

# Electrical Stimulation of the Nucleus Accumbens Shell Reduces Voluntary Ethanol Consumption in Bulbectomized Rats

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## Abstract

**Background:** Alcoholism is often accompanied by depression. It has been shown that deep electrical stimulation of the nucleus accumbens is effective in the treatment of alcoholism.

**Objective:** Despite the promising results of clinical trials, the mechanisms underlying its effects are still unclear. To elucidate these mechanisms, valid animal models are needed.

**Methods:** We investigated the effects of electrical stimulation of the nucleus accumbens shell (NAS) and nucleus accumbens core (NAC) on alcohol intake in bulbectomized rats. Bilateral removal of the olfactory bulbs (OBX) is considered a valid animal model of depression.

**Results:** It was shown that electrical stimulation of the NAS with the described stimulus parameters significantly reduced voluntary ethanol intake in OBX rats. After the cessation of stimulation, this effect became insignificant. In contrast, stimulation of the NAC did not modify voluntary ethanol intake. In sham-bulbectomized animals, voluntary ethanol intake was negligible and ethanol consumption did not alter as a result of electrical stimulation of the NAC or NAS.

**Conclusions:** Our study underlines the relevance of the OBX model in the study of depression comorbid alcohol intake. Moreover, it might be useful in the study of the mechanisms underlying the clinical effectiveness of electrical stimulation of the NAS in the treatment of alcoholism.

**Keywords:** Depression; Alcoholism; Bulbectomy; Electrical Stimulation; Nucleus accumbens; Rat

## Introduction

Alcohol dependence is a chronic disease which is characterized by physical and psychological addiction to alcohol. It is associated with a wide range of harmful physical, mental, and social problems. The pharmacological treatment of alcoholism remains a major challenge. The aims of pharmacological strategies are to treat the symptoms of alcohol withdrawal, reduce the consumption of and craving for alcohol, prevent relapse and treat associated psychiatric problems [1].

A number of neurotransmitters and neuromodulators are reportedly involved in the manifestation of alcoholism. Moreover, chronic alcohol intake induces plastic adaptive morphological changes within the central nervous system, leading to altered motivational, affective, and cognitive behavior [2,3]. With special regard to plastic-adaptive neuromorphological alterations, neuromodulation techniques such as noninvasive transcranial magnetic stimulation, transcranial direct current stimulation, and more invasive techniques such as deep brain stimulation (DBS) appear to offer hope for the treatment of alcoholism. DBS seems to be a therapeutic option for those patients suffering from severe alcohol dependence for whom other treatment options have failed [4-9]. Moreover, in preclinical and clinical investigations the therapeutical effectiveness of DBS in the treatment of depression was shown [10-14]. The mechanisms underlying its therapeutic usefulness on a cellular and circuit level are far from being understood. Thus more research – including animal experiments – is needed to elucidate the mechanisms underlying the effectiveness of DBS.

Alcoholism is influenced by genetic, psychological, and cultural

factors. This heterogeneous nature of alcoholism might explain why the efficacy of recent treatment approaches has been modest at best [15,16]. Much effort is spent optimizing recent approaches and discovering the mechanisms underlying approaches in clinical use such as DBS, based on animal experiments. However, rodents have a natural aversion to alcohol [17]. Therefore, in DBS studies genetically selected alcohol-preferring rats [16,18] or a saccharin-fading procedure [14] were used. Clinical observation revealed a comorbidity of depression and alcoholism [19-21]. After removal of the olfactory bulbs – which is a validated model in depression research – elevated alcohol consumption was found [22]. Therefore, we used OBX rats to investigate the effect of DBS of the NAC and NAS on voluntary depression comorbid ethanol consumption.

In alcohol-preferring rats, DBS in the nucleus accumbens (NA) reduced alcohol preference [18]. This shows that DBS is effective in both humans and animals, and provides a reliable basis for further studies aimed at discovering the mechanisms underlying the effects of

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DBS. This is all the more interesting in the case of comorbid psychiatric diseases and addiction. Alcoholism is often accompanied by depression, and may contribute to the etiology of depression and vice versa [19-21].

Bilateral removal of the olfactory bulbs (OBX) is an accepted and validated animal model in experimental depression research [23-31]. Six weeks after surgery, animals exhibit typical depression-like behavior such as reduced grooming behavior, anhedonia, alterations in nociception, and a deficit in learning performance. These depression-like symptoms are responsive to treatment with different antidepressant drugs [19,24,31] and vagus nerve stimulation [32], thus demonstrating the predictive validity of this model. In bulbectomized mice, an elevated increase in voluntary ethanol consumption was found [22]. This suggests that OBX might also be a useful model for investigating alcohol abuse disorders and therapeutic options such as DBS with the aim of optimizing neuromodulation techniques as well as investigate the mechanism underlying their effectiveness.

The NA is a region of the brain which is crucially involved in functions such as motivation, reward, and drug addiction [33]. Therefore, the NA seems to be a relevant target in the treatment of alcoholism. It can be divided into two substructures, i.e. the nucleus accumbens core (NAC) and the nucleus accumbens shell (NAS). These substructures have different morphologies and functions in terms of behavior and addiction [34]. In the present study, we investigated the effect of DBS of the NAC and NAS on depression comorbid voluntary ethanol consumption in OBX rats to test the hypothesis whether this attempt is suitable for the elucidation of the comprehensive neuronal network underlying DBS effects in ethanol consumption. Voluntary consumption presents a useful model for investigating natural patterns of consumption and their underlying neurochemical or behavioral mechanisms [35].

## Materials and Methods

The work reported here was conducted in accordance with EC regulations and those of the National Act on the Use of Experimental Animals (Germany). The protocol was approved by the Saxony-Anhalt Committee on Animal Care.

### Animals

The animals used were male Wistar rats (RjHan:WI, Janvier, St. Berthevin, France). The rats were kept under controlled laboratory conditions with a light/dark cycle of 12:12 (lights on at 6 a.m.), temperature  $20 \pm 2^\circ\text{C}$ , and air humidity 55 - 60%. The animals were fed with commercial rat pellets (ssniff R/M-H, ssniff Spezialdiäten GmbH, Soest, Germany) and tap water ad libitum. The animals were housed in groups of 5 in Macrolon IV cages. Studies on voluntary ethanol intake in adult Wistar rats report varying intake of around 1-4 g/kg/day using continuous access models [36,37]. OBX or sham surgery (OBS) was performed on 8-week-old rats.

### Olfactory bulbectomy

Bilateral olfactory bulbectomy (OBX) was performed as described by O'Connor and Leonard [38]. Briefly, rats were deeply anesthetized with sodium pentobarbital (40 mg/kg body weight, intraperitoneally i.p., injection volume 10 ml/kg body weight) and a midline skin incision was made to expose the skull overlying the bulbs. Two holes (diameter 2 mm) were drilled above the bulbs (6.5 mm anterior to the bregma, 2 mm laterally on both sides of the midline). The olfactory bulbs were cut and removed by aspiration using a deflected pipette. The resulting space was filled with hemostatic sponges (Gelitaspon<sup>®</sup>, Gelida

Medical, Amsterdam, The Netherlands), and the skin was closed with tissue adhesive (Histoacryl<sup>®</sup>, B. Braun Aesculap, Tuttlingen, Germany). Sham-operated rats (OBS) were treated in the same manner, including piercing of the dura, but the bulbs were left intact.

### Voluntary ethanol consumption

Five weeks after OBX or OBS, the animals were housed singly in Macrolon III cages which were equipped with 3 bottles, and were given access to tap water, 5% ethanol, and 10% ethanol solution respectively. Individual fluid intake was calculated based on bottle weight measured twice a week. Total fluid intake was defined as water intake + ethanol solution intake and total ethanol intake was defined as 5% + 10% ethanol solution intake or as absolute ethanol intake. These measures were expressed as g/kg body weight. The position of the bottles was changed at weighing. Ethanol and water intake were measured over a period of 5 weeks. The cages were located together in racks so that auditory and olfactory contact was maintained.

### Electrode implantation

OBX rats showing stable ethanol intake after a period of 5 weeks and OBS rats received 40 mg/kg body weight sodium pentobarbital i.p. as anesthesia. After the induction of anesthesia, rats were placed into a stereotaxic apparatus (Stoelting, Wood Dale, USA). Two bipolar stainless steel electrodes were then implanted into either the NAS (1.6 mm anterior to bregma, 0.9 mm lateral to the midline, and 7.5 mm ventral to the surface of the skull) or the NAC (2.2 mm anterior to bregma, 0.9 mm lateral to the midline, and 7.0 mm ventral to the surface of the skull) of the NA according to Paxinos and Watson [39] and modified according to Lopez [40]. Electrodes were affixed to the skull using dental cement (Paladur, Heraeus Kulzer, Hanau, Germany). Electrode placement is illustrated in Figure 1.

### Deep brain stimulation

After a 1-week period for convalescence, the rats received 3 h per day DBS (biphasic rectangular pulses with a phase duration of 0.06 ms delivered at 130 Hz in trains of 1 min duration and with a 1 min intertrain interval), using a 2100 Isolated Pulse Stimulator (A-M systems Inc., Carlsborg, USA). The current was adapted to the individual sensitivity of the animals. Stimulation began with 50  $\mu\text{A}$ . If there were no signs of vibrissae erection, eye blinking, or head twitching, the current was increased in steps of 10  $\mu\text{A}$  up to a maximum current

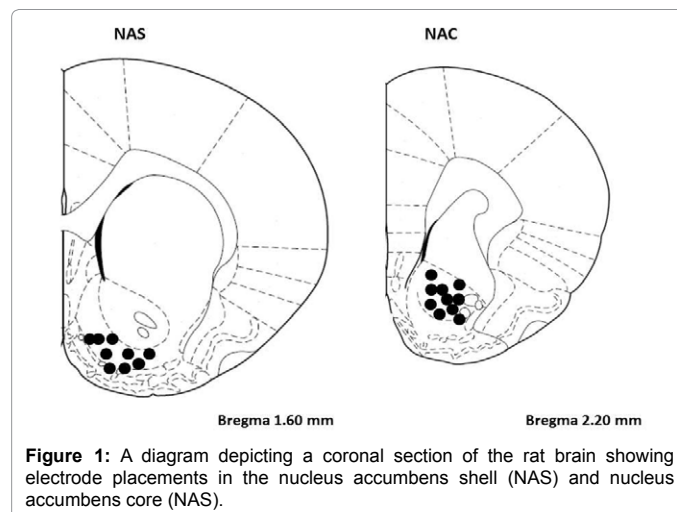


Figure 1: A diagram depicting a coronal section of the rat brain showing electrode placements in the nucleus accumbens shell (NAS) and nucleus accumbens core (NAC).

of 70  $\mu$ A. Sham-stimulated animals were connected to the stimulator but did not receive current. The stimulation period lasted for 3 weeks, during which voluntary ethanol intake was quantified as described.

### Post-stimulation voluntary ethanol intake

Following the stimulation period, voluntary ethanol intake was quantified for another 2-week period to investigate the persistence of a possible DBS effect.

### Verification of olfactory bulbectomy and electrode placements

Following the completion of all experiments, the animals were anesthetized with sodium pentobarbital (40 mg/kg) and decapitated. Prior to removing the brain from the cranium, macroscopic inspection was used to confirm that the olfactory bulbectomy had been carried out correctly. The brain was frozen in methyl butane on dry ice, and coronal sections (60  $\mu$ m) were taken at the level of the NA using a Cryocut (Leica CM 3050, Leica Microsystems, Nussloch, Germany). After Nissl staining, the correct placement of the electrode was microscopically verified. Animals with incomplete olfactory bulbectomy, electrode placements outside of the areas of interest, or with excessive mechanical damage, were excluded from the subsequent data analysis.

### Statistics

Ethanol and total fluid intake were analyzed using the repeated measures ANOVA model with the within-subjects variable of time (pre-stimulation, stimulation, and post-stimulation period) and the between-subjects factor of groups (OBS, OBX-stimulated; OBS, OBX

sham-stimulated) with SPSS Statistics, Version 21. A  $p < 0.05$  was considered statistically significant.

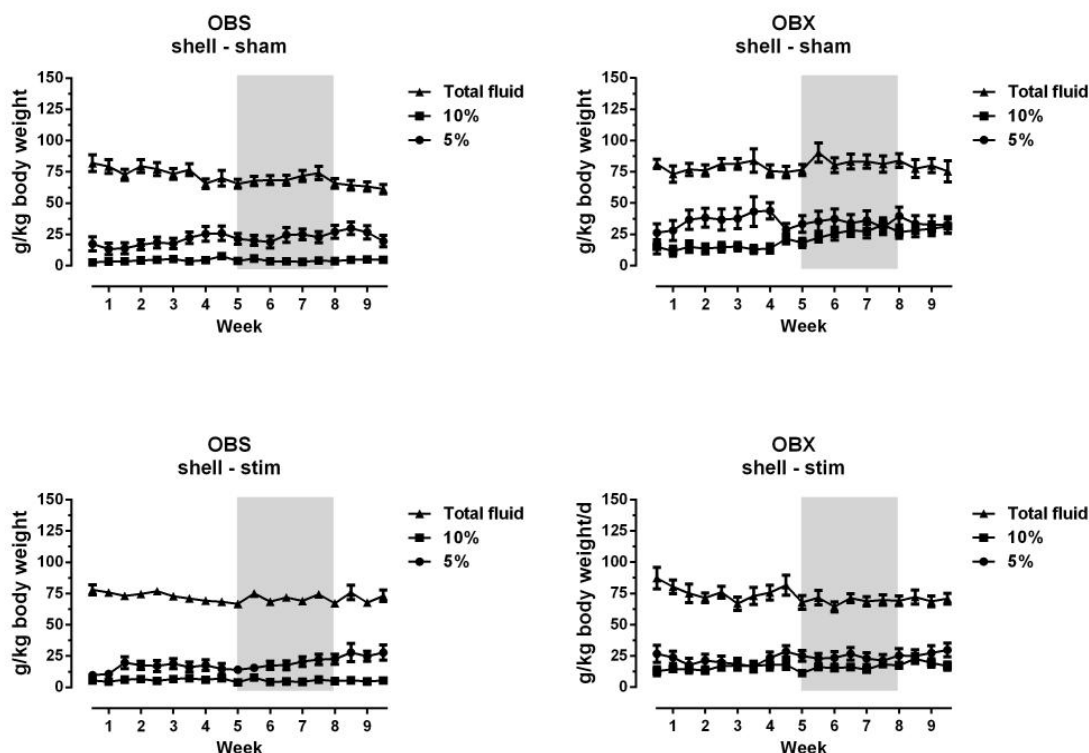
## Results

### Stimulation in the nucleus accumbens shell (NAS)

The 4 experimental groups did not differ in total fluid intake (i.e. water + 5% ethanol solution + 10% ethanol solution) over the three periods of the experiment (pre-stimulation, stimulation, post-stimulation,  $F = 1.22$ ,  $df = 3$ ,  $p = 0.31$ ), Figure 2. The animals' 5% and 10% ethanol solution intake showed a high inter-individual variation. Therefore, statistical evaluation was based on total ethanol, i.e. absolute ethanol intake (Figure 3). Compared with OBS rats, in both groups of OBX rats the total ethanol intake was significantly enhanced ( $F = 21.51$ ,  $df = 1$ ,  $p < 0.001$ ) but there was no difference between the groups to be stimulated and to be sham-stimulated ( $F = 1.96$ ,  $df = 1$ ,  $p = 0.17$ ) in the pre-stimulation period. Electrical stimulation in the NAS had no effect on the ethanol intake of OBS animals. However, in OBX rats, ethanol intake was reduced significantly as a result of stimulation. This effect was not statistically detectable in the post-stimulation period ( $F = 3.41$ ,  $df = 1$ ,  $p = 0.08$ ), (Figure 3).

### Stimulation in the nucleus accumbens core (NAC)

There was a significant difference in total fluid intake over the three periods of the experiment ( $F = 10.85$ ,  $df = 3$ ,  $p < 0.001$ ), (Figure 4). Total fluid intake was higher in the bulbectomized NAC group. In the pre-stimulation period, OBX rats had a higher ethanol intake



**Figure 2:** Daily intake of 5% and 10% ethanol solution and total fluid intake in sham-bulbectomized (OBS) and bulbectomized (OBX) rats 5 weeks prior to bilateral implantation of bipolar electrodes in the nucleus accumbens shell, during the 3-week stimulation period (grey background), and 2 weeks after electrical stimulation (stim) or sham stimulation (sham). 14 - 20 animals per group.

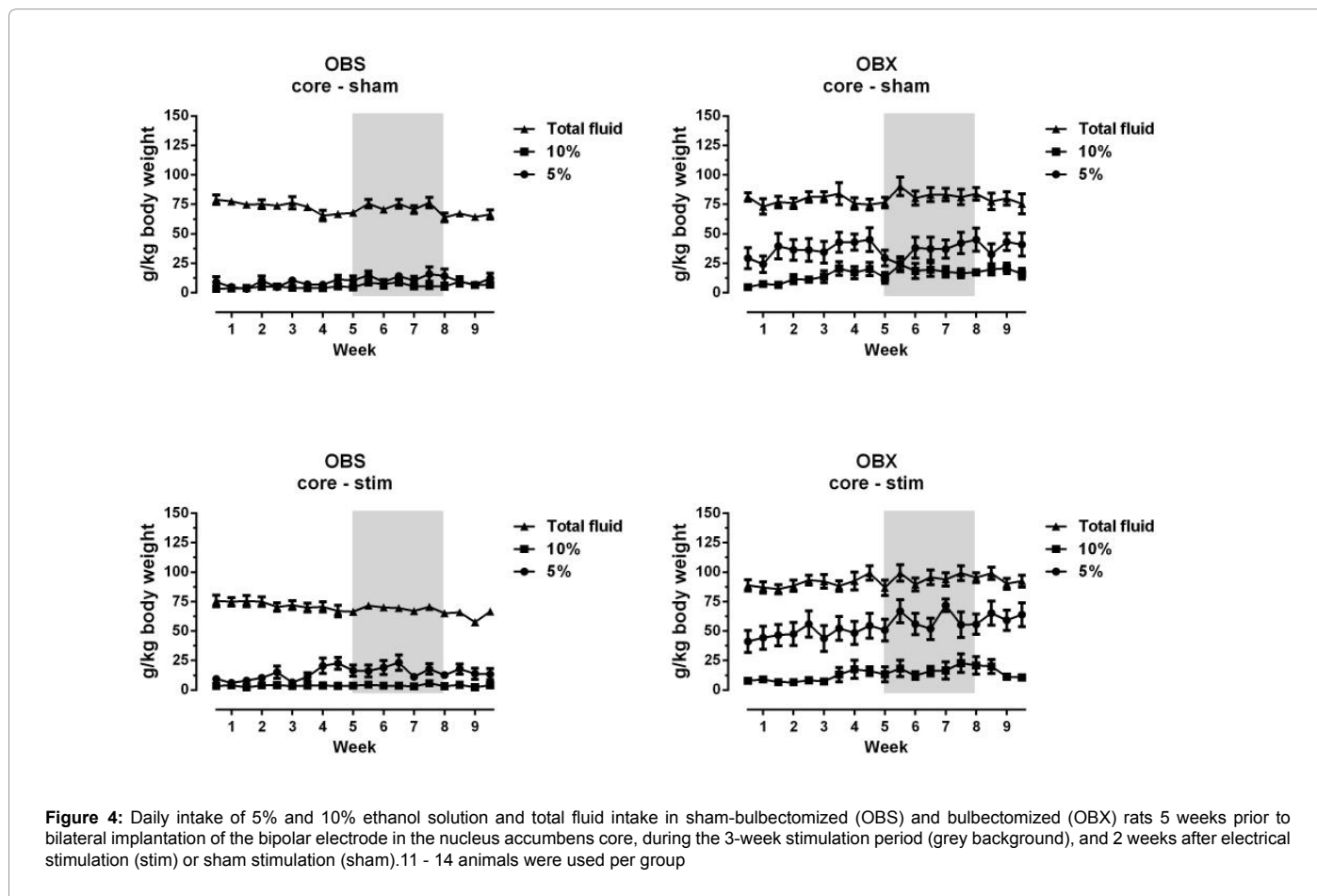
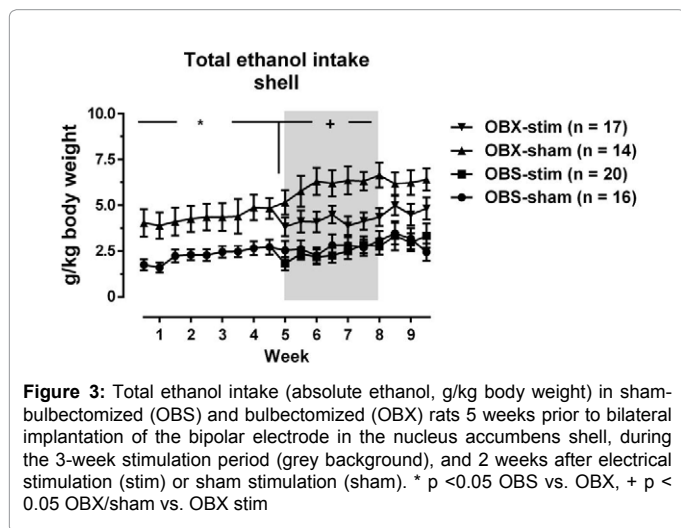
compared with OBS animals ( $F = 58.39$ ,  $df = 1$ ,  $p < 0.001$ ). As found in the previous experiment, there was no difference between the OBX rats which were to be stimulated and those which were to be sham-stimulated ( $F = 0.87$ ,  $df = 1$ ,  $p = 0.36$ ), Figure 5. Stimulation of the NAC had no effect on ethanol consumption in the OBS ( $F = 0.013$ ,  $df = 1$ ,  $p = 0.91$ ) or OBX rats ( $F = 2.2$ ,  $df = 1$ ,  $p = 0.11$ ), (Figure 5).

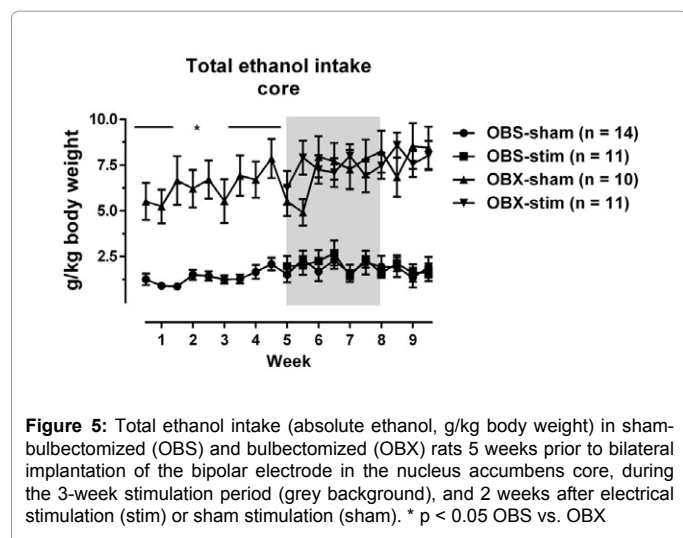
## Discussion

Our study shows that voluntary ethanol consumption is elevated in OBX rats. Electrical stimulation of the NAC with the described stimulus parameters did not modify ethanol intake in OBS and OBX rats. The experimental conditions surgery and electrical stimulation did not affect total fluid intake. This is the first study showing that stimulation of the NAS significantly reduced voluntary ethanol intake in OBX rats. After the cessation of stimulation, this effect became insignificant. These results support our hypothesis that DBS in OBX rats represents a useful tool to investigate the mechanisms underlying DBS effects in ethanol consumption.

OBX is an animal model which is characterised by a high predictive validity to investigate the possible biochemical or neurobiological mechanism(s) of depression, as well as the antidepressant-like property of test molecules [29]. Previously we have shown that rats showed depression-like behavior 5 weeks after OBX [28, 42, 45]. It is well-known that handling can modify rodent behavior [41-44]. Therefore no additional experiments were included to avoid modifying effects on ethanol intake.

As shown in Figures 2-5, ethanol intake in OBS is low. The ethanol intake measured in the present experiments is in good accordance with data obtained in previous experiments [45]. Therefore we can only conclude that DBS of the NAC and NAS with the parameters used in the present experiment did not increase voluntary ethanol consumption in the rats.





**Figure 5:** Total ethanol intake (absolute ethanol, g/kg body weight) in sham-bulbectomized (OBS) and bulbectomized (OBX) rats 5 weeks prior to bilateral implantation of the bipolar electrode in the nucleus accumbens core, during the 3-week stimulation period (grey background), and 2 weeks after electrical stimulation (stim) or sham stimulation (sham). \*  $p < 0.05$  OBS vs. OBX

Before interpreting the results, one should consider the technical limitations of our study, because the alcohol preference did vary. Our intention was to evaluate preference based on ethanol concentration. Interestingly, inter-individual variation was very high. As can be seen in the Figures, the animals differed in the daily voluntary consumption of ethanol. Although ethanol intake did gradually increase in the OBX groups, some animals preferred 5% ethanol, others preferred 10% ethanol, and some rats switched between the two concentrations. Therefore, we focused on total ethanol, i.e. absolute ethanol consumption. However, total ethanol consumption differed between the NAC and the NAS experiments. Since these experiments could not be carried out in parallel, we speculate that circannual rhythms contributed to the difference in total ethanol consumption. In clinical studies, seasonal influences on bipolar affective disorders have been well documented [46,47]. It has been speculated that seasonal changes in mood and behavior may also be closely related to alcoholism in humans [48] and animals [49]. Such seasonal interferences might also contribute to the higher total fluid intake in OBX rats in the experiment with NAC stimulation.

One might argue that the increase in ethanol intake in OBX rats may be due the loss of smell. Using a 3-bottle free choice paradigm and the constant change of the bottles' position makes such an assumption less likely.

Another limitation might derive from housing conditions. To measure individual ethanol and fluid intake, the animals were housed singly. Single housing is a model used in depression research which is also linked to increased ethanol consumption [50,51]. To minimize the effect of isolation, the duration of the 3 experimental periods (pre-stimulation, stimulation, and post-stimulation periods) was limited, but we cannot exclude the possibility of an overlap of isolation and OBX effects.

The NA can be divided into NAS and NAC which differ significantly in anatomical input-out characteristics [52] and functional aspects. A plethora of animal and human studies have shown that ethanol increases the release of dopamine in the NA (for a review, cf. [53]), preferentially in the NAS rather than in the NAC [54]. Recently, heterogeneity in the behavioral relevance of NAS sub-regions to reward-seeking behavior was described [55]. Microdialysis experiments suggest that after injection of addictive drugs there is an increase in the levels of dopamine in the extracellular area of the NA.

Beside dopamine, different neurotransmitter systems at the level of the NAC circuitry have been linked to different phenomena related to drug addiction, such as compulsive use and relapse [55-58].

We selected stereotactic coordinates aimed at the NAC and NAS and found that NAS stimulation reduced the voluntary ethanol intake in OBX rats. In this experiment the OBX-stim animals showed a non-significant tendency to have lower ethanol intake values than OBX-sham animals already in the prestimulation period (Figure 3).

The effect of DBS is dependent on two variables: the need of accurate estimates of effective current spread and its effects on the excitable elements of the tissue [59]. Stimulus strengths – distance relationships were discussed by others [60]. As shown in Figures 3 and 5, the effects of DBS on voluntary ethanol intake differed. This was really unexpected, because it is unlikely that stimulation was restricted to one target alone. The current may spread out, so stimulation of one target probably affected the closely adjacent tissue of the other target. Such a spread might explain the results by [14]. These authors reported that DBS delivered to either the NAC or NAS sub-regions can significantly reduce the rat's ethanol intake. This difference between the results obtained by [14] and our own may be due to different stimulation protocols.

Pharmacological investigations revealed that Acamprostate (N-acetyl homotaurine) is effective in the relapse prevention of alcoholism. Its effectiveness was explained in terms of antagonism on NMDA receptors [61-66]. In the study by Henderson et al. [18] and in the present results it was shown that DBS in the NA reduced voluntary ethanol intake in rats. Moreover, NAC stimulation inhibited the morphine-induced rats associated hyperactivation of glutamatergic excitatory neurotransmission in the mesocorticolimbic reward circuit [67]. This led to the assumption that at least in part the suppression of neuronal activity via the activation of inhibitory interneurons and/or depolarization inactivation contributes to the effectiveness of DBS in the treatment of drug craving and relapse [68-70].

There are different explanations for the effect of DBS on voluntary ethanol intake. First, it is questionable whether the decrease in ethanol consumption by DBS in the NAS originates from the reduction in the reinforcing properties of ethanol or instead by remission of depression related symptoms. In the latter case one would argue that DBS in the NAS would alleviate hedonic-like symptoms in OBX rats. In a recent study it was shown that DBS in the NAC did not affect sucrose preference or the consumption of freely available chow. Stimulation in the NAS revealed functional dissociation between different shell regions [71]. Second, anhedonic effects of OBX have been well described in the literature [72-74]. This anhedonic effect may be due to the lack of motivation to switch the bottles when the animals found the liquid unpleasant. Thus, the effect of NAS stimulation may also be explained as a restoration of the motivation to avoid alcohol. For the moment this question remains open.

A number of hypotheses which are in part contradictory were developed to explain the possible mode of action of DBS in the treatment of addiction (for a review, cf. 4). The use of a set of three different animal models, i.e. genetically alcohol-preferring rats, intake of sweetened alcohol solution, and increased voluntary ethanol consumption in OBX rats, might provide a basis upon which to study the mechanisms underlying the clinical effects of DBS in addicts.

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