Ethanol Withdrawal Reduces the Number of Spontaneously Active Ventral Tegmental Area Dopamine Neurons in Conscious Animals

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ABSTRACT
Withdrawal from chronic ethanol treatment leads to a reduction in the electrical activity in dopamine (DA) neurons in the ventral tegmental area (VTA). However, there is disagreement on how the electrical activity is reduced (i.e., in the number of spontaneously active DA neurons or their firing rates and burst firing activity) and on the underlying mechanisms. The use of general anesthesia has been suggested to cause this discrepancy. In the present study, we demonstrate that ethanol withdrawal, in conscious animals, causes a reduction in the number of spontaneously active VTA DA neurons, but not in their firing rate or burst firing activity. Similar results were obtained in a previous study using anesthetized preparation, showing that general anesthesia does not cause this difference. Ethanol withdrawal-induced reduction in a number of spontaneously active VTA DA neurons could be mediated by depolarization inactivation because this effect was reversed by systemic administration of amphetamine, which inhibits VTA DA neurons by hyperpolarization. In addition, the withdrawal effect was normalized by acute ethanol administration, suggesting that the decrease in the number of spontaneously active VTA DA neurons represents an adaptational change to chronic ethanol treatment. Because the electrical activity of DA neurons controls the release of DA, it is possible that the decreased DA release during ethanol withdrawal observed in previous studies is caused by the reduction in the electrical activity of VTA DA neurons.

Similar to many substances of abuse, ethanol acutely increases the activity of dopamine (DA) neurons in the ventral tegmental area (VTA; Gessa et al., 1985; Brodie et al., 1990) and DA release in the nucleus accumbens (Acb) (Weiss et al., 1993; Yim and Gonzales, 2000). These actions are thought to mediate the rewarding properties of ethanol (Koob and Weiss, 1992). Evidence from microdialysis studies suggests that the increased DA release in the Acb after acute systemic ethanol administration is mediated by an excitation of VTA DA neurons and not by a direct effect of ethanol on DA terminals (Yim and Gonzales, 2000).

In contrast to the acute effect of ethanol, withdrawal from repeated ethanol administration is associated with decreased DA neurotransmission, which has been proposed to mediate ethanol craving during abstinence (Weiss and Porrino, 2002). Specifically, the release of DA (inferred by DA levels in dialysate) is decreased during ethanol withdrawal (Hunt and Majchrowicz, 1974; Darden and Hunt, 1977; Diana et al., 1993; Weiss et al., 1996). DA turnover is reported to be decreased (Trulson, 1985) or unchanged (Fadda et al., 1980; Reggiani et al., 1980). The electrical activity of VTA DA neurons is also reduced during ethanol withdrawal (Diana et al., 1992, 1993; Shen and Chiodo, 1993, 1995). This raises the possibility that reduced DA release and turnover is the result of decreased electrical activity of VTA DA neurons.

An understanding of the cause of decreased electrical activity of VTA DA neurons during withdrawal would represent an important step toward delineating the neural mechanism of alcoholism. However, this goal has been hampered by a lack of agreement in the current literature on how reduced activity in VTA DA neurons during ethanol withdrawal is manifested. Diana et al. (1992, 1993, 1995) have observed a decreased firing rate and burst firing activity in spontaneously active DA neurons in withdrawing animals. Shen and Chiodo (1993) have reported a reduction in the number of spontaneously active VTA DA neurons, but no change in firing rate and burst firing activity in withdrawing animals. These observations have lead to the proposal of different underlying mechanisms. Diana et al. (1993) propose that a decreased excitation is responsible for a reduction in the firing rate of VTA DA neurons during ethanol withdrawal...
Treatment was carried out by two intubations at 0 or 5 g/kg (5 g/kg ethanol [20% (v/v) in 0.9% saline] except during weekends. Male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing between 125 and 150 g, were used for the study. To mimic the binge drinking behavior that produces high blood ethanol concentrations in humans, rats were administered ethanol via intragastric intubation for 8 weeks. Animals were treated for 8 weeks with a daily dose of 0 (0.9% saline) or 10 g/kg ethanol [20% (v/v) in 0.9% saline] except during weekends. Treatment was carried out by two intubations at 0 or 5 g/kg [5–6 h apart; between 10:00 AM and 5:00 PM]. The blood ethanol concentration measured 1.5 h after the second daily intubation of ethanol was between 280 and 341 mg/dl. The blood ethanol concentration returned to 0 mg/dl 12 h after the second daily intubation. Rats in the ethanol group also received thiamine injections (8 mg/kg i.m. twice a week) to avoid thiamine deficiency induced by ethanol treatment. After 8 weeks of treatment, animals underwent surgical and recording procedures during the acute withdrawal stage, 17 to 24 h after the second daily intubation.

**Surgical Procedures.** The electrophysiological recordings of DA neurons were performed in conscious (paralyzed, locally anesthetized) rats. Surgical procedures were performed under temporary halothane anesthesia. All incision sites and blunt pressure points were then infiltrated with a long-acting local anesthetic (morpaine, 0.25%, Abbott Labs, North Chicago, IL). While anesthetized with halothane, each rat was tracheotomized, cannulated with a tracheal tube, and mounted in a stereotaxic apparatus. The skull and dura overlapping the VTA were removed and tail vein cannulated. Each rat was respired immediately with a mixture of O₂/N₂O (70%:30%) by connecting the tracheal cannula with a rodent ventilator (Edo Scientific Inc., Chapel Hills, NC). N₂O provided sedative and analgesic effects (Balster, 1998). In the rat, N₂O administered at 30% does not lead to a loss of consciousness (Kuiyla et al., 2003). Each rat was respired for a minimum of 20 min before each electrophysiological recording. Expired CO₂ levels were continuously monitored with a CO₂ monitor (Biochem 9000 capnometer; Biochem, Wankesha, WI) and maintained between 28 and 43%. Body temperature was monitored and maintained between 36 and 37°C. Heart rate and blood oxygen saturation were monitored with an oximeter (Nonin 8600V; Nonin Medical, Inc., Plymouth, MN). Heart rate was maintained between 280 and 400/min. Blood oxygen was maintained above 90%. Gallamine triethiodide supplement was administered every 30 min.

**Electrophysiology.** Electrophysiological recordings were carried out as described previously (Shen et al., 1994). Extracellular action potentials were recorded with single-barrel glass micropipettes (1.5 mm o.d.; Sutter Instrument Co., Novato, CA) filled with 2 M NaCl (in vitro impedance, 2–4 MΩ at 135 Hz). The micropipette was lowered into the VTA and monitored with a high-input impedance amplifier (bandpass filter settings, 0.3–3 kHz). The output was sent to an analog oscilloscope, audiomonitor, window discriminator, and a 486 personal computer. To perform the cells-per-track technique, the recording electrode was passed systematically 12 times through a stereotaxically defined block in the VTA. The electrode tracks were separated by 200 μm (coordinates, 2.8–3.4 mm anterior to lambda; 6.0–9.0 mm below the brain surface; 0.6–1.0 mm lateral to the midline). Spontaneously active DA neurons were identified by their characteristic waveforms and firing patterns (Chiodo, 1988). Average firing rates of DA neurons were determined from all DA neurons sampled within each grid.

To test the acute effects of amphetamine (d-amphetamine sulfate, 0.5 mg/kg i.v.; Sigma-Aldrich) or ethanol (3 g/kg i.p.) administration on the number of spontaneously active DA neurons in animals undergoing ethanol withdrawal, cells-per-track experiments were performed in the right VTA first. VTA DA neurons in the left midbrain were sampled in the same animals beginning 20 min after the injection.

To analyze the firing pattern, interspike intervals of individual DA neurons were determined from 500 consecutive action potentials obtained from each DA neuron. Firing pattern analysis was performed on the interspike interval data with the Burstan program (Shen et al., 1994). The onset of a burst was defined by an interspike interval of less than 80 ms and a burst termination with the next interspike interval of 160 ms or greater. Burst firing patterns were compared by burst number (percentage of the 500 spikes that were present within the burst), burst length (mean number of spikes within each burst), interburst interval (mean interval between bursts), within-burst interval (mean interval between spikes within each burst), and percentage of bursting cells in each group (a bursting cell was defined as a neuron with a burst number greater than 10%).

**Materials and Methods**

**Chronic Ethanol Administration.** Male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing between 125 and 150 g, were used for the study. To mimic the binge drinking behavior that produces high blood ethanol concentrations in humans, rats were administered ethanol via intragastric intubation for 8 weeks. Animals were treated for 8 weeks with a daily dose of 0 (0.9% saline) or 10 g/kg ethanol [20% (v/v) in 0.9% saline] except during weekends. Treatment was carried out by two intubations at 0 or 5 g/kg [5–6 h apart; between 10:00 AM and 5:00 PM]. The blood ethanol concentration measured 1.5 h after the second daily intubation of ethanol was between 280 and 341 mg/dl. The blood ethanol concentration returned to 0 mg/dl 12 h after the second daily intubation. Rats in the ethanol group also received thiamine injections (8 mg/kg i.m. twice a week) to avoid thiamine deficiency induced by ethanol treatment. After 8 weeks of treatment, animals underwent surgical and recording procedures during the acute withdrawal stage, 17 to 24 h after the second daily intubation.
which was the EC50 to obtain total inhibition of firing rates in systemically administering amphetamine at 0.5 mg/kg i.v., during ethanol withdrawal. We tested this possibility by VTA DA neurons. Therefore, it is possible that amphetamine exerted opposite effects on the number of spontaneously active VTA DA neurons, we conducted the cells-per-track experiments before and after an acute ethanol (3 g/kg i.p.; a high dose) treatment in control animals and animals undergoing ethanol withdrawal. The results show that acute ethanol administration exerted opposite effects on the number of spontaneously active VTA DA neurons in animals undergoing ethanol withdrawal were slightly but significantly decreased after acute amphetamine treatment (Tukey’s HSD post hoc test; \( P < 0.05 \)). The firing rates were 4.43 ± 0.15 (\( n = 46 \)) and 3.90 ± 0.13 (\( n = 79 \)) spikes/s before and after amphetamine treatment, respectively. This difference is also reflected in a significant interaction effect between chronic ethanol treatment and acute amphetamine administration on the firing rate of VTA DA neurons (\( F_{1,27} = 5.15, P < 0.05, \) two-way ANOVA).

**Acute Ethanol Administration Restores the Number of Spontaneously Active VTA DA Neurons.** To test the hypothesis that ethanol intake can reverse the reduced number of spontaneously active VTA DA neurons, we conducted the cells-per-track experiments before and after an acute ethanol (3 g/kg i.p.; a high dose) treatment in control animals and animals undergoing ethanol withdrawal. The results show that acute ethanol administration exerted opposite effects on the number of spontaneously active VTA DA neurons in these two groups of animals. This is reflected in a significant interaction (two-way ANOVA; \( F_{1,10} = 37.39, P < 0.01 \)). After acute ethanol treatment, the number of spontaneous VTA DA neurons was decreased in control animals (from

**Results**

**Reduction in the Number of Spontaneously Active VTA DA Neurons during Ethanol Withdrawal in Conscious Animals.** The results show that the decrease in the electrical activity of VTA DA neurons during ethanol withdrawal was mediated by a decrease in the number of spontaneously active VTA DA neurons, and not in the firing rates or firing pattern. The number of spontaneously active VTA DA neurons decreased by approximately 50%. The numbers of cells per track in control animals and animals undergoing ethanol withdrawal were 1.03 ± 0.04 (\( n = 22 \)) and 0.51 ± 0.04 (\( n = 24 \)), respectively (Fig. 1). The firing rates and the firing pattern of the spontaneously active VTA DA neurons remained unchanged. They were 4.30 ± 0.08 and 4.31 ± 0.09 spikes/s (Fig. 1), respectively, for the control animals and animals undergoing ethanol withdrawal. The burst firing patterns of VTA DA neurons did not differ in control animals and in animals undergoing ethanol withdrawal (Table 1).

**Acutely Administered Amphetamine Reverses Ethanol Withdrawal-Induced Effect.** Apomorphine has been shown to be effective in reversing the reduced spontaneously active DA neurons in our previous study (Shen and Chiolo, 1993). By increasing extracellular DA in the VTA, low doses of systematically administered amphetamine could exert similar inhibitory effects as apomorphine on the firing rate of VTA DA neurons. Therefore, it is possible that amphetamine could also restore the electrical activity of VTA DA neurons during ethanol withdrawal. We tested this possibility by systemically administering amphetamine at 0.5 mg/kg i.v., which was the EC50 to obtain total inhibition of firing rates in individual VTA DA neurons in normal rats in our previous study (Xu and Shen, 2001). The result shows that amphetamine exerted opposite effects on the number of spontaneously active VTA DA neurons in control and animals undergoing ethanol withdrawal. In control animals, amphetamine decreased cells-per-track from 1.13 ± 0.06 to 0.60 ± 0.05 (60% reduction; \( n = 5 \)). In contrast, in animals undergoing ethanol withdrawal, amphetamine administration increased the number of spontaneously active DA neurons by 74% (from 0.54 ± 0.07 to 0.94 ± 0.08; \( n = 7 \); Fig. 2, A and B). The opposite effects of amphetamine were reflected in a significant interaction effect between ethanol treatment and acute amphetamine administration (two-way ANOVA; \( F_{1,10} = 102.5, P < 0.001 \)).

Despite a decrease in the number of spontaneously active VTA DA neurons in control animals, acute amphetamine treatment did not significantly change the average firing rate in the remaining VTA DA neurons in control animals (Fig. 2A). The firing rates were 3.99 ± 0.14 (\( n = 67 \)) and 4.14 ± 0.16 (\( n = 36 \)) before and after amphetamine treatment, respectively. The average firing rates of spontaneously active VTA DA neurons in animals undergoing ethanol withdrawal were slightly but significantly decreased after acute amphetamine treatment (Tukey’s HSD post hoc test; \( P < 0.05 \)). The firing rates were 4.43 ± 0.15 (\( n = 46 \)) and 3.90 ± 0.13 (\( n = 79 \)) spikes/s before and after amphetamine treatment, respectively. This difference is also reflected in a significant interaction effect between chronic ethanol treatment and acute amphetamine administration on the firing rate of VTA DA neurons (\( F_{1,27} = 5.15, P < 0.05, \) two-way ANOVA).

**TABLE 1**

<table>
<thead>
<tr>
<th>No. of Cells</th>
<th>Coefficient of Variation</th>
<th>Burst No.</th>
<th>No. of Doublets</th>
<th>Interburst Interval</th>
<th>Withing-Burst Interval</th>
<th>Burst Length</th>
<th>Burst Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>102.5</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>568 Shen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

![Fig. 1](https://example.com/figure1.png)

**Fig. 1.** Acute withdrawal from chronic ethanol treatment decreased the number of spontaneously active VTA DA neurons in conscious animals. The numbers of cells per track in control and ethanol-treated animals were 1.03 ± 0.04 and 0.51 ± 0.04, respectively. The average firing rates of spontaneously active VTA DA neurons in control and ethanol treated animals were not different. The number inside each bar indicates group size. **+,** \( P < 0.01 \).
1.11 ± 0.07 to 0.46 ± 0.05 cells per track) and increased in animals undergoing ethanol withdrawal (from 0.49 ± 0.07 to 0.86 ± 0.18 cells per track; Fig. 3). Acute ethanol administration also significantly decreased the average firing rates of the remaining spontaneously active neurons in the control animals; from 4.24 ± 0.13 (n = 80) to 3.37 ± 0.17 spikes/s (n = 33; Tukey's HSD post hoc test, \( P < 0.05 \)), but did not change the firing rates of VTA DA neurons recorded from animals undergoing ethanol withdrawal. The average firing rates before and after acute ethanol treatment were 3.89 ± 0.17 (n = 35) and 3.96 ± 0.16 (n = 61), respectively, in animals undergoing ethanol withdrawal.

We also examined the effect of acute ethanol administration on the change in firing rates in individual spontaneously active VTA DA neurons. The result is presented in Table 2. In all VTA DA neurons, acute ethanol administered in cumulative doses first increased the firing rate of VTA DA neurons and then caused a sudden cessation of firing. Acute ethanol treatment at doses ranging from 0.5 to 2 g/kg did not produce significant differences in firing rate changes between control animals (n = 12) and animals undergoing ethanol withdrawal (n = 8; two-way ANOVA), although there was a trend toward greater increase in firing rate by acute ethanol in control animals. On the other hand, the acute ethanol doses that caused a cessation of firing are significantly different between these two groups (chi square test, \( \chi^2 = 1964; df = 3; P < 0.001 \)), with more neurons stopped firing at lowered doses in control animals (Table 2).

**Discussion**

The first goal of the present study was to address the possibility that reduced numbers of spontaneously active VTA DA neurons during ethanol withdrawal observed previously may have been influenced by the use of general anesthetics (Shen and Chiodo, 1993). The results demonstrate that in conscious (paralyzed, locally anesthetized), ethanol-withdrawing animals, there is still a reduction in the number of spontaneously active VTA DA neurons but no change in their firing rate and burst firing pattern. These findings are consistent with other studies showing a lack of impact of general anesthesia on how the electrical activity is reduced in...
DA neurons after chronic drug treatment (Bunney and Grace, 1978; Xu and Shen, 2001), and therefore they do not support the suggestion made by Diana et al. (1995) that the reduction in the number of spontaneously active VTA DA neurons during ethanol withdrawal is an artifact of general anesthetics.

Clarification of how the electrical activity of VTA DA neurons is reduced during ethanol withdrawal is critical to the understanding of the underlying cellular mechanisms mediating this effect. Based on the observation that ethanol withdrawal decreases firing rate and burst firing pattern, but not the number of spontaneously active VTA DA neurons, Diana et al. (1992, 1993, 1995) suggest that there is a decreased excitation in VTA DA neurons due to changes in input or membrane excitability. In contrast, based on our observations, we speculate that excessive excitation leading to impaired spike generation (depolarization inactivation; Grace et al., 1997) mediates the reduced number of spontaneously active VTA DA neurons and no changes in the firing rate or burst firing pattern. Depolarization inactivation was originally proposed to be the underlying mechanism for the reduction in the spontaneously active DA neurons after chronic antipsychotic treatment (Bunney and Grace, 1978; White and Wang, 1982; Chiodo and Bunney, 1983; Grace et al., 1997). The supporting evidence for an inactivation by excessive depolarization includes the finding that individual "quiescent (not spontaneously active)" DA neurons can be made to discharge with inhibitory agents, which decrease individual DA neuron firing rate by hyperpolarization, such as locally applied GABA or DA, but not excitatory agents like glutamate (Grace et al., 1997). In addition, the decreased number of spontaneously active DA neurons can be normalized to control levels by systemically applied apomorphine, which in control animals inhibits the firing rate of individual DA neurons and decreases the number of spontaneously active neurons (Grace et al., 1997). We previously used these strategies in anesthetized, ethanol withdrawing animals to confirm that depolarization inactivation was indeed the mechanism for reduced number of spontaneously active DA neurons. Specifically, we observed that "quiescent" VTA DA neurons resumed firing with microiontophoretically applied GABA and that the reduced number of spontaneously active VTA DA neurons was normalized by systemic application of apomorphine (Shen and Chiodo, 1993). In the present study, we further demonstrate this general phenomenon in conscious animals. We used an inhibitory agent, amphetamine, to normalize the reduction in the number of spontaneously active VTA DA neurons during ethanol withdrawal in conscious animals. Like apomorphine, amphetamine in control animals reduced the number of spontaneously active VTA DA neurons. Such opposite effects of a systemically administered inhibitory agent on the number of spontaneously active VTA DA neurons in control and ethanol withdrawing animals strongly suggest that the decreased number of spontaneously active VTA DA neurons during ethanol withdrawal is caused by depolarization inactivation. Although the cellular mechanism for depolarization inactivation is currently unknown, changes in the inputs to DA neurons may be required because a reduced number of spontaneously active DA neurons can no longer be observed after forebrain inputs to DA neurons are severed (Grace et al., 1997). Ethanol withdrawal is known to cause hyperexcitability largely by an up-regulation in NMDA receptor function and a down-regulation of GABA<sub>A</sub> receptors (Crews et al., 1996). If hyperexcitability occurs in VTA DA neurons due to these changes during ethanol withdrawal, it may lead to depolarization inactivation and a reduced number of spontaneously active VTA DA neurons. Our results also show that a low dose of systemically administered amphetamine (0.5 mg/kg i.v.) is sufficient to reverse the reduction in the spontaneously active VTA DA neurons during ethanol withdrawal. This is consistent with our previous observation that low doses of DA agonists (e.g., apomorphine, 20 μg/kg i.v.) can restore the number of spontaneously active DA neurons during ethanol withdrawal (Shen and Chiodo, 1993). The low-dose requirement of apomorphine and amphetamine is mediated by the activation of somatodendritic DA autoreceptors, which are more sensitive than postsynaptic DA receptors (Chiodo, 1988). However, amphetamine works as an indirect agonist. It increases DA release in the cell body area and then activates somatodendritic DA autoreceptors (Mercuri et al., 1985). In addition, amphetamine also increases DA release in the forebrain terminal areas and activates postsynaptic DA receptors. This effect then leads to further inhibition in DA neurons via a feedback pathway (Bunney and Aghajanian, 1976).

The ability of DA agonists such as apomorphine and amphetamine to normalize ethanol withdrawal-induced reduction in the spontaneously active VTA DA neurons may have clinical implications. Because the electrical activity of DA neurons controls the release and synthesis of DA (Suaud-Chagny et al., 1992), it is possible that ethanol withdrawal-induced decrease in extracellular DA level in the Acb (Diana et al., 1993; Weiss et al., 1996) is caused by the reduction in the number of spontaneously active VTA DA neurons. Because DA hypofunction has been proposed to be the underlying neuronal mechanism for ethanol craving during abstinence (Weiss and Porrino, 2002), DA agonists such as apomorphine or amphetamine may be effective in treating ethanol craving by normalizing DA function via reversing the activity of DA neurons and DA synthesis/release. Although there is a general concern regarding using stimulants to treat

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**TABLE 2**

Effects of acute administration of ethanol on the firing rate of individual spontaneously active VTA DA neurons during withdrawal from chronic ethanol treatment.

<table>
<thead>
<tr>
<th>Cumulative Doses of Acute Ethanol Dose (g/kg i.p.)</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5-2.0</th>
<th>2.5-3.5</th>
<th>4.5-5.5</th>
<th>&gt;6.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firing rate (% baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 g/kg</td>
<td>112.5 ± 11.9</td>
<td>120.45 ± 9.4</td>
<td>175.8 ± 12.6</td>
<td>168.2 ± 5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 g/kg</td>
<td>119.8 ± 6.7</td>
<td>117.85 ± 3.6</td>
<td>125.2 ± 12.3</td>
<td>139.6 ± 43.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Cells ceased firing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 g/kg</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>40</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>10 g/kg***</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>36</td>
<td>36</td>
<td>14</td>
</tr>
</tbody>
</table>

***p < 0.001, χ² test.
substance abuse, recent evidence has shown such treatment can decrease cocaine use when close monitoring is provided (Levin et al., 1998).

Our results confirm a reduction in the number of spontaneously active VTA DA neurons during ethanol withdrawal is not likely to be caused by using general anesthetics. This discrepancy between our results and those of Diana et al. (1992, 1993, 1995) does not seem to be caused by the difference in rat strains. There are other methodological issues that may cause the discrepancy. For example, the paralyzing agent d-tubocurarine used by Diana et al. (1992, 1993, 1995) has been suggested to exert central effects and influence the outcome of DA neuron activity after long-term drug treatment (Grace et al., 1997). On the other hand, N2O used in the present study could also have central effects similar to ethanol (Kaiyala et al., 2003), although N2O was not used in the anesthetized preparation while similar results were obtained. Furthermore, pattern of chronic ethanol exposure may dictate the nature of the neural adaptation. To simulate binge-drinking pattern in humans, animals in our studies received ethanol for longer periods of time (3–8 weeks) and underwent repeated daily ethanol withdrawal. This intermittent treatment paradigm has been shown to generate strong ethanol withdrawal symptoms (Riihioja et al., 1999). A continuous intoxication paradigm with shorter exposure time (5–7 days) was adopted by Diana et al. (1992, 1993, 1995). In addition, it seems that a different population of VTA DA neurons was sampled by Diana et al. (1992, 1993, 1995). They focused on a more posterior population of VTA DA neurons than that by us and other investigators. Also, they sampled only VTA DA neurons identified by antidromic spikes elicited by stimulating Acb. This procedure only identifies 30 to 60% of the spontaneously active VTA DA neurons during recording (Wang, 1981). Stimulation in the Acb is known to inhibit the firing rate of VTA DA neurons, presumably by increasing DA release in the cell body area, which leads to hyperpolarization by somatodendritic autoreceptors activation (Wang, 1981). Therefore, it is possible that hyperpolarization generated by Acb stimulation can restore the number of spontaneously active DA neurons by the removal of excessive depolarization (Chiodo, 1988; Grace et al., 1997) during ethanol withdrawal and results in no difference in the number of spontaneously active VTA DA neurons similar to that observed by Diana et al. (1992, 1993, 1995). This notion is supported by our recent observation that stimulation of the Acb to elicit antidromic spikes in VTA DA neurons indeed reversed the reduction in the number of spontaneously active VTA DA neurons in adult animals with prenatal ethanol exposure (unpublished observation).

The goal of the third experiment was to investigate whether the reduction in the number of spontaneously active VTA reflects an adaptational change due to ethanol withdrawal. This possibility was confirmed by demonstrating a reversal in the number of spontaneously active VTA DA neurons after acute ethanol administration. Because the acute ethanol-induced recovery in Acb DA level during ethanol withdrawal has also been observed in microdialysis studies (Diana et al., 1993; Weis et al., 1996), it is likely this effect is contributed by the reversal of VTA DA neuron activity. Contrary to the reversal effect observed in withdrawing animals, 3 g/kg acute ethanol treatment reduced the number of spontaneously active VTA DA neu-
ber of spontaneously active VTA DA neurons during ethanol withdrawal.

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