

Effects of high-frequency stimulation of the nucleus accumbens on the development and expression of ethanol sensitization in mice

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The behavioural effects of high-frequency electrical stimulation (HFS) are often similar to the effects of lesions, with the advantage of being reversible. The present study examined the effects of HFS of the nucleus accumbens (NAc), an area that has been shown to be important for sensitization to several psychostimulants, on the development and expression of EtOH sensitization. Male DBA/2 mice received five biweekly injections of EtOH (2.2 g/kg, intraperitoneally) or saline (SAL) immediately before assessments of locomotor activity (LMA). For some of the mice, each EtOH or SAL injection was preceded by 2 h of bilateral NAc HFS, whereas the remaining mice received no stimulation. Seven days after the last injection, LMA was again measured after the mice received a challenge dose of EtOH (1.8 g/kg, intraperitoneally) or SAL, either preceded or not preceded by 2 h of HFS. Mice receiving NAc HFS before EtOH injections during the sensitization period showed progressive increases in LMA that were not different from the LMA scores of EtOH-injected mice which had received no HFS. However, when the latter group was subsequently challenged after receiving HFS, a strong suppression of LMA was observed, in comparison with their own previous LMA scores (–72%) and compared with EtOH-sensitized groups challenged in the absence of HFS (–70%). A separate cohort of mice that were surgically implanted but not stimulated showed a robust EtOH sensitization response that did not differ from that of EtOH-treated mice

without electrodes, demonstrating that HFS behavioural effects were not merely a result of the presence of electrodes in the NAc. These results suggest that NAc HFS may have different effects at different stages of the EtOH sensitization process, specifically suppressing expression, but not the development of EtOH sensitization. This pattern of distinct effects of NAc manipulations on different aspects of sensitization is similar to what has been reported for other drugs of abuse, suggesting a commonality of mechanisms. Our findings also suggest that the sensitization may provide a useful paradigm for the investigation of mechanisms of clinical effectiveness of HFS in humans. *Behavioural Pharmacology* 26:184–192 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

In laboratory animals, repeated exposure to psychostimulants produces a long-lasting enhancement of locomotor activity (LMA) (Robinson and Berridge, 1993; Stewart and Badiani, 1993; Vanderschuren *et al.*, 2001). This phenomenon, known as behavioural sensitization, is considered to produce various neurobiological changes that have also been implicated in addiction processes (Robinson and Berridge, 2000; Steketee and Kalivas, 2011). Sensitization has been shown to persist for weeks and months after the last drug treatment, and this has led to its classification as a form of pathological experience-dependent plasticity (Robinson and Berridge, 2000; Thomas *et al.*, 2001; Nona *et al.*, 2013). Indeed, sensitizing regimens of psychostimulant administration have been shown to induce changes in synaptic plasticity in brain

regions involved in reward (Taepavarapruk *et al.*, 2000; Thomas *et al.*, 2001; Li and Kauer, 2004; Borgland *et al.*, 2006; Abrahao *et al.*, 2013).

Brain processes underlying sensitization are often divided into two temporally distinct phases. The development phase refers to transient neuroadaptations induced by intermittent drug administration, which can then lead to more persistent changes in the subsequent expression phase (Jones and Bonci, 2005). The latter phase occurs after cessation of drug exposure and refers to the long-lasting changes in synaptic function of key neural circuits that mediate the persistent sensitivity to subsequent drug exposure (Vanderschuren and Kalivas, 2000; Jones and Bonci, 2005).

Studies aimed at identifying key neuroanatomical regions involved in the maintenance of sensitization have

primarily focused on cocaine and amphetamine (AMPH) as inducers. Although sensitization occurs with other drugs of abuse, including ethanol (EtOH), the literature addressing neuroanatomical substrates of EtOH sensitization is lacking in comparison with other drugs. Data from cocaine and AMPH sensitization studies have shown structural and neurophysiological alterations of the mesolimbic dopamine (DA) system (Kalivas *et al.*, 1993; Jones and Bonci, 2005). In particular, such studies have shown that drug-induced synaptic plasticity in the ventral tegmental area (VTA) appears to be critical in the development phase of sensitization, whereas such plasticity in the nucleus accumbens (NAc) is crucial to the expression of sensitization (Pierce and Kalivas, 1997; Wolf, 1998; Everitt and Wolf, 2002; Jones and Bonci, 2005). It is unclear, however, whether this also applies in the case of EtOH sensitization. EtOH sensitization differs from sensitization to other abused drugs in that it occurs in mice, but not as readily in rats; it is more pronounced in the DBA/2J strain compared with C57BL/6 and other strains, and it occurs only within a narrow dose range (1.5–3.0 g/kg) (Masur and Boerngen, 1980; Phillips *et al.*, 1997; Broadbent *et al.*, 2003; Camarini and Hodge, 2004; Harrison and Nobrega, 2009a; Melón and Boehm, 2011). Given these differences, it is conceivable that neuroanatomical regions critical to the development or expression of EtOH sensitization may be different from those mediating sensitization to cocaine and AMPH.

Recent work from our laboratory has indicated that high-frequency electrical stimulation (HFS) of localized brain areas often produces effects that are similar to those of lesions or tissue inactivation in rodents (Hamani *et al.*, 2010; Creed *et al.*, 2012; Pushparaj *et al.*, 2013), in general agreement with clinical observations. In comparison with lesions, HFS has the advantage of being a reversible procedure. In order to help elucidate the neural circuitry involved in the development and expression of EtOH sensitization, we examined the effects of HFS of the NAc in these two phases.

Methods

Subjects

Male DBA/2 mice ($n = 86$), aged 7 weeks at the beginning of the experiment, were obtained from Charles River (Quebec, Canada). Mice were individually housed in polycarbonate cages ($32 \times 14 \times 12$ cm) in a room controlled for temperature, humidity (21.1°C, 30% humidity) and photoperiod (12:12; lights on at 7 a.m. and off at 7 p.m.). Food and water were freely available throughout the experiment. All procedures were approved by the Animal Care Committee at the Centre for Addiction and Mental Health and were in keeping with the guidelines and practices outlined by the Canadian Council on Animal Care.

Test apparatus

Measurements of LMA were carried out in $40 \times 40 \times 35$ cm Plexiglas activity monitor chambers (MED Associates,

St Albans, Vermont, USA) that automatically detect LMA by horizontal beam breaks. All activity testing was performed between 10:00 and 15:00 h. As in previous work (Harrison and Nobrega, 2009a, 2009b), 15-min sessions were used throughout. This test duration was chosen because the stimulant effects of EtOH are observed within the first 0–15 min of drug administration, after which sedative effects dominate the behavioural profile (Crabbe *et al.*, 1982).

Drugs

Anhydrous ethyl alcohol (Commercial Alcohols, Brampton, Ontario, Canada) was diluted with physiological saline (0.9% NaCl) to a concentration of 15%w/v. Mice received 2.2 g/kg of EtOH intraperitoneally (15 ml/kg) or an equal volume of saline (SAL) during the development phase of the study. For the challenge, mice received 1.8 g/kg (intraperitoneally) of EtOH or an equal volume of SAL. These doses were chosen for the sensitization and challenge phases of the experiment, respectively, because they elicit locomotor stimulant rather than sedative effects in mice (Phillips *et al.*, 1994; Quadros *et al.*, 2002a; Harrison and Nobrega, 2009a).

EtOH sensitization procedure

Following 7 days of acclimatization to the colony room, mice received 3 daily habituation sessions to the test chambers, where LMA was measured in the absence of drug or stimulation. Following habituation sessions, mice were counterbalanced for baseline LMA and subsequently assigned to receive either EtOH ($n = 34$), SAL ($n = 22$), HFS before EtOH injections (HFS + EtOH; $n = 21$) or HFS before SAL injections (HFS + SAL; $n = 9$) groups. On test days (i.e. injections 1, 3 and 5), mice were transported in their home cages to the test room and allowed to acclimatize for 30 min. They were then injected with EtOH or SAL and LMA was immediately measured for 15 min, after which mice were returned to the colony room. For the HFS + EtOH and HFS + SAL groups, each EtOH or SAL injection was immediately preceded by 2 h of bilateral NAc core HFS, as described below. On days when LMA was not measured (injections 2 and 4), mice were brought to the testing room for 30 min, injected with EtOH or SAL and returned to their cages. The cages were left in the testing room for an additional 15 min before being returned to the colony room.

Challenge experiment

Following a 7-day drug-free period after the completion of the EtOH sensitization protocol, EtOH, SAL, HFS + EtOH and HFS + SAL mice were further assigned to receive either SAL or a challenge dose of EtOH (1.8 g/kg, intraperitoneally) either preceded or not preceded by 2 h of HFS and immediately followed by a 15 min LMA session.

HFS surgery and protocol

Electrode implantation surgery was performed 1 week before the first habituation session for the groups that received HFS during the sensitization phase and 1 week before the challenge session for groups that received HFS only during the challenge phase. Mice were anaesthetized with isoflurane vapour and had stainless-steel, polyimide-insulated electrodes (0.125 mm diameter with 0.25 mm of the tip exposed) implanted bilaterally into the NAc core (A–P: +1.4, M–L: 1.3, D–V: –4.7 mm, using the Franklin and Paxinos atlas (Franklin and Paxinos, 1997). A ground electrode was wrapped around a screw implanted over the somatosensory cortex, and the preparation was secured to the skull using 3M/ESPE Keta-cem dental cement (K-Dental, Toronto, Ontario, Canada). Mice that were not receiving electrodes were anaesthetized with isoflurane vapour for the same period of time as those receiving electrode implantations.

At least 5 days after surgery, HFS was applied using a portable stimulator (model 6510; St Jude Medical, Plano, Texas, USA) set to deliver a current of 37.5 μ A (biphasic pulses, 90 μ s width, 130 Hz frequency). During the HFS sessions, mice were connected to the tether in their home cages and received bilateral stimulation for 2 h before an injection of EtOH or SAL and subsequent placement in the activity monitor chamber. In all cases, HFS was turned off and animals were disconnected from the stimulator before being placed in the activity monitoring boxes.

Blood ethanol concentrations

Immediately after LMA was assessed on the challenge day, mice were killed by cervical dislocation, brains were harvested and trunk blood was collected. Blood samples from EtOH-treated mice were immediately centrifuged and plasma was decanted and stored at –80°C until the time of blood ethanol concentration (BEC) determination. BECs were determined using the BioVision Ethanol Assay Kit (BioVision Inc., Milpitas, California, USA).

Verification of electrode placements

Harvested brains were sectioned coronally (20 μ m) on a Leica CM-3000 cryostat (Leica Microsystems, Richmond Hill, Ontario, Canada) microtome at –20°C, thaw-mounted onto Fisher Scientific Positive Charge glass microscope slides (Whitby, Ontario, Canada) and stored at –30°C. Slides were postfixed in 10% formalin vapour, stained with cresyl violet and then examined at \times 10 magnification under a Nikon E-600 microscope (Nikon Canada Inc, Mississauga, Ontario, Canada).

Statistical analysis

For the development phase, LMA data were analysed by a mixed-model analysis of variance (ANOVA), with injection day as a repeated-measures factor and drug treatment (EtOH or saline) and HFS (present vs. absent) as two between-subjects factors, followed, where necessary, by

Bonferroni's post-hoc tests. Data for the challenge phase were analysed by a repeated-measures ANOVA using injection 5 versus challenge injection as a within-subjects factor and drug/HFS condition as a between-subjects factor, followed by Bonferroni's tests for between-group comparisons and paired *t*-tests for within-group comparisons. All statistical analyses were carried out using SPSS 17.0 software (SPSS Inc., Chicago, Illinois, USA).

Results

Electrode localization

Figure 1 shows all electrode placements in the NAc for animals that received NAc core HFS, or that were implanted with electrodes in the NAc core but did not receive HFS. Only mice with both electrodes correctly placed in the NAc core were included in the data analyses. For the 13 mice receiving HFS during the development phase, 11 had bilateral NAc core placements; of the 18 HFS mice in the challenge experiment, 12 had bilateral NAc core implants.

Effects of HFS on the development of EtOH sensitization

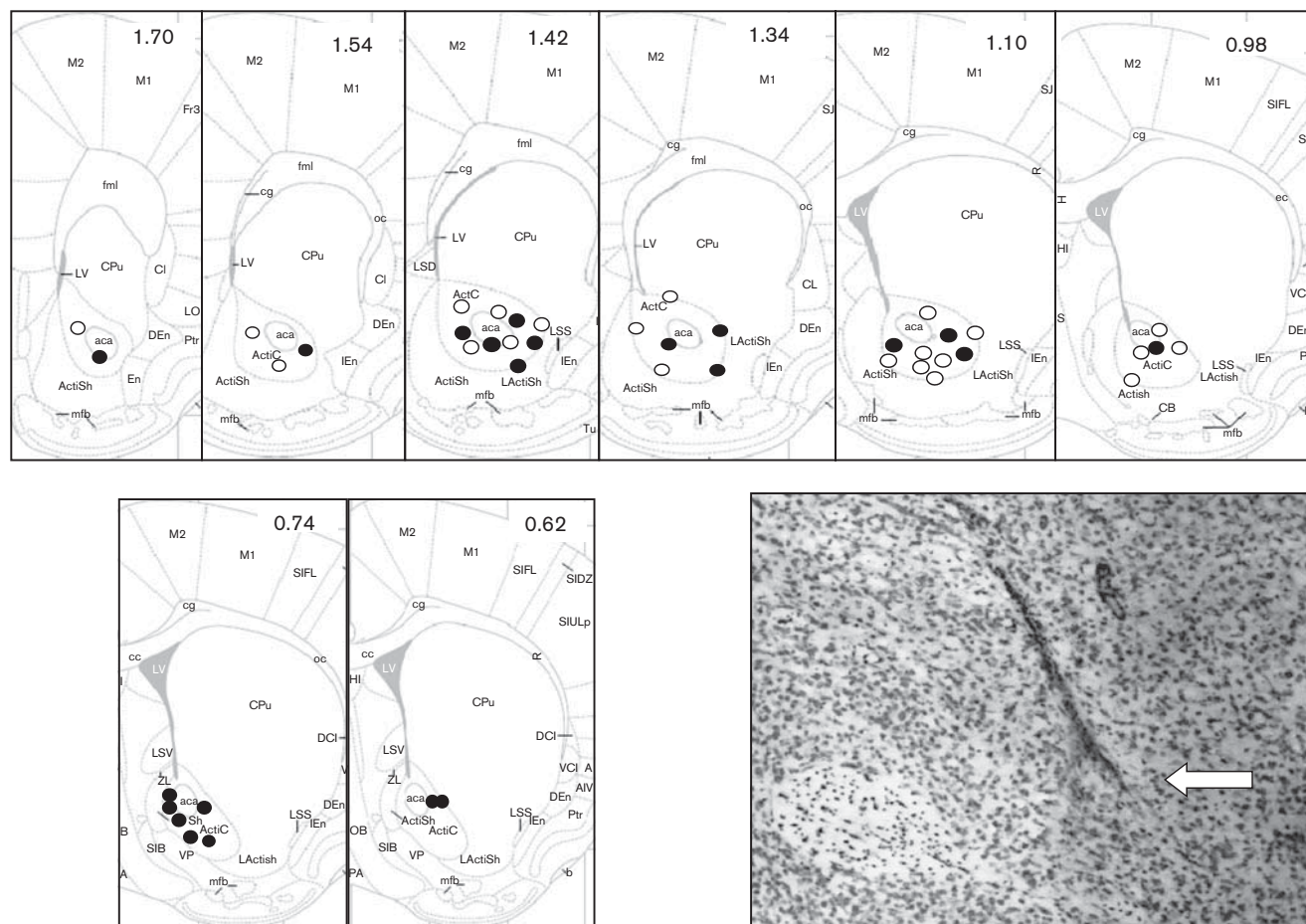
Data for the development and expression of EtOH sensitization and HFS effects are shown in Fig. 2. A repeated-measures ANOVA indicated significant main effects of injection day [$F(2, 102) = 6.90, P < 0.002$] and drug treatment [$F(1, 51) = 47.89, P < 0.001$], but no significant main effect of HFS. The drug treatment \times injection day interaction was also significant [$F(2, 102) = 4.03, P < 0.025$], but not the interaction between injection day and HFS or between drug treatment and HFS. The drug treatment \times injection day \times HFS interaction was also not statistically significant.

As shown in Fig. 2, LMA scores in the EtOH and HFS + EtOH groups were significantly higher than those of SAL controls on injection days 3 and 5 ($P < 0.01$, in both cases). In both EtOH groups, scores on day 5 were also significantly different from each mouse's own baseline score, further confirming that sensitization had occurred in both groups. LMA scores in the HFS + EtOH group were also significantly higher than those of HFS + SAL mice on injection 1 ($P = 0.001$), injection 3 ($P < 0.01$) and injection 5 ($P < 0.01$). EtOH and HFS + EtOH groups did not differ from one another at any point. Of note, HFS + SAL mice showed a pronounced decrease in LMA after injection 1 compared with the SAL group. This difference just missed statistical significance ($P < 0.059$) in Bonferroni's-corrected comparisons.

Effects of acute HFS on the expression of EtOH sensitization

The effects of HFS immediately, followed by an acute EtOH challenge are shown in Fig. 2b. To capture the changes in LMA between injection 5 and challenge, a repeated-measures ANOVA was performed using these

Fig. 1



Electrode placements. In the upper panel, filled circles indicate bilateral high-frequency electrical stimulation applied in the NAc core, whereas open circles indicate electrode placements in which no stimulation was delivered. Only animals with both electrodes correctly placed in the NAc core were included in the final data analyses. The number at the top of each panel corresponds to the distance from the bregma in mm according to the Franklin and Paxinos 2007 atlas. The lower panel shows a $\times 10$ photomicrograph showing the tip of the stimulating electrode in the NAc core (arrow). NAc, nucleus accumbens.

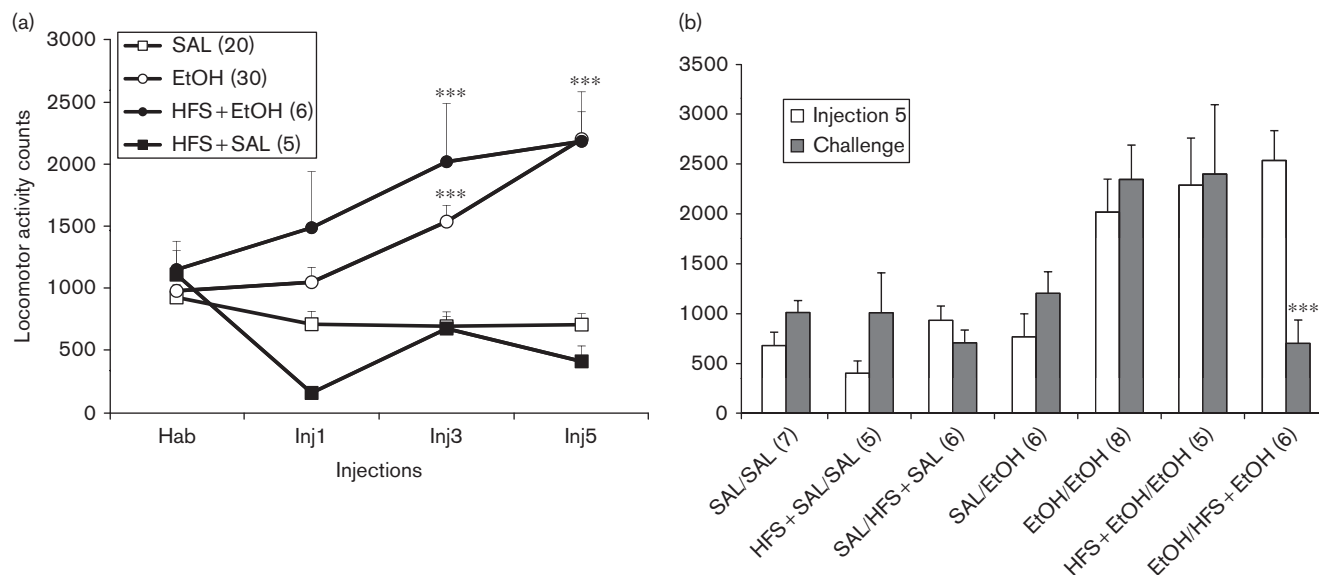
two time points as the repeated-measures factor and drug/HFS treatment as a between-subjects factor. The analysis indicated a significant main effect of challenge treatment [$F(6, 35) = 7.07, P < 0.001$] and a significant treatment \times injection interaction [$F(6, 35) = 4.47, P < 0.002$]. The group that had received EtOH during development and was challenged with EtOH (the EtOH/EtOH group) showed a 19% increase in LMA. The group that had been administered HFS before EtOH during the development phase and was then challenged with EtOH alone (HFS + EtOH/EtOH) showed a smaller LMA increase (5%). The group that had been administered EtOH alone and was then challenged after HFS (EtOH/HFS + EtOH) showed a 72% decrease in LMA ($P < 0.01$). Further, differences between injection 5 and challenge LMA values for the EtOH/HFS + EtOH group were larger than for any other group ($P < 0.05$). Challenge LMA scores for this group were also smaller

than those in the EtOH/EtOH and HFS + EtOH/EtOH groups ($P < 0.05$). The smaller sample sizes in the HFS groups were partly because of loss of electrode caps and postsurgical complications. Health problems in DBA mice after surgical manipulations and consequent removal from experiments have been observed previously (Gremel and Cunningham, 2008).

Effects of electrode implantation

To ascertain whether the effects of NAc HFS could be because of an effect of surgery or the mere presence of electrodes, the experiment was repeated as described above, except that these implanted mice never received stimulation and were therefore designated as Sham controls. The results are shown in Fig. 3. A repeated-measures ANOVA indicated significant main effects of injection day [$F(2, 112) = 22.07, P < 0.001$] and drug treatment [$F(1, 56) = 57.25, P < 0.001$], but no significant

Fig. 2



Development and expression of EtOH sensitization. (a) Development phase. Locomotor activity scores in EtOH-treated and high-frequency electrical stimulation (HFS) + EtOH-treated mice were significantly higher than in saline (SAL) controls on injection days 3 and 5 ($***P < 0.01$; Bonferroni's-adjusted comparisons). In both EtOH groups, scores on day 5 were also significantly different from each mouse's own baseline score. (b) Challenge phase. Following a 7-day drug-free period, mice were challenged with either EtOH (1.8 g/kg, intraperitoneally) or an equal volume of SAL, preceded or not by 2 h of HFS. Groups are designated by treatment during the development phase/treatment during the challenge phase. $***P < 0.01$, paired *t*-comparisons between injection 5 and challenge score for each animal. In both (a) and (b), data points are means \pm SEM for 15 min test sessions of locomotor activity measurements, and numbers in parentheses indicate sample size.

main effect of electrode implantation. The drug treatment \times injection day interaction was also significant [$F(2, 112) = 14.28$, $P < 0.001$]. As shown in Fig. 3, LMA scores of the EtOH group were greater than those of the SAL group on injection days 3 ($P < 0.005$), and 5 ($P < 0.001$). The Sham + EtOH group had higher LMA scores than both the SAL and Sham + SAL groups on injection days 3 ($P < 0.001$ in both cases) and 5 ($P < 0.001$ in both cases). The EtOH group also had greater LMA scores than the Sham + SAL group at injection days 3 ($P < 0.005$) and 5 ($P < 0.001$). EtOH and Sham + EtOH groups did not differ from one another at any time point.

As in the previous experiment, after a 7-day drug-free period following the EtOH sensitization regimen, the groups were further divided into groups receiving either SAL or a challenge dose of EtOH, with some groups having NAc-implanted electrodes after the 5th injection of the EtOH sensitization protocol. The effects of electrode implantation before the acute EtOH challenge are shown in Fig. 3b. A repeated-measures ANOVA showed a significant main effect of challenge treatment [$F(6, 56) = 9.19$, $P < 0.001$] and treatment \times injection interaction [$F(6, 56) = 2.9$, $P < 0.02$]. As can be seen, the presence of electrodes did not affect the sensitization response as all groups reacted normally to the EtOH challenge. In particular, the group receiving EtOH

during the development phase and then challenged with EtOH in the presence of implanted electrodes (the EtOH/Sham + EtOH group) showed a 38% increase in LMA ($P < 0.05$), thus confirming that the observed decrease in the corresponding HFS group in the first experiment was not because of surgery or the mere presence of electrodes in brain tissue.

Blood ethanol concentrations

BEC (in mg of EtOH/ml blood) collected immediately after LMA was assessed on the challenge day for mice receiving EtOH and are shown in Fig. 4. A nonsignificant correlation coefficient ($r = -0.398$; $P = 0.1$) was obtained between challenge LMA and BEC values. Inspection of the scatterplot suggested the presence of a single outlier (circled in Fig. 4). When this single point was removed, the correlation coefficient decreased to 0.054, further confirming that LMA scores in sensitized animals were not dependent on blood EtOH levels.

Discussion

In the present study, HFS applied to the NAc core did not interfere with the development of EtOH sensitization in mice tested immediately after 2 h of bilateral stimulation. However, acute HFS appeared to block the

