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Sensitization to the Stimulant Motor Effects of Ethanol Is Not Dependent On Tolerance to Ataxic or Sedative Properties of Ethanol in Female Mice

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Abstract

Ethanol (EtOH)-induced behavioral sensitization (EIBS) is defined as an enhancement of locomotor activity following repeated EtOH exposure and is proposed to reflect an increase in EtOH "wanting". However, the reliability of the sensitization model in studying addiction is still a matter of debate. One major criticism is that the increase in locomotion occurring during sensitization may be a by-product of tolerance to the ataxic and/or sedative effects of EtOH.

We investigated the relationship between EIBS amplitude and sensitivity to EtOH-induced ataxia and sedation after the development of tolerance to EtOH depressant effects in adult female DBA/2J mice. After receiving daily injection of saline or 2 g/kg EtOH during 10 consecutive days to induce EIBS, recovery from acute motor incoordination produced by ethanol (2 g/kg EtOH using rotarod) and from loss of righting reflex (4 g/kg EtOH) was measured.

We showed that induction of EtOH sensitization after repeated administration of EtOH is associated with a more rapid recovery from acute motor incoordination and from sedation produced by ethanol when compared to the acute groups, suggesting the development of tolerance to the ataxic and sedative effects of EtOH. However, correlational analyses failed to detect any relationship between EIBS amplitude and the response to EtOH ataxic or sedative effects.

Altogether, our results confirm and extend previous data showing a tolerance to the ataxic and sedative properties of EtOH after repeated exposure to EtOH and suggest that this tolerance is not related to the amplitude of EIBS.

Keywords: Ataxia; Behavioral sensitization; Chronic tolerance; Ethanol; Loss of righting reflex; Rotarod; Sedation

Abbreviations

BEC: Blood Ethanol Concentration; EIBS: Ethanol-Induced Behavioral Sensitization; EtOH: Ethanol; LORR: Loss of Righting Reflex

Introduction

Repeated ethanol (EtOH) exposure can result in the development of two opposite phenomena. The first characterized, tolerance, is defined as a decrease in the sensitivity to EtOH effects at a constant dose or as a need to raise the dose to maintain the same level of response. Tolerance develops to various EtOH-induced depressant effects such as hypothermia [1], ataxia [2] and sedation [3]. EtOH tolerance occurs within a single exposure (acute tolerance; [4]), between two injections (rapid tolerance; [5]) or after repeated administrations or chronic exposure (chronic tolerance; [6]) and persists for several weeks [7,8]. Conversely, repeated exposure to EtOH may induce an enhancement of EtOH-induced excitatory effects, i.e., behavioral sensitization. EtOH-induced behavioral sensitization (EIBS) is classically observed for the locomotor activating effect of EtOH [9,10]. EIBS can develop between two injections (acute sensitization [11]) or after repeated exposure on an extended period of time [10] and has been shown to last for weeks to months [12-15].

Both tolerance and sensitization theoretically participate to the progressive development of excessive drinking in humans and experimental animals [16-19]. Tolerance to EtOH aversive effects may thus allow individuals to drink more without experiencing an enhancement in the negative, undesirable effects of EtOH [7,20-22]. Conversely, tolerance to the euphorigenic effects of EtOH may require individuals to increase their consumption in order maintain the pleasant effects of EtOH. However, no evidence for the development of tolerance to the reinforcing effects of EtOH has been demonstrated to our knowledge [21-24]. Repeated intermittent exposure to drugs like EtOH is hypothesized to result in sensitization of mechanisms underlying a pathological incentive motivation ("wanting") reflected by EIBS. Therefore, EIBS could reflect mechanisms that play an important role in both early and recurring steps of addiction [25-27]. While the role of tolerance to the depressant effect of EtOH in addiction is well documented, the role of sensitization in EtOH dependence is less clear [13,27,28]. The development of locomotor sensitization is not fully understood and its reliability as a model of addiction-like behavior remains controversial. It is believed that the increase in locomotion following repeated exposure to EtOH may be a by-product of tolerance to the depressant effect of EtOH that would progressively unmask the locomotor stimulant effect of EtOH [10,29].

Although EIBS and tolerance to the depressant effect of EtOH can occur concomitantly [30-32], some studies suggest a dissociation of these phenomena in rodents. The first evidence comes from a study

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by Masur and colleagues who showed that chronic tolerance to the depressant motor actions of EtOH develops in rats after a chronic EtOH treatment (20 days) that does not induce locomotor sensitization [9]. Similarly, it has been observed that high alcohol preferring (HAP) mice voluntarily drinking intoxicating levels of EtOH would eventually develop tolerance to ataxia but not locomotor sensitization [33]. On the other hand, EIBS can occur in the absence of tolerance to the depressant effect of EtOH. As an example, HAP mice developed locomotor sensitization after repeated EtOH treatment without any development of tolerance to the ataxic effect of EtOH measured by the static dowel test [16]. Moreover, EIBS can be induced by EtOH doses as low as 0.5 g/kg and 1 g/kg which do not produce sedation in C57BL/6 and DBA/2J mice, respectively [34,35]. Furthermore, we previously showed in DBA/2J mice that the sensitized stimulant locomotor effect of EtOH occurs predominantly during the first 15 min following an EtOH challenge and that no locomotor tolerance is detected after 15 min, when EtOH depressant effect arises [35]. In addition, the NMDA antagonist MK-801 alters EIBS in DBA/2J mice but has no effect on the development of tolerance to ataxia [36].

A major insight into this debate emerged from a study by Phillips and colleagues who revealed, using quantitative trait loci analysis, the lack of a genetic correlation between tolerance to EtOH-induced ataxia, measured by the grid test, and sensitization to the locomotor effect of EtOH [37]. This study indirectly supports the hypothesis that EIBS and tolerance to the depressant effect of EtOH are separate phenomena. More recently, it has been shown that chronic exposure to high ethanol doses induced sensitization with higher magnitude in adolescent mice compared to adult mice [38]. Interestingly, this difference was not associated with age-related variations in chronic tolerance to the sedative effect of ethanol, measured by the latency and duration of loss of righting reflex (LORR) [39]. This latter study, however, did not directly compare the magnitude of EIBS and sedation tolerance in the same animals.

Here, we proposed to assess EtOH-induced ataxic and sedative effects using respectively the rotarod and LORR tests in DBA/2J adult female mice previously submitted to an EIBS procedure. This approach allowed us to directly assess the relationship between the development of chronic tolerance to EtOH-induced ataxia/sedation and sensitization to the hyperlocomotor effect of EtOH in the same animals.

Materials and Methods

Subjects

Adult female DBA/2J mice were purchased from Janvier (Le Genest Saint Isle, France). Mice were housed in groups of 10 in clear plastic cages (24 × 42 ×15 cm) and kept in a temperature (21 ± 0.5°C) and humidity-controlled (55 ± 10%) environment under an established photoperiod (07.00 - 19.00 hours) with free access to food (Mouse and Rats, Maintenance, Extrudate; Provimi Kliba, Kaiseraugst, Switzerland) and tap water. In the present study, we used female mice in continuity with our previous work [13,35-41] and also to keep consistency with the most relevant reports supporting our working hypothesis [37,39]. The number of animals was kept to a minimum, and all efforts were made to limit stress and to avoid animal suffering. Experiments comply with both the guidelines for Care and Use of Laboratory Animals (NIH), the European Community regulations for animal use in research (CEE No 86/609) and our local ethics committee C.R.E.M.E.A.P. (Comité Régional d'Ethique en Matière d'Expérimentation Animale de Picardie).

Drugs

EtOH (96%, v/v), obtained from Prolabo (Fontenay-sous-Bois, France), was diluted to 20% (v/v) in saline solution 0.9%. All injections were made via the intraperitoneal (i.p.) route in volumes of 1.25 ml per 100 g of body weight.

EIBS procedure

On the habituation day (day H), all mice (n = 70) received a single i.p. injection of saline solution and were immediately placed into the center of the locomotor monitoring chamber. Locomotor activity was recorded for the next 5 min in the LE 8811 IR motor activity monitor (Bioseb, Vitrolles, France) to specifically capture the stimulant effects occurring during the ascending limb of the blood alcohol concentrations [42]. Mice were then divided into Saline- (n = 30) and EtOH- (n = 40) treated groups that were equated in terms of horizontal locomotion on day H. During 10 days (day 1 - day 10), mice received one daily i.p. injection of saline (Acute (R) and (L) groups, for rotarod and LORR experiments, respectively) or 2 g/kg EtOH (EtOH group) solution immediately followed by locomotor activity measurements every two to three days. Mice were then left undisturbed for 5 days (day 11-day 15) in their home cages (Figure 1). To estimate the level of sensitization of each mouse, we decided to use two different methods that are commonly used in literature. This dual approach would eventually strengthen the accuracy of our results. The first sensitization score, hereafter mentioned as the "Delta Score" was calculated as [(locomotor activity on day 10) – (locomotor activity on day 1)] [13,41,43]. The second score, or "D10 Score", was the distance travelled during the 10th and last sensitization session [44,45]. Delta and D10 scores were referred as "locomotion scores" for mice from the acute group which did not experienced repeated EtOH injections and did not exhibit EIBS.

Assessment of the ataxic effect of EtOH in the rotarod paradigm

On day 16, mice were trained to the rotarod (IITC Life Science, Woodland Hills, CA, USA) until they could walk twice for an arbitrarily selected time of 60 seconds without a fall (fixed speed of 8 rpm). On day 17, mice were first given two trials on the rotarod with a limited time of 5 min. The baseline was calculated as the average of the latency to fall during both trials. Then, Acute (R) (n = 15) and EtOH (n = 40) groups of mice were i.p. injected with 2 g/kg EtOH (Figure 1). Thus, rotarod training and baseline were performed away from EtOH injection so that motor learning unlikely interacted with the incoordination effect of EtOH. Following the injection, the latency to fall was determined every 15 min for 2 hours with a limited time of 3 min. For practical reasons considering the number of animals and the limited space on the rotarod apparatus, the cut-off of 5 min used to assess the baseline was brought to 3 min after EtOH administration. The rotarod experiments were conducted between 8:00AM and 11:30AM to avoid any effect of diurnal variation on the motor behavior of animals.

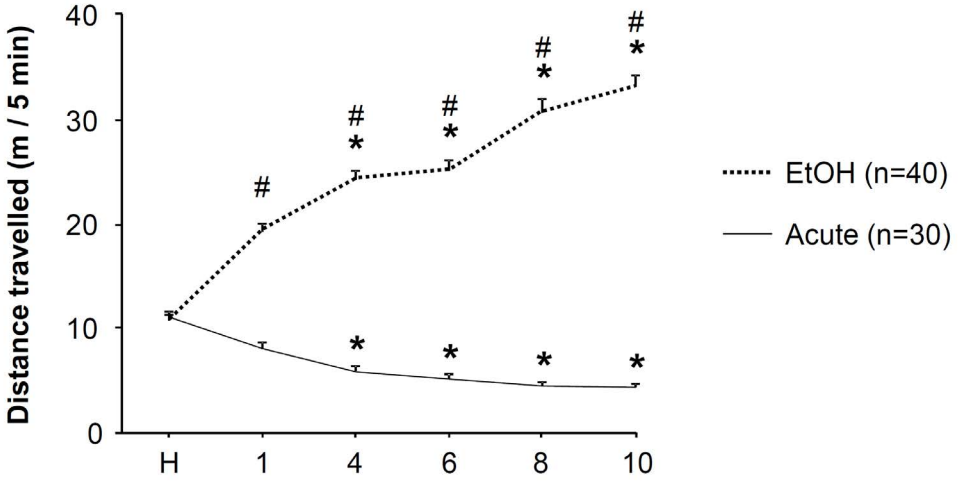
Assessment of the sedative effect of EtOH in the LORR paradigm

On day 18, Acute (L) (n = 15) and EtOH (n = 40) groups of mice were i.p. injected with 4 g/kg EtOH and placed individually in a supine position into a flat surface box (Figure1). The righting reflex was defined as the postural reaction that allows the animal to right itself onto all 4 paws three times in 30 seconds after being placed on its back. The latency to LORR (time elapsed between EtOH injection and LORR),

a

	Day H Habituation	Days 1 - 10 EIBS	Day 16 Rotarod training	Day 17 Rotarod	Day 18 LORR
Acute (R) n=15	Saline	Saline	No treatment	2 g/kg EtOH	
Acute (L) n=15		Saline		Saline	4 g/kg EtOH
EtOH n=40		2 g/kg EtOH		2 g/kg EtOH	4 g/kg EtOH

b



(a) Experimental schedule. (b) On the first day of the experiment (habituation day, H), all mice (n = 70) were i.p. injected with saline solution. Then, from day 1 to day 10, mice received 10 consecutive once-daily i.p. injection of saline (Acute group; n=30) or 2 g/kg ethanol (EtOH group; n = 40) immediately followed by a 5 min-long locomotor activity recording session every two to three days. Data are represented as means ± SEM of distance travelled during 5-min tests. *P < 0.001 vs. Day 1; #P < 0.001 vs. Acute group. EIBS: ethanol-induced behavioral sensitization; LORR: Loss of Righting Reflex.

Figure 1: Ethanol-treated mice display variability in the development of ethanol-induced behavioral sensitization.

the LORR duration (time elapsed between LORR and regain) and the blood ethanol concentrations (BECs) at regain were determined. The dose of 4 g/kg was chosen based on literature [29,46]. Linear regression analyses were performed to investigate correlations between both sensitization scores and latency to LORR, LORR duration and BECs at regain. Four animals that did not lose righting reflex within 3 min after EtOH injection, probably because of incomplete EtOH injection, were excluded from the analysis.

Blood EtOH concentration

On day 18 immediately upon recovery of the righting reflex, mice were euthanized by decapitation and blood was collected. Blood EtOH concentrations were measured in plasma with the AM1 Alcohol Analyser (Analox Instruments, IMLAB, Lille, France) as previously described [40].

Statistical analysis

All data analyses were conducted using SigmaStat2.0 software (LogiLabo, Paris, France) except the Spearman correlations that were performed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). The EIBS data were analyzed by a two-way repeated measure (RM-) Analysis Of Variance (ANOVA) followed by Tukey's post-hoc tests. Data from the rotarod experiment were not following a normal distribution because some animals reached the 3 min cut-off, particularly during late recovery. Therefore, Mann-Whitney U tests were computed on data from Acute (R) and EtOH groups at each time-point of the rotarod experiment. Since 9 time-points were examined, it was necessary to operate an adjustment of the level of significance so that a difference was considered significant when $P < 0.0056$ (i.e., 0.05 divided by 9 tests performed). We used Spearman correlations to investigate the relationship between both sensitization scores and the latency to fall during the baseline trial and during the early (60 min) and late (120 min) recovery of normal motor coordination. Again, an adjustment of the level of significance was performed and P was set at 0.0083 (i.e., 0.05 divided by 6 tests performed). Finally, two-tailed unpaired t tests were used to test the difference between Acute (L) and EtOH groups in the LORR experiment and the relationships between both sensitization scores and the LORR variables were explored using linear regression analyses. An adjustment of the level of significance was performed for the multiple linear regression analyses and P was set at 0.0083 (i.e., 0.05 divided by 6 tests performed). Except when otherwise specified, statistical significance was set at $P < 0.05$.

Results

EtOH-treated DBA/2J mice display variability in the development of EIBS

On day H, 70 mice received an i.p. injection of saline solution and were then divided into Acute (n = 30) and EtOH (n = 40) groups

(Figure 1) that were equated in terms of horizontal locomotion (two-tailed unpaired t test; $P > 0.05$). From day 1 to day 10, mice were daily injected with saline (Acute group) or 2 g/kg EtOH (EtOH group) followed by a locomotor activity measurement every two or three days. A 2-way RM-ANOVA focused on data from day 1 to day 10 showed an effect of group ($F1,73 = 395.603$; $P < 0.001$), an effect of day ($F4,272 = 12.651$; $P < 0.001$) and a significant interaction group x day ($F4,272 = 161.740$; $P < 0.001$). Post-hoc tests revealed a significant increase in locomotor activity with repeated EtOH injections (EtOH group; $P < 0.001$) as well as a difference between the Acute and EtOH groups all along the sensitization procedure.

Amplitude of EIBS is not associated with tolerance to the ataxic effect of EtOH

On day 17, Acute (R) (n = 15) and EtOH (n = 40) groups of mice were submitted to the rotarod test before and after a 2 g/kg EtOH injection to assess EtOH-induced ataxic effect. Analysis of data during the baseline trials failed to detect a difference between Acute (R) and EtOH groups (Mann-Whitney U test; $P > 0.05$; Figure 2a). A Mann-Whitney U test was performed to compare the latency to fall between Acute (R) and EtOH groups at each time-point. These tests revealed a significant difference between groups from the 45 min to the 90 min time-point ($P < 0.001$).

Spearman correlations failed to detect a correlation between both sensitization scores and latency to fall at baseline within the EtOH group (n = 40) ($R = 0.057$ and $P = 0.736$ with the Delta Score; $R = 0.058$ and $P = 0.73$ with the D10 Score; Figure 2b), early recovery (60 min; $R = 0.119$ and $P = 0.466$ with the Delta Score; $R = 0.063$ and $P = 0.70$ with the D10 Score; Figure 2c) and late recovery (120 min; $R = 0.175$ and $P = 0.281$ with the Delta Score; $R = 0.110$ and $P = 0.498$ with the D10 Score; Figure 2d). As a control, we confirmed the absence of correlation within the Acute (R) group between locomotion scores and latency to fall at baseline ($R = -0.339$ and $P = 0.216$ with the Delta Score; $R = -0.332$ and $P = 0.226$ with the D10 Score), early recovery (60 min; $R = 0.056$ and $P = 0.844$ with the Delta Score; $R = -0.291$ and $P = 0.293$ with the D10 Score) and late recovery (120 min; $R = 0.274$ and $P = 0.324$ with the Delta Score; $R = -0.173$ and $P = 0.536$ with the D10 Score).

These results suggest that mice chronically treated with EtOH developed tolerance to the ataxic effect of EtOH and that tolerance to ataxia was not associated with the level of EIBS.

Amplitude of EIBS is not associated with tolerance to the sedative effect of EtOH

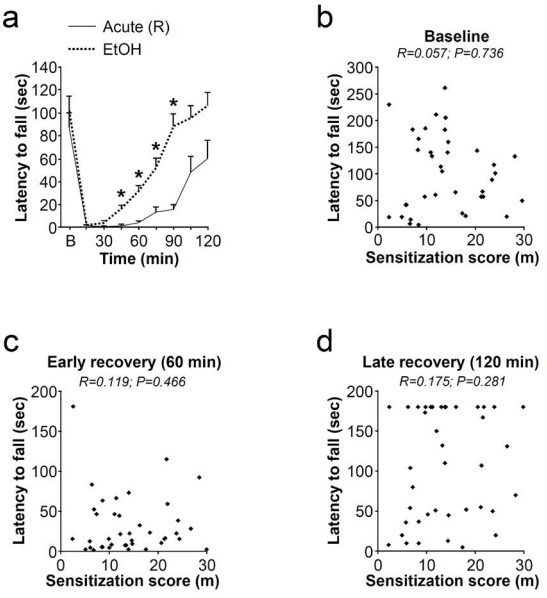
On day 18, immediately after a 4 g/kg EtOH injection, Acute (L) (n = 15) and EtOH (n = 40) groups of mice were submitted to the LORR test to assess EtOH-induced sedative effect. Four animals that did not exhibit LORR within the first 3 min were excluded from the analysis. Analysis of data from Acute (L) and EtOH groups showed no difference in the latency to LORR (two-tailed unpaired t test; $P > 0.05$; Figure 3a), a significant decrease of LORR duration in the EtOH group (two-tailed unpaired t-test; $P < 0.05$; Figure. 3b) and no difference in the BECs at regain (two-tailed unpaired t test; $P > 0.05$; Figure 3c).

Linear regression analyses of data from the LORR test within the EtOH group (n = 36) failed to detect a correlation between both sensitization scores and latency to LORR ($R2 = 0.009$ and $P = 0.573$ with the Delta Score; $R2 = 0.0024$ and $P = 0.775$ with the D10 Score; Figure 3d), duration of LORR ($R2 = 0.001$ and $P = 0.855$ with the Delta Score; $R2 = 0.026$ and $P = 0.346$ with the D10 Score; Figure 3e) and BECs at regain of the righting reflex ($R2 = 0.048$ and $P = 0.198$ with the Delta Score; $R2 = 0.130$ and $P = 0.03$ with the D10 Score; Figure 3f). As a control, we confirmed the absence of correlation within the Acute (L) group between locomotion score and latency to LORR ($R^2 = 0.045$ and $P = 0.506$ with the Delta Score; $R^2 = 0.263$ and $P = 0.088$ with the D10 Score), LORR duration ($R^2 = 0.081$ and $P = 0.371$ with the Delta Score; $R^2 = 0.0032$ and $P = 0.862$ with the D10 Score) and BECs at regain ($R2 = 0.071$ and $P = 0.404$ with the Delta Score; $R^2 = 0.153$ and $P = 0.209$ with the D10 Score).

These results suggest that mice chronically treated with EtOH developed tolerance to the sedative effect of EtOH and that tolerance to sedation was not associated with the level of EIBS.

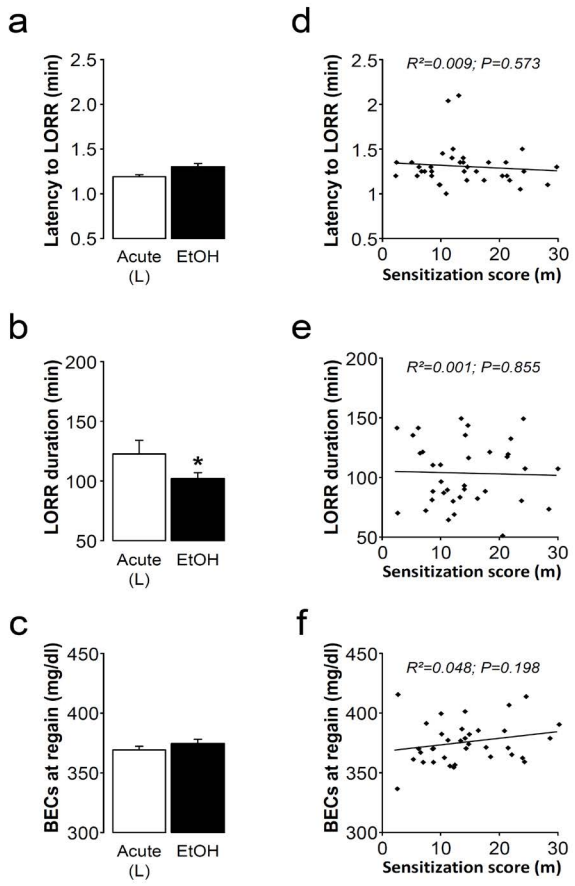
Discussion

While sensitization to the stimulant motor effects of drugs of abuse is widely used in the addiction field as a neuroplasticity model, its exact role in addictive behaviors is still an open question. There is also a debate on whether locomotor sensitization to EtOH emerges from tolerance to EtOH depressant effects. The purpose of the present study was to directly determine the relationship between the levels of chronic tolerance to EtOH-induced ataxia/sedation and the amplitude of EIBS in female mice. Our data strongly suggest dissociation between tolerance to the depressant properties of EtOH and sensitization to the locomotor activating effect of EtOH. This study indicates that the enhancement of locomotor activity following repeated administrations



On day 17, Acute (R) (n = 15) and EtOH (n = 40) groups of mice were submitted to the rotarod test before (baseline; B) and after (recovery) a 2 g/kg ethanol (EtOH) injection. (a) The latency to fall was determined at baseline and every 15 min for 2 hours following injection. Data are represented as mean ± SEM. Spearman correlations were performed to investigate the relationship between the sensitization score and the latency to fall during (b) baseline, (c) early recovery and (d) late recovery. Data are represented as scatter diagrams. *P < 0.001 vs. Acute (R) group.

Figure 2: Amplitude of ethanol-induced behavioral sensitization is not significantly correlated with tolerance to the ataxic effect of ethanol.



On day 18, immediately after a 4 g/kg ethanol (EtOH) injection, Acute (L) (n = 15) and EtOH (n = 40) groups of mice were submitted to the loss of righting reflex (LORR) test. Data are represented as mean ± SEM of (a) latency to LORR, (b) LORR duration and (c) Blood Ethanol Concentrations (BECs) at regain in Acute (L) and EtOH mice. Linear regression analyses were performed to investigate the relationship between the sensitization score and (d) latency to LORR, (e) LORR duration and (f) BECs at regain. Data are represented as scatter diagrams. *P < 0.05 vs. Acute (L) group.
Figure 3: Amplitude of ethanol-induced behavioral sensitization is not significantly correlated with tolerance to the sedative effect of ethanol.

of EtOH unlikely results from a tolerance to the ataxic or sedative properties of EtOH.

Chronic tolerance to the ataxic [31,37,47] and sedative [29,48,49] effects of EtOH has been extensively demonstrated in rodents. In line with these reports, we confirmed that a chronic regimen of EtOH administration induced tolerance to both ataxic and sedative effects of EtOH in adult DBA/2J mice. Interestingly, both tolerance to the depressant effects of EtOH and sensitization to its locomotor activating properties occur concomitantly. One part of the scientific community legitimately raised the question of whether EIBS could derive from tolerance to the depressant effects of EtOH [10,29]. According to this hypothesis, if tolerance to the depressant properties of EtOH was responsible for the increase in locomotion following administrations of EtOH, one would expect that individuals who exhibit the most tolerance would also develop the highest sensitization levels. An important point of the present study is its dimensional, rather than categorical approach. Indeed, we based our analysis on the individual vulnerability to the adaptations induced by chronic EtOH exposure. To do so, we measured the amplitude of EIBS reflected by two different sensitization scores in lieu of using binary variable as classically observed in literature (e.g., chronic EtOH exposure versus chronic saline exposure). Considerable work on EIBS, including ours, studied the outcomes of chronic EtOH exposure by dividing the group of EtOH-treated mice into "non-sensitized" (or resistant) and "sensitized" (or respondent) mice [40,44]. This common classification allows a simplified interpretation of results but has been frequently criticized because it may result in a loss of information and reduced statistical power, residual confounding and even spurious interactions [50,51]. For these reasons, we performed correlational analysis to avoid dichotomizing continuous variables and therefore optimizing our data, not to mention that this strategy has been successfully adopted in two very recent and relevant studies in the EIBS field [43,52]. This is, to our knowledge, the first time that this approach is used in a study on this topic. We showed that both sensitization scores did not correlate neither with the latency to fall in the rotarod test at different time-points after EtOH injection, nor with the latency and duration of the LORR. Our results indicate that animals that achieved the highest levels of sensitization were not the same as those that developed the highest tolerance to the depressant effect of EtOH. This study is in line with recent reports by Quoilin and colleagues who showed in outbred mice that adolescents require high ethanol doses to develop EIBS [38], and do not display any chronic tolerance to the sedative effect of EtOH using the same schedule of EtOH administrations [39]. They concluded that the development of EIBS in adolescent mice is not related to tolerance to the sedative effect of EtOH. Our study also extends a previous report by Phillips and colleagues (1996) that highlighted the absence of a genetic correlation between tolerance to EtOH-induced ataxia and EIBS [37]. Specifically, they measured EIBS and tolerance to the ataxic effects of EtOH in BXD/Ty recombinant inbred mice and showed that the strains more vulnerable to EIBS differed from the strains that developed the higher tolerance. Altogether, these results indicate that chronic tolerance to the depressant properties of EtOH does not underlay the development of EIBS.

One limitation of the present study is that we did not directly assess individual levels of tolerance development to the depressant effects of EtOH, by performing, for instance, the rotarod and/or LORR tests before and after repeated EtOH exposure (within-group comparisons). However, this limitation may have been minimized by the use of a second sensitization score based on individual locomotion during the last sensitization session (D10 Score) rather than the Delta Score. This approach enhanced the consistency in the estimation of both EtOH-induced sensitization and tolerance. Indeed, the individual levels of tolerance development were estimated based exclusively on the levels of tolerance after its development, so were the individual estimations of sensitization using the D10 Score. Moreover, our inter-group results show a robust tolerance to EtOH depressant effects. It is therefore likely that individual sensitivity to the rotarod and LORR tests after repeated EtOH exposure reflects the individual tolerance to the depressant effects of EtOH so that individuals with the lowest sensitivity to the depressant effects of EtOH are the one who developed the highest tolerance. Another limitation of our study is the 3-min-cut-off used during the recovery of normal locomotion in the rotarod experiment. As a consequence of this early cut-off, parts of the data from this experiment were censored. This technical issue may have limited the sight of our statistical analyses.

Chronic tolerance is classically divided into metabolic (pharmacokinetic) and functional (pharmacodynamic) tolerance. We repeatedly showed that the protocol used in the present study (daily

injection of 2 g/kg EtOH for 10 days) did not result in metabolic tolerance in DBA/2J mice [35,40,53]. It is therefore unlikely that metabolic tolerance occurred in the present experiment. Yet, our results showed that the decrease in LORR duration after repeated EtOH injections was not paralleled by higher BECs at regain. Chronic tolerance to LORR is expected to lead to an earlier regain of righting reflex together with higher BECs at regain [29]. Therefore, we cannot exclude that a metabolic tolerance may participate in tolerance to the sedative effect of EtOH as commonly described in the literature [29,48,49,54]. We think that the long interval between EtOH injection and BECs measurements (about 100 min herein) may have facilitated the detection of metabolic tolerance as BECs are mostly dependent on EtOH metabolism whereas absorption and distribution are negligible at this time-point. Also, we cannot exclude a role for behavioral (or contingent) tolerance in the tolerance to ataxic and sedative effects of EtOH observed in the present study. Behavioral tolerance describes "the diminution of a drug-induced disruption of a goal-oriented behavior that is dependent upon learning processes" [55]. Specifically in our experiment, learning to coordinate movements under EtOH exposure even in the home cage might transfer to the tests conditions (rotarod and LORR tests) and thus increase the latency to fall from the rotarod or decrease the LORR duration. Even though tolerance to sedative properties of EtOH may rely in part on metabolic or behavioral tolerance, our results strongly suggest that this phenomenon did not interfere with the development of EIBS.

Our study does not support the hypothesis that sensitization to EtOH locomotor stimulant properties emerges from tolerance to the depressant effects of EtOH. Rather, our results suggest that they are two unrelated phenomena. It is therefore conceivable that both processes contribute independently to the development of excessive drinking and ultimately to the acquisition of addiction. However, both phenomena are rarely studied together. It would be therefore of great interest to further study the individual contribution of each phenomenon in the development of EtOH addiction.

Disclosures/Conflict of Interest

All authors reported no biomedical financial interests or potential conflicts of interest.

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