA assay kit (Thermo Scientific, Waltham, MA), 50–70 μg of protein were boiled for 3 min in the presence of β-mercaptoethanol, separated electrophoretically, and then transferred to a PVDF membrane (Bio-Rad, Hercules, CA). Membranes were blocked in nonfat dry milk and then incubated with the following either an anti-Sig-1R rabbit polyclonal antibody (1:1,000 recognizing C-terminal 143-165aa Sig-1R or an anti-β-tubulin mouse monoclonal antibody, Santa Cruz Biotechnology, Dallas, TX) [8]. After washing, membranes were incubated with secondary antibodies (Santa Cruz Biotechnology, Inc., Dallas, TX). The blots were then developed with the SuperSignal West Femto detection system according to the manufacturer's instructions (Thermo Scientific, Waltham, MA) Sig-1R expression levels were calculated as percentage relative to β-tubulin expression.

2.7. Statistical analysis

Data from the acquisition experiment were analyzed using a two-way mixed design ANOVA with Treatment as between-subject factor and Time as within-subject factor. Data from the alcohol-seeking experiment and Sigma-1 receptor protein expression were analyzed using one-way ANOVAs. Pairwise post-hoc comparisons were made using the Student Newman Keuls test; student-*t* test was used to compare two groups. Significance was set at $p<0.05$. The software/graphic packages used were Systat 11.0, InStat 3.0, Statistica 7.0, and SigmaPlot 11.0.

3. RESULTS

3.1. BD-1063 blocks the ethanol home cage acquisition in Scr:sP rats

As shown in Fig. 1, panel A, chronic administration of BD-1063 decreased 24-h ethanol intake in Scr:sP rats (Treatment: $F(1,14)=10.41$, $p< 0.01$), with a more pronounced effect towards the first part of the 14-day treatment period (Treatment X day: $F(13,182)=1.75$, $p= 0.05$). Both the vehicle and the BD-1063 groups increased their alcohol intake over the course of the 14 days; however, rats treated with vehicle increased their intake from 1.88±0.42 g/kg on day 1 to 5.47±0.35 g/kg on day 14, while the intake of BD-1063-treated rats increased from 0.89±0.16 g/kg on day 1 to only 3.57±0.91 g/kg on day 14.

Water intake was also significantly affected by the treatment (Treatment: $F(1,14)=11.34$, $p< 0.01$; Treatment X day: $F(13,182)=0.63$, *n.s.*), with BD-1063-treated rats drinking more water than vehicle-treated rats during the entire treatment period, as shown in Fig. 1, panel B. This resulted in a significant reduction in ethanol preference on all 14 days of treatment (Treatment: $F(1,14)=11.77$, $p<0.01$; Treatment X Day: $F(13,182)=1.55$, *n.s.* Fig. 1, panel C) and no change in total fluid intake (Treatment: $F(1,14)=2.03$, *n.s.*; Treatment X Day: $F(13,182)=1.36$, *n.s.*; Fig. 1, panel D).

The treatment with BD-1063 did not affect food intake (Treatment: $F(1,14)=4.07$, *n.s.*; Treatment X Day: $F(13,182)=1.13$, *n.s.*, Figure 2, panel A), total caloric intake (comprised of the calories coming from both food and ethanol; Treatment: $F(1,14)=0.79$, *n.s*; Treatment X Day: $F(13,182)=1.13$ *n.s.*; Figure 2, panel B). In addition, cumulative body weight gain was not affected by the treatment (Treatment: $F(1,14)=1.76$, *n.s.*; Treatment X Day: $F(13,182)=1.21$, *n.s.;* Fig. 2, panel C).

3.2. BD-1063 reduces alcohol-seeking behavior in Scr:sP rats

A separate set of animals ($n=7$) was used to assess the effects of the Sig-1R antagonist on alcohol-seeking behavior. As shown in Fig. 3, panel A, BD-1063 (0, 3, 10, 30 mg/kg s.c.) decreased alcohol-seeking behavior in Scr:sP rats ($F(3,27)= 24.28$, $p< 0.001$) in a second-order schedule of reinforcement, in which an alcohol-associated incentive stimulus maintains alcohol-seeking behavior. All three doses of BD-1063 significantly decreased the BD-1063 number of lever presses during the first (pre-ingestive) interval (79.2% reduction with the highest dose, compared to vehicle treatment). The treatment had no effect on inactive lever presses ($F(3,27)=1.61$, *n.s.*; Fig. 3, panel B).

3.3. Protein expression of Sig-1 receptor is elevated in NAcc, but not the CeA, of ethanol-naive Scr:sP rats

Sig-1R protein levels were measured in ethanol naïve outbred Wistar, ethanol naïve Scr:sP, and Scr:sP rats under continuous access to ethanol (24h/day for 4 weeks). One-way ANOVA showed a significant difference in protein levels among groups in the NAcc ($F(2,18)= 6.91$, $p< 0.01$). Post-hoc analysis revealed an increase of Sig-1R protein levels in ethanol-naïve Scr:sP rats compared to ethanol-naïve Wistar rats; in addition, Scr:sP rats which underwent continuous voluntary intake of ethanol for 4 consecutive weeks showed control-levels of Sig-1R protein expression (Fig. 4 panel A), as this group significantly differed from the ethanol-naïve Scr:sP but not from the ethanol-naïve Wistar. Sig-1R protein levels did not differ among groups in the CeA ($F(2,18)= 0.25$, *n.s.;* Fig. 4 panel B).

4. DISCUSSION

This series of studies demonstrates that pretreatment with BD-1063, a selective antagonist of Sig1-R [42], significantly reduces the acquisition of alcohol-drinking behavior in alcohol-preferring Scr:sP rats. The Sig-1R antagonist also increases the intake of the concurrently available water, resulting in a marked reduction of alcohol preference, while not reducing food intake or body weight. Importantly, treatment with BD-1063 is also able to reduce alcohol-seeking behavior in Scr:sP rats. Finally, we show that ethanol naïve Scr:sP rats display increased levels of Sig-1R expression in the NAcc, compared to ethanol naïve control Wistar rats, and that chronic voluntary alcohol consumption normalize the altered expression to control Wistar rats levels.

Alcohol-preferring rats are a well-established model for alcoholism with a strong face, predictive and construct validity [32, 45–47]. In the first part of our study, alcohol-naïve Scr:sP rats were treated daily with the Sig-1R antagonist BD-1063 for 2 weeks, concomitantly with the initiation of alcohol access. BD-1063 significantly reduced the

acquisition of alcohol drinking (~1.5 g/kg reduction of intake compared to vehicle-treated rats on the last day of injection), suggesting a decrease as well as a delay of the expression of the genetic predisposition toward high alcohol intake. BD-1063 increased water intake and did not reduce food intake, demonstrating that the reduction in alcohol intake was not due to motor impairment, inability to drink, induction of nausea or another aversive state. The increase in water intake in BD-1063-treated rats can be interpreted as a compensatory behavior for the diminished ethanol intake, as the total fluid intake remained unchanged compared to vehicle-treated rats. Similar increases in water intake have been previously shown for several other drugs which are effective in reducing concurrent alcohol intake [48–51]. The results are also consistent with previous reports of lack of motor impairing effects of this dose of BD-1063 [52].

The second main finding of this study was that the Sig-1R antagonist BD-1063 decreased the alcohol-seeking behavior, suggesting a role for Sig-1R in incentive motivational mechanisms controlling ethanol-seeking and intake. This is a novel and highly relevant observation, as it proves that SigR antagonists can not only reduce alcohol consumption, but also alcohol-seeking behavior. We demonstrated this by means of a second-order schedule of reinforcement, a procedure widely used with psychostimulants and opiates [43, 53], which is characterized by the maintenance of responding not only by the self-administered drug, but also by the contingent presentation of drug-paired stimuli that serve as conditioned reinforcers of instrumental behavior [38]. BD-1063 was able to decrease the active lever presses during the first interval as a function of the dose administered; the first interval is the “drug-free” one, and is therefore not affected by the pharmacological effects of recently administered alcohol. Importantly, the effect was selective for alcohol-seeking, as responses on the inactive lever were unaffected by the treatment. Our results therefore suggest a major role for Sig-1R in either the incentive motivational mechanisms governing alcohol-seeking and intake or in the impact of alcohol-associated conditioned reinforcer (environmental cues) to maintain responding [38, 43].

Previously we and others have shown that Sig-1R antagonism reduces the consummatory and motivational behavior to work for alcohol in both operant and home-cage situations. Acute treatment with BD-1063 is indeed able to reduce ethanol self-administration in a fixed ratio-1 schedule of reinforcement as well as the motivation to work to obtain ethanol in a progressive ratio schedule of reinforcement in Scr:sP rats [29]. In addition, the selective Sig-1R antagonist NE-100 was shown to be able to suppress home-cage ethanol drinking in Scr:sP rats under continuous access conditions [28]. The present study adds further evidence supporting a key role for Sig-1Rs in alcohol drinking, and expands the previous findings by demonstrating that Sig-1R is also involved in the initial acceptance of an ethanol solution, i.e. the early acquisition phase of drinking, as well as in alcohol-seeking behavior. Blocking Sig-1Rs may therefore reduce the elevated probability of drinking that accompanies certain genetic backgrounds, thus allowing individuals with a genetic predisposition toward alcohol abuse to resist the impulse to start drinking heavily as well as to seek alcohol later in the condition.

Previous studies have shown that Sig-1R antagonists work to decrease alcohol intake selectively in alcohol-preferring rats, while the low to moderate drinking levels of outbred

rats is not affected by the treatment [29]. In the present study we therefore aimed at identifying potential innate differences in the expression levels of the Sig-1R protein in Scr:sP rats in reward-related brain areas. Indeed, ethanol-naïve Scr:sP rats showed innately enhanced levels of Sig-1R protein in the NAcc, compared to ethanol-naïve Wistar rats. On the other hand, no differences were observed among groups in expression of Sig-1R protein in the CeA. The NAcc and the CeA both are intimately related to reward-related processes and play a major role in the reinforcing effects of drugs, including alcohol [54–56]. In particular, the NAcc integrates limbic information related to memory, drive, and motivation with the generation of goal-directed behaviors [57–59]. The NAcc also is implicated in excessive alcohol drinking [60, 61]. The NAcc shows moderately concentrated staining of Sig-1R [62–64]. The alteration in the NAcc expression of Sig-1R displayed by Scr:sP rats may therefore explain the previously reported higher sensitivity of these alcohol-preferring rats to pharmacological blockade with Sig-1R antagonists [29]. In addition, considering that chronic treatment with a SigR agonist has been shown to induce binge-like drinking in rats [27], we argue that an overexpression of Sig-1R in the NAcc may therefore mediate the susceptibility to excessive drinking, both innate (as in alcohol-preferring rats) and induced by chronic alcohol exposure (as in alcohol-dependent animals). The present study did not discriminate between the Core and the Shell of the NAcc, as the brain punches in which the Sig-1R levels were measured included both sub-regions; future studies will determine whether one of the two contributes more than the other to the effect observed. Our laboratory has previously demonstrated innate differences in the mRNA expression of Sig-1R in alcohol-preferring rats compared to outbred rats [29]; the present study, therefore, extends those findings to the Sig-1R protein, adding important information about the genetic basis of high alcohol drinking.

To investigate whether voluntary alcohol drinking could alter Sig-1R expression, we also measured Sig-1R protein levels in the NAcc and CeA of Scr:sP rats continuously exposed to voluntary alcohol drinking. We found that a 4-week consumption of relatively high amounts of ethanol (~5.5 g/kg/day) by Scr:sP rats was associated with reduced (normalized) Sig-1R protein levels in the NAcc, a region-specific effect (no effect in the CeA). Our observation is in line with several reports showing that ethanol drinking –especially chronic– can differentially affect ethanol-naive alcohol-preferring rats, eliminating or introducing neurochemical behavioral differences [65–68]. The now decreased Sig-1R levels, which may appear counter-intuitive at a first glance, may instead be in part responsible for the reduced motivation to drink alcohol which follows recent alcohol consumption; future studies can assess whether an abstinence period, which increases the motivation to drink and can drive relapse [69–72], can restore Sig-1R levels. This hypothesis is consistent with the proven ability of a Sig-1R antagonist to reduce alcohol-deprivation effect in Scr:sP rats after a 7-day abstinence [28]. In this study we recorded the alcohol intake of each individual cage, but not each individual rat; it will be interesting in future studies to assess whether the mean daily alcohol intake over the exposure period shows a correlation with NAcc Sig-1R protein levels, i.e. whether Scr:sP rats which drink more alcohol show also lower levels.

Previous studies in limbic areas have shown the interaction between Sig-1R and dopamine system [73, 74]. Sig-1R is indeed localized in brain areas in which dopamine innervation is highly distributed [62, 75, 76]. Sig-1R has been indeed demonstrated to affect the whole

process of dopamine production, from synthesis to release, and uptake [77–79], with the activation of Sig-1R generally increased the firing rate of dopaminergic pathways [80, 81]. Therefore, the blockade of Sig-1R may attenuate dopamine neurotransmission in reward-related areas, which in turn may suppress the acquisition of ethanol drinking behavior as well as the seeking behavior. The dopamine mesolimbic system has been shown to be essential for acquisition of alcohol drinking behavior, as lesions with 6-hydroxy dopamine in the NAcc for example attenuate the acquisition of ethanol consumption in Indiana preferring rats [82]. Dopamine release in the NAcc has also been shown to be increased by non-contingent presentations of drug-associated conditioned stimuli [83]. Nevertheless, the potential involvement of other neurotransmitter systems (e.g. glutamatergic) in the observed effects of the Sig-1R antagonist cannot be ruled out, especially considering the involvement of glutamatergic transmission in the NAcc in drug-seeking behavior [84] and the interaction reported between the Sig-1R and the glutamatergic system, mainly NMDA [85–87].

The data here presented give novel, important insights regarding the potential genetic basis of excessive alcohol consumption and seeking, suggesting that innate differences in the Sig-1R system are associated with higher alcohol preference in Scr:sP rats and that the modulation of this system regulates acquisition and seeking of alcohol. The findings also indicate a modulation of Sig-1R levels following chronic alcohol consumption, suggesting that the NAcc may be a key brain region for the Sig-1R modulation of alcohol-drinking behavior observed after systemic administration of ligands.

Altogether these results suggest that SigR system plays a major role in the susceptibility to alcohol addiction and strengthen the notion that Sig-1Rs are a very important therapeutic target for alcohol abuse and dependence.

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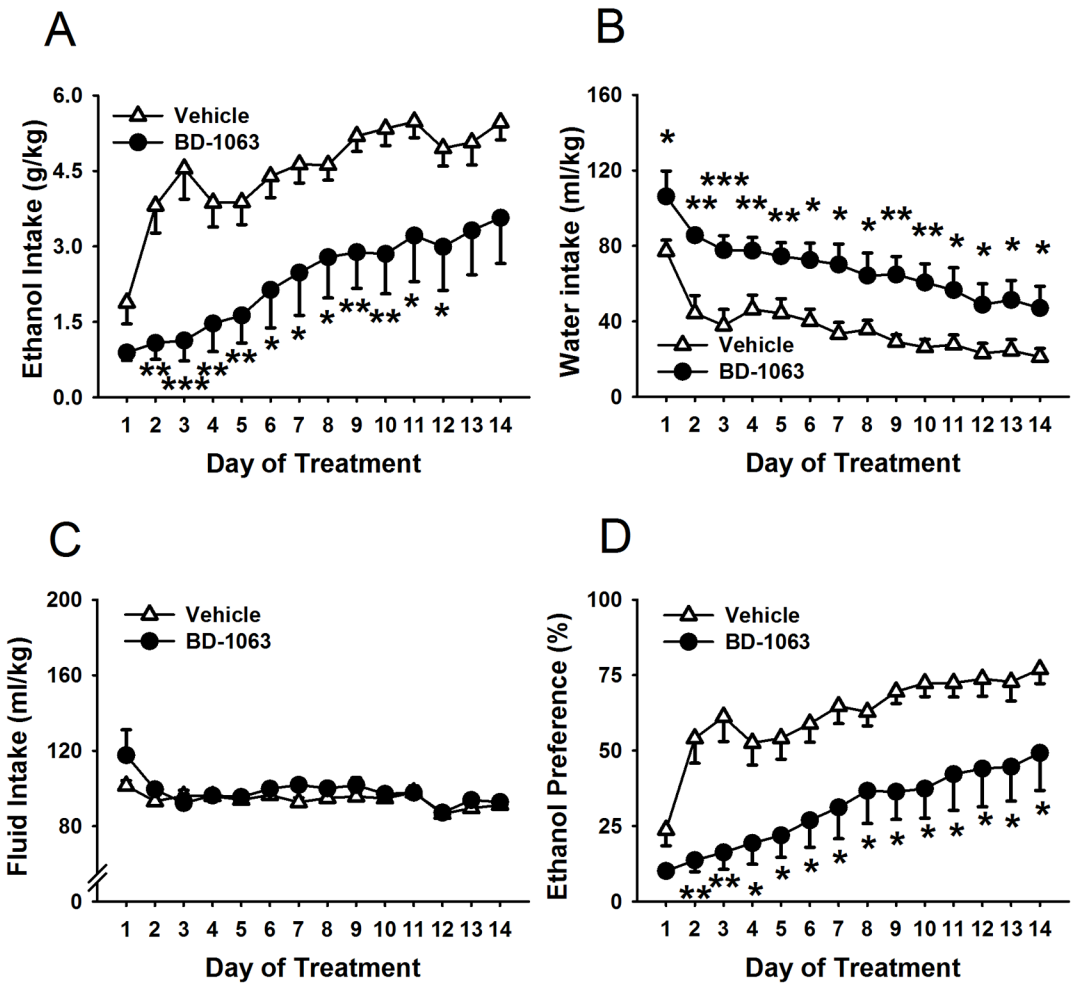

Figure 1.

Effect of chronic administration (14 days) of the Sig-1R antagonist BD-1063 (0, 30 mg/kg, *s.c.*) on daily ethanol intake (g/kg) (panel A), water intake (ml/kg) (panel B), ethanol preference (%) (panel C) and total fluid intake (ml/kg) (panel D) in Scr:sP rats (n=18). Data represent Mean ± SEM; * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$ vs. vehicle-treated group.

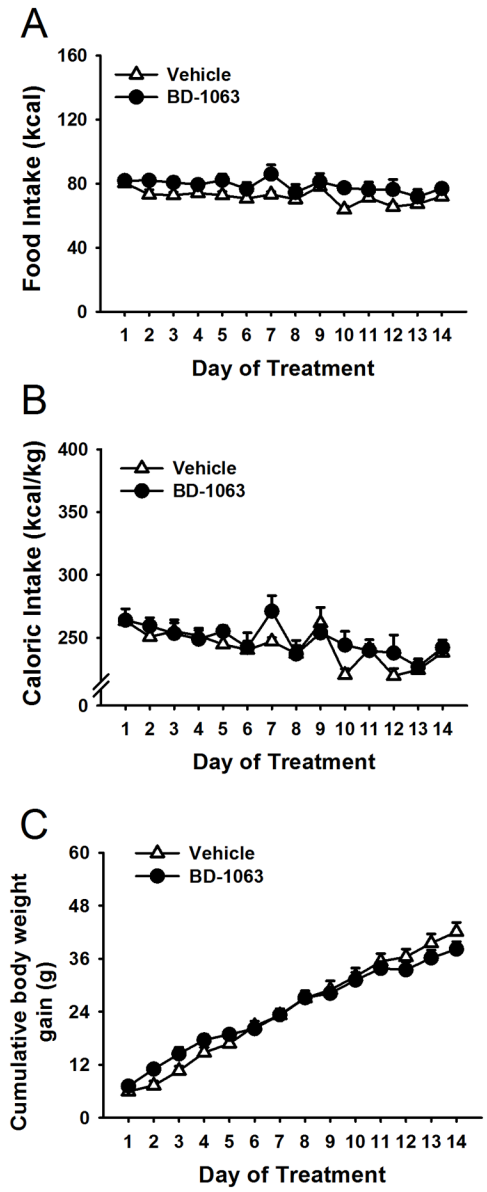

Figure 2.

Effect of chronic administration (14 days) of the Sig-1R antagonist BD-1063 (0, 30 mg/kg, *s.c.*) on food intake (panel A), total caloric intake (panel B) and cumulative body weight gain (panel C) in Scr:sP rats exposed 24h/day to the ethanol-water choice (*n*=18). Data represent Mean ± SEM.

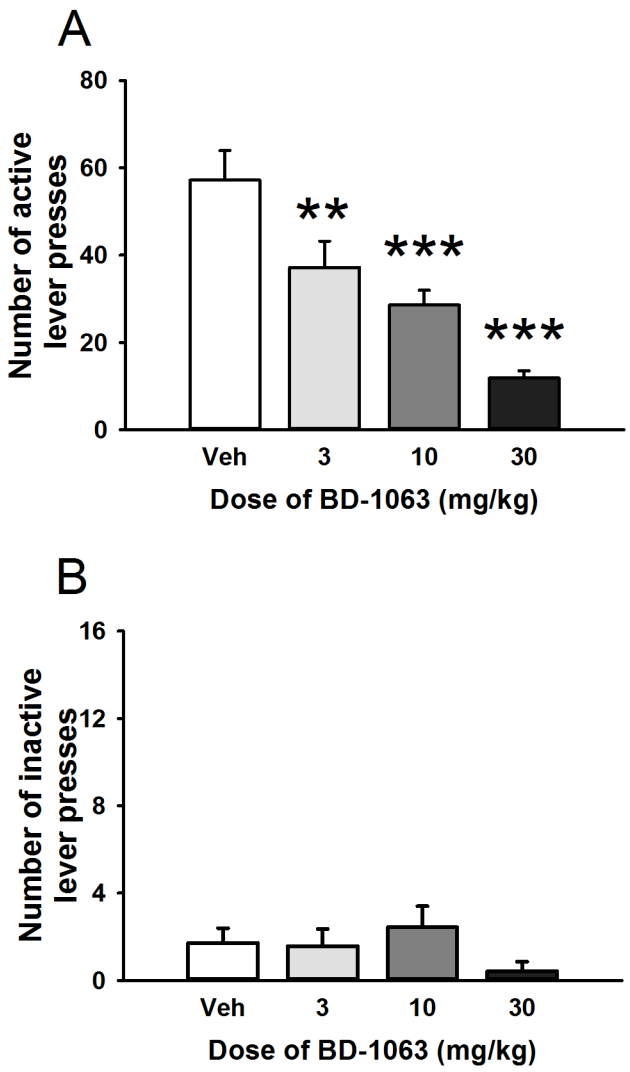

Figure 3.

Effect of acute administration of the Sig-1R antagonist BD-1063 (0, 3, 10, 30 mg/kg) on an operant second-order schedule of reinforcement to assess alcohol-seeking behavior in Scr:sP rats (*n*=7). The number of active lever presses following BD-1063 administration is shown in panel A, the number of lever presses on the inactive lever in panel B. Data represent Mean ± SEM; ** $p < 0.01$, *** $p < 0.001$ vs. vehicle-treated group.

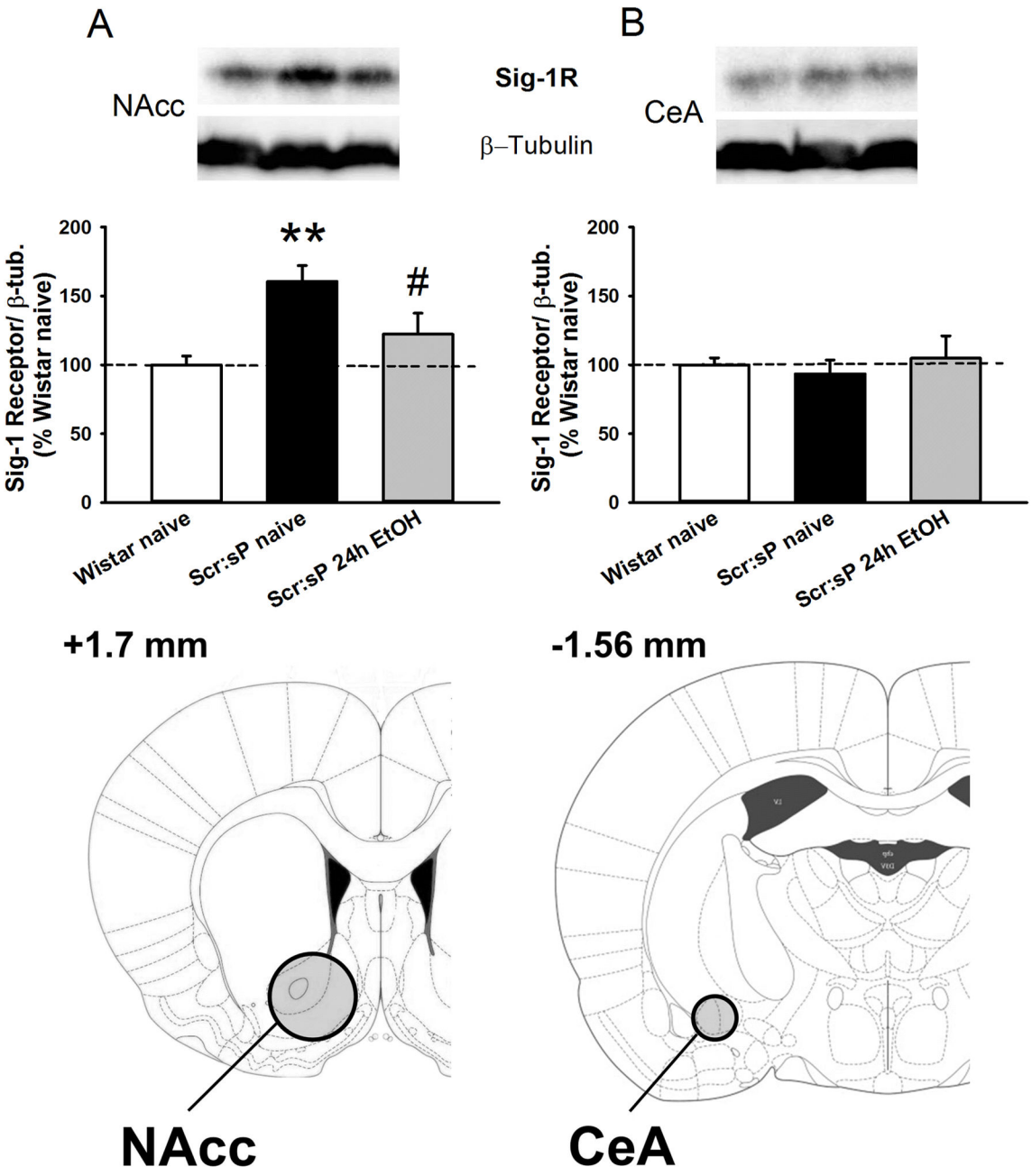

Figure 4.

Sig-1 receptor protein expression normalized to β-tubulin in the nucleus accumbens (NAcc) (panel A) and the central nucleus of the amygdala (CeA) (panel B) (*n*=14). Panel C contains drawings of rat brain coronal slices, where circles show brain regions that were punched out for the western blot. Values are expressed as % of the control Wistar group. Data represent Mean ± SEM; ** $p < 0.01$ vs. Wistar group, # $p < 0.05$ vs. Scr:sP naïve group.