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Environmental novelty and illumination modify ethanol-induced open-field behavioral effects in mice ☆

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ABSTRACT

Both spontaneous and drug-induced animal behaviors can be modified by exposure to novel stimuli or different levels of environmental illumination. However, research into how these factors specifically impact ethanol (ETH)-induced behavioral effects is currently lacking. We aimed to investigate the effects of these two factors, considered separately or in conjunction, on ETH-induced acute hyperlocomotor effect and its sensitization in adult male Swiss mice. Mice were placed in a novel or familiar open-field under normal light (200 lx) or low light (9 lx) immediately after receiving an ip injection of either 1.8 g/kg ETH or saline (SAL). After 7 days, all animals received an ip challenge injection of 1.8 g/kg ETH, and were placed in the open-field under the same light conditions described above. Novelty increased central locomotion and decreased grooming, while low light increased grooming. Acute ETH administration increased both total and peripheral locomotion and these effects were potentiated by low light. Both low light and novelty were able to facilitate ETH-induced locomotor sensitization, which was detected by the central locomotion parameter. However, there was no synergism between the effects of these two modulating factors on ETH-induced behavioral sensitization. We conclude that both the acute behavioral effects of ETH and behavioral sensitization induced by previous administration of this drug can be critically modified by environmental factors. In addition, our study stresses the importance of using different behavioral parameters to evaluate the interaction between environmental factors and ETH effects.

1. Introduction

Ethanol (ETH) is among the drugs most frequently abused by humans. In rodents it stimulates locomotion mainly via the activation of the mesolimbic dopamine pathway (Di Chiara and Imperato, 1988; Koob, 1992; Phillips et al., 1997). As reviewed by Phillips et al. (1997), it is thought that animal models of drug-induced locomotor stimulation would model human drug-induced euphoria and thus the study of such hyperlocomotion would be important to elucidate the mechanisms involved in such human drug effects.

Repeated treatment with ETH can produce behavioral sensitization in rodents (Araujo et al., 2005, 2006b, 2009; Camarini et al., 2000a,b; Pastor and Aragon, 2006; Phillips et al., 1997), characterized by a progressive increase in the drug-elicited behavioral responses. This phenomenon has been considered an important pharmacological tool with which to study plasticity in the mesolimbic dopamine reward circuitry that may underlie drug craving and drug-seeking behavior in humans (Robinson and Becker, 1986; Robinson and Berridge, 1993, 2000, 2001) and is usually measured in terms of changes in locomotion or stereotyped behavior in rodents (Robinson and Becker, 1986).

Several different neural systems have been demonstrated to be involved in ETH-induced behavioral sensitization. Examples are the dopamine (Araujo et al., 2009; Broadbent et al., 2005; Harrison and Nobrega, 2009; Nestby et al., 1997), GABA (Broadbent and Harless, 1999), opioid (Camarini et al., 2000a; Pastor and Aragon, 2006), glutamate (Broadbent et al., 2003; Camarini et al., 2000b; Kotlinska et al., 2006), adenosine (Houchi et al., 2008) and taurine (Ginsburg and Lamb, 2008) systems. Enzymes of metabolism of ETH in the brain (Correa et al., 2004, 2009), protein kinases (Fee et al., 2006) and homer proteins (Szumlinski et al., 2005) have also been studied in the context of ETH sensitization.

Much evidence indicates that the effects of ETH on dopamine neurotransmission seem to be of great relevance to its locomotor stimulant effect as well as its reinforcing properties (see Nestler and Self, 1997), but some authors have reported that these effects of ETH may not depend on dopamine (Broadbent et al., 1995; Lanteri et al., 2008; Zapata et al., 2006).

Although behavioral sensitization is usually observed after repeated treatment with drugs of abuse, it has been shown that it is not necessary to repeatedly administer a drug for long periods of time to produce such

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a phenomenon. Indeed, a single injection of amphetamine, for instance, has been reported to enhance both stereotypy (Browne and Segal, 1977; Chinen et al., 2006; Ellison and Morris, 1981) and locomotor stimulation (Costa et al., 2001; Vanderschuren et al., 1999) produced by a subsequent injection of amphetamine given hours or weeks later.

It has been extensively demonstrated that environmental manipulations that modify dopamine transmission in the reward mesotelencephalic systems can affect the phenomenon of behavioral sensitization. One important and widely applied manipulation that is known to activate brain dopamine transmission is the exposure of an animal to a novel stimulus, for instance, to a novel environment or novel objects (Hooks and Kalivas, 1995; Legault and Wise, 2001; Rebec et al., 1997a, b). Within this context, Badiani et al. (1995a, b, c, 1997) demonstrated in a series of studies that repeated treatment of rats with amphetamine in a relatively novel environment potentiated the development of sensitization to its behavioral effects (when observing rotational behavior in 6-OHDA-lesioned rats and hyperlocomotion). However, Carey et al. (2005), comparing open-field habituated and non-habituated rats with respect to their responses to repeated injections of cocaine, verified that habituated rats developed a sensitization-like increase in locomotor activity, while non-habituated animals exhibited a tolerance-like decrease in locomotor activity. We have demonstrated previously that the administration of amphetamine in a completely novel environment can potentiate both the development and the expression of amphetamine-induced behavioral sensitization in mice (Alvarez et al., 2006; Fukushiro et al., submitted). However, to our knowledge there are no studies in the literature examining the effects of exposure to a novel environment on behavioral sensitization induced by ETH administration.

From a clinical point of view, novelty has been proposed as a major contributing factor to drug-craving in humans (Kosten et al., 1994; Zuckerman, 1996). In addition, novel stimuli and drugs of abuse seem to activate, at least in part, the same dopaminergic neuronal substrates (Bardo et al., 1996).

In parallel, it has been suggested that the dark phase of the circadian cycle, as well as exposure to sudden darkness, can also evoke an increase in brain dopamine transmission in rodents (Bert et al., 2005; Nasello et al., 1998, 2003; Paulson and Robinson, 1994; Smith et al., 1992). Consequently, it has been demonstrated that the illumination level of an environment can modify both physiological and biological processes as well as drug-induced behaviors in these animals.

Within this context, Hlinák and Rozmarová (1986) described that rats kept under a reversed light regime and tested during their dark phase showed greater behavioral activity under dark experimental conditions than under bright illumination. In addition, exposure to sudden darkness has been reported to increase motor activity and to decrease anxiety of rodents observed either in the open-field or in the elevated plus-maze (Bert et al., 2005; Nasello et al., 1998, 2003). It has also been demonstrated that sudden darkness is able to modulate several apomorphine-induced behavioral effects in rats (Nasello et al., 2003). Likewise, apomorphine-induced yawning is increased in the dark phase of the circadian cycle of rats (Nasello et al., 1995). It is important to point out that, since rodents are nocturnal animals, most of their behaviors are increased in dark surroundings. Therefore, exposure of these animals to darkness would be the equivalent to exposure of humans to light.

In light of the fact that novel stimuli, environmental illumination and ETH administration share the ability to affect dopaminergic transmission in the brain, the aim of the present study was to investigate whether novelty exposure and different illumination conditions, presented separately or in conjunction, would modify the hyperlocomotor effect of ETH and the sensitization to it in mice. Since it has been demonstrated that other behavioral parameters may be of great value to evaluate the effects of environmental modifications on the response to ETH (Araujo et al. 2005, 2006a) and other drugs of abuse (Alvarez et al., 2006), grooming behavior was also evaluated in the present study. In this regard, grooming behavior of rodents has been shown to be affected by stimulation of dopamine D_1 receptors (Beninger et al., 1991) and stress (Moody et al., 1988) and consists in an important feature of a range of neuropsychiatric diseases (Crawley, 2007; Graybiel and Saka, 2002).

2. Methods

2.1. Subjects

Three-month-old Swiss EPM-M1 male mice from our own colony were housed under conditions of controlled temperature (22–23 °C) and lighting (12/12 h light/dark, lights on at 06:45 h) in polypropylene cages (32 cm × 42 cm × 18 cm). Food and water were available ad libitum throughout the experiment.

The experimental protocol was approved by the Committee for the use of animal subjects from our Institution (UNIFESP). The animals used in this study were maintained in accordance with the guidelines of the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). All efforts were taken to minimize pain and discomfort of the animals.

2.2. Drugs

ETH (MERCK®) was freshly diluted in saline solution (1.8 g/kg, 23% v/v, 10 ml/kg). Saline (SAL) was used as the control solution. The solutions were given intraperitoneally at a volume of 10 ml/kg body weight.

2.3. Open-field test

Immediately after SAL or ETH injection, animals were individually placed in the center of the open-field arena for direct quantification of locomotor activity and grooming behavior for 10 min, with registration of each behavior every 5 min. The open-field apparatus used in the present study was a circular wooden box (40 cm in diameter and 50 cm high) with an open top and floor divided into 19 squares. Hand-operated counters were used to score the following behavioral parameters: total locomotion frequency (number of any floor units entered), peripheral locomotion frequency (number of entrances into the floor units close to the walls of the apparatus), central locomotion frequency (number of entrances into any floor unit not close to the walls of the apparatus) and grooming duration (total seconds of mouth or paws on the body and on the head).

Quantification of locomotion frequency in the open-field apparatus has been demonstrated to be a very effective method to evaluate behavioral sensitization induced by ETH (Araujo et al., 2005; Bellot et al., 1996). In addition, open-field locomotion of rodents has been extensively proven to be a very sensitive behavioral parameter with which to evaluate the effects of drugs acting on dopaminergic systems (Frussa-Filho and Palermo-Neto, 1990, 1991; Frussa-Filho et al., 1996; Fukushiro et al., 2007, 2008).

Due to the short-lasting stimulant effect of ETH on mouse locomotor activity, the quantification of this behavioral parameter for even less than 10 min has been shown to be effective and sufficient to demonstrate ETH-induced hyperlocomotion and its sensitization under the conditions used here (Araujo et al., 2005, 2006a, b).

3. Experimental procedure

Forty-seven mice received an intraperitoneal (ip) injection of SAL or 1.8 g/kg ETH and were immediately placed in a novel (NOV) open-field under normal light (200 lx — NL) or low light (dimmer, 9 lx — LL) for 10 min for activity quantification (session 1). These animals were divided into 4 groups: NOV–NL–SAL ($N = 12$), NOV–LL–SAL ($N = 11$), NOV–NL–ETH ($N = 12$) and NOV–LL–ETH ($N = 12$).

After 7 days, all mice received an ip challenge injection of 1.8 g/kg ETH (/ETH) in order to assess behavioral sensitization. Immediately after the injection, they were placed in the open-field for 10 min for activity quantification in the same light conditions described above (session 2). Therefore, NL animals were tested under normal light in both sessions and LL animals were tested under low light in both sessions.

Other 47 mice were previously habituated (HAB) to the open-field (20-min sessions) under normal light (200 lx — NL) or low light (dimmer, 9 lx — LL) over the course of 3 consecutive days. These animals were also allocated to 4 groups: HAB–NL–SAL ($N=11$), HAB–LL–SAL ($N=12$), HAB–NL–ETH ($N=12$) and HAB–LL–ETH ($N=12$) and the same protocol of treatment and exposure to the open-field described for the NOV groups were then followed.

The design of the experiment is illustrated in Table 1.

The ETH dose was chosen on the basis of previous studies of our research group, which succeeded in characterizing its locomotor stimulant effects and behavioral sensitization in mice (Araujo et al., 2005, 2006b, 2009; Bellot et al., 1996).

4. Statistical analysis

Data from session 1 and session 2 regarding the entire session (10 min) were analyzed by a 2×2×2 (novelty×light intensity×drug factors) three-way ANOVA. Multiple comparisons were performed using the Duncan post hoc test when necessary. Data regarding different time intervals (0–5 and 5–10 min) of each session were analyzed by one-way ANOVA with repeated measures (time interval×groups). When a significant time×groups interaction was found, an additional 2×2×2 (novelty×light intensity×drug factors) three-way ANOVA with repeated measures was performed. A p value less than 0.05 was considered as a statistically significant difference.

5. Results

Panels A, B, C and D of Fig. 1 show, respectively, the total, peripheral and central locomotion frequencies and grooming duration of mice after SAL or ETH acute administration during session 1. For both total (Panel A) and peripheral (Panel B) locomotion, three-way ANOVA revealed significant effects of light intensity (normal×low) [$F(1,86)=6.62$, p 0.01 for total locomotion and $F(1,86)=9.29$, p 0.00 for peripheral locomotion] and drug (SAL×ETH) [$F(1,86)=59.81$, p 0.00 for total locomotion and $F(1,86)=74.21$, p 0.00 for peripheral locomotion] factors, as well as a significant interaction between light intensity and drug administration [$F(1,86)=4.74$, p 0.03 for total locomotion and $F(1,86)=6.19$, p 0.02 for peripheral locomotion]. The Duncan test showed that all animals acutely treated with ETH (HAB–NL–ETH, HAB–LL–ETH, NOV–NL–ETH and NOV–LL–ETH) presented significantly higher total and peripheral locomotion frequencies than their respective SAL-treated control groups, confirming the stimulatory effect of ETH on locomotor activity. Importantly, the peripheral locomotion frequencies of the HAB–LL–ETH and the NOV–LL–ETH groups were significantly higher than those exhibited by the HAB–NL–ETH and the NOV–NL–ETH groups, respectively, indicating that conditions of low-light potentiated the hyperlocomotor effect induced by ETH. This potentiation was also detected in total locomotion behavior, although the increased frequency of the NOV–LL–ETH group when compared to the NOV–NL–ETH group just missed statistical significance.

As for central locomotion (Panel C), three-way ANOVA revealed that only the factor of novelty (habituation×novelty) was associated with any significant effects [$F(1,86)=10.60$, p 0.00]. These data thus indicate an enhancement in this behavioral parameter induced by novelty exposure.

Concerning the grooming behavior of mice during session 1 (Panel D), three-way ANOVA revealed significant effects due to the factors of novelty (habituation×novelty) [$F(1,86)=8.82$, p 0.00] and light intensity (normal×low) [$F(1,86)=5.34$, p 0.02], indicating a decrease in grooming duration during novelty exposure and an increase in this behavioral parameter during exposure to the low-light condition.

Panels A, B, C and D of Fig. 2 show respectively the total, peripheral and central locomotion frequencies and grooming duration of mice after ETH challenge injection (session 2), administered 7 days after the first injection of SAL or ETH. For total and peripheral locomotion (Panels A and B, respectively), three-way ANOVA revealed only significant effects of light intensity factor (normal×low) [$F(1,86)=$ 24,87, p 0.00 for total locomotion and $F(1,86)=24.88$, p 0.00 for peripheral locomotion]. These data seem to replicate the potentiating effect of the low-light condition on ETH-induced hyperlocomotion observed during session 1 and indicate that behavioral sensitization to ETH did not develop for these parameters.

Regarding central locomotion (Panel C), three-way ANOVA detected significant effects of novelty (habituation×novelty in session 1) [$F(1,86)=4.11$, p 0.04] and drug (ETH×SAL in session 1) [$F(1,86)=15.89$, p 0.00] factors as well as significant novelty×drug [$F(1,86)=4.01$, p 0.04] and novelty×light intensity×drug [$F(1,86)=$ 7.68, p 0.01] interactions. The Duncan test showed that the ETH pre-treated animals submitted to novelty and/or low light presented a significant increase in central locomotion frequency in response to ETH challenge when compared to all of the other groups, suggesting the development of behavioral sensitization in these groups (HAB–LL–ETH/ETH, NOV–NL–ETH/ETH and NOV–LL–ETH/ETH). Conversely, central locomotion presented by the ETH pre-treated mice submitted to normal light and previous environmental habituation (HAB–NL–ETH/ETH group) did not differ from that exhibited by the SAL pre-treated mice, indicating that these animals did not develop behavioral sensitization.

Panel D of Fig. 2 shows grooming behavior of mice in session 2. Three-way ANOVA revealed no significant effects due to any of the factors that were analyzed, individually or in conjunction, on this behavior.

Figs. 3 and 4 show the effects of ETH, novelty and illumination on total, peripheral and central locomotion as well as on grooming behavior within-session habituation (which was evaluated by the comparison between data obtained in the first vs. in the last 5 min of the 10-min session).

Panels A, B, C and D of Fig. 3 show respectively the total, peripheral and central locomotion frequencies and grooming duration of mice during the 5-min intervals of session 1. With respect to total (Panel A) and peripheral (Panel B) locomotion as well as grooming behavior (Panel D), one-way ANOVA with repeated measures revealed only significant effects of the time interval [$F(1,86)=67.1$, 43.4, 20.8, p 0.00 for total locomotion, peripheral locomotion and grooming behavior,

Table 1
Experimental design.

Groups	3-day habituation in the OF	Session 1			Session 2	
		Light intensity	Priming injection		Light intensity	Challenge injection
HAB–NL–SAL	NL	NL	SAL		NL	
HAB–LL–SAL	LL	LL	SAL		LL	
HAB–NL–ETH	NL	NL	ETH		NL	
HAB–LL–ETH	LL	LL	ETH		LL	
				7 days		ETH
NOV–NL–SAL	–	NL	SAL		NL	
NOV–LL–SAL	–	LL	SAL		LL	
NOV–NL–ETH	–	NL	ETH		NL	
NOV–LL–ETH	–	LL	ETH		LL	
		↓ OFQ			↓ OFQ	

HAB = previous habituation to the open-field; NOV = first exposure to the open-field; NL = normal-light condition; LL = low-light condition; SAL = saline i.p. injection; ETH = 1.8 g/kg ethanol i.p. injection; OFQ = open-field quantification for 10 min.

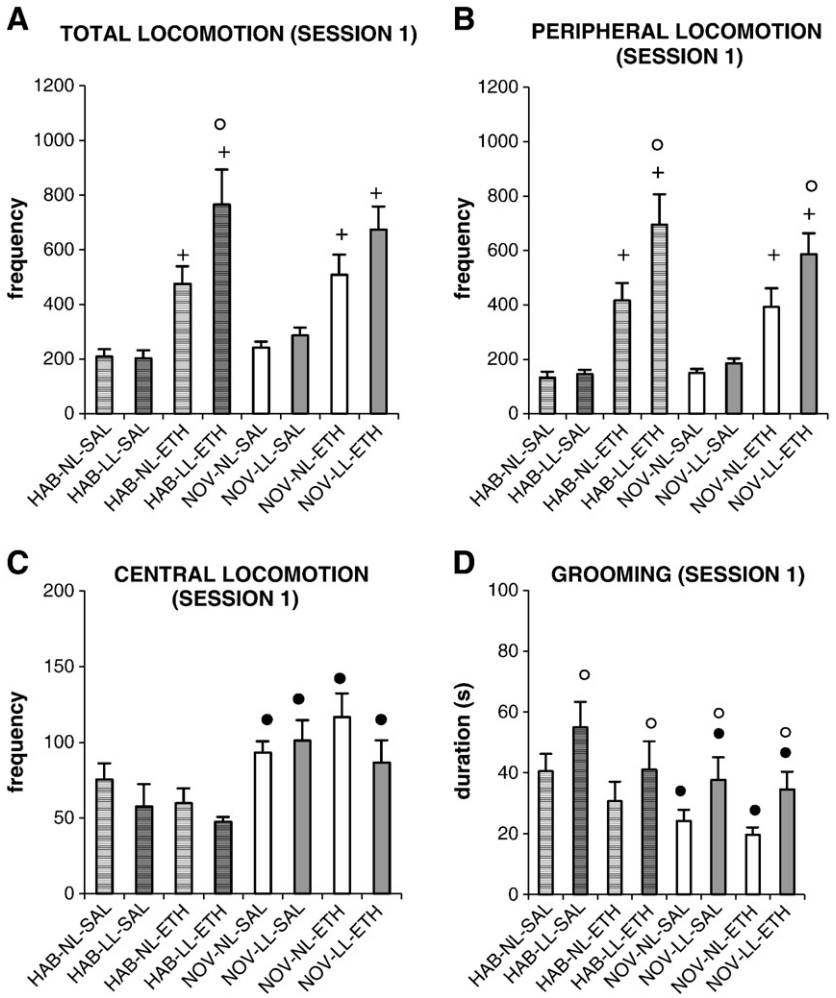

Fig. 1. Total locomotion frequency (A), peripheral locomotion frequency (B), central locomotion frequency (C) and grooming duration (D) of mice which received either an injection of SAL (SAL) or 1.8 g/kg ethanol (ETH) and were immediately exposed to a novel (NOV) or a familiar (HAB) open-field under normal-light (NL) or low-light (LL) conditions for 10 min (session 1). Data represent means ± E.P. Three-way ANOVA followed by Duncan test when necessary. + $p<0.05$ compared to the group submitted to the same novelty and illumination conditions, but acutely treated with SAL (SAL). ○ $p<0.05$ compared to the group submitted to the same novelty condition and pharmacological treatment, but exposed to a normal-light (NL) open-field. • $p<0.05$ compared to the group submitted to the same illumination condition and pharmacological treatment, but exposed to a familiar (HAB) open-field.

respectively] and the groups [$F(7,86)=10.5$, 2.9, 13.2, p 0.00, 0.00 and 0.01 for total locomotion, peripheral locomotion and grooming behavior, respectively]. Concerning central locomotion (Panel C), one-way ANOVA with repeated measures revealed significant effects due to the time interval [$F(1,86)=18.5$, p 0.00] and the groups [$F(7,86)=4.1$, p 0.00] as well as a significant time interval × groups interaction [$F(7,86)=4.0$, p 0.00]. The additional three-way ANOVA with repeated measures showed significant effects of the time interval [$F(1,86)=18.5$, p 0.00] and of the factor of novelty (habituation × novelty) [$F(1,86)=21.9$, p 0.00] as well as a significant time interval × drug (SAL × ETH) interaction [$F(1,86)=24.5$, p 0.00].

Panels A, B, C and D of Fig. 4 show respectively the total, peripheral and central locomotion frequencies and grooming duration of mice during the 5-min intervals of session 2. For total and peripheral locomotion (Panels A and B, respectively), one-way ANOVA with repeated measures revealed only significant effects of the time interval [$F(1,86)=7.0$, 6.4, p 0.01, for total locomotion and peripheral locomotion, respectively] and the groups [$F(7,86)=3.7$, 3.8, p 0.00, for total locomotion and peripheral locomotion, respectively]. Concerning central locomotion (Panel C), one-way ANOVA with repeated measures revealed a significant effect of the groups [$F(7,86)=5.3$, p 0.00] and a significant time interval × groups interaction [$F(7,86)=2.3$, p 0.03]. The additional three-way ANOVA with repeated measures showed significant effects of the factors of novelty (habituation x novelty in session 1) [$F(1,86)=4.1$, p 0.04] and drug (ETH × SAL in session 1) [$F(1,86)=15.9$, p 0.00] as well as significant time interval × novelty [$F(1,86)=6.8$, p 0.01], novelty × drug [$F(1,86)=4.0$, p 0.04] and novelty × light intensity × drug [$F(1,86)=7.7$, p 0.01] interactions. For grooming behavior, one-way ANOVA with repeated measures revealed only a significant time interval × groups interaction [$F(7,86)=2.4$, p 0.03]. The additional three-way ANOVA with repeated measures revealed only a significant interaction between time interval and the factor of novelty [$F(1,86)=10.6$, p 0.00].

6. Discussion

In the present study, we demonstrated that: 1) novelty exposure increased spontaneous central locomotion and decreased spontaneous grooming behavior, whereas sudden exposure to low light increased spontaneous grooming behavior in mice; 2) sudden exposure to a low level of environmental illumination produced a marked and very reliable potentiation of the hyperlocomotor effect induced by acute administration of ETH and increased its sensitization; 3) novelty exposure only facilitated ETH-induced behavioral sensitization, without modifying the acute behavioral effects of ETH; 4) there was no synergism between the effects of sudden exposure to low light and novelty on ETH-induced behavioral sensitization; 5) compared to total and peripheral locomotion, central locomotion is a more sensitive open-field behavioral parameter to evaluate between-session habituation as

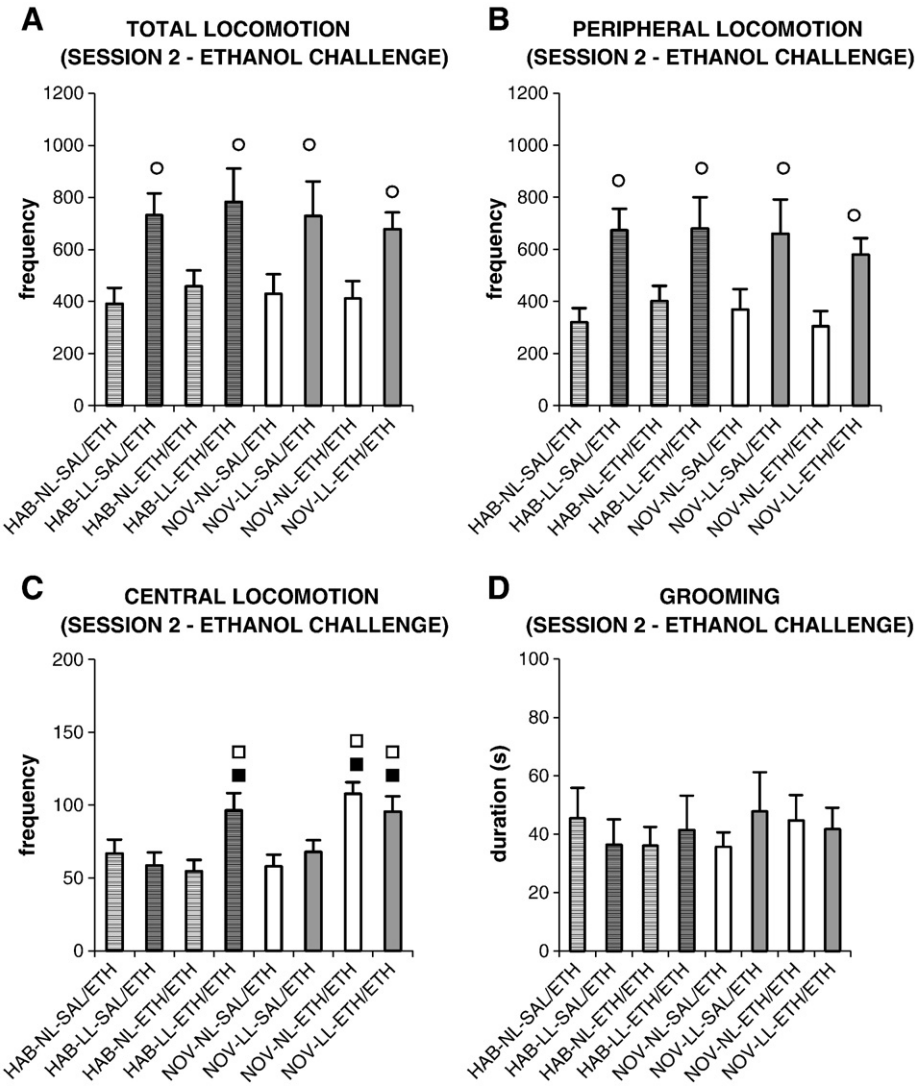

Fig. 2. Total locomotion frequency (A), peripheral locomotion frequency (B), central locomotion frequency (C) and grooming duration (D) of mice challenged with an injection of 1.8 g/kg ethanol (/ETH), administered 7 days after the initial saline (SAL) or 1.8 g/kg ethanol (ETH) injection. Immediately after the ethanol challenge injection, mice were exposed to the open-field for 10 min (session 2) in the same light conditions as described for the first injection. Data represent means ± E.P. Three-way ANOVA followed by Duncan test when necessary. ○ $p<0.05$ compared to the group submitted to the same novelty condition and pharmacological treatment, but exposed to a normal-light (NL) open-field. ■ $p<0.05$ compared to all the groups acutely treated with SAL in session 1. □ $p<0.05$ compared to the HAB–NL–ETH/ETH group.

well as the inhibitory effect of ETH on within-session habituation and 6) compared to total and peripheral locomotion, central locomotion is also a more sensitive open-field behavioral parameter to evaluate behavioral sensitization induced by single ETH injection.

In regard to the effects of sudden dim light in session 1, the exposure of mice to low levels of environmental illumination increased spontaneous grooming behavior without modifying spontaneous locomotion. However, previous studies have demonstrated an increase in the spontaneous motor activity of rodents during the dark period of the day (Hlinák and Rozmarová, 1986) and also during exposure to sudden darkness (Bert et al., 2005; Crawley, 1988; Nasello et al., 1998, 2003). This phenomenon has been linked by these authors to an increase in dopamine transmission (Berger and Lemmer, 1976; Feenstra et al., 2000; Nasello et al., 2003) or to a decrease in anxiety of animals (Bert et al., 2005; Nasello et al., 1998) during exposure to darkness.

The discrepancy found between the present study and the previous ones with respect to these findings may be due to several factors, such as different subjects and apparatus. More importantly, although the design of the present study is similar to the studies with respect to the introduction of sudden darkness (i.e., light intensity was lowered at the same moment animals were placed in the apparatus), we should note that our animals were not observed in the total absence of light, as was the case in the previous studies, but in an apparatus under a low level of illumination. Therefore, it is possible that the release of dopamine induced by low light exposure was not sufficient to increase the spontaneous locomotion of mice in our experimental conditions (in contrast to the dopamine release induced by total darkness in the previous studies). Nevertheless, the fact that low light exposure increased the duration of spontaneous grooming suggests that at least a slight enhancement of dopamine transmission was induced by the dim light, as evidence exists that the activation of dopamine D_1 receptors is partially responsible for regulating grooming behavior (Beninger et al., 1991; Chinen and Frussa-Filho, 1999; Downes and Waddington, 1993; Eilam et al., 1992; Starr and Starr, 1986).

Following this interpretation, this possible slight increase in dopamine transmission induced by the sudden exposure to dim light seemed to be sufficient to potentiate the hyperlocomotor effect of ETH on total and peripheral locomotion. Indeed, we verified that mice acutely treated with ETH and suddenly exposed to the low-light condition (HAB–LL–ETH and NOV–LL–ETH groups) presented higher

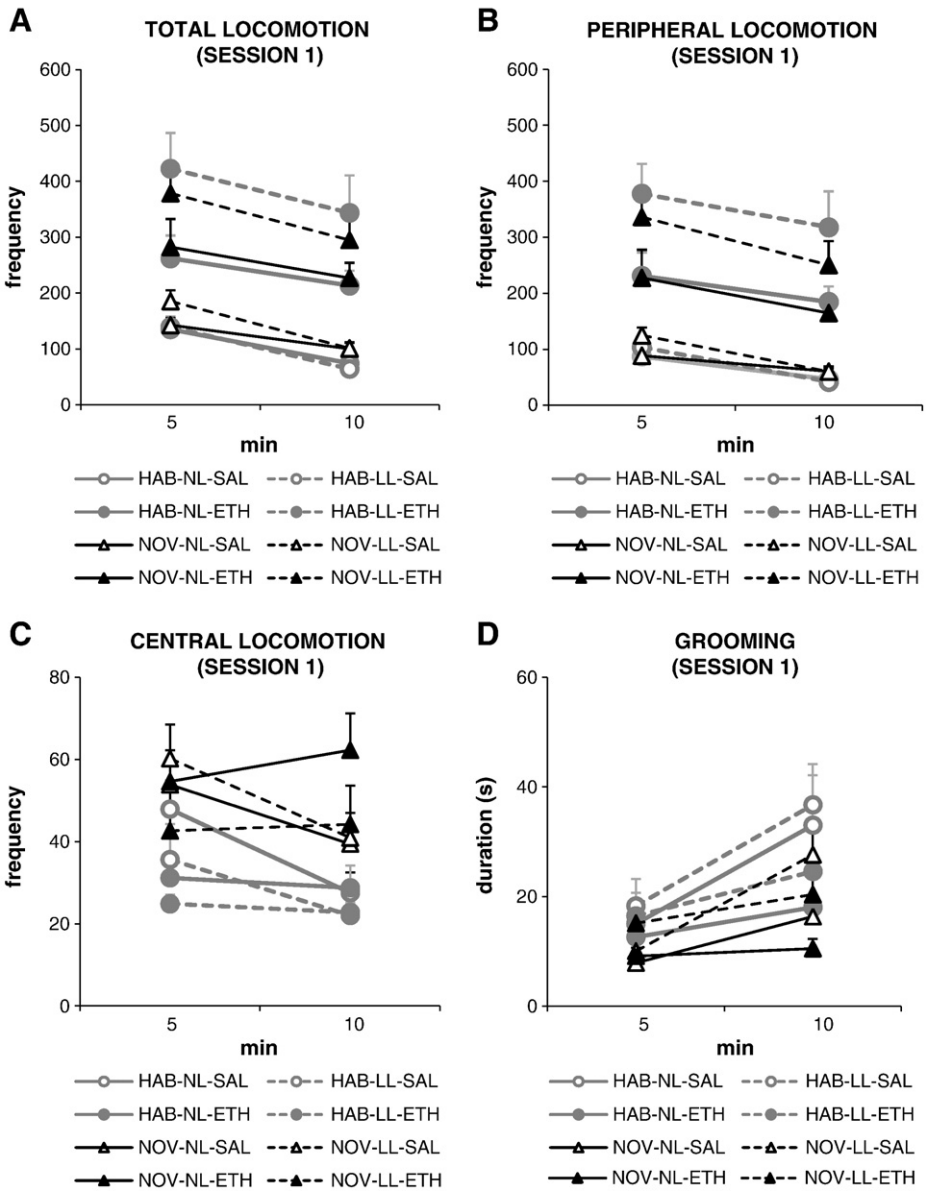

Fig. 3. Total locomotion frequency (A), peripheral locomotion frequency (B), central locomotion frequency (C) and grooming duration (D) during the 5-min intervals (0–5 and 5–10 min) of the 10-min session of mice which received either an injection of SAL (SAL) or 1.8 g/kg ethanol (ETH) and were immediately exposed to a novel (NOV) or a familiar (HAB) open-field under normal-light (NL) or low-light (LL) conditions (session 1). Data represent means ± E.P. One-way and three-way ANOVA with repeated measures.

total and peripheral locomotion frequencies than the animals that also received ETH but were exposed to normal light (HAB–NL–ETH and NOV–NL–ETH groups).

Taking into account that both sudden darkness and ETH are known to increase dopamine release (Di Chiara and Imperato, 1985; Nasello et al., 2003) and that dopamine transmission in the nucleus accumbens is related to locomotion in rodents (Kelly and Iversen, 1976; Kelly et al., 1975), it could be suggested that sudden exposure to a low-light environment and acute ETH administration acted in an additive fashion on dopamine release in the nucleus accumbens, thereby leading to increased locomotor activity.

However, the potentiating effect of dim light on ETH-induced hyperlocomotion could also be explained by a change in the anxiety-like state of the animals. Previously, it has been demonstrated that sudden darkness decreases the anxiety-like state of both mice and rats in the elevated plus-maze test (Bert et al., 2005; Nasello et al., 1998). In parallel, the anxiolytic effects of ETH have been well described in the literature (Aston-Jones et al., 1984; Blanchard et al., 1993; Kameda et al., 2007; Wilson et al., 2004). Thus, it could also be suggested that the anxiolytic effect produced by the sudden exposure to dim light acted in concert with the anxiolytic effect induced by acute ETH administration, thereby inhibiting the well known phenomenon of anxiety-induced hypolocomotion in the open-field apparatus (Broadhurst, 1960).

It has been shown that ETH-induced hyperlocomotion is increased during the dark phase of hamsters (Phillips, 1982). However, the study conducted by Phillips (1982) investigated the effects of ETH administered at different periods of the circadian cycle of rodents. Therefore, animals were tested in their dark phase, which usually requires a reversed light regime and may lead to hormonal changes. Only Nasello et al. (2003) have studied the effects of sudden darkness on the drug-induced behavioral responses of rats. In that study, the authors found that sudden darkness diminished pre-synaptic responses (yawning) to apomorphine and increased lower post-synaptic responses (motor activity) without modifying higher post-synaptic responses (stereotypy). Notwithstanding, the effects of an immediate light change on the animal behaviors elicited by drugs of abuse had not, until now, been investigated. Future studies could

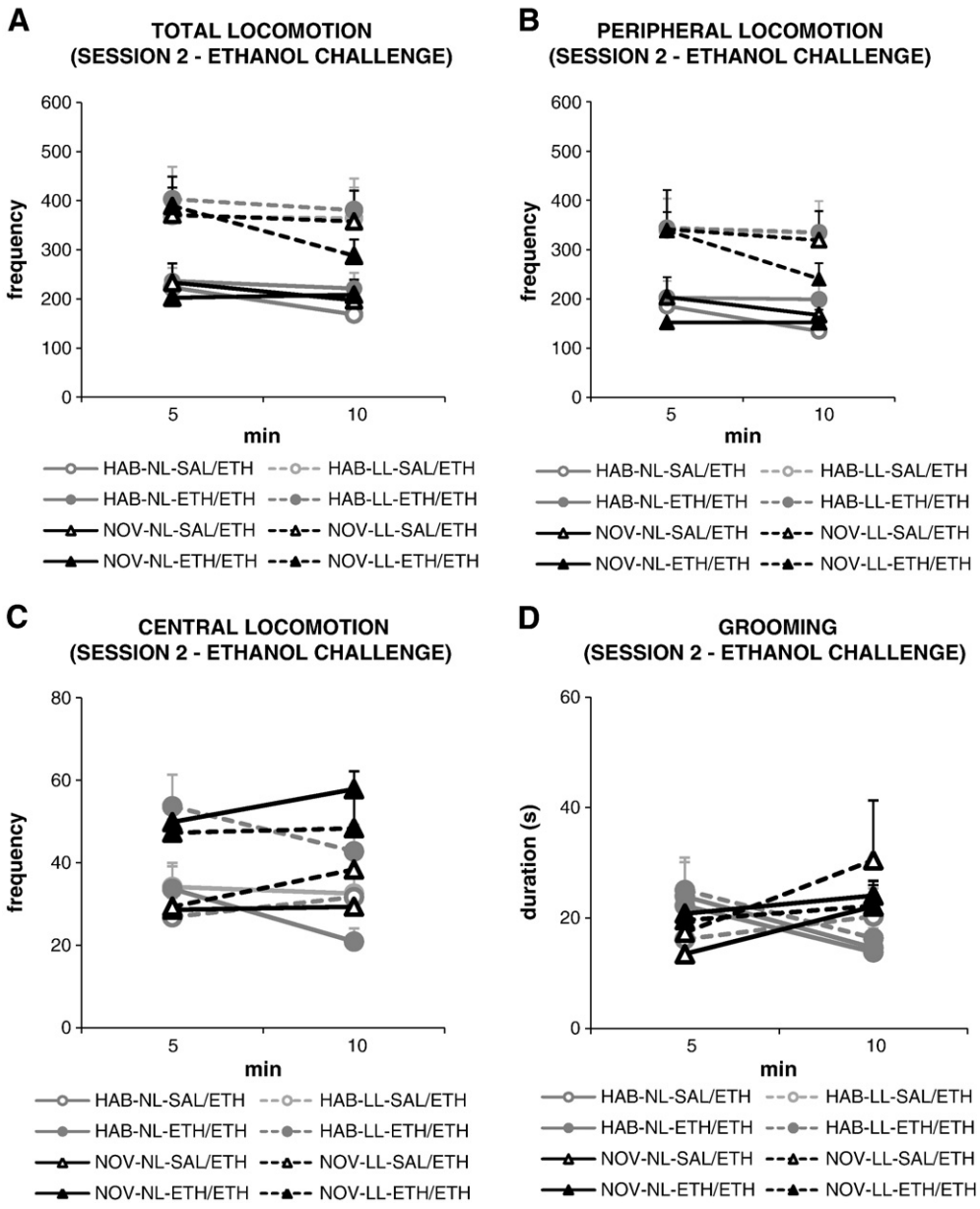

Fig. 4. Total locomotion frequency (A), peripheral locomotion frequency (B), central locomotion frequency (C) and grooming duration (D) during the 5-min intervals (0–5 and 5–10 min) of the 10-min session of mice challenged with an injection of 1.8 g/kg ethanol (/ETH), administered 7 days after the initial saline (SAL) or 1.8 g/kg ethanol (ETH) injection. Immediately after the ethanol challenge injection, mice were exposed to the open-field for 10 min (session 2) in the same light conditions as described for the first injection. Data represent means ± E.P. One-way and three-way ANOVA with repeated measures.

provide information about whether sudden exposure to low levels of environmental illumination would also modify the behavioral effects and sensitization of other drugs of abuse. This would be an interesting working hypothesis since it is well known that most, if not all, drugs of abuse increase dopamine levels in the nucleus accumbens (Di Chiara and Imperato, 1988).

Studying the effects of exposure to a novel environment in session 1, we verified that this condition increased spontaneous central locomotion and decreased the spontaneous grooming behavior of mice. This modulation by novelty on the spontaneous behavior of mice was likely due to an enhancement of dopamine transmission in the brain (Hooks and Kalivas, 1995; Legault and Wise, 2001; Rebec et al., 1997a, b).

Still in session 1, novelty exposure had no effects on hyperlocomotion produced by acute administration of ETH. These data seem to be in line with the findings of Pastor et al. (2005) in the only but elegant paper we found in the literature investigating the effects of novelty exposure on ETH-induced locomotor stimulation. These authors compared the response to a single injection of ETH into male Swiss mice previously habituated to an open-field with mice non-habituated to the apparatus, in an experimental protocol very similar to ours. As a result, they verified that hyperlocomotion induced by acute administration of several doses of ETH was not modified in either group.

These results are different from those found in previous studies for amphetamine (Badiani et al., 1995a, b, c, 1997; Pastor et al., 2005), cocaine (Carey et al., 2005) and morphine (Pastor et al., 2005), in which novelty exposure potentiated the behavioral effects elicited by a single injection of these drugs (hyperlocomotion or rotational behavior induced by 6-OHDA lesion of the mesostriatum). As previously proposed by Pastor et al (2005), it is possible that ETH-induced behavior is less sensitive to the changes in dopamine neurotransmission caused by novelty.

When dim light conditions were combined with novelty, no further increase in spontaneous behaviors or further potentiation of ETH-induced hyperlocomotion was observed. This suggests that there was no

synergism in the facilitating or potentiating effects of dim light or novelty on either spontaneous or ETH-induced behaviors of mice.

Altogether, the results from session 1 indicate that each factor (low-light conditions, novelty and ETH) has specific effects on the general activity of mice and that acute ETH administration and low-light conditions may interact to affect total and peripheral locomotion.

In session 2, we replicated the potentiating effect of low light on ETH-induced hyperlocomotor effect that was previously detected in session 1 on total and peripheral locomotion (see LL groups).

Notably, in session 2, behavioral sensitization to ETH was only detected in the central locomotion parameter (and only in mice that had either been exposed to novelty in session 1 and/or suddenly exposed to the low-light condition in sessions 1 and 2). Indeed, only the HAB–LL–ETH/ETH, NOV–NL–ETH/ETH and NOV–LL–ETH/ETH groups exhibited an enhancement of central locomotion related to their respective controls pre-treated with SAL (–SAL/ETH groups) or to the group that had also been pre-treated and challenged with ETH but was exposed to a familiar open-field under normal-light condition (HAB–NL–ETH/ETH). However, as seen for the acute effects of ETH, there was no synergism between the effects of novelty and dim light on ETH-induced behavioral sensitization.

It is important to state here that in our study ETH-induced behavioral sensitization was demonstrated by between groups comparisons (–ETH/ETH-treated groups × -SAL/ETH-treated groups on the ETH challenge day — session 2) rather than by within groups comparisons between session 1 and session 2 in order to avoid the influence of different environmental conditions in our results (novelty in session 1 and habituation process in session 2, in which mice were exposed to the apparatus for the second time).

Concerning the potentiating effect of dim light on behavioral sensitization, it is possible that this phenomenon was not detected in the total and peripheral locomotion parameters because of a "ceiling effect". Indeed, as has already been discussed, all of the animals suddenly exposed to the low-light open-field conditions presented a very robust response in these parameters to the ETH challenge injection. Nevertheless, it is worth noting the relative lack of sensitivity of these specific parameters in detecting the potentiating effect of novelty on behavioral sensitization (Alvarez et al., 2006).

Within this context, a very intriguing result of the present and previous (Alvarez et al., 2006) studies is the lack of effect of our habituation treatment on total and peripheral locomotor activity and the lack of sensitivity of these behavioral parameters to reveal the behavioral sensitization phenomenon induced by single drug injection. Indeed, both the habituation and the behavioral sensitization phenomena were specifically demonstrated by the central locomotion parameter. In order to further investigate these results we analyzed our data from a within-session habituation point of view (Figs. 3 and 4). Interestingly, while between-session habituation was detected only in central locomotion and grooming (as discussed above), within-session habituation was demonstrated in all the open-field behavioral parameters quantified (including total and peripheral locomotion). Thus, concerning the between-session habituation, the possibility may be raised that although the animals had already been exposed to the apparatus, the novelty of the new situation (transference from home-cage to another environment) could have produced a maximum stimulant effect on the total and peripheral locomotion parameters. Indeed, three-way ANOVA (novelty × light intensity × drug) with repeated measures (within-session habituation) showed no interaction between novelty and within-session habituation evaluated by any of the open-field parameters used. This was also the case for the factor of light intensity. However, within-session habituation in central locomotion and grooming behavior (but not in total and peripheral locomotion) was significantly inhibited by ETH acute administration.

Taken together, these results suggest that central locomotion is a more sensitive behavioral parameter than total and peripheral locomotion to evaluate not only between-session habituation but also drug interference on within-session habituation. The higher sensitivity of the central locomotion parameter when compared to the total and peripheral locomotion parameters may be related to the higher aversiveness of the central area of the apparatus. In line with this hypothesis, it has been extensively demonstrated that rodents show higher habituation to the open (aversive) arms of an elevated plus-maze when compared to the closed (less aversive) arms (Dawson et al., 1994; Rodgers et al., 1992, 1996). In this regard, the inhibitory effect of ETH on central locomotion and grooming within-session habituation could be related to the increased environmental salience produced by dopamine release in the nucleus accumbens, a critical phenomenon related to both drug dependence in humans and behavioral sensitization in rodents.

Interestingly, the facilitation of ETH-induced central locomotor sensitization by dim light and/or novelty was independent of the acute psychomotor response to the drug, since neither novelty nor dim light modified the acute effects of ETH on central locomotion during session 1. Therefore, the facilitation of sensitization seen in animals previously exposed to novelty in session 1 and/or suddenly exposed to the low-light condition in sessions 1 and 2 was not simply due to a general enhancement of the psychomotor response to ETH due to these factors.

Within this context, the lack of evidence of behavioral sensitization in peripheral (or total) locomotion, the only measure that had indicated ETH-induced activity during the first session, could be interpreted as contrary to the dopamine hypothesis.

To the extent that ETH, environmental novelty and low levels of environmental illumination share the ability to enhance dopamine transmission, especially in the mesolimbic dopamine system, we believe that ETH-induced acute hyperlocomotion and locomotor sensitization were potentiated by the sudden dim light and/or novelty through activation of this system. However, although evidence exists demonstrating that dopamine neurotransmission is necessary for ETH sensitization (Araujo et al., 2009; Broadbent et al., 2005; Harrison and Nobrega, 2009; Nestby et al., 1997), we should state that there are conflicting results in the literature on the role of dopamine in this process. Within this context, Broadbent et al. (1995) showed that systemic administration of haloperidol failed to prevent the development of ETH-induced locomotor sensitization in DBA/2j mice and Zapata et al. (2006) found no enhanced dopamine response in the nucleus accumbens to a subsequent ETH challenge in ETH experienced C57BL/6j and DBA/2j mice 2 weeks after withdrawal, despite the observation of clear behavioral sensitization at this time point.

Future studies are necessary to determine the specific role of dopamine neurotransmission in the facilitating effects of sudden dim light on locomotor stimulation induced by acute ETH and of sudden dim light and/or novelty on ETH-induced behavioral sensitization. Nevertheless, results from those studies should be interpreted with caution, since novelty, low levels of environmental illumination and ETH-induced acute locomotor stimulation and behavioral sensitization that develops to this effect seem to be related to increased dopamine transmission. Therefore, if ETH-induced hyperlocomotion or behavioral sensitization were reduced or blunted by a dopamine antagonist, it would be difficult to know whether it would be really blocking the mechanisms underlying hyperlocomotion and sensitization or the mechanisms by which novelty or the sudden dim light facilitates these processes.

In view that the specific mechanisms underlying ETH-induced behavioral sensitization are still unclear and that different genes and mouse strains have been studied for their effects on ETH responses (see Crabbe et al., 2006), the present results have important implication for future pre-clinical studies in the pharmacology and genetics of ETH-induced sensitization.

7. Conclusion

Our study provides new information about the influences of environmental illumination and exposure to novel stimuli in an animal

model of ETH abuse. The data presented here demonstrate that the ETH-induced hyperlocomotor effect was potentiated only by low levels of environmental illumination but that behavioral sensitization to ETH was influenced by both novelty and low-light conditions. From a clinical standpoint, we therefore speculate that both these environmental factors may modify the magnitude of ETH dependence.

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