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SUBSTANTIA NIGRA PARS RETICULATA IS CRUCIALLY INVOLVED IN BARBITURATE AND ETHANOL WITHDRAWAL IN MICE

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Abstract

Sedative-hypnotic CNS depressant drugs are widely prescribed to treat a variety of disorders, and are abused for their sedative and euphoric effects. Physiological dependence and associated withdrawal episodes are thought to constitute a motivational force that sustains their use/abuse and may contribute to relapse in dependent individuals. Although no animal model duplicates depressant dependence, models for specific factors, like withdrawal, are useful for identifying potential neural determinants of liability in humans. Recent analyses implicate the caudolateral substantia nigra pars reticulata (clSNr) in withdrawal following acute and repeated ethanol exposures in mice, but did not assess its impact on withdrawal from other sedative-hypnotics or whether intrinsic neurons or fibers of passage are involved. Here, we demonstrate that bilateral chemical (ibotenic acid) lesions of the clSNr attenuate barbiturate (pentobarbital) and ethanol withdrawal. Chemical lesions did not affect convulsions in response to pentylenetetrazol, which blocks $GABA_A$ receptor-mediated transmission. Our results demonstrate that the clSNr nucleus itself rather than fibers of passage is crucial to its effects on barbiturate and ethanol withdrawal. These findings support suggest that clSNr could be one of the shared neural substrates mediating withdrawal from sedative-hypnotic drugs.

Keywords

seizure; sedative-hypnotic; lesion; ibotenic acid; convulsion

1. Introduction

Sedative-hypnotic (SH) drugs are commonly prescribed to treat insomnia, anxiety, and seizure disorders and are widely abused for their sedative and euphoric actions. In fact, abuse of prescription SH medications (including benzodiazepines and barbiturates) and other SH agents (including ethanol) is among the top five health problems in the U.S. [1].

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SH physiological dependence and associated withdrawal episodes are thought to constitute a powerful motivational force that perpetuates SH use and abuse [2]. Unfortunately, current understanding of the neural substrates that contribute to SH withdrawal is limited, hindering treatment and resulting in a lack of alternatives for dependent individuals.

Although no animal model duplicates clinically-defined SH dependence, models for specific factors, including the withdrawal syndrome, are useful for identifying potential genetic determinants of liability in humans. It is well-established that there is common genetic influence on withdrawal from a variety of SH drugs in mice [3–12]. While there are more common signs of SH withdrawal in humans, a genetic contribution to individual differences in withdrawal convulsions is apparent in humans and animal models [8, 13, 14]. In mice, withdrawal hyperexcitability following both acute and chronic exposure to short and long-acting SH drugs is frequently characterized using the handling-induced convulsion [4–6, 12, 15–18].

Previously, we identified a quantitative trait locus (QTL) on chromosome 4 with large effects on withdrawal from a barbiturate (pentobarbital [PB]) and alcohol (ethanol) in mice [10, 19]. Using c-Fos as a high-resolution histological marker of neuronal stimulation [20, 21], we found that animals congenic for the chromosome 4 QTLs for ethanol and PB withdrawal exhibit significantly less ethanol withdrawal-associated neuronal activation than background strain mice within the basal ganglia [22]. This was particularly evident in the substantia nigra pars reticulata (SNr). Bilateral electrolytic lesions of subregions of the SNr implicated the caudolateral SNr (clSNr) in withdrawal following acute and repeated ethanol exposure [22]. Based on our finding that the QTLs for ethanol and PB withdrawal are both located within the same small (<2 Mb) interval of chromosome 4, we tested the hypothesis that the clSNr may also influence PB withdrawal and be part of a shared neural substrate crucial for PB and ethanol withdrawal.

Electrolytic lesions impact both intrinsic nuclei and fibers of passage [23], whereas ibotenic acid lesions produce little or no damage to fibers of passage at appropriate doses [24–29]. Here, by utilizing both approaches, we sought to dissociate the effects of intrinsic neurons from those of fibers of passage on PB and ethanol withdrawal convulsions. Additionally, to investigate whether clSNr lesions influence CNS excitability more generally, chemically-lesioned mice were also tested for pentylenetetrazole-enhanced seizures. Our results indicate that the clSNr is intrinsically and crucially involved in both PB and ethanol withdrawal.

2. Materials and methods

2.1 Animals

DBA/2J inbred strain breeders were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were group-housed 2–4 per cage by strain and sex. Mouse chow (Purina #5001) and water were available *ad libitum*. Procedure and colony rooms were kept at 21±1°C and lights were on from 06:00–18:00. Behavioral testing was initiated between 07:00 and 09:00. All procedures were approved by the Oregon Health & Science University and VA Medical Center Care and Use Committees in accordance with USDA and USPHS guidelines. A total of 84 male DBA/2J mice were used for the lesion analyses, and were 50–70 days old at the time of surgery.

2.2. Electrolytic and ibotenic acid lesions

Mice were anesthetized by i.p. injection of anesthetic cocktail (10 mg xylazine, 100 mg ketamine, 2 mg acepromazine per kg body weight in 0.9% saline) and then placed in a stereotaxic instrument (Cartesian Research Inc. Sandy, Oregon). The skull surface was exposed and a burr hole was drilled. The coordinates for the clSNr were estimated based

upon coordinates for the C57BL/6J strain [30] and determined empirically for DBA/2J strain mice [22], and were as follows: 3.0 mm caudal to bregma [anterior-posterior (AP) = −3.0], 1.55 mm lateral to midsaggital suture [medial-lateral (ML) = ±1.55], and 4.5 mm deep from the skull surface [dorsal-ventral (DV) = −4.5]. For electrolytic lesions, an insulated 0.1 mm O.D. tungsten wire electrode with a conductive tip was lowered to the lesion site. Bilateral lesions were performed using a 0.4 mA current for 4 sec. The procedure for sham-lesioned animals was identical except that no current was passed. For ibotenic acid lesions, a fine glass pipette (diameter = 40 μm) glued to a 1 μl Hamilton syringe was lowered to the lesion sites. Bilateral lesions were performed using 0.8 μg ibotenic acid per side (80 nl of 10 μg ibotenic acid/μl in 0.01 M phosphate buffered saline). This dose was chosen in order to lesion an area of the appropriate size without disruption of the fibers of passage. Electron microscopic analyses indicate that, at doses up to 7 μg ibotenic acid, the projecting nerve terminals as well as axons traversing the SNr remain intact [27, 28]. Sham lesioned mice received bilateral injections of an equivalent volume of vehicle. An additional group of animals had no surgical procedure (unoperated control group).

2.3. PB and ethanol withdrawal

Withdrawal seizures are one of the primary characteristics of withdrawal from SH drugs including barbiturates and ethanol [31, 32], and are a useful index of withdrawal because they are displayed in humans and in rodent models. In order to focus on CNS mechanisms of physiological dependence, withdrawal was assessed following an acute injection of PB or ethanol, because chronic treatment induces metabolizing enzymes resulting in metabolic tolerance [33, 34]. McQuarrie and Fengl [35] and Crabbe et al [6] first demonstrated a state of withdrawal CNS hyperexcitability after acute ethanol or PB administration, respectively. Withdrawal severity was examined by monitoring handling-induced convulsions (HICs) associated with withdrawal, which is a sensitive index of SH withdrawal severity [6]. Details of the acute PB and ethanol withdrawal procedures have been published [19, 36], and the same 7-point HIC scoring procedure (Table 1) was used in the present studies. Individual baseline HICs were measured twice on the day before surgery. 7–10 days post-lesion, baseline (pre-PB) HICs were measured immediately before PB administration (60 mg/kg, i.p., in sterile physiological saline). HIC testing continued hourly between 1 and 8 hr post-PB administration. Two weeks later, the mice were tested for ethanol withdrawal. Baseline (pre-ethanol) HICs were scored immediately before ethanol (4 g/kg, i.p., 20% v/v in saline) administration. HIC testing continued hourly between 2 and 12 hr post-ethanol administration. The last HIC test was performed at hr 24 to confirm that HIC scores had returned to pre-drug baseline levels. Previous empirical observations show that prior testing for PB withdrawal does not affect severity of HICs associated with ethanol withdrawal tested 1–2 weeks later.

In order to create indices of PB and ethanol withdrawal response that are independent of individual differences in baseline HIC scores and reflect differences in withdrawal convulsion severity, post-PB and post-ethanol HIC scores were corrected for the individual's average baseline (pre-drug) HIC score as in previous work [19, 36]. PB and ethanol withdrawal severity scores were calculated as the area under the curve (AUC; the summed post-PB or post-ethanol HIC scores) over 8 hr post-PB and 12 hr post-ethanol. Individual withdrawal severity scores correspond to these corrected AUC values.

2.4. Pentylenetetrazole (PTZ)-enhanced HICs

The mice were assessed for PTZ-enhanced HICs one week after being tested for ethanol withdrawal. Previous studies show that the severity of PTZ-enhanced HICs is not influenced by prior testing for ethanol withdrawal or surgery [22]. The mice were tested for baseline (pre-PTZ) HICs immediately before i.p. administration of 30 mg/kg PTZ. PTZ-enhanced

HICs were measured 1, 3, 5, 8, 10, 15, 20, 35, 50 and 65 min post-PTZ as in our previous work [22]. This time period was selected based upon the time course of PTZ elicited convulsions in a panel of mouse strains [37]. This PTZ dose increases HIC intensity, but does not induce other types of convulsions (e.g., tonic hindlimb extensor) that are associated with higher doses of PTZ. To create an index of PTZ response that is independent of individual differences in baseline HIC scores, all PTZ-enhanced HIC scores were corrected for the individual's baseline (pre-PTZ) scores. PTZ-enhanced HIC severity scores were calculated as the area under the curve as in previous work [22].

2.5. Histology

Within 2 hr after behavioral testing was completed, the animals were euthanized using an overdose of mouse anesthetic cocktail. Thionin staining was used to confirm lesion locations as well as determine the rostrocaudal extent of the lesions. Only behavioral results from animals with confirmed bilateral lesions of the clSNr are included in the statistical analyses presented.

2.6. Statistics

The behavioral data were not normally distributed based upon a Shapiro-Wilks test, and were therefore analyzed using non-parametric statistical tests. We generated a Mann-Whitney *U* statistic for a comparison of two groups, or an *H* statistic for a comparison of more than two groups. All data were analyzed using Systat 12 statistical software version 12.00.08 (Systat Statistical Inc.). Unless noted otherwise, the significance level was $p<0.05$ (two-tailed).

3. Results

3.1. Lesions

Figure 1 shows the extent of chemical ibotenic acid (Fig 1A) and electrolytic (Fig 1B) lesions of the clSNr, and representative photomicrographs of the lesion sites (Fig 1C–D). Based upon coordinates from a mouse brain atlas [30], confirmed electrolytic lesions of clSNr extended approximately −3.1 to −4.0 mm AP from Bregma. The chemical lesions were generally smaller in size than electrolytic lesions, with the latter occasionally extending into the adjacent substantia nigra pars lateris (SNl) and/or cerebral peduncles. Additionally, necrotic cavities were frequently observed at electrolytic lesion sites (Fig 1B), but were not detected at ibotenic acid lesion sites (Fig 1D).

3.2. Electrolytic lesions of the clSNr attenuate PB withdrawal

Bilateral electrolytic lesions of clSNr significantly attenuate withdrawal following both acute and repeated ethanol administration [22]. Here, we expanded our analyses to assess the role of the clSNr in PB withdrawal. Only behavioral results from animals with confirmed bilateral lesions of the clSNr are included in the statistical analyses presented. Because baseline (pre-PB) HIC scores were comparable in sham-lesioned and unoperated control animals ($U_{(1,25)}=73$, $p=0.26$, NS), as were their PB withdrawal severities ($U_{(1,25)}=59.5$, $p=0.83$, NS), the data from sham-lesioned and unoperated control animals were collapsed into one control group in order to increase the statistical power of our analyses.

Baseline (pre-PB) HIC scores did not differ between clSNr-lesioned and control animals 7 days post-surgery ($U_{(1,36)}=154$, $p=0.24$, NS). However, bilateral lesions of clSNr significantly reduced PB withdrawal severity compared to control animals ($U_{(1,36)}=233.5$, $p=0.001$) (Fig. 2). Overall, the electrolytically lesioned mice showed an 82% reduction in PB withdrawal severity compared with control mice. Neither unilateral lesions of clSNr nor lesions located near but not within the SNr altered PB withdrawal convulsion severity

compared to control animals (data not shown). These results, taken together with our previous electrolytic lesion analyses assessing ethanol withdrawn mice [22], implicate the clSNr in both PB and ethanol withdrawal.

3.3. Bilateral ibotenic acid lesions of the clSNr attenuate PB and ethanol withdrawal

Because electrolytic lesions destroy intrinsic cells as well as fibers of passage, we performed chemical lesions of the clSNr and tested their effect on PB and ethanol withdrawal. Appropriate sham-lesioned and unoperated control mice exhibited comparable baseline (pre-PB) HIC scores ($U_{(1,16)}$=73.5, p=0.26, NS) and PB withdrawal severities ($U_{(1,16)}$=23, p=0.45, NS), so the data from sham-lesioned and unoperated animals were collapsed into a single control group to increase the statistical power of our analyses. Baseline (pre-PB) HIC scores did not differ between clSNr-lesioned and control animals ($U_{(1,28)}$=101, p=0.73, NS). Bilateral ibotenic acid lesions of the clSNr reduced PB withdrawal severity by 72% compared with control animals ($U_{(1,28)}$=161, p=0.002) (Fig. 3A).

Two weeks post-PB withdrawal testing, the mice were tested for ethanol withdrawal. In order to assess the potential influence of prior PB withdrawal testing on ethanol withdrawal severity, a separate naïve group of animals were also tested for ethanol withdrawal. Ethanol withdrawal severity did not differ based on prior treatment (i.e., among naïve, PB-treated unoperated control, or PB-treated sham-lesioned control animals; $H_{(2,26)}$=0.15, p=0.93, NS), indicating no effect of prior PB withdrawal testing on ethanol withdrawal convulsions. We therefore collapsed the data from all of these control animals into a single control group in order to increase the statistical power of our analyses. Baseline (pre-ethanol) HIC scores did not differ between clSNr-lesioned and control animals ($U_{(1,38)}$=155, p=0.97, NS). Bilateral ibotenic acid lesions of clSNr reduced ethanol withdrawal severity by 58% compared with control animals ($U_{(1,38)}$=248, p=0.004, Fig. 3B).

Neither unilateral lesions of the clSNr nor lesions located near but not within the SNr altered PB or ethanol withdrawal convulsion severity compared to control animals (data not shown). These results indicate that intrinsic cells within the clSNr have a significant role in PB and ethanol withdrawal.

3.4. PTZ-enhanced HICs

SNr-lesioned mice were compared to control mice for their HIC severities following administration of PTZ (30 mg/kg, i.p.), a chemiconvulsant that exerts its effects by impairing $GABA_A$ receptor-mediated transmission [38, 39]. Baseline (pre-PTZ) HIC scores did not differ from the other ‘baseline’ scores obtained pre-ethanol ($H_{(3,74)}$=3.11, p=0.38, NS) or pre-PB ($H_{(3,64)}$=3.55, p=0.32, NS). No differences in PTZ-enhanced HICs between clSNr-lesioned and control mice were detected ($U_{(1,37)}$=113, p=0.32, NS). These results are consistent with and expand upon our data showing that electrolytic lesions of the clSNr do not affect PTZ-enhanced convulsions [22].

4. Discussion

Currently, the structures responsible for the onset, propagation, and cessation of withdrawal convulsions are not known. The present studies are the first to show that the clSNr plays a crucial role in barbiturate and ethanol withdrawal and that intrinsic neurons rather than fibers of passage are essential to its role in withdrawal. These findings demonstrate that the clSNr plays a crucial role within a brain circuit involved in withdrawal from PB and ethanol and potentially other drugs.

Recently, we identified the SNr as a brain region that exhibits genotype-dependent neuronal activation associated with ethanol withdrawal [22, 40]. Although electrolytic lesions of the

clSNr implicated this structure in ethanol withdrawal [22], they also damage fibers of passage [23]. In contrast, ibotenic acid lesions produce little or no damage to fibers of passage at appropriate doses [23–29]. Consistent with these reports, we found in the present studies that necrotic tissue was frequently observed at electrolytic lesion sites, but was not detected at the chemical lesion sites. Our results demonstrate that the reduction in PB withdrawal severity resulting from bilateral chemical lesions of the clSNr was comparable in magnitude to that resulting from bilateral electrolytic lesions of this region. These results demonstrate that intrinsic clSNr cells play a critical role in PB withdrawal.

Ethanol withdrawal was also substantially attenuated by bilateral chemical lesions of the clSNr, indicating that intrinsic cells are crucial to the role of the clSNr on both ethanol and PB withdrawal. The degree to which ethanol withdrawal was reduced by ibotenic acid lesions (~60%) may be less than we reported previously after bilateral electrolytic lesions (which reduced ethanol withdrawal by 65–95% using acute and repeated ethanol exposure models [22]. A potentially smaller effect of chemical lesions might be influenced by the more limited extent of the chemical lesions, which was apparent in the present studies. It might also reflect potential differences in injury to regions remote to the target sites when different lesion methods are used [24]. Finally, a potentially smaller effect of chemical lesions could also suggest that, in addition to intrinsic clSNr cells, fibers passing through the clSNr that terminate in other brain regions may also contribute to ethanol withdrawal. However, this was not apparent for PB withdrawal.

Our results confirm that the clSNr is critical for both PB and alcohol withdrawal, but additional brains regions are certainly involved in withdrawal [40, 41, 42]. The SNr receives afferents from other basal ganglia regions including the striatum and subthalamic nucleus [43], which are also implicated in CNS hyperexcitability phenotypes and might plausibly play a role in SH drug withdrawal. Interestingly, bilateral electrolytic lesions of the striatal ventral caudate putamen exacerbate ethanol withdrawal [44], while electrolytic lesions of the subthalamic nucleus do not affect ethanol withdrawal convulsions [22]. The deep layers of the superior colliculus (DLSC) receive a significant projection from the SNr as well as multimodal sensory information including visual, acoustic, somato-vesticular inputs [45]. SNr projections to the DLSC may gate the sensorimotor association and influence the motor centers for expression of convulsions, such as motor neurons in the nucleus reticularis pontis oralis [46]. The mesopontine tegmentum receives projections from the SNr as well as other basal ganglia regions [47], and microinjection of PB into this brain area depresses CNS activity [48], so it is plausible that rebound hyperexcitability in this region might contribute to withdrawal from PB and other SH drugs. Much future work will be needed to fully elucidate the neural networks modulating the SH drug withdrawal syndrome.

The acute actions of both ethanol and barbiturates include the enhancement of inhibitory transmission mediated by GABA, including effects on $GABA_A$ receptors and GABA release in many brain regions [49–52]. Withdrawal from these drugs represents the manifestation of physiological compensation to their effects and is thought to involve changes in GABAergic transmission [53, 54]. It is therefore interesting, though not surprising, that the clSNr lesions reduced ethanol and PB withdrawal convulsions, but did not affect convulsions in response to PTZ, which blocks $GABA_A$ receptor-mediated transmission [37, 38]. Consistent with this observation, mice congenic for a QTL on chromosome 4 QTL affecting PB and ethanol withdrawal do not differ in PTZ-enhanced HICs [55]. This may suggest that the chromosome 4 QTL affects GABAergic ‘tone’ rather than $GABA_A$ receptor function *per se*. In the SNr, activation of presynaptic $GABA_B$ receptors reduces GABA release and suppresses the frequency of synaptic currents mediated by postsynaptic $GABA_A$ receptors [56]. Also in the SNr, 5-HT_{2C} receptor activation increases the frequency and amplitude of these $GABA_A$ receptor mediated synaptic currents, suggesting an effect on GABA release

[56]. Both $GABA_B$ and 5-HT_{2C} receptors physically associate with the multi-PDZ domain protein (MPDZ, also called MUPP1 [57, 58]) encoded by *Mpdz*, a quantitative trait gene for the ethanol and PB withdrawal QTLs on chromosome 4 [10]. PDZ domain proteins affect the function/expression of proteins with which they physically associate [59], so is plausible that MPDZ affects $GABA_B$ and/or 5-HT_{2C} receptor function/expression and thereby affects GABAergic tone and withdrawal. Indeed, preliminary analyses suggest that central administration of baclofen exacerbates withdrawal in mice (Kruse L and Buck K, unpublished data), and that microinjection of a preferential 5-HT_{2C} receptor agonist into the clSNr attenuates withdrawal severity (Chen G and Buck K, unpublished data). Diminished 5-HT_{2C} receptor expression may be functionally related to the increased susceptibility to respiratory arrest induced by generalized convulsive seizures in DBA/2 compared to C57BL/6J mice [60]. Future studies will be needed to test the hypothesis that MPDZ affects $GABA_B$ and 5-HT_{2C} receptor function in the SNr and other brain regions and thereby affects withdrawal.

Our findings advance our understanding of the neural determination of drug withdrawal. To date, we have assessed the impact of SNr lesions on two drugs, so it remains to be determined to what extent our results generalize to additional drugs. Second, this study focuses on withdrawal HICs. While many signs of the withdrawal syndrome are genetically correlated with HIC severity (i.e., tremors, hypoactivity, emotionality; [3, 18, 61], others are not [i.e., tail stiffness; 18]. Future studies will be needed to determine to what degree the clSNr and associated brain circuitry affect other withdrawal signs. Despite these limitations, the current study demonstrates that the clSNr is crucially involved in barbiturate and ethanol withdrawal.

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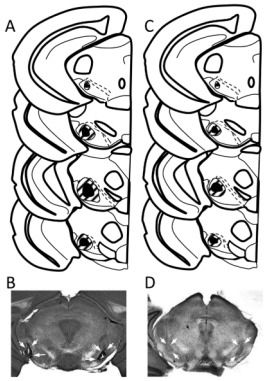

Fig. 1.

Photomicrographs and representative reconstructions of electrolytic and ibotenic acid (IBO) lesions of the clSNr. ***(A,C)*** Schematic representations of the minimal (black) and maximal (striped) extent of electrolytic and IBO lesions of the clSNr, respectively. Coordinates of the coronal sections are indicated with reference to Bregma according to the stereotaxic atlas of Paxinos and Franklin (2001). ***(B,D)*** Low magnification (2X) photomicrographs of representative brain sections for bilateral electrolytic and IBO lesions of the clSNr, respectively. The white arrows point to the bilateral lesion areas. Scale bar = 500 μm.

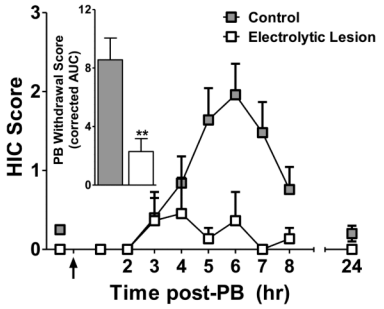

Fig. 2.

Attenuation of PB withdrawal by bilateral electrolytic lesions of the clSNr. Bilateral lesioned (*open squares; n=11*) and control (*closed squares; n=25*). D2 strain mice were scored twice for baseline HICs immediately before administration of 60 mg/kg PB (the *arrow* marks PB injection at time 0), and hourly between 1 and 8 hr post-PB administration. After 3 hr, HIC scores increase above baseline, indicating a state of withdrawal hyperexcitability, which peaks approximately 6 hr post-PB administration. Data represent the group mean scores + SEM for baseline and post-PB HICs. *Inset:* clSNr lesions significantly reduced PB withdrawal scores (corrected area under the curve, AUC) compared to control animals. $^{**}p<0.01$.

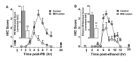

Fig. 3.

Attenuation of PB and ethanol withdrawal by ibotenic acid lesions of the clSNr. **(*A*)** PB withdrawal was indexed using the HIC in lesioned (*open squares, n=12*) and control (*closed squares, n=16*) D2 mice. The mice were scored twice for baseline HICs immediately before administration of 60 mg/kg PB (the *arrow* marks PB injection at time 0), and hourly between 1 and 8 hr post-PB administration. *Inset:* clSNr lesions significantly reduce PB withdrawal scores (corrected AUC) compared to control animals. **(*B*)** Ethanol withdrawal was indexed using the HIC in lesioned (*open squares; n=12*) and control (*closed squares; n=26*) D2 mice. The mice were scored twice for baseline HICs immediately before administration of 4 g/kg ethanol (the *arrow* marks ethanol injection at time 0), and hourly between 2 and 12 hr post-ethanol administration. After 4 hr, HIC scores increase above baseline, indicating a state of withdrawal hyperexcitability, which peaks approximately 6–8 hr post-ethanol administration. *Inset:* clSNr lesions significantly reduce ethanol withdrawal scores (corrected AUC) compared to control animals. ** $p<0.01$.

TABLE 1

Handling-induced convulsion (HIC) rating scale

Symptom	Score
No convulsion or facial grimace after gentle 180° spin	0
A facial grimace is seen after gentle 180° spin	1
No convulsion when lifted by the tail, but a tonic convulsion is elicited by a gentle 180° spin	2
Tonic-clonic convulsion after a gentle 180° spin	3
Tonic convulsion upon lifting by the tail	4
Tonic-clonic convulsion when lifted by the tail, often with the onset delayed up to 1–2 seconds	5
Severe, tonic-clonic convulsion when lifted by the tail, with a quick onset and long duration, often continuing for several seconds after the mouse is released	6
Severe, tonic-clonic convulsion elicited prior to lifting by the tail, with a quick onset and long duration, often continuing for several seconds after the mouse is released	7