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Electrolytic lesions of the medial nucleus accumbens shell selectively decrease ethanol consumption without altering preference in a limited access procedure in C57BL/6J mice ☆

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ABSTRACT

The central extended amygdala (cExtA) is a limbic region proposed to play a key role in drug and alcohol addiction and to contain the medial nucleus accumbens shell (MNAc shell). The aim of this study was to examine the involvement of the MNAc shell in ethanol and sucrose consumption in a limited and free access procedure in the C57BL/6J (B6) mouse. Separate groups of mice received bilateral electrolytic lesions of the MNAc shell or sham surgery, and following recovery from surgery, were allowed to voluntarily consume ethanol (15% v/v) in a 2 h limited access 2-bottle-choice procedure. Following 1 week of limited access ethanol consumption, mice were given 1 week of limited access sucrose consumption. A separate group of lesioned and sham mice were given free access (24 h) to ethanol in a 2-bottle choice procedure and were run in parallel to the mice receiving limited access consumption. Electrolytic lesions of the MNAc shell decreased ethanol (but not sucrose) consumption in a limited access procedure, but did not alter free access ethanol consumption. These results suggest that the MNAc shell is a component of the underlying neural circuitry contributing to limited access alcohol consumption in the B6 mouse.

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1. Introduction

Alcohol-related disorders remain major public health problems (Kranzler and Rosenthal, 2003). Although the physiological mechanisms and neural circuits associated with these disorders are poorly understood (Mann, 2004; Mariani and Levin, 2004), the central extended amygdala (cExtA) has been proposed to play a key role in drug addiction and alcohol dependence (Koob and Le Moal, 2005). The cExtA is mainly comprised of the central nucleus of the amygdala (CeA) and the lateral bed nucleus of the stria terminalis (LBNST), but it continues into the nucleus accumbens shell (Nac shell) [more specifically, the medial portion (MNAc shell)]. Pharmacological manipulations of these three nuclei have been shown to alter ethanol consumption and operant self-administration of ethanol in rats (Hyytia and Koob, 1995; Heyser et al., 1999; Eiler et al., 2003; Funk et al., 2006; Foster et al., 2004; Roberts et al., 1996), and in C57BL/6J (B6) mice (Finn et al., 2007), confirming that this brain region is associated with alcohol-related measures of reward and reinforcement.

Numerous neurotoxic lesions and pharmacological manipulations made to the NAc have implicated its involvement in ethanol consumption. For example, lesioning the dopaminergic terminals of the NAc with the neurotoxin 6-hydroxydopamine (6-OHDA) produced the following variable results: a blockade in the acquisition, but not in the maintenance of ethanol consumption in a free access procedure in female alcohol-preferring P rats (Ikemoto et al., 1997); no effect on either the acquisition or the maintenance of ethanol consumption in male alcohol-preferring AA rats (Koistinen et al., 2001); no effect on the maintenance of limited access self-administration (Rassnick et al., 1993) and free access consumption (Fahlke et al., 1994) of ethanol in Wistar rats; and increased consumption and preference for ethanol during maintenance in a free access procedure in Sprague Dawley rats (Quarfordt et al., 1991). It is possible that the difference in genetic background might account for the variation in results, as was recently demonstrated in dopamine receptor 2 (DRD2) knockout mice that had been backcrossed onto a B6 background (Thanos et al., 2005). Viral vector-mediated overexpression of DRD2 specifically in the NAc decreased acquisition of ethanol consumption in a free access procedure in wild-type mice, but increased ethanol consumption in the DRD2 knockout mice (Thanos et al., 2005). Overall, these results suggest that the dopaminergic system of the NAc can modulate limited and free

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access ethanol consumption in rats and mice, and that genetic background can influence the effect of dopamine deficiency or increased dopaminergic sensitivity in the NAc on ethanol consumption.

In addition to manipulations of the dopaminergic system, pharmacological manipulations of the GABAergic, glutamatergic and serotonergic systems of the NAc have also been shown to affect ethanol consumption in rats. The GABA-A receptor antagonist, SR95531, administered into the NAc shell decreased self-administration of ethanol, but not water intake, demonstrating that the decrease in self-administration was selective for ethanol (Hyytia and Koob, 1995). Intra-NAc injection of the *N*-methyl-D-aspartate (NMDA) receptor partial agonist, 1-aminocyclopropanecarboxylic acid (ACPC), or the competitive NMDA receptor antagonist, 2-amino-5-phosphopentanoic acid (AP-5), decreased the maintenance of limited access ethanol consumption (Stromberg et al., 1999) and free access ethanol consumption (Rassnick et al., 1992), respectively. A recent study by Kapasova and Szumlinski (2008) carried out in B6 mice also showed that intra-MNAc shell microinjections of the glutamate reuptake inhibitor dl-threo-beta-benzyloxyaspartic acid (TBOA) can enhance alcohol intake under free access conditions while the local infusion of the mGluR2/3 agonist (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC) reduced this measure. Injection of the 5-HT_3 antagonist, tropisetron, into the MNAc shell decreased the maintenance of ethanol consumption in a limited access procedure (Jankowska and Kostowski, 1995), whereas viral vector mediated over-expression of 5-HT_{1B} receptors in the MNAc shell increased ethanol consumption in a free access procedure (Hoplight et al., 2006).

Outside of the studies noted above, there have been no attempts to specifically test the role of the MNAc shell in ethanol consumption. Thus, the purpose of the current study was to examine the effects of electrolytic lesions of the MNAc shell on ethanol and sucrose consumption. Data were acquired in B6 mice using both continuous and limited access procedures. Our working hypothesis was that MNAc shell lesions would decrease ethanol, but not sucrose consumption, in both a limited and free access consumption procedure. We chose to use an electrolytic lesion rather than temporary inactivation of this region, as it would allow us to examine the effects on daily ethanol intake without the procedural and interpretational difficulties inherent with multiple (daily) microinjections. Additionally, we recently documented that electrolytic lesions of the CeA and LBNST significantly decreased limited access ethanol intake in B6 mice (Dhaher et al., 2008).

2. Materials and methods

2.1. Animals

Male B6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Upon arrival, mice were group housed (4 mice/cage) and given at least 1 week of acclimation prior to surgery. They were maintained in a temperature-controlled colony room (21 °C–23 °C) on a 12 h light–dark cycle and were allowed free access to food and water. Mice were 10–12 weeks of age at the time of surgery and were given 7–16 days of recovery following surgery and prior to measuring consumption. All procedures were approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University and were conducted in accordance with US National Institute of Health guidelines.

2.2. Electrolytic lesions

Animals were anesthetized with ketamine (220 mg/kg)/xylazine (44 mg/kg)/acepromazine (22 mg/kg) and placed on a Cartesian stereotaxic instrument (Cartesian Research, Inc. Sandy, Oregon). A 50 mm monopolar electrode with a 0.25 mm uninsulated tip (SNE-300, Rhodes Medical Instruments, Summerland, CA) was lowered into the following stereotaxic coordinates (in mm) for the NAc shell, with bregma marking zero for the mediolateral (ML) and anterioposterial (AP) directions and the top of the skull marking zero for the dorsoventral (DV) direction: AP=1.10, ML=±0.60, DV=4.60. A 0.4 mA current was applied for 8 s. Sham lesions were produced by inserting the electrode into the stated coordinates without passing current.

2.3. Verification of lesion location

Mice were euthanized by cervical dislocation, and brains were collected and frozen by placing in an isopentane solution chilled with isopropanol and dry ice. The brains were stored at −80 °C. Brain slices were cut to a thickness of 40 μm and stained with thionin. The extent of tissue damage was examined microscopically.

2.4. Limited and free access ethanol consumption

Fluids were presented in two 25 mL graduated cylinders placed on a stainless steel cage top. Food was placed on the left side, and the bottles were placed on the right side. For the first 2 days of the drinking procedure, tap water was available in both tubes for all animals. For the limited access group, one water bottle was replaced with one ethanol bottle beginning at 3 h after lights off for 2 h following this acclimation. The duration of 2 h ethanol consumption lasted for 7 days. For the free access group, one ethanol bottle replaced one water bottle continuously for 6 days. Mice in the limited access group were tested in parallel with mice in the free access group. The ethanol concentration was 15% v/v (Pharmco Products, Brookfield, CT). Fluid consumption was measured by recording the meniscus level. The minimal unit of measurement was 0.1 ml. Tubes placed on empty cages served as controls for spillage and/or evaporation. Mice were weighed once every 6 days.

2.5. Limited access sucrose consumption

Following 7 days of limited access ethanol consumption, animals had continuous access to tap water in both tubes over a period of 12 days. Following this ethanol-free drinking period, one water bottle was replaced with one bottle containing a 3% sucrose solution beginning at 3 h after lights off for 2 h over a period of 6 days. Fluid consumption and mouse weights were measured in a manner similar to measurements taken during limited access ethanol consumption. Due to the high limited access sucrose intake, sucrose consumption during a 24 h period was not measured.

2.6. Data analysis

Analyses were conducted to compare mice that received sham surgery ($N=12$) and only mice with confirmed lesions of the medial portion of the NAc shell ($N=7$ each for the limited access and free access groups). Unless otherwise noted, all data are presented as the mean±SEM. Daily outcome measures were total fluid consumed (mL), ethanol dose consumed (g/kg), water consumed (mL), sucrose dose consumed (g/kg), ethanol preference (ethanol volume/total volume) and sucrose preference (sucrose volume/total volume) ratios. Standard analysis of variance (ANOVA) techniques were used to determine the effect of lesion on the outcome measures, and this analysis was conducted over both average and daily intake. For the post-hoc analyses, Tukey's test was used. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Lesions of the NAc shell

Fig. 1 illustrates a representative animal with a NAc shell lesion at 4× magnification; data are presented at 3 different anterior–posterior

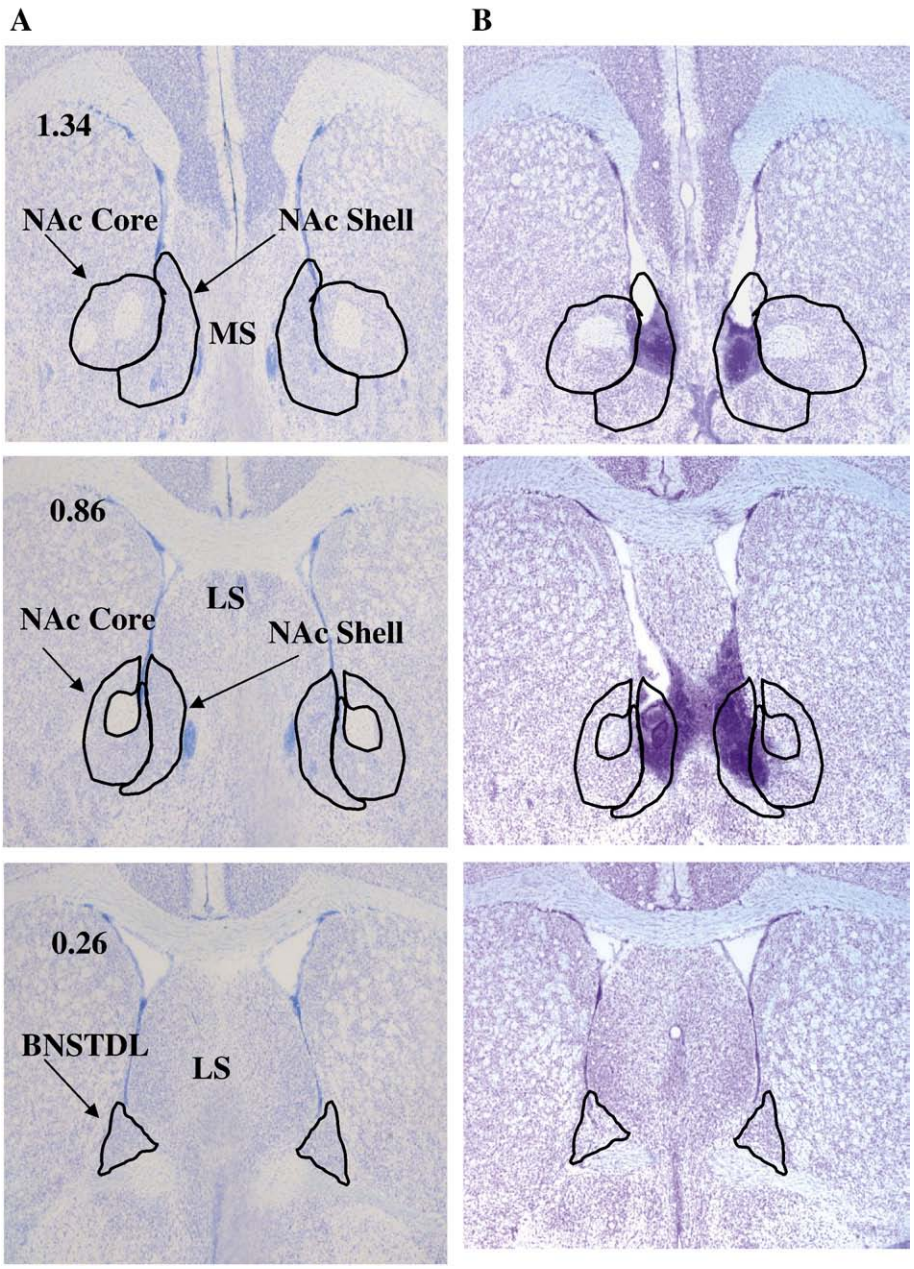

Fig. 1. Representative photomicrographs of the MNAc shell lesions at 3 different A/P positions at 4× magnification. Sham and electrolytic lesions are illustrated in columns A and B, respectively. The A/P positions are relative to bregma.

(A/P) levels, comparing the Sham controls (left panels, Fig. 1A) and lesioned animals (right panels, Fig. 1B). The sections at 1.34 and 0.86 mm anterior to bregma illustrate the extent of damage to the NAc shell. Brain slices at these coordinates demonstrate that while the NAc core and the lateral portion of the NAc shell were left intact, the medial portion of the NAc shell was lesioned with partial damage to the medial septum (MS). Brain slices at 0.26 mm demonstrate that the lateral septum (LS) and the dorsolateral portion of the bed nucleus of the stria terminalis (BNSTDL) were left intact.

Body weight measurements averaged over 18 days (3 weight measurements) were 25.05±0.29 g for the Sham lesioned mice and 25.15±0.47 g for the NAc shell lesioned mice. The ANOVA revealed no effect of lesion, a significant effect of days [$F(2,36)=64.91$, $p<0.00001$], and no lesion × days of interaction. During the first 2 days of acquisition to the two bottle choice procedure that occurred prior to receiving ethanol, water consumption was measured during the 2 h period between 3 and 5 h into the dark cycle. This is depicted in Fig. 2, Panel A. Water consumption was also measured during the 22 h period that occurred between these two 2 h readings. This is depicted in Fig. 2, Panel B. Water consumption during the 2 h period averaged over 2 days was 1.19±0.06 ml for the Sham control group and 0.75±0.07 ml for NAc shell lesioned group. The ANOVA revealed a significant effect of lesion [$F(1,17)=17.83$, $p<0.0001$], a significant effect of day [$F(1,18)=8.23$, $p<0.001$], and a significant lesion × days of interaction [$F(1,18)=8.23$, $p<0.001$]. Post-hoc tests indicate that water consumption increased over the days in the Sham surgery control group, but not in the NAc shell lesioned group. Water consumption during the 22 h period between these two measurements did not differ between groups; 4.36±0.17 ml for the Sham control group and 4.33±0.18 ml for the NAc shell lesioned group.

Fig. 3, panels A and B, illustrate the results for daily and averaged limited access ethanol consumption (g/kg) respectively. The analysis of daily intake revealed a significant effect of lesion [$F(1,17)=3.83$, $p<0.05$], a significant effect of days [$F(6,108)=6.34$, $p<0.00001$], and

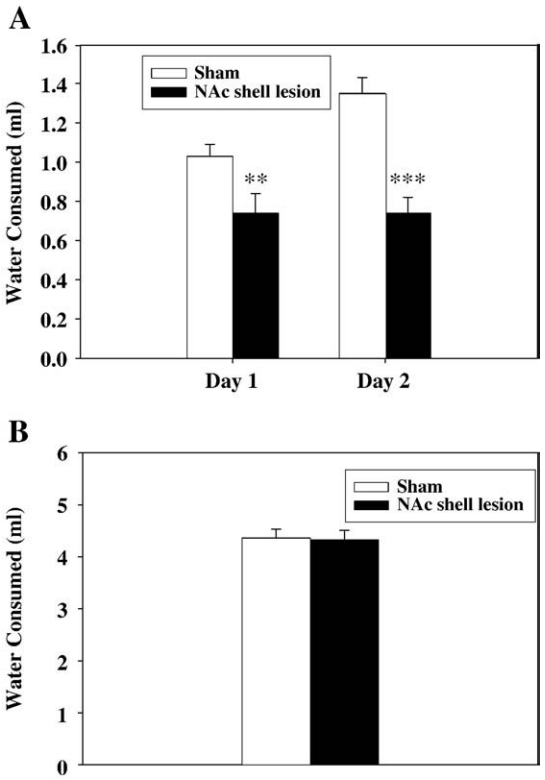

Fig. 2. Effect of MNAc shell lesion on total water consumed in B6 mice. The mice received sham (N=24) or MNAc shell (N=14) lesions prior to beginning the limited access and free access ethanol preference drinking procedures. (A) Averaged water intake during the two h period between the 3rd and 5th hours into the dark cycle. Water intake was significantly lower in the MNAc shell lesioned group, when compared to the sham surgery control group on both days prior to ethanol exposure. (B) Averaged water intake during the 22 h period prior to the exposure to ethanol consumption. The 22 h water intake did not differ between the MNAc shell lesioned group and the sham surgery control group. All values are the mean±SEM. $^{**}p<0.01$, $^{***}p<0.001$ versus the sham surgery control group.

no lesion × days of interaction. Analysis of averaged intake confirmed that ethanol consumption was higher in the Sham lesioned mice (3.03±0.20 g/kg) than in the NAc shell lesioned mice (2.41±0.13 g/kg).

Fig. 3, panels C and D, illustrate the effects of NAc shell lesions on daily and average preference for ethanol during the limited access sessions, respectively. Daily ethanol preference ratio was not significantly affected by lesion, but it did change significantly across days [$F(6,108)=4.13$, $p<0.01$]. However, there was no lesion × days of interaction. The averaged ethanol preference ratio was 0.92±0.02 for the NAc shell lesioned group and was 0.85±0.04 for the Sham lesioned mice.

Fig. 3, panels E and F, illustrate the effect of the NAc shell lesion on daily and averaged total volume of fluid consumed during the limited access session, respectively. ANOVA revealed a significant effect of lesion [$F(1,17)=11.87$, $p<0.001$], a significant effect of days [$F(6,108)=4.81$, $p<0.001$], but no significant lesion × days of interaction. Analysis of the averaged intake indicated that total volume of fluid consumed was higher in the Sham lesioned mice (0.79±0.04 ml) than in the NAc shell lesioned mice (0.52±0.03 ml).

Fig. 4, panel A and B, illustrate the results for daily and averaged 24 h ethanol consumption (g/kg), respectively. ANOVA revealed no significant effect of lesion. Averaged ethanol consumption was 9.86±1.06 g/kg for the Sham control mice, and 11.78±1.20 g/kg for the NAc shell lesioned mice.

Fig. 4, panel C and D, illustrate the effects of NAc shell lesions on daily and averaged 24 h ethanol preference, respectively. There was no significant effect of lesion or days, and no significant lesion × days of interaction. The averaged ethanol preference ratio was 0.46±0.06 for the Sham lesioned mice and 0.62±0.08 for the NAc shell lesioned mice.

Fig. 4, panel E and F, illustrate the effect of the NAc shell lesion on daily and averaged total volume of fluid consumed during free access, respectively. ANOVA revealed no significant effect of lesion, a significant effect of days [$F(5,90)=7.39$, $p<0.00001$], and no significant lesion × days of interaction. Total fluid consumed was 4.56±0.27 ml for the Sham lesioned mice and 4.10±0.36 ml for the NAc shell lesioned mice. These values are similar to the baseline (22 h) water consumption in the Sham and lesioned mice in the limited access procedure.

Limited access sucrose consumption, averaged over 6 days, was 4.56±0.38 g/kg for the Sham control group and 5.16±0.58 g/kg for the NAc shell lesioned group. The ANOVA revealed no significant effect of lesion or days and no significant lesion × days of interaction. Preference for sucrose was 0.92±0.02 for the Sham control group and 0.89±0.05 for the NAc shell lesioned mice. Again, the ANOVA revealed no significant effect of lesion or days and no significant lesion × days of interaction. Total volume consumed was 4.11±0.32 ml for the Sham control group and 4.39±0.43 ml for the NAc shell lesioned mice. The ANOVA revealed no significant effect of lesion or days and no significant lesion × days of interaction.

4. Discussion

During the present studies, we lesioned the MNAc shell in B6 mice and determined the impact on ethanol and sucrose consumption. The key finding from these experiments was that lesions of the MNAc shell significantly decreased limited access, but not free access, ethanol intake without altering ethanol preference. These results were not due to the extent of damage, as lesion size did not differ between the limited and free access groups. Additionally, lesions of the MNAc shell did not significantly alter sucrose consumption in a limited access procedure. These results suggest that the MNAc shell is selectively involved in the acquisition of ethanol consumption in a limited access procedure.

The selectivity of the MNAc shell lesion to reduce limited access ethanol but not sucrose intake is consistent with data from metabolic mapping and dopamine microdialysis studies. The metabolic mapping studies demonstrated that voluntary ethanol consumption, but not sucrose consumption, increased the rate of glucose utilization in the rostral pole and shell of the nucleus accumbens in Wistar rats (Porrino et al., 1998). With respect to accumbal microdialysis studies, significant correlations were found between ethanol (but not sucrose) intake and dopamine concentrations in the NAc during the initial 10 min of consumption (Doyon et al., 2005) in Long–Evans rats. Consistent with these results, a separate microdialysis study indicated that rats experienced with operant ethanol self-administration exhibited a significant increase in NAc dopamine efflux during the first 10 min of an anticipation period (i.e., prior to the regular self-administration session). There was no effect of anticipation for saccharin self-administration on dopamine efflux in the NAc. Additionally, self-administration of ethanol, but not saccharin, significantly increased NAc dopamine efflux during the 20 and 30 min time points of the self-administration session and during the first 10 min of the post-administration period (Melendez et al., 2002).

The putative selective involvement of the MNAc shell in limited access ethanol consumption, but not free access consumption, is consistent with other studies whereby pharmacological manipulations produced different effects on limited and free access ethanol consumption. The literature on the 5-HT_3 receptor antagonists serves to illustrate this point. In general, 5-HT_3 receptor antagonists have been shown to decrease ethanol consumption in a free access procedure, but only under certain situations in a limited access procedure (Fadda et al., 1991; Knapp and Pohorecky, 1992; Svensson

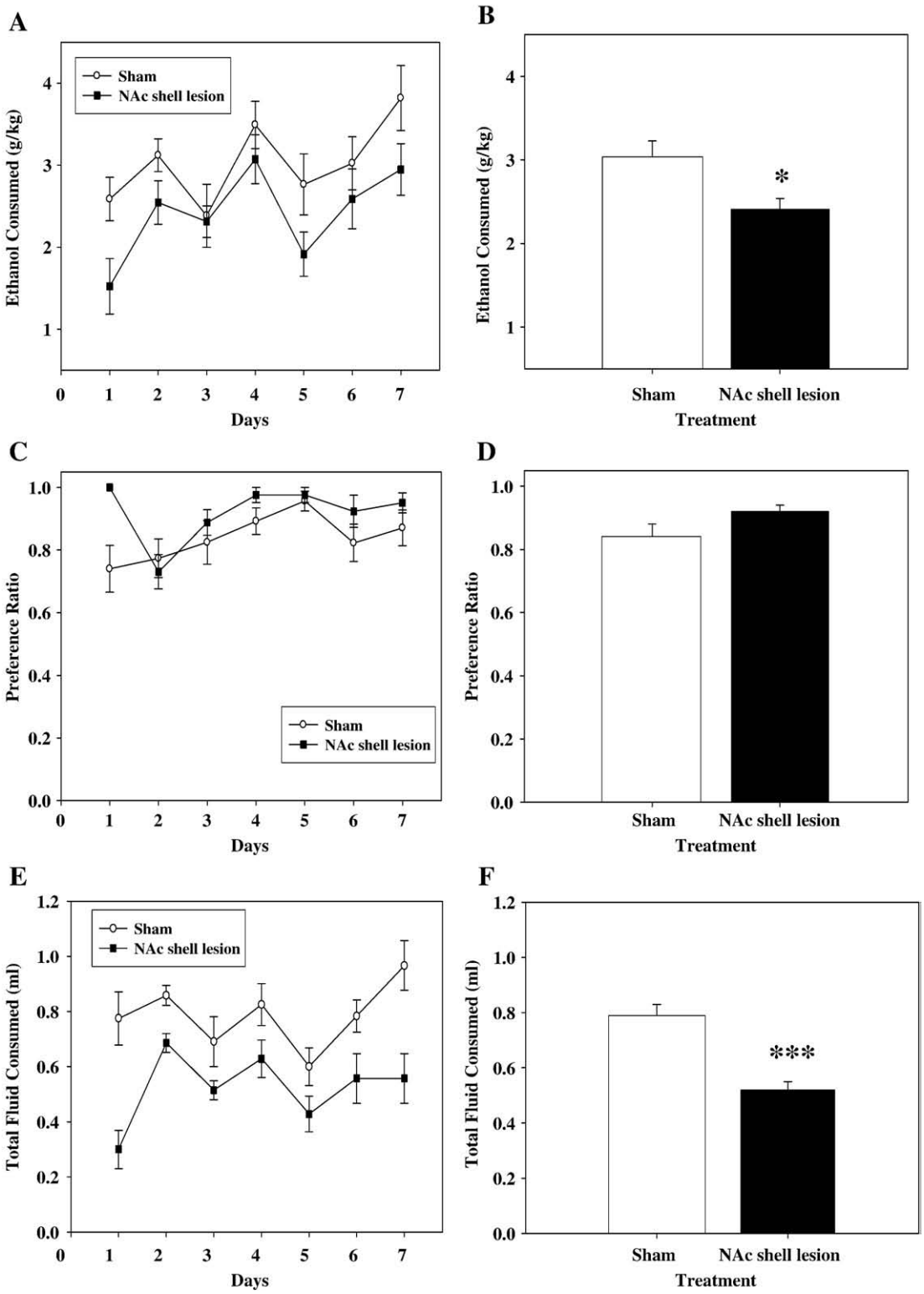

Fig. 3. Effect of MNAc shell lesion on limited access ethanol consumption (Panels A & B), ethanol preference ratio (Panels C & D), and total fluid consumed (Panels E & F) in B6 mice. The mice received sham ($N=12$) or MNAc shell ($N=7$) lesions prior to beginning the limited access ethanol preference drinking procedure. (A) Daily ethanol consumption (g/kg/2 h); (B) Averaged ethanol consumption. Ethanol intake was significantly decreased in the MNAc shell lesioned group, when compared to the sham surgery control group. (C) Daily ethanol preference ratio (volume of ethanol consumed/total volume consumed); (D) Averaged ethanol preference ratio. Preference for ethanol did not differ between the MNAc shell lesioned group and the sham surgery control group. (E) Daily total volume of fluid consumed (mL/2 h); (F) Averaged total volume of fluid consumed. The MNAc shell lesioned group showed a lower level of total fluid consumption than the sham surgery control group. All values are the mean ± SEM. $^{*}p<0.05$, $^{***}p<0.001$ versus the sham surgery control group.

et al., 1993; Hodge et al., 1993; Beardsley et al., 1994; Tomkins et al., 1995). It has been suggested (McKinzie et al., 1998) that a loss of temporal and contextual associations due to variability in the time that the ethanol was presented during the dark cycle might contribute to the difference in the responsivity of the limited and free access groups to the 5-HT_3 receptor antagonists.

Contextual and temporal conditioning may differ in procedures involving limited access versus free access ethanol consumption. In a

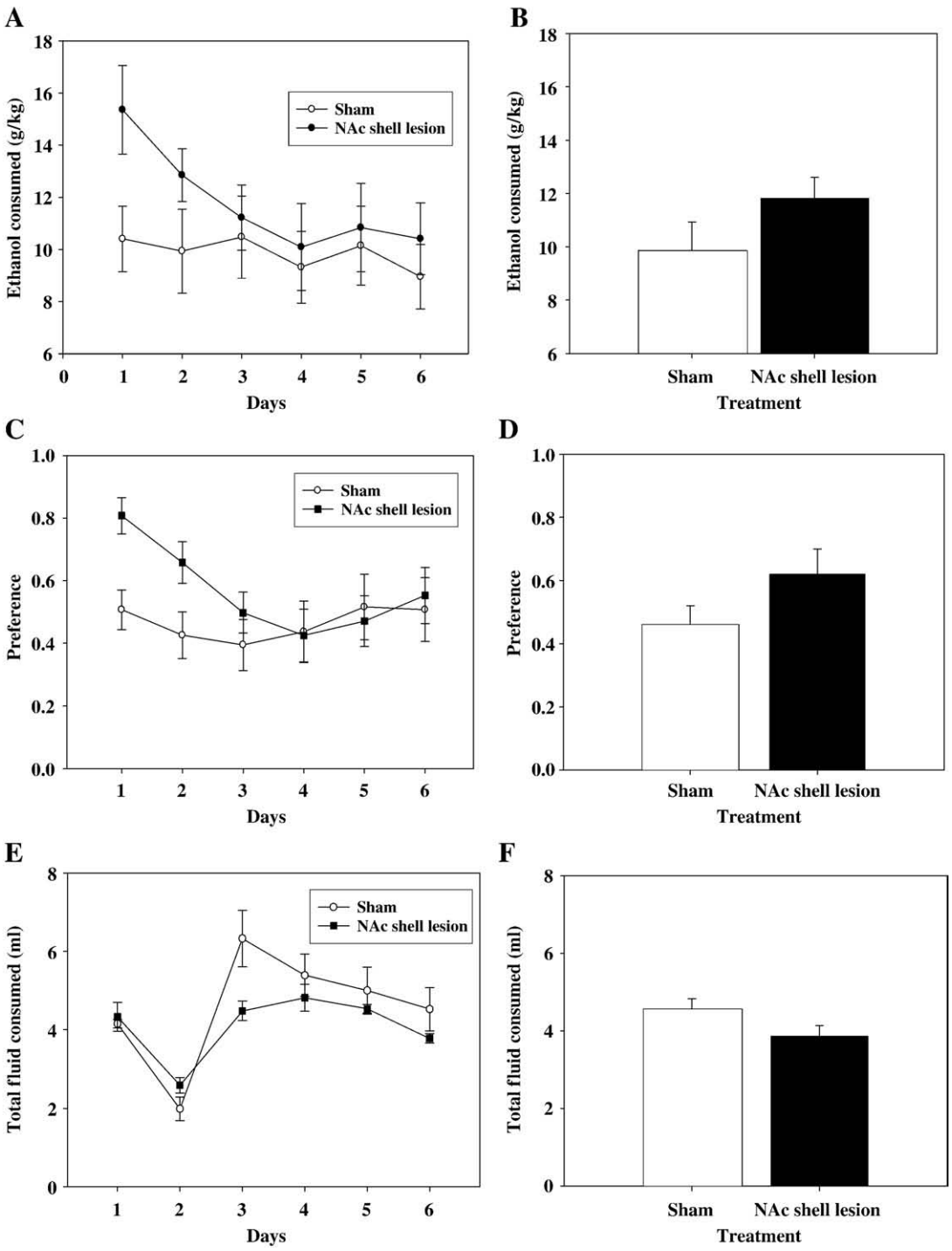

Fig. 4. Effect of MNAc shell lesion on 24 h access ethanol consumption (Panels A & B), ethanol preference ratio (Panels C & D), and total fluid consumed (Panels E & F) in B6 mice. The mice received sham (N=12) or MNAc shell (N=7) lesions prior to beginning the free access ethanol preference drinking procedure; (A) Daily ethanol consumption (g/kg/24 h); (B) Averaged ethanol consumption. There is a range difference between Figs. 3 and 4 on panels A and B. There was no difference in 24 h ethanol consumption between the MNAc shell lesioned group and the sham surgery control group. (C) Daily ethanol preference ratio (volume of ethanol consumed/total volume consumed). (D) Averaged ethanol preference. Preference for ethanol did not differ between the MNAc shell lesioned group and the surgery control group. (E) Daily total volume of fluid consumed (mL/24 h). (F) Averaged total volume of fluid consumed. Total fluid intake did not differ between the MNAc shell lesioned group and the sham surgery control group. All values are the mean ± SEM.

limited access procedure, the presentation of ethanol can be associated with contextual and temporal cues that may not be as apparent in a free access drinking procedure. Consider the following example: McKinzie et al. (1998) observed that the 5-HT_3 receptor antagonist, MDL 72222, did not decrease ethanol consumption when ethanol was presented during a 4 h limited access procedure at fixed times in the dark cycle. However, when the time of limited access varied during the dark cycle, MDL 72222 was able to decrease ethanol consumption. Although this effect dissipated over time, McKinzie et al. (1998) interpreted the weakening of the drug effect to the strengthening of conditioning of contextual cues over trials. In conjunction with the present findings, the involvement of the MNAc shell in ethanol consumption may be dependent on the access to ethanol (i.e., limited access with conditioning or free access without conditioning).

From this perspective, it is of interest to note that the serotonergic system of the MNAc shell has been shown to be involved in contextual

place preference conditioning (CPP) to certain drugs of abuse that include cocaine (Barot et al., 2007; Neumaier et al., 2002; Harris et al., 2001) and morphine (Harris and Aston-Jones, 2001). Other neurotransmitters including the glutamatergic, dopaminergic, opioid, and cannabinoid systems of the NAc shell have been shown to play a role in drug-conditioning. For instance, overexpression of the glutamate-1 transporter in the NAc shell attenuated both morphine and amphetamine-induced CPP (Fujio et al., 2005), and morphine microinjected into the posterior NAc shell produced a CPP (Terashvili et al., 2008). Microinjection of other drugs of abuse into the NAc shell also induced CPP. For example, microinjection of Δ^9-tetrahydrocannabinol, the active ingredient in marijuana, into the NAc shell, produced CPP (Zangen et al., 2006), as did a microinjection of amphetamine (McBride et al., 1999). With regard to the dopaminergic system of the NAc shell; activation of dopamine D1-family receptors in the medial and lateral NAc shell mediated context-induced reinstatement of heroin seeking (Bossert et al., 2007); blockade of D1 receptors in the NAc shell attenuated nicotine-induced CPP (Spina et al., 2006); and blockade of dopamine receptors in the NAc shell attenuated the development of an intracerebroventricular ethanol-induced CPP (Walker and Ettenberg, 2007). With regard to lesioning studies and conditioned drug effects, lesions of the NAc disrupted amphetamine-induced CPP (Olmstead and Franklin, 1996), disrupted the effect of amphetamine on conditioned reinforcement (Parkinson et al., 1999), did not affect cocaine-induced CPP (Spyraki et al., 1982), produced mixed effects on morphine-induced CPP (Kelsey et al., 1989; Shippenberg et al., 1993; Olmstead and Franklin, 1996; White et al., 2005), and disrupted the acquisition of ethanol-induced CPP (Gremel and Cunningham, 2008). Even though these lesion studies did not distinguish between NAc core and shell, in conjunction with the more specific pharmacological manipulations as well as our present findings, it is possible that the MNAc shell may be implicated in the contextual conditioning involved with limited access ethanol consumption. Consistent with this idea, it has been proposed that the NAc shell versus core play different roles in drug-conditioned incentive related to the development of addiction, in that dopamine transmission in the NAc shell strengthened stimulus-drug associations (reviewed in Di Chiara, 2002).

Here it should be noted that the preference for ethanol was much higher in the limited access procedure than in the free access procedure in our studies. Although the preference reported in our free access procedure was lower than what has normally been reported, it is typical for ethanol preference in a free access procedure to be lower than that seen in a limited access procedure (Belknap et al., 1993; Grahame et al., 1999; Sharpe et al., 2005; Rhodes et al., 2005). Related to this point, differences in the microstructure of ethanol drinking (e.g., bout frequency) have been reported in limited versus free access procedures (Samson, 2000).

Like preference, ethanol consumed during the 3rd to 5th hours after lights off was higher in the limited access group than the free access group (data not shown), suggesting that the limited access group achieved higher blood ethanol concentrations (BECs) than the free access group during this time period, thus resulting in differing pharmacological effects of ethanol on the neural circuits involved with limited and free access ethanol consumption. The possibility exists that the different BECs achieved with the two procedures may activate different neural circuits involving limited and free access ethanol consumption.

With regard to the free access ethanol consumption, it is of interest to note that ethanol drinking and preference during the 1st day of ethanol exposure was higher in the MNAc shell lesioned group than in the sham control group. This was a nonspecific effect, since it was also seen with sucrose consumption (data not shown). The role that the MNAc shell plays in novelty may provide an explanation for such results. For instance, while sucrose consumption had no effect on dopamine release in the NAc shell, addition of an unfamiliar appetitive taste, such as chocolate, increased dopamine release in the NAc shell. A habituation of the dopamine response occurred with repeated exposure to the novel taste (Bassareo et al., 2002). It is unclear as to why, in the case of ethanol, on the first day of exposure, the MNAc shell lesioned group drank less than the sham control group, and with free access consumption, the MNAc shell lesioned group drank more ethanol. This result is further evidence that the neural circuitry involved with free access ethanol consumption is different from that involved with limited access ethanol consumption.

In the limited access procedure, the significant decrease in ethanol dose was due to a decrease in total volume consumed, rather than a change in ethanol preference. Fluid consumption was lower in the MNAc shell lesioned mice when compared to the sham controls, during the 3rd to 5th hour after lights off, both on the 2 days prior to ethanol being available (i.e., water intake), and during the ethanol preference procedure (i.e., ethanol and water intake). These two findings suggest that MNAc shell lesions could have nonspecific effects on limited access drinking. The nonspecific effect was limited to the 3rd to 5th hour after lights off, since total volume of fluid consumed over the 24 h free access period did not differ between groups. The nonspecific effect was also limited to water and ethanol, since sucrose consumption did not differ between groups, possibly because of the difference in the reinforcing properties of water, ethanol, and sucrose. These results are in contrast with those in female P rats whereby a decrease in preference for ethanol and total water intake during a 48 h period was observed following 6-OHDA lesions in the NAc (Ikemoto et al., 1997). The discrepancy between results may well be due to genetic differences between the rat and mouse species, as well as to the difference in specificity of the lesion.

One consideration for the interpretation of studies utilizing electrolytic lesions is that an electrolytic lesion destroys fibers of passage. Thus, it is possible that the ability of the lesion to decrease ethanol consumption in the limited access procedure was due to destruction of fibers of passage and not to damage done to the MNAc shell. While a potential explanation, the background studies noted in the Introduction, which manipulated the region pharmacologically and spared fibers of passage (via the use of viral vectors and excitotoxic lesions), provide strong evidence that it is the nucleus itself that is involved in alcohol consuming behavior. We chose to use an electrolytic lesion in the present study because; first, we have successfully used this strategy in previous studies lesioning other components of the cExtA (Dhaher et al., 2008); second, temporary inaction of the MNAc shell would require daily microinjections that would lead to potential stress effects; and finally, with an excitotoxic lesion, the angle of syringe insertion necessary to avoid the ventricle has the potential to lead to mechanical and excitotoxic damage to the NAc core. Since damage due to an electrolytic lesion is limited to the uninsulated tip, we reasoned that there would be no need to avoid the ventricle, and thus, no need to go through the NAc core to get to the MNAc shell. Thus one can do less damage and be more anatomically specific to one region with the electrolytic lesion.

Electrolytic lesion studies carried out in our lab have demonstrated that the two major nuclei of the cExtA, the CeA and LBNST, are involved in ethanol consumption in B6 mice in a limited access procedure (Dhaher et al., 2008). Even with the proviso that electrolytic lesions in the present (and our recent) study may not allow the interpretation of direct effects on specific nuclei, the results provide evidence for a role of the CeA, LBNST and MNAc in the modulation of limited access ethanol intake. Notably, the results found in our present study indicate that the MNAc shell holds functional characteristics similar to the two major nuclei of the cExtA, providing further support for the concept of the cExtA as including the MNAc shell.

In conclusion, the results of our study indicate that the MNAc shell is selectively involved in limited access, but not free access ethanol consumption, with sucrose consumption remaining unaffected. Notably, the significant decrease in ethanol intake was not accompanied by a significant decrease in ethanol preference. Overall, the

present findings are consistent with the results from a number of studies indicating that the MNAc shell is involved in ethanol consummatory behavior. The results have implications for understanding the neural circuitry that underlies ethanol consumption in alcohol dependent people, particularly with regard to differences found between those that drink continuously throughout the day and those that consume alcohol in a large bout in a limited amount of time. Differentiation between these forms of alcohol use and abuse and the underlying neural circuitry will lead to better success in developing pharmacotherapeutic treatments for different forms of alcoholism.

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