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Running wheel activity protects against increased seizure susceptibility in ethanol withdrawn male rats

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ABSTRACT

Ethanol withdrawal is a dysphoric condition that arises from termination of ethanol intake by dependent individuals. Common withdrawal symptoms include anxiety, increased reactivity to stimuli and increased seizure susceptibility as well as the risk of increased seizure severity. We use an animal model of dependence and withdrawal to study withdrawal behaviors and potential underlying neurobiological mechanisms. For a number of years, we have quantified pentylenetetrazol seizure thresholds as an assessment of ethanol withdrawal at both one day and three days of withdrawal. Typically, we see a significant decrease in seizure threshold (increased sensitivity to seizure induction) that persists through three days of withdrawal for male rats. Increasing evidence indicates that voluntary exercise affords protection against various challenges to physical and psychological health, including ethanol-related challenges. Therefore, the current study investigated the effect of voluntary wheel running on seizure susceptibility following chronic ethanol administration and withdrawal. We found that voluntary wheel running attenuated the increased sensitivity to pentylenetetrazol-induced seizures observed with ethanol withdrawal, at both the one-day and three-day time points. This result was especially interesting as animals with access to the running wheels consumed more of the ethanol-containing diet. These findings showed that chronic voluntary wheel running reduces the severity of ethanol withdrawal in our animal model and suggest that exercise-based interventions may have some utility in the clinical management of heavy drinking and alcohol withdrawal.

1. Introduction

Alcohol (ethanol) withdrawal arises when an ethanol-dependent individual stops consumption. Ethanol withdrawal (EW) symptoms reflect a rebound central nervous system hyperexcitability resulting from removal of this central nervous system depressant, and include anxiety, agitation, insomnia, general dysphoria and tremors that may progress to seizures (Ballenger and Post, 1978; Goldstein and Pal, 1971). Preclinical studies with laboratory rats and mice have shown them to be useful models for studying ethanol dependence and withdrawal, as EW animals display a significant increase in seizure susceptibility and severity during early EW (Becker et al., 1997; Devaud et al., 1995a; Devaud and Morrow, 1999; Finn et al., 1995; Finn and Crabbe, 1999; Veatch et al., 2007). Seizure susceptibility measurements are used in these animal models as a quantifiable reflection of the rebound hyperexcitability that is unmasked during EW.

The CNS hyperexcitability of EW is believed to arise from neuroadaptations engendered by persistent ethanol intake. A number of brain adaptations in key neurotransmitter systems and cellular modulators occur (see Moonat et al., 2010; Olsen et al., 2007; Spanagal, 2009; Vengeliene et al., 2008 for review). Ethanol acts as a CNS depressant, largely by enhancing GABAergic transmission and inhibiting glutamatergic activity. Therefore, the homeostatic drive to limit the effects of persistent ethanol exposure results in a reduced responsiveness of $GABA_A$ receptors and increased responsiveness of glutamatergic systems, particularly NMDA receptors. These chronic ethanol-induced adaptations are believed to involve alterations in subunit composition of both of these receptor types (Alele and Devaud, 2005; Cagetti et al., 2003; Devaud et al., 1995b; 1998; Devaud and Morrow, 1999; Devaud and Alele, 2004; Mhatre and Ticku, 1994; Mehta and Ticku, 2005). While these adaptations are believed to contribute to several manifestations of withdrawal, such as the increased anxiety and seizure susceptibility, it is likely that the stress of withdrawal itself also exacerbates seizure sensitivity (Friedman et al., 2011).

Increasing evidence indicates that exercise exerts protective effects against a variety of challenges to physical and psychological health. In rats and mice, these effects can be effectively modeled by housing animals with free access to running wheels. Studies have

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shown that adaptation to running wheels promotes neuronal health by enhancing synaptic plasticity and neurogenesis, even in adult animals (Cotman and Berchold, 2002; Stranahan et al., 2007; van Praag et al., 1999a, 1999b). Voluntary wheel running improves the ability to manage stress exposure by reducing the HPA response and increasing production of brain growth factors, such as BDNF (Nyhius et al., 2010). These effects appear to account for the anxiolytic and antidepressant effects of running-wheel activity (Duman et al., 2008; Salam et al., 2009). Chronic voluntary wheel running has also been shown to reduce the intoxicating effects of acute ethanol administration in a mouse model (Mollenauer et al., 1991, 1992) while antagonizing both the antiproliferative (Crews et al., 2004) and neurotoxic effects of repeated binge-like ethanol administration in rats (Leasure and Nixon, 2010). Further, chronic intermittent ethanol exposure reduced running, especially during the active (night) phase (Logan et al., 2010), a finding that suggests ethanol dependence and withdrawal may reduce the beneficial effects of voluntary wheel running. Pentylenetetrazol (PTZ) is a chemoconvulsant and has been used in numerous investigations to assess seizure susceptibility in animal models. We have published a number of reports studying drug effects on PTZ seizure thresholds during EW and have now extended this approach to determine whether free access to running wheels modulates the increased sensitivity to pentylenetetrazole-induced seizures seen during EW.

2. Methods

2.1. Animals

Male CR rats (Charles Rivers Lab) were approximately 42 days old at the start of experimental procedures.

2.2. Materials

Pentylenetetrazol (PTZ) from Sigma-Aldrich (St. Louis, MO) was dissolved in normal saline at a concentration of 5 mg/ml.

2.3. Activity wheel procedure

Animals were individually housed and randomly assigned to one of three running wheel conditions: (1) standard rat cages without wheels (No Wheel), (2) standard rat cages with wheels that are locked (Locked Wheel) or (3) standard rat cages with functioning running wheels (Free Wheel). The locked wheel condition was included to separate the possible effects of environmental complexity from exercise. The wheel condition was constant for 24 h each day throughout the course of the experiment. Running wheel activity was recorded by use of an external electronic LCD counter that was attached to the side of each free running wheel cage. Activity was recorded twice daily at 7:00 a.m. (start of rest phase) and at 5:00 p.m. (start of active phase). We chose these two times as lights on (7:00 a.m.) and approaching lights off (7:00 p.m.). Counters were manually reset after obtaining counts. All animals were housed under their respective conditions for 10 days prior to introduction of the liquid diets to allow for acclimation and adaptation to running wheels in Free Wheel animals.

2.4. Liquid diet procedure

Animals were made ethanol-dependent by administration of 6% ethanol, v/v, in a nutritionally complete liquid diet, which was slightly modified from the Frye liquid diet (Frye et al., 1983). Diet components were purchased individually with diet made at least twice per week and fresh diet was provided daily (MP Biomedical, Costa Mesa, CA) and administered for 14 days as previously described (Devaud and Morrow, 1994; Devaud et al., 1995a). Control animals were pair-fed the same liquid diet but with dextrose substituted isocalorically for the ethanol to ensure equivalent caloric intake and comparable nutritional status. The amount of liquid diet consumed was recorded daily.

After 14 days of liquid diet administration, the liquid diet was removed and regular lab chow provided ad libitum to all animals to maintain equivalent diet conditions. Seizure threshold testing was scheduled at 1 day or 3 days EW. All procedures were conducted in accordance with approved University of Maine Animal Welfare Protocols and NIH guidelines for the humane care and use of animals in an AAALAC-accredited facility.

2.5. PTZ Seizure threshold procedure

Constant tail vein infusion of the chemoconvulsant was used for the induction of seizures. A 25 g butterfly needle was inserted into a lateral tail vein while the animals were gently restrained and needle taped into place. The animal was then allowed to move freely while the observer gently held the tip of its tail. PTZ was infused at 1.6 ml/min and the time to the first myoclonic twitch of the face and/or neck indicated the endpoint of infusion (Alele and Devaud, 2007). Seizure thresholds were calculated from the time of infusion (minutes) times the dose (5 mg/ml × 1.6 ml/min) of PTZ infused per body weight of the animal and are presented as mg PTZ per kilogram body weight.

2.6. Data analysis

Data were analyzed using one-way and two-way ANOVA, and post-hoc pairwise comparisons were performed using the least-significant difference (LSD) procedure to control type-1 error rate (SPSS, Chicago IL, USA).

3. Results

3.1. Body weights and ethanol consumption

Animals in all groups showed substantial weight gain over the course of the experiment from initial weights (day 1) until final weight determinations (day 25 or 27) (Table 1). Two-factor ANOVA conducted on body weights revealed a significant main effect of housing conditions (No Wheel, Locked Wheel, Free Wheel) [$F_{(2,72)} = 16.64$, $P < 0.001$] but there was no effect of ethanol treatment group (1 day EW, 3 day EW, control) nor a housing by treatment interaction. Post-hoc pairwise comparisons indicated that Free Wheel animals weighed less than both No Wheel and Locked Wheel animals, which did not differ from each other.

Despite the fact that Free Wheel animals gained less weight relative to the other two housing conditions, Table 2 shows that Free Wheel animals actually consumed nearly 10% more of the ethanol-containing liquid diet than either No Wheel or Locked Wheel animals. One-way ANOVA showed a significant effect of housing conditions on

Table 1
Starting and ending body weights for rats across diet and wheel conditions.

	Initial body weight (g)	Final body weight (g)	% Increase in body weight
Control			
No Wheel	169.2 ± 8.0	377.4 ± 13.4	123%
Locked Wheel	176.7 ± 7.0	381.0 ± 13.6	116%
Free Wheel	163.7 ± 11.6	307.6 ± 17.5*	88%
Ethanol fed			
No Wheel	165.6 ± 7.9	379.6 ± 12.6	129%
Locked Wheel	171.4 ± 9.1	366.1 ± 12.3	114%
Free Wheel	164.4 ± 6.1	335.8 ± 20.2*	104%

* $P < 0.001$ compared to both No Wheel and Locked Wheel conditions.