

The selective dopamine D3 receptor antagonist SB-277011-A attenuates ethanol consumption in ethanol preferring (P) and non-preferring (NP) rats

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Received 22 September 2004; received in revised form 11 March 2005; accepted 21 March 2005

Available online 28 April 2005

Abstract

The mesolimbic dopamine (DA) system plays an important role in mediating addiction to alcohol and other drugs of abuse. Recent evidence points toward the role of the DA D3 receptor (D3R) in drug-induced reward, drug-taking, as well as cue-, drug-, and stress-triggered relapse to drug-seeking behavior. Accordingly, the present study examined the effects of acute selective antagonism of the D3R on ethanol consumption in alcohol Preferring (P) and Non-Preferring (NP) rats. We employed the two-bottle choice paradigm to monitor ethanol consumption in these rats before and after treatment with 3, 10, and 30 mg/kg (i.p.) of the selective D3R antagonist SB-277011-A. Results indicated a significant attenuation in ethanol preference, intake and lick responses in P rats treated with 10 and 30 mg/kg SB-277011-A. A similar, though not as robust effect was observed in ethanol consumption in the NP rats when treated with 30 mg/kg SB-277011-A. Finally, the acute administration of SB-277011-A did not produce extrapyramidal side effects, as indicated by stable lick response–volume ratios and lick response time distributions. These results further support the notion that the D3R is important in mediating the addictive properties of alcohol and suggest that selective blockade of the D3R may constitute a new and useful target for prospective pharmacotherapeutic approaches to alcoholism.

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Keywords: Alcoholism; Addiction; Dopamine; Drinking

1. Introduction

Alcoholism in the United States is a serious health concern. Fourteen million Americans, or 7.5% of the population, classify as alcoholics or alcohol abusers according to DSM-IV criteria (Grant et al., 1994). In 1998, projected annual costs associated with alcoholism

totalled \$185 billion, accounting for 60% of the total cost of drug abuse (Harwood, 1998, 2000).

A number of neurochemical pathways have been implicated in alcoholism, including the DA, GABA, norepinephrine, serotonin, opioid, and cannabinoid neurotransmitter systems. The dopaminergic pathway, in particular, is associated with emotive, cognitive, endocrine, and motor functions, and reinforcement of addictive substances or behaviors (Volkow et al., 2002). The dopaminergic pathway consists of five receptor subtypes, D₁–D₅, which are classified in D₁-like (D₁ and D₅ receptors) and D₂-like (D₂, D₃ and D₄ receptors) families. Previous studies have

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implicated the D₂ family of receptors and in particular the D2 receptor (D2R), as playing an important role in alcohol and drug addiction (Blum et al., 2000, 1996, 1990; Thanos et al., *in press*, 2004, 2001; Volkow et al., 1993, 1996, 2002).

Recently, a growing body of evidence has strengthened the likelihood that the D3R is also significantly involved in mechanisms of drug dependence and abuse. It has been shown that the D3R is primarily localized in brain areas associated with the mesolimbic DA system and the rewarding properties of alcohol (Heidbreder et al., 2004). Furthermore, associations between gene variants at the D3R and alcoholism, as well as functional pharmacological aspects of different D3R and mixed D3R/D2R agonists and antagonists were recently reviewed in the context of alcohol intake and relapse (Heidbreder et al., 2004).

In humans, D3Rs have been localized in the mesolimbic regions of the human forebrain, including the nucleus

accumbens, ventral striatum, and islands of Calleja, and are present in low to moderate levels in the cerebral cortex, amygdala, and substantia nigra (Suzuki, 1998). Homologous D3R distribution has been observed in various species of rodents (Sokoloff et al., 1990).

Recent studies have demonstrated that SB-277011-A, a highly selective D3 receptor antagonist, significantly attenuates cocaine seeking behavior (Di Ciano et al., 2003; Vorel et al., 2002), cocaine-induced place preference (Gyertyan and Gal, 2003), nicotine conditioning (Le Foll et al., 2003), nicotine-triggered relapse (Andreoli et al., 2003), and heroin-induced place preference (Ashby et al., 2003). Thus, one may suggest that the selective D3R antagonist SB-277011-A (Reavill et al., 2000) putatively mitigates pleasure reinforcement by modulating activity of midbrain dopaminergic neurons (Ashby et al., 2000) and hence drug and ethanol self-administration. In the present study we

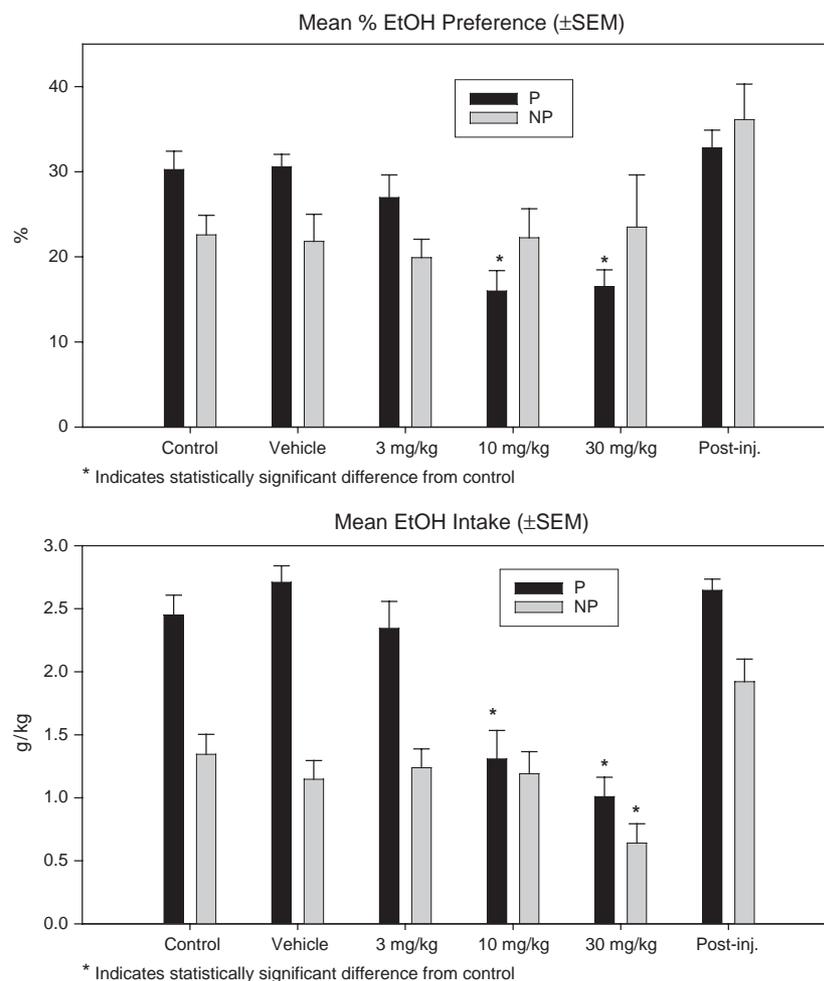


Fig. 1. The dopamine D3R antagonist SB-277011-A significantly (Tukey test, $p < 0.05$) diminished mean percent ethanol preference for P rats [one-way ANOVA ($F = 11.776$, $df = 5, 54$; $p < 0.001$) pairwise multiple comparisons using the Tukey test revealed significant differences between vehicle and 10 and 30 mg/kg; $p < 0.001$] but not for NP rats [one-way ANOVA ($F = 2.393$, $df = 5, 54$; ns)]. SB-277011-A significantly reduced ethanol intake for ethanol-preferring P rats [one-way ANOVA ($F = 18.786$, $df = 5, 54$; $p < 0.001$) pairwise multiple comparisons using the Tukey test revealed significant differences between vehicle and 10 and 30 mg/kg; $p < 0.001$] and for NP rats [one-way ANOVA ($F = 6.532$, $df = 5, 54$; $p < 0.001$) pairwise multiple comparisons using the Tukey test revealed significant differences between vehicle and 30 mg/kg; $p < 0.05$]. For both P and NP rats, resurgence in ethanol consumption was observed during the 3-day post-injection period, indicating the effect of the SB-277011-A was acute. In addition, a two-way ANOVA between strain (P, NP) and the effects of the D3R antagonist found a significant interaction for ethanol intake ($F = 3.889$, $df = 2, 54$; $p < 0.05$) but not for preference ($F = 2.816$, $df = 2, 54$; ns).

examined ethanol self-administration behavior in a rat paradigm of alcoholism and the effects of acute treatment with SB-277011-A. The ethanol-preferring P and non-preferring NP rat strains are well-established models of human alcoholism (Lumeng and Hawkins, 1977; McBride and Li, 1998). In an unrestricted access, two-bottle (water and ethanol) paradigm, P rats typically consume in excess of 6 g/kg/d ethanol, whereas NP rats typically consume less than 0.5 g/kg/d (Lumeng et al., 1982; McBride and Li, 1998), reflecting dichotomous behaviors between alcoholics and non-alcoholics. We hypothesized that SB-277011-A would attenuate ethanol intake, preference and ethanol-seeking behavior in P and NP rats.

2. Materials and methods

2.1. Animals

Ethanol-preferring (P, $n=10$) and non-preferring (NP, $n=10$) male adult rats (375–500 g) selectively inbred 50

generations (Wistar background) were used. Rats were individually housed in a controlled environment (22 ± 2 °C, $50 \pm 10\%$ relative humidity) and subjected to a 24-h reverse light cycle (lights off at 0800 hours) to maintain activity during daytime. Weights were obtained daily for calculation of ethanol intake and SB-277011-A doses. Food was provided ad libitum and water access was restricted to 4-h operant sessions. Experiments were conducted in conformity with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals (1996) and Brookhaven National Laboratory Institutional Animal Care and Use Committee protocols.

2.2. Operant ethanol self-administration

In order to characterize the behavioral effects of the SB-277011-A, a two-bottle (water and 15% v/v ethanol) self-administration paradigm was employed. Animals were introduced to computer automated operant chambers (Mini-Mitter) equipped with lickometer sensors and VitalView software for 4 h daily at 1000 hours. All

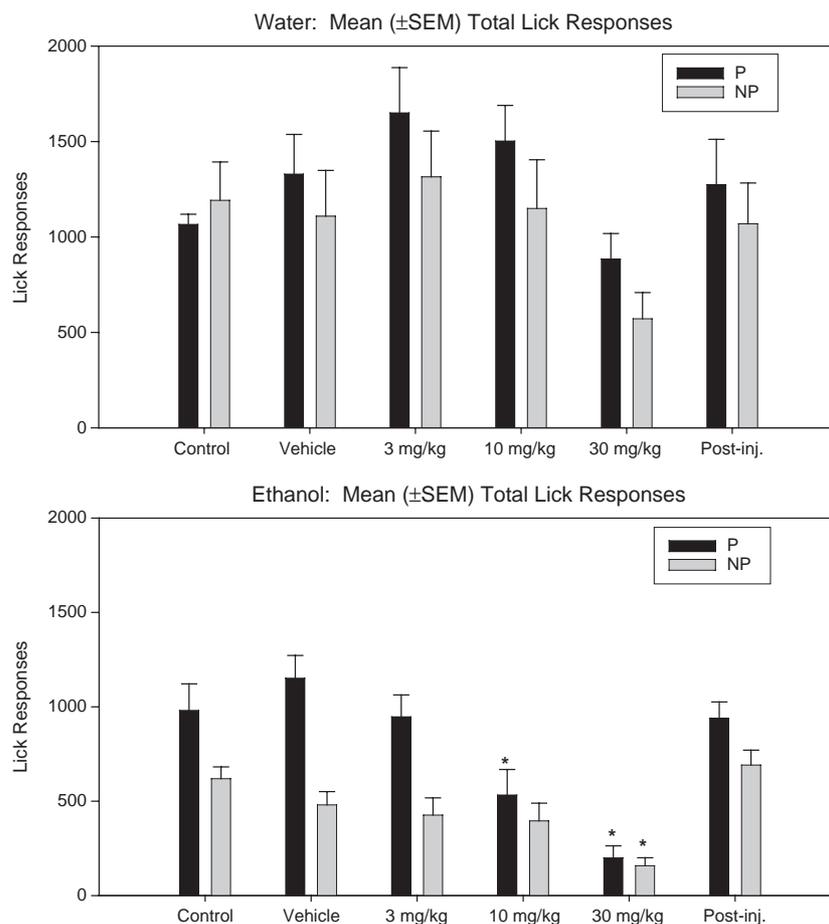


Fig. 2. Water versus ethanol total lick responses. SB-277011-A did not significantly influence total water lick responses for P rats [one-way ANOVA ($F=2.222$, $df=5, 54$; ns)] or NP rats [one-way ANOVA ($F=1.397$, $df=5, 54$; ns)]. SB-277011-A however did significantly influence total ethanol lick responses for P rats [one-way ANOVA ($F=9.74$, $df=5, 54$; $p<0.001$) pairwise multiple comparisons using the Tukey test revealed significant differences between vehicle and 10 mg/kg ($p<0.01$) and 30 mg/kg; $p<0.001$] and NP rats [one-way ANOVA ($F=6.204$, $df=5, 54$; $p<0.001$) pairwise multiple comparisons using the Tukey test revealed significant differences between vehicle and 30 mg/kg; $p<0.05$]. * indicates statistically significant difference from control.

experiments were performed in the dark. The positions of ethanol and water bottles were interchanged daily, and contents were flushed and refilled daily. Volume consumption (adjusting for spill volume) and time-specific lick response data were collected daily.

2.3. Control and vehicle baseline criteria

Control baseline criteria were satisfied when an individual exhibited less than $\pm 20\%$ deviation in mean percent ethanol preference for 5 consecutive days. Subsequently, animals were administered vehicle injections of saline (0.9% NaCl) intraperitoneally (IP) immediately prior to drinking sessions. Vehicle baseline criteria were achieved when an animal exhibited less than $\pm 20\%$ deviation from its 5-day control mean preference for 3 consecutive days.

2.4. Drug

SB-277011-A, *trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydro-isoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecar-

boxamide (GlaxoSmithKline Pharmaceuticals), was dissolved in 40% (w/w) 2-hydroxypropyl- β -cyclodextrin (HBC) in 0.9% NaCl in 3, 10, and 15 mg/ml concentrations. Following satisfaction of vehicle criteria, rats were administered IP 3, 10, and 30 mg/kg doses of SB-277011-A on consecutive days immediately prior to drinking sessions. Operant self-administration was monitored up to 3 days post-drug injection.

2.5. Data analysis

Volume consumption, percent preference, cumulative lick response, and lick response–volume ratio data were collected from all animals. Normalized and non-normalized data of these main factors were analyzed by one-way ANOVA and subsequent multiple pairwise comparisons using the Tukey test. In all analyses, mean values (and associated standard error values) represent a group composite of individual mean 5-day control, 3–6-day vehicle, and 2–3-day post-injection data.

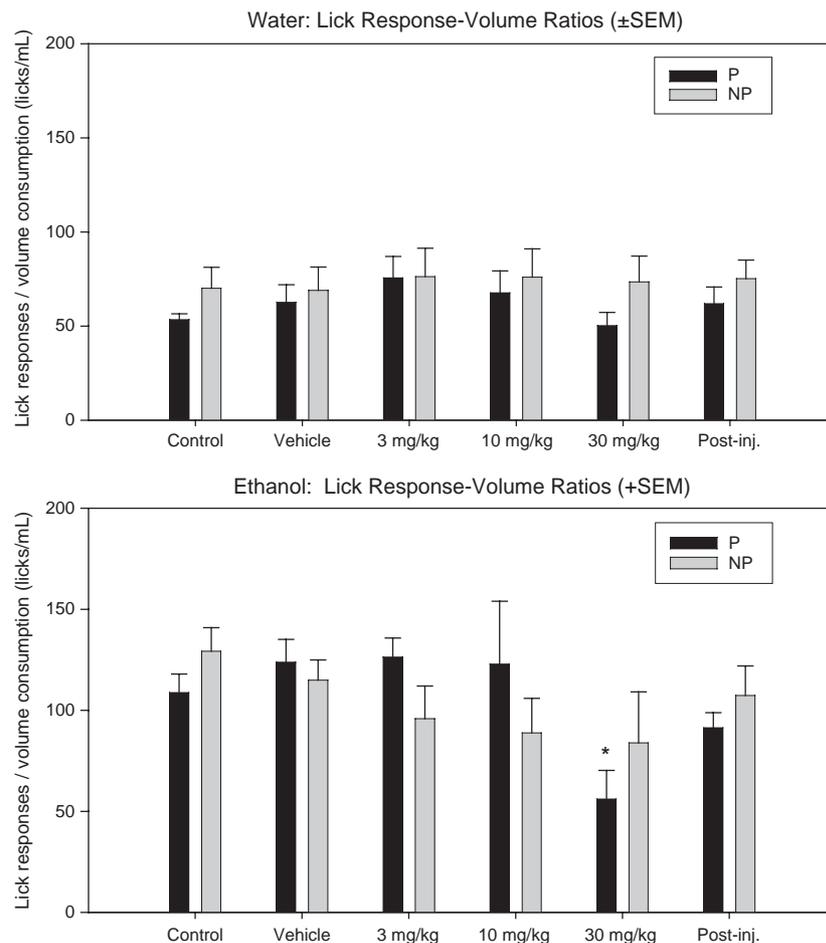


Fig. 3. Lick response–volume ratio for water and ethanol. SB-277011-A did not significantly influence water lick response–volume ratio for P rats [one-way ANOVA ($F=1.012$, $df=5$, 54 ; ns)] or NP rats [one-way ANOVA ($F=0.057$, $df=5$, 54 ; ns)]. SB-277011-A significantly changed ethanol lick response–volume ratio for P rats [one-way ANOVA ($F=2.806$, $df=5$, 54 ; $p<0.02$)] pairwise multiple comparisons using the Tukey test revealed significant differences between vehicle and 30 mg/kg; $p<0.05$] but not NP rats [one-way ANOVA ($F=1.115$, $df=5$, 54 ; ns)]. * indicates statistically significant difference from control.

3. Results

Control and vehicle baseline ethanol self-administration between P and NP rats revealed significantly greater ethanol preference and intake in P rats when compared to NP rats (Fig. 1). SB-277011-A significantly reduced ethanol preference and ethanol intake (g/kg) in P rats at the 10 and 30 mg/kg doses, and ethanol intake in NP rats at the 30 mg/kg dose (Fig. 1). For both P and NP rats, a return in ethanol consumption was observed during the 3-day post-injection period. No such effects were observed at the 3 and 10 mg/kg doses. Results revealed a strong drug discontinuation effect in NP rats, with an increase in post-injection NP ethanol preference (143% control baseline) and ethanol intake (130% control baseline) in Fig. 1.

Operant behavior (lick responses) was assessed for both groups of rats in the two-bottle choice paradigm (Fig. 2). Water lick responses were constant across treatment, but ethanol lick responses decreased sequentially in concurrence with percent ethanol preference and ethanol intake trends (Fig. 2). SB-277011-A significantly attenuated total

ethanol lick responses for P and NP rats at the 30 mg/kg dose.

Lick responses were also examined with respect to volume consumed of ethanol or water (licks/ml). Quantitative assessment of these ratios (Fig. 3) revealed a homogeneous drinking profile (no significant differences) with regard to water consumption for both P and NP rats across treatment condition. The drinking profile with regard to ethanol again yielded a homogeneous pattern across treatment for both P and NP rats. However, comparison of water versus ethanol, lick response/volume ratios revealed approximately a 2-fold greater ratio to ethanol by both groups of rats (Fig. 3). When the data were normalized to control values (Fig. 4) the ratio data was similar and showed a significant decrease to ethanol in licks/ml for P rats treated with 30 mg/kg SB-277011-A.

Cumulative lick responses over time were examined in both P and NP rats to both water and ethanol. Specifically, distribution of water and ethanol lick responses over 15-min time intervals, as cumulative and rate functions, respectively were plotted in Fig. 5. In both

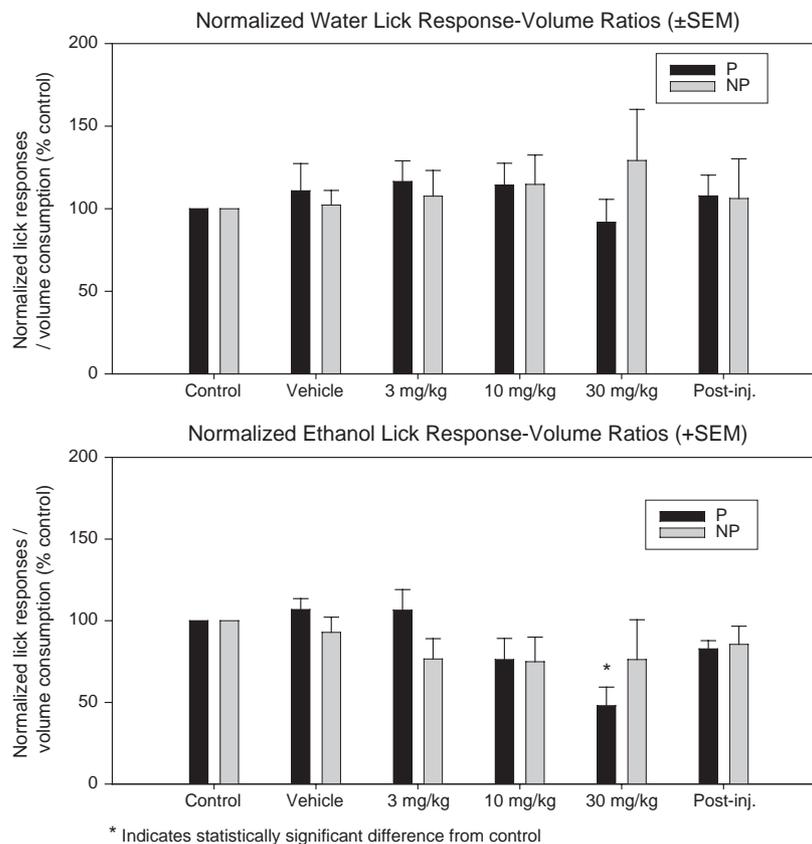


Fig. 4. Normalized lick response–volume ratios for water and ethanol (from Fig. 3). SB-277011-A did not significantly influence normalized water lick response–volume ratio for P rats [Kruskal–Wallis one-way ANOVA on ranks ($H=2.172$; ns)] or NP rats [Kruskal–Wallis one-way ANOVA on ranks ($H=1.644$; ns)]. SB-277011-A significantly altered normalized ethanol lick response–volume ratio for P rats [Kruskal–Wallis one-way ANOVA on ranks ($H=19.235$; $p<0.01$)] pairwise multiple comparisons using Dunn’s method revealed significant differences between vehicle and 30 mg/kg; $p<0.05$] but not NP rats [one-way ANOVA ($F=0.59$; ns)].

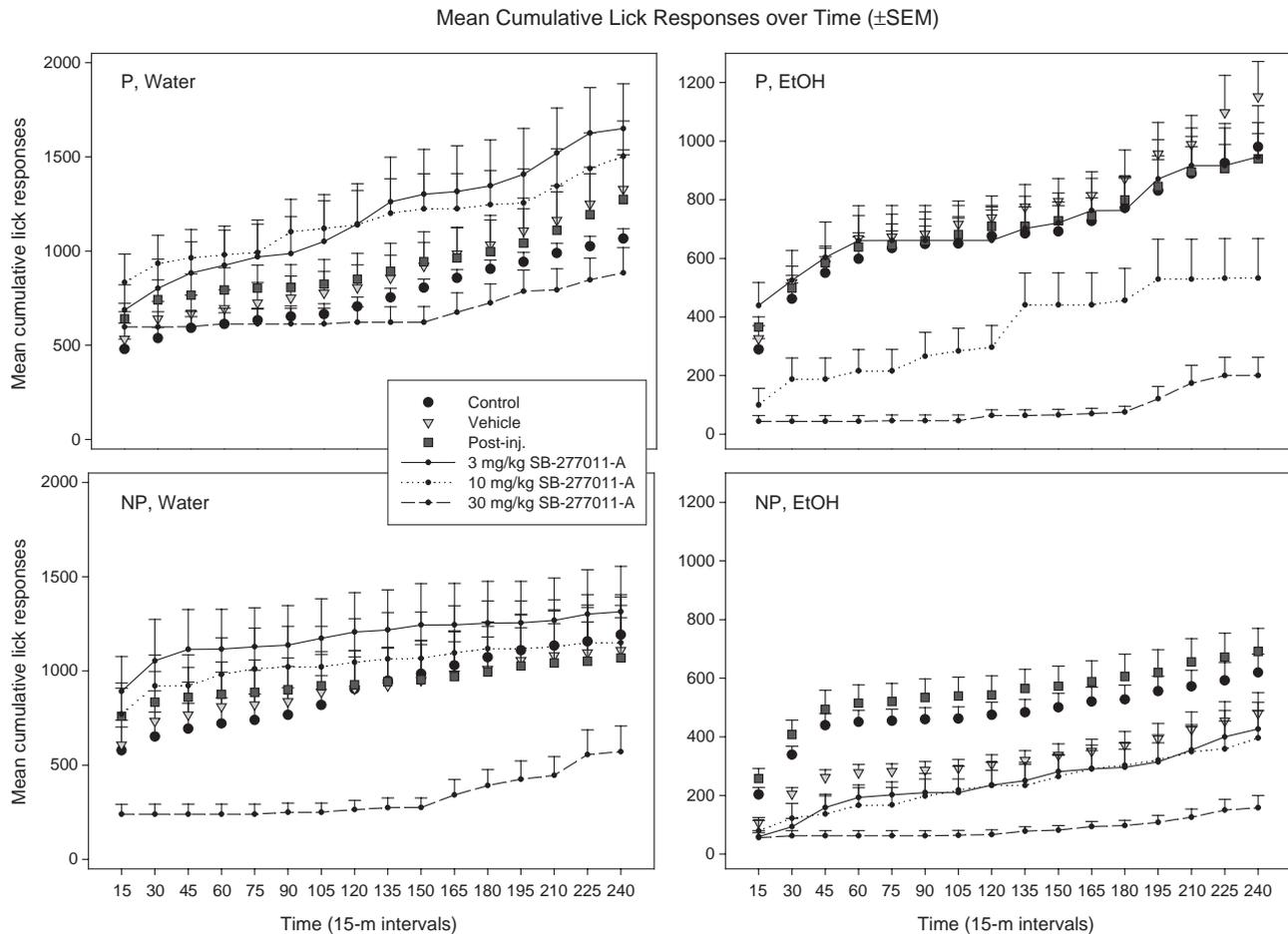


Fig. 5. Mean water and ethanol lick responses per 15-min time interval, preceding, during, and following SB-277011-A. A two-way repeated measure ANOVA was conducted on both groups of rats for both water and ethanol lick responses over time. Specifically, the pattern of ethanol drinking over time for P rats revealed a significant Treatment ($F=9.74$, $df=5$, 810; $p<0.0001$), Time ($F=37.13$, $df=15$, 810; $p<0.0001$) and Interaction ($F=3.41$, $df=75$, 810; $p<0.0001$) effects. The pattern of water drinking over time for P rats revealed no significant Treatment ($F=2.22$, $df=5$, 810; $p=ns$), and a significant Time ($F=121.65$, $df=15$, 810; $p<0.0001$) and interaction effect ($F=1.41$, $df=75$, 810; $p<0.01$). NP rats: the pattern of ethanol drinking revealed a significant Treatment ($F=6.2$, $df=5$, 810; $p<0.001$), Time ($F=36.63$, $df=15$, 810; $p<0.0001$) and Interaction ($F=4.26$, $df=75$, 810; $p<0.0001$) effect. The pattern of water drinking revealed no significant Treatment ($F=1.4$, $df=5$, 810; $p=ns$), but did show a significant Time ($F=98.13$, $df=15$, 810; $p<0.0001$) and Interaction ($F=2.61$, $df=75$, 810; $p<0.0001$) effect.

groups of rats, SB-277011-A dose-dependently influenced lick responses.

4. Discussion

In the present study, behavioral analysis of P rats treated with SB-277011-A showed decreased percent ethanol preference, decreased ethanol intake, decreased ethanol lick responses and sustained water lick responses. Although the 3 mg/kg dose was not found to produce any significant changes in ethanol consumption, 10 and 30 mg/kg SB-277011-A significantly attenuated preference, intake and ethanol seeking behavior. The effect of SB-277011-A was particularly evident when examining ethanol mean cumulative lick responses over time (Fig. 5).

Ethanol consumption in NP rats, while limited in comparison to P rats, showed a similar though not as

robust effect in response to SB-277011-A. NP ethanol preference however showed no statistical change, perhaps due in part to greater variability in water lick responses, including a reduction in water lick responses at the 30 mg/kg dose of SB-277011-A (Fig. 5). NP ethanol intake (Fig. 1), and ethanol lick responses (Fig. 2) however, decreased linearly with drug dose. Assessment of the interaction between strain (P, NP) and the effects of the D3R antagonist found a significant interaction for ethanol intake, revealing that the ethanol intake in the two groups of rats was dependant upon the D3R antagonist. Thus, SB-277011-A had a significant effect on attenuating alcohol consumption but the magnitude of this decrease was also dependent upon the strain (P or NP).

Following treatment with SB-277011-A, all rats were monitored for an additional 3 days (post-injection). The data showed a strong drug discontinuation effect in NP rats, with an increase in post-injection NP ethanol

preference (143% control baseline) and ethanol intake (130% control baseline) as compared to P rats in Fig. 1. In addition, this drug discontinuation effect (post-injection) was observed in the cumulative lick response data (Fig. 5). This phenomenon was not observed in P rats. Post-injection behavior for these animals reverted back to previous control baseline percent ethanol preference and ethanol intake (Fig. 1). This return in post-injection ethanol consumption indicated that the attenuating effects of SB-277011-A were acute. Previous autoradiography studies using [3H]-7-OH-DPAT have shown that there were no differences in D3R binding between P and NP rats (McBride et al., 1997). However, these results must be interpreted with caution since 7-OH DPAT may also label D2R depending on experimental conditions and protocols (Gonzalez and Sibley, 1995).

Examination of the lick responses/volume consumption data revealed another interesting observation in that P and NP rats displayed approximately two fold greater ratio for ethanol compared to water (Fig. 3). This would suggest differences in the way both groups of rats responded to ethanol compared to water. Furthermore, similar results for P and NP rats suggest both lines ingest water and ethanol in a similar manner.

A transgenic and gene knockout (*Drd3*^{-/-} mice) approach recently examined and concluded that elimination of the D3R (*Drd3*^{-/-}) had little influence on ethanol intake (Boyce-Rustay and Risinger, 2003) or a decrease in ethanol intake in a chronic (7% v/v) ethanol diet though these animals had a longer loss of righting reflex to a 3.5-g/kg dose of ethanol, indicating an increase in sensitivity to ethanol (Narita et al., 2002). These results are in contrast to those of pharmacological studies employing the mixed D2/D3 receptor antagonist U99194A that enhanced (Boyce and Risinger, 2002) ethanol-induced place conditioning. The discrepancies could reflect differences in the mice strains used by these two previous studies since it has been shown that mouse strain background influences phenotypic expression of genotypes (Phillips et al., 1999; Xu et al., 1997), but also brain adaptations in knockouts, which are not observed in wild-type animals in response to a pharmacological challenge. The findings with the nonselective D2/D3 receptor antagonist U99194A also differ from our results showing decreases in alcohol consumption with the selective D3R antagonist SB-277011-A. This could reflect the fact that U99194A is not selective for D3R (10–15-fold more selective) vs. D2R (Waters et al., 1994), compared with SB-277011-A (100-fold higher selectivity for D3R over D2R). In contrast with U99194A and other mixed D2/D3 receptor antagonists, SB-277011-A is highly selective and does not produce catalepsy, does not alter locomotion in habituated or non-habituated animals, does not increase plasma prolactin levels, and fails to alter amphetamine-induced hyperlocomotion (Reavill et al., 2000).

In conclusion, these findings further support the concept that the D3R is significant in mediating the addictive

properties of alcohol and propose that selective blockade of the D3R may represent a new and useful target for prospective pharmacotherapeutic approaches to alcoholism. Furthermore, these results demonstrated that highly selective antagonism of D3R could attenuate alcohol-seeking behaviors without significant side effects. While further studies are necessary to better examine this new potential pharmacotherapeutic strategy for alcoholism, the present results are encouraging.

Acknowledgment

We thank the SULI program at Brookhaven National Laboratory for supporting JMK. This work was supported by the NIAAA (AA 11034 and AA07574, AA07611) and by the U.S. Department of Energy under contract DE-AC02-98CH10886.

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