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Energy drink constituents (caffeine and taurine) selectively potentiate ethanol-induced locomotion in mice

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ABSTRACT

Mixing alcohol with energy drinks has emerged as a popular trend over the last decade. However, epidemiological studies have found this consumption to be associated with increased hazards, such as binge drinking, increased alcohol-related harm and risk of developing alcohol use disorder. The mechanisms underlying these effects are not clear, but much attention has been attributed to caffeine. However, taurine, another common ingredient in energy drinks, has also been associated with the dopamine elevating properties of ethanol, and may in this respect contribute to the increased liability associated with the mixture of alcohol and energy drinks. In the present study we measured locomotor activity, a phenomenon previously linked to the dopamine activating and reinforcing properties of the drug, following acute systemic administration with caffeine (1, 5, 15, 30 mg/kg), taurine (30, 60, 300, 600 mg/kg) and ethanol (1.75, 2.5, 3.25 g/kg), alone or in combination. We found that ethanol and caffeine, but not taurine, increased locomotion compared to vehicle. In addition, when combined with ethanol, caffeine, but not taurine, increased the locomotor stimulatory effect of ethanol. Furthermore, the combination of caffeine and taurine were able to further enhance the ethanol-induced locomotor response. Eleven days of intermittent caffeine exposure produced a sensitized response to the caffeine-induced locomotion, but did not alter the additive effect produced by the combination of caffeine and taurine on ethanol-induced locomotion. Based on the present study we suggest that the combination of caffeine and taurine, at a specific dose range, enhances the locomotor stimulatory properties of ethanol, a phenomenon previously linked to the reinforcing properties of the drug.

1. Introduction

A growing trend during the last decade has been to consume alcohol mixed with energy drinks (Oteri et al., 2007; Marczinski, 2011; Seifert et al., 2011). However, there are reports indicating that this consumption is associated with risks, such as increased probability of binge drinking, alcohol-related harm and development of alcohol use disorders (AUDs) (Price et al., 2010; Arria et al., 2011; Marczinski et al., 2011; Caviness et al., 2017; Oh et al., 2019). Clinical studies also indicate that people consuming alcohol mixed with energy drinks display an impaired ability to assess not only their own, but also other people's level of intoxication, thereby exposing themselves and others for risks (Ferreira et al., 2006; O'Brien et al., 2008; Marczinski et al., 2012). Whether the impaired judgment is the result of a pharmacological interaction, and if so, which substances that are responsible for the increased hazardous behavior, are not yet defined. Therefore, it is essential to determine whether the common energy drink ingredients caffeine and taurine have the ability to pharmacologically influence the effects of ethanol.

Caffeine has been suggested to be the major pharmacologically active ingredient in energy drinks (Marczinski and Fillmore, 2006; Hilbert et al., 2013; Lopez-Cruz et al., 2013; May et al., 2015). Preclinical studies performed on rodents have shown that a systemic injection with caffeine increases voluntary ethanol intake (Kunin et al., 2000) and enhances ethanol-induced locomotion (Hilbert et al., 2013), indicating that these two substances interact with regard to ethanol-induced dopamine-related behaviors. Another common ingredient in energy drinks is taurine (Seifert et al., 2011). Interestingly, taurine is released following ethanol exposure (Dahchour et al., 1996; Ericson et al., 2011) and may also increase extracellular dopamine levels in nucleus

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accumbens (nAc) by itself (Ericson et al., 2006). Moreover, we have proposed that an elevation of extracellular taurine levels is required for ethanol to induce dopamine release in the nAc (Adermark et al., 2011; Ericson et al., 2011). Therefore, the role of taurine as a pharmacologically active ingredient in energy drinks should be considered, and a possible interaction with the reinforcing effects of ethanol needs to be further investigated. Further supporting an interaction, Aragon and colleagues proposed that taurine exerts a biphasic effect on ethanol-induced locomotion (Aragon et al., 1992). In addition, taurine was found to produce opposite effects on ethanol-induced sleep-time in mice, depending on the length of pretreatment (McBroom et al., 1986; Ferko and Bobyock, 1988). Taurine pre-treatment has also been shown to reduce ethanol-induced increases of acetaldehyde in blood and liver (Watanabe et al., 1985), indicating that taurine may influence some of the aversive effects of ethanol.

Taken together, there are studies indicating interactive effects of caffeine or taurine on ethanol-induced behaviors and neurotransmission, but few that have explored tentative interactions between these two substances alone, or in combination with ethanol. Thus, in the present study we aimed to investigate the effects of the combination of caffeine and taurine on ethanol-induced locomotor activity in naïve and caffeine experienced animals. It is known that ethanol in moderate doses increases locomotor activity in mice, and that this phenomenon is related to ethanol-induced activation of the mesolimbic dopamine system (Carlsson et al., 1974; Liljequist et al., 1981; Wise and Bozarth, 1987; Engel et al., 1988). Therefore, monitoring locomotor activity in animals is frequently used for the study of drug-induced behavioral effects related to the brain reward system. We hypothesized that the combination of caffeine and taurine would enhance ethanol-induced locomotor activation in outbred mice.

2. Material and methods

2.1. Animals

A total of 582 male NMRI mice (Charles River, Sulzfeld, Germany) were used in the study. The animals weighted 21–25 g at arrival and were kept at a regular 12/12-hour light/dark cycle (lights on at 7 AM and light off at 7 PM). All mice were group housed (Greenline GR900) and allowed at least one week of acclimatization to the animal facilities (19–21 °C and a humidity of 40–70%) before any experimental procedures were initiated. All of the mice had ad libitum access to tap water and regular chow (Harlan Teklad, Norfolk, England). The experiments were approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden.

2.2. Drugs and doses

Ethanol (95% Kemetyl AB, Haninge, Sweden) was diluted in saline (0.9% NaCl) to a concentration of 15% for intraperitoneal (i.p.) injections (1.75, 2.5 or 3.25 g/kg). Two moderate ethanol doses (1.75 and 2.5 g/kg) were used as these have been shown to increase locomotion in mice (Carlsson et al., 1974; Liljequist, 1991; Blomqvist et al., 1994; Hilbert et al., 2013; Vallof et al., 2017). A higher, sedative dose of ethanol (3.25 g/kg) (Hilbert et al., 2013) was administrated to account for the possibility that caffeine and/or taurine would diminish the sedative effects of ethanol. Caffeine (Sigma-Aldrich, Stockholm, Sweden) was dissolved in saline (0.9% NaCl, 85 °C) and administrated i.p. (1, 5, 15 or 30 mg/kg). The threshold dose for caffeine-induced locomotor stimulation has been shown to be approximately 3 mg/kg, with a maximum effect at 20–30 mg/kg (Buckholtz and Middaugh, 1987; Hsu et al., 2009; Hilbert et al., 2013). The lowest dose (1 mg/kg) of caffeine was used to identify possible additive effects when administrated in combination with ethanol and/or taurine. Taurine (Sigma-Aldrich, Stockholm, Sweden) was dissolved in saline (0.9% NaCl) and administrated i.p. (30, 60, 300 or 600 mg/kg). These doses were selected as it was previously demonstrated that either low or high doses of taurine alone or in combination with ethanol may affect locomotion (Aragon et al., 1992; Ginsburg and Lamb, 2008; Whirley and Einat, 2008).

2.3. Locomotor activity

All of the animals were monitored in the test box for 60 min (habituation) before they received the acute treatment and were immediately returned to the boxes for another 60 min of recording. Locomotor activity was measured using open-field arenas that were placed in six identical sound attenuated, ventilated and dim lit boxes. The open-field arena was equipped with a two-layer grid, consisting of rows of photocell beams (40 × 40 cm, Med Assoc., Fairfax, VT, USA), which allowed a computer-based system (Activity Monitor 7, Med Assoc., St. Albans, VT, USA) to register the activity of the mice. Total ambulatory counts were measured during the entire experiment. Locomotor activity was defined as the accumulated number of consecutive photocell beams interrupted during a 5-minute period.

In the first part of the study, locomotor activity was monitored in drug-naive animals after acute administration of ethanol (1.75, 2.5 or 3.25 g/kg, i.p.), caffeine (1, 5, 15 or 30 mg/kg, i.p.), and/or taurine (30, 60, 300 or 600 mg/kg, i.p.). This part was performed in a between-group design. In the second part of the study, a new set of drug-naïve animals was used. They were administrated caffeine (15 mg/kg, i.p.) or vehicle for eighteen days in an intermittent treatment regime, resulting in a total of eleven injections. Locomotor activity was measured on the first and last day of this treatment period. On day nineteen, animals were administrated the combination of ethanol (2.5 g/kg, i.p.), caffeine (15 mg/kg, i.p.) and taurine (300 mg/kg, i.p.) or vehicle, and locomotor activity was monitored.

2.4. Statistics

Statistics were performed using GraphPad Prism, version 7.0b for Mac OS X (GraphPad Software, Inc., San Diego, CA, USA). Locomotor activity was evaluated by a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, or by a two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test or unpaired *t*-test. All values are presented as mean ± SEM. A probability value ($p < 0.05$) was considered statistically significant.

3. Results

3.1. Acute administration of ethanol or caffeine, but not taurine, increases locomotor activity

As outlined in the method section, the drugs were administered in doses that were in congruence with the literature and previous experience. Following one hour of habituation, mice that were administered with ethanol (2.5 g/kg) significantly increased their locomotor activity as compared to vehicle-treated control (one-way ANOVA: $F_{(3, 61)} = 3.54$, $p = 0.019$, followed by Tukey's post hoc analysis: $p = 0.018$; Fig. 1B). None of the other ethanol doses significantly increased locomotion when compared to vehicle treatment alone. Following an acute injection with caffeine, mice treated with either one of the three highest doses (5, 15 or 30 mg/kg) significantly increased their locomotor activity compared to vehicle-treated mice ($F_{(4, 54)} = 11.33$, $p < 0.001$, followed by Tukey's post hoc analysis: caffeine 5 mg/kg ($p = 0.031$), 15 mg/kg ($p < 0.001$) and 30 mg/kg ($p < 0.001$); Fig. 1D). The lowest dose of caffeine (1 mg/kg) did not increase locomotion as compared to vehicle. None of the chosen doses of taurine influenced locomotor activity in relation to vehicle treatment ($F_{(4, 58)} = 0.23$, $p = 0.915$; Fig. 1F).

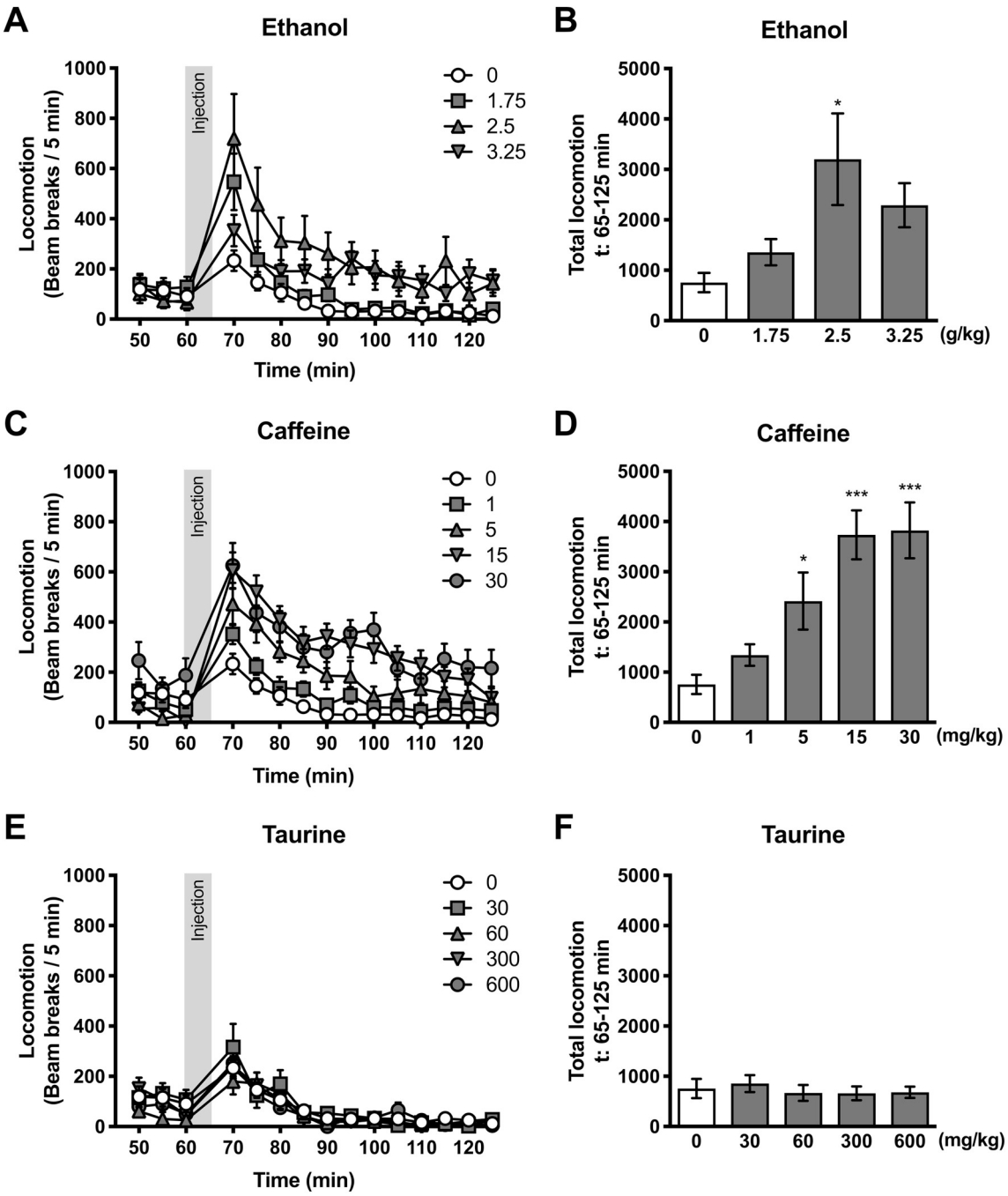

Fig. 1. Acute administration of ethanol or caffeine, but not taurine, dose-dependently increases locomotor activity. Locomotion during habituation and after acute administration with A, B) ethanol (0, 1.75, 2.5 or 3.25 g/kg, i.p.) (n = 15–18), C, D) caffeine (0, 1, 5, 15 or 30 mg/kg, i.p.) (n = 10–15) or E, F) taurine (0, 30, 60, 300 or 600 mg/kg, i.p.) (n = 11–15). Data are presented as mean ± SEM. *p < 0.05 vs. vehicle; ***p < 0.001 vs. vehicle.

3.2. Caffeine and ethanol produce additive effects on locomotor activity

In order to evaluate the influence of caffeine on ethanol-induced locomotion caffeine (0, 1, 5, 15 or 30 mg/kg) was administrated together with ethanol (0, 1.75, 2.5, 3.25 g/kg), in all possible combinations (Fig. 2). Following one hour of habituation, mice treated with the combination of caffeine 5 mg/kg and ethanol 3.25 g/kg showed a significant increase in locomotor activity compared to either drug alone, and vehicle treatment (Table 1, Fig. 2B). An additive increase in locomotion was also found following co-administration of the intermediate dose of caffeine (15 mg/kg) and ethanol (1.75 g/kg or 3.25 g/kg) (Fig. 2C), as well as after the highest caffeine dose (30 mg/kg) combined with the intermediate ethanol dose (2.5 g/kg) (Table 1, Fig. 2D). None of the other drug combinations had an additive effect on locomotor activity (Tables S1, S2, S3, S4).

3.3. Taurine does not increase ethanol-induced locomotor activity

Taurine (0, 30, 60, 300, 600 mg/kg) and ethanol (0, 1.75, 2.5, 3.25 g/kg) were administrated in all possible combinations in order to evaluate whether taurine would influence ethanol-induced locomotion. In a subset of subjects, a trend for increased ethanol-induced locomotor activity was observed after administration of 300 mg/kg taurine and the intermediate dose of ethanol (2.5 g/kg) (Fig. 3B). Nevertheless, following one hour of habituation, a two-way ANOVA followed by Tukey's post hoc analysis demonstrated that the effect of this

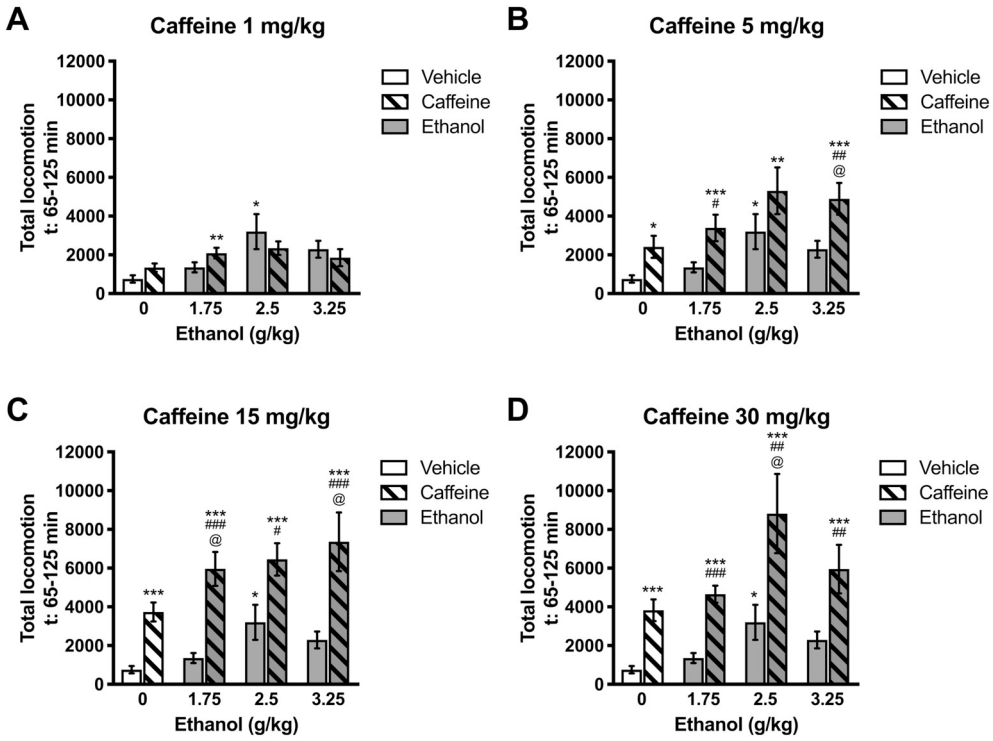

Fig. 2. Effects of caffeine and ethanol on locomotor activity. Locomotor activity after acute administration with ethanol (0, 1.75, 2.5 or 3.25 g/kg, i.p.) and caffeine, i.p. A) 1 mg/kg (n = 10–18), B) 5 mg/kg (n = 11–18), C) 15 mg/kg (n = 10–18) or D) 30 mg/kg (n = 10–18). The same control groups (vehicle or ethanol) are used in all graphs (A–D). Data are presented as mean ± SEM. *p < 0.05 vs. vehicle; **p < 0.01 vs. vehicle; ***p < 0.001 vs. vehicle; #p < 0.05 vs. ethanol; ##p < 0.01 vs. ethanol; ###p < 0.001 vs. ethanol; @p < 0.05 vs. caffeine.

combination (taurine 300 mg/kg and ethanol 2.5 g/kg) did not significantly differ from that produced by ethanol per se (taurine effect: F(1, 51) = 0.61, p = 0.437; ethanol effect: F(1, 51) = 9.60, p = 0.003; interaction effect: F(1, 51) = 0.45, p = 0.508; followed by Tukey's post hoc analysis: taurine + ethanol vs. vehicle (p = 0.008), taurine + ethanol vs. ethanol (p = 0.7105), taurine + ethanol vs. taurine p = 0.008); Fig. 3, Table 2). Taurine did not significantly influence ethanol-induced locomotion at any of the other drug combinations used (Fig. 3, Tables S5, S6, S7). As locomotion was evaluated at different time points in previous studies (Aragon et al., 1992; Ginsburg and Lamb, 2008; Whirley and Einat, 2008), we performed additional analyses to assess locomotor activity at 5, 15 or 30 min after drug challenge. However, taurine still had no effect on ethanol-induced locomotor activity (data not shown).

3.4. The combination of caffeine and taurine potentiates ethanol-induced locomotor activity

Since the overall aim of the study was to evaluate if the combination of caffeine and taurine potentiates the locomotor stimulatory effect of ethanol in mice, we performed an interim analysis (n = 5–6) in order to determine which combinations of caffeine and taurine to include. We found that 5 mg/kg caffeine modestly increased locomotor activity compared to vehicle treatment, indicating that the threshold value for increased locomotion after caffeine is approximately 5 mg/kg. The interim analysis also revealed that mice treated with 15 mg/kg caffeine produced a maximum increase of locomotor activity since administration of 30 mg/kg did not increase the locomotion further. Based on these observations 5 and 15 mg/kg caffeine were chosen for further testing in combination with taurine and ethanol. Taurine alone or in combination with ethanol did not significantly influence locomotion following any of the administered doses. However, a trend for increased ethanol-induced locomotor activity was observed after 300 mg/kg taurine, at least in some animals, which qualified this dose for experiments using the triple combination of caffeine, taurine and ethanol. Co-administration of taurine 300 mg/kg and caffeine 5 or 15 mg/kg did not have a significant effect on caffeine-induced locomotion (caffeine 5 mg/kg vs. taurine 300 mg/kg + caffeine 5 mg/kg: unpaired *t*-test: t = 0.15, df = 18.65, p = 0.883; caffeine 15 mg/kg vs. taurine 300 mg/kg + caffeine 15 mg/kg: unpaired *t*-test: t = 1.53, df = 18.99,

Table 1
Statistical analysis of the effect of an acute injection with a combination of ethanol (0, 1.75, 2.5 or 3.25 g/kg, i.p.) and caffeine (0, 15, or 30 mg/kg, i.p.) on locomotor activity in mice (two-way ANOVA followed by Tukey's post hoc analysis). Locomotor activity was recorded during the subsequent 60 min after drug administration. E = ethanol; C = caffeine; veh = vehicle.

Treatment	Caffeine 5 mg/kg & Ethanol 3.25 g/kg	Caffeine 15 mg/kg & Ethanol 1.75 g/kg	Caffeine 15 mg/kg & Ethanol 3.25 g/kg	Caffeine 30 mg/kg & Ethanol 2.5 g/kg
Two-way ANOVA				
EtOH effect	F(1, 51) = 15.55	F(1, 49) = 8.58	F(1, 52) = 10.64	F(1, 51) = 11.03
	p < 0.001	p = 0.005	p = 0.002	p = 0.002
Caffeine effect	F(1, 51) = 17.55	F(1, 49) = 61.61	F(1, 52) = 25.98	F(1, 51) = 15.03
	p < 0.001	p < 0.001	p < 0.001	p < 0.001
Interaction effect	F(1, 51) = 0.87	F(1, 49) = 2.84	F(1, 52) = 1.75	F(1, 51) = 1.29
	p = 0.356	p = 0.099	p = 0.192	p = 0.261
Tukey's post hoc analysis				
E + C vs. veh	p < 0.001 ***	p < 0.001 ***	p < 0.001 ***	p < 0.001 ***
E + C vs. E	p = 0.004 ##	p < 0.001 ###	p < 0.001 ###	p = 0.003 ##
E + C vs. C	p = 0.012 @	p = 0.018 @	p = 0.019 @	p = 0.029 @

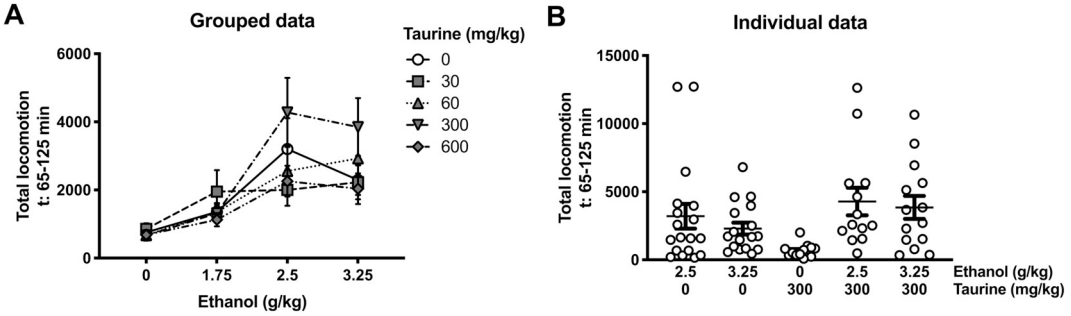

Fig. 3. Taurine does not enhance ethanol-induced locomotor activity. A) Locomotor activity after acute administration of the combination of ethanol (0, 1.75, 2.5 or 3.25 g/kg, i.p.) and taurine (0, 30, 60, 300 or 600 mg/kg, i.p.) (n = 11–18). Data are presented as mean ± SEM. B) Locomotion for each individual after challenge with ethanol (0, 2.5 or 3.25 g/kg, i.p.) and taurine (0 or 300 mg/kg, i.p.).

Table 2
Statistical analysis of the effect of an acute injection with a combination of ethanol (0, 1.75, 2.5 or 3.25 g/kg, i.p.) and taurine (300 mg/kg, i.p.) on locomotor activity in mice (two-way ANOVA followed by Tukey's post hoc analysis). Locomotor activity was recorded during the subsequent 60 min after drug administration. E = ethanol; T = taurine; veh = vehicle.

Treatment	Taurine 300 mg/kg & Ethanol 1.75 g/kg	Taurine 300 mg/kg & Ethanol 2.5 g/kg	Taurine 300 mg/kg & Ethanol 3.25 g/kg
Two-way ANOVA			
EtOH effect	F(1, 48) = 6.10 p = 0.017	F(1, 51) = 9.60 p = 0.003	F(1, 51) = 16.18 p < 0.001
Taurine effect	F(1, 48) = 0.52 p = 0.476	F(1, 51) = 0.61 p = 0.437	F(1, 51) = 0.21 p = 0.651
Interaction effect	F(1, 48) = 0.13 p = 0.718	F(1, 51) = 0.45 p = 0.508	F(1, 51) = 0.06 p = 0.808
Tukey's post hoc analysis			
E + T vs. veh	p = 0.2533	p = 0.008 **	p < 0.001***
E + T vs. E	p = 0.9991	p = 0.7105	p = 0.1107
E + T vs. T	p = 0.1619	p = 0.008 @@	p < 0.001 @@@

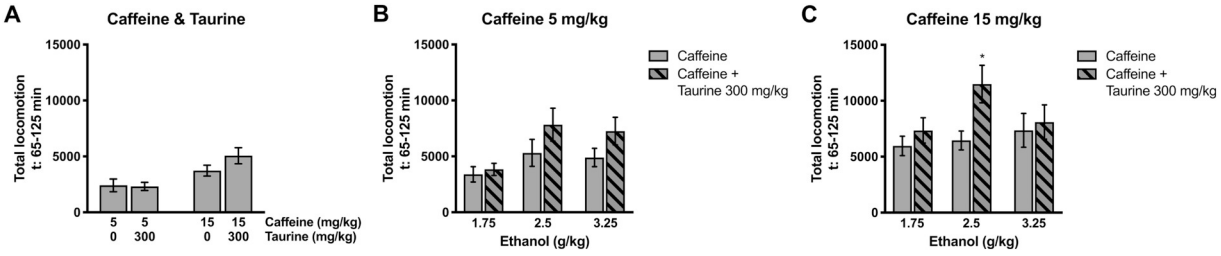

Fig. 4. Effects of combinations of caffeine, taurine and ethanol on locomotor activity. A) Locomotor activity after acute administration with caffeine (5 or 15 mg/kg, ip) and taurine (0 or 300 mg/kg, ip) (n = 11–12). B) Locomotor activity after acute administration with ethanol (1.75, 2.5 or 3.25 g/kg, ip) and caffeine (5 mg/kg, ip) or the combination with taurine (300 mg/kg, ip) (n = 11–17). **C)** Locomotor activity after acute administration with ethanol (1.75, 2.5 or 3.25 g/kg, ip) and caffeine (15 mg/kg, ip) or the combination with taurine (300 mg/kg, ip) (n = 10–16). Data are presented as mean ± SEM. *p = 0.05 vs. vehicle.

p = 0.143; Fig. 4A). Acute administration of taurine 300 mg/kg selectively potentiated locomotion elicited by the combination of 15 mg/kg caffeine and 2.5 g/kg ethanol (unpaired *t*-test: t = 2.69, df = 19.94, p = 0.014; Fig. 4C), but did not modulate locomotion induced by any of the other concentrations of caffeine and ethanol (Fig. 4B and C).

3.5. Additive influence of caffeine and taurine on ethanol-induced locomotion remains in the caffeine experienced individual

In order to evaluate whether sub-chronic caffeine treatment would alter the additive effect produced by the combination of caffeine and taurine on ethanol-induced locomotion, caffeine (15 mg/kg) or vehicle was administrated intermittently for three weeks, resulting in a total of eleven injections.

Caffeine-induced locomotor activity was tested on the first and last day of injection. On day one, as well as on the last day of injection, caffeine treated animals showed an increase in locomotion as compared to vehicle treated animals (one-way ANOVA: $F_{(3,\ 30)} = 41.67$ p < 0.001, followed by Tukey's post hoc analysis: day 1 (p > 0.001), day 18 (p > 0.001); Fig. 5B). Furthermore, on the last day of injection, caffeine had an increased locomotor stimulatory effect on animals repeatedly receiving caffeine, showing that caffeine produces behavioral sensitization (one-way ANOVA: ($F_{(3,\ 30)} = 41.67$, p < 0.001, followed by Tukey's post hoc analysis: p = 0.001; Fig. 5B). Vehicle treated mice did not alter their locomotor activity following repeated vehicle administrations (one-way ANOVA: ($F_{(3,\ 30)} = 41.67$, p < 0.001, followed by Tukey's post hoc analysis p = 0.747; Fig. 5B). However, locomotion induced by acute administration of the combination of ethanol (2.5 g/kg), caffeine (15 mg/kg) and taurine (300 mg/kg) was not further enhanced by caffeine sensitization (unpaired *t*-test: t = 0.26, df = 12, p = 0.799; Fig. 5B, C).

4. Discussion

In the present study we show that caffeine increases locomotor behavior and produces an additive effect on ethanol-induced locomotor activity. In addition, although not having a substantial effect on locomotion by itself, or in combination with ethanol, we found that the amino acid taurine could further potentiate this effect at specific doses. This additive effect was not altered in animals repeatedly exposed to caffeine, even though a behavioral sensitization towards caffeine was detected.

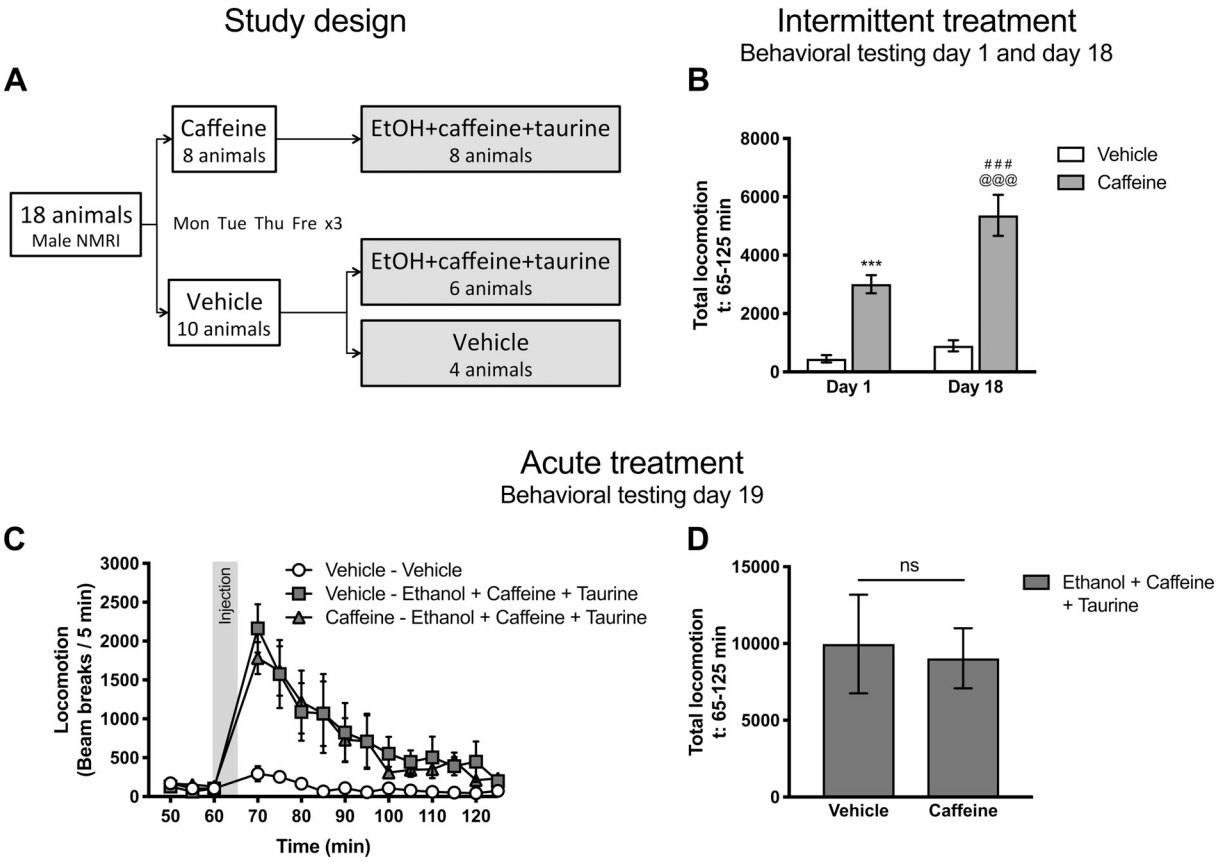

Fig. 5. Additive influence of caffeine and taurine on ethanol-induced locomotion remains in caffeine pre-treated animals. A) Schematic illustration of the second part of the study where animals were intermittently administrated caffeine or vehicle for eighteen days. Locomotor activity was monitored on day one and day eighteen. On day nineteen, after completion of the intermittent treatment, the animals were administrated with the combination of ethanol, caffeine and taurine or vehicle and locomotor activity was measured. B) Locomotor activity after acute and eighteen days of intermittent pre-treatment with caffeine (15 mg/kg, i.p.) or vehicle (n = 7–10). C, D) Locomotion during habituation and after acute administration with the combination of ethanol (0 or 2.5 g/kg, i.p.), caffeine (0 or 15 mg/kg, i.p.) and taurine (0 or 300 mg/kg, i.p.) in caffeine or vehicle pre-treated animals (n = 4–8). Data are presented as mean ± SEM. ***p < 0.001 vs. vehicle day 1, ###p < 0.001 vs. vehicle day 18, @@@p < 0.001 vs. caffeine day 1. EtOH = ethanol.

In order to maximize the potential outcome of the study we used several different doses of ethanol, caffeine and taurine. The ethanol doses were chosen based on previous experience from us and others, where moderate doses of ethanol increased locomotor activity in mice (1.75–2.5 g/kg) (Liljequist, 1991; Blomqvist et al., 1994; Hilbert et al., 2013; Vallof et al., 2017). A higher ethanol dose (3.25 g/kg), previously shown not to stimulate locomotion (Hilbert et al., 2013), was also included to account for the possibility that caffeine and/or taurine would diminish the sedative effects of ethanol. In the present study only the intermediate dose of ethanol increased locomotor activity. It has been shown that various strains of mice (NMRI, C57 and CBA mice) differ in their sensitivity to ethanol including its locomotor stimulating effect, where NMRI mice appears to exhibit a greater ethanol-induced locomotor activity than C57 and CBA mice (Liljequist and Ossowska, 1994). Therefore, a possible explanation to discrepancies between studies when measuring locomotor stimulatory effects by ethanol could be the use of different suppliers or strains. The threshold dose for caffeine-induced locomotor stimulation has been shown to be approximately 3 mg/kg, with a maximum effect at 20–30 mg/kg (Buckholtz and Middaugh, 1987; Hsu et al., 2009; Hilbert et al., 2013). In our study, caffeine was administrated in four different doses with the aim of obtaining low, threshold and robust locomotor stimulatory effects. We found all but the lowest dose of caffeine to significantly increase locomotor activity. These effects may be connected to the ergogenic and hyperthermic effects displayed by caffeine, which in turn could be related to changes in dopamine transmission, although this is debated (Quarta et al., 2004; De Luca et al., 2007; Zheng et al., 2014).

When reviewing the literature it appears that the effects of systemically administrated taurine on rodent behavior, much like ethanol, display a high concentration-dependent variability, where either very low or high doses were found to be effective. Low doses of taurine were shown both to decrease (1 g/kg ethanol) and increase (2 g/kg ethanol) ethanol-induced locomotor activity (Aragon et al., 1992). Low doses of taurine have also been shown to decrease ethanol-induced sleeping time, while increasing the time spent in the open arms of the elevated plus-maze (Boggan et al., 1978; Chen et al., 2004). Very high doses of taurine (> 400 mg/kg) have been shown to decrease locomotor activity, and may, depending on route of administration and time-point of taurine treatment, both increase as well as decrease ethanol-induced sleeping time (McBroom et al., 1986; Ginsburg and Lamb, 2008; Whirley and Einat, 2008). Additionally, very high doses of taurine decrease cocaine-induced conditioned place preference, and locomotor sensitization elicited by repeated cocaine exposure (Hruska et al., 1975; Banerjee et al., 2013). High levels of taurine might thus reduce the reinforcing effects of drugs of abuse. Consequently, in the present study we used two low and two substantially higher doses of taurine. In our study, none of the administered doses of taurine influenced locomotion, a phenomenon that also is supported by previous studies (Aragon et al., 1992; Ginsburg and Lamb, 2008), although decreased locomotion has been reported at different time points (Whirley and Einat, 2008). When sub-analyzing our data in 5, 15 or 30 min bins we found no such effects (data not shown). As for ethanol, mouse strain and/or supplier of the animals could influence the outcome.

When the combination of caffeine and ethanol was administered, we

found that caffeine increased ethanol-induced locomotion, which is in line with previous studies (Waldeck, 1974; Hilbert et al., 2013). Here, only acute experiments were conducted with the combination of caffeine and ethanol. We did not explore the effect by the combination in caffeine-experienced animals. But, May and colleagues demonstrated that repeated intake of caffeine altered the locomotor response to ethanol (May et al., 2015). Interestingly they also found that repeated administration of the combination of caffeine and ethanol produced a robust locomotor sensitization, 2.5 times greater than what was produced by caffeine or ethanol alone, suggesting that co-administration of these two substances exerts synergistic effects on the development of locomotor sensitization. The mechanisms underlying the interaction of ethanol and caffeine have not been addressed in this study but others have demonstrated that caffeine mainly acts as a nonselective antagonist at adenosine (A1 and A2A) receptors (Daly and Fredholm, 1998; Fredholm et al., 1999). Thus, the locomotor stimulatory effect of caffeine has been suggested to derive from enhanced dopaminergic neurotransmission in the nAc, mediated by antagonism of A1 and A2A receptors (El Yacoubi et al., 2000; Karcz-Kubicha et al., 2003; Quarta et al., 2004; Kuzmin et al., 2006). Ethanol, like most drugs of abuse, is believed to increase locomotor activity as a consequence of dopamine elevation in the nAc (Wise and Bozarth, 1987), and may also exert some of its behavioral effects through the adenosine-system (Nagy et al., 1990; Krauss et al., 1993; Arolfo et al., 2004; Lopez-Cruz et al., 2013; Nam et al., 2013). Thus, the interactive locomotor stimulatory effect of ethanol and caffeine could be explained by the drugs' combined effects in the mesolimbic dopamine system, e.g. by modulating adenosine-signaling.

The effects of taurine on ethanol-induced locomotion have only been examined in a few studies. Here acute administration of taurine did not impact ethanol-induced locomotion after any of the doses used. This is in contrast to one previous study showing that taurine decreases ethanol-induced locomotion after low doses of ethanol and increases it after higher doses of ethanol (Aragon et al., 1992), and another study showing that taurine attenuates ethanol-induced locomotion 5 min after ethanol administration (Ginsburg and Lamb, 2008). The discrepancies between the studies could be explained by different treatment protocols and the use of different strains of mice (Liljequist and Ossowska, 1994). Nevertheless, it can be concluded that the influence of taurine on ethanol-induced locomotion appears to be very subtle.

An interesting finding of the present study is that, at a particular dose combination, co-administration of caffeine and taurine increases ethanol-induced locomotion to a greater extent than any drug administered alone or in combination. This is especially interesting since this potentiating effect of taurine and caffeine on ethanol-induced locomotor activity remained in caffeine-experienced animals displaying neuroadaptations manifested as behavioral sensitization. Thus, even though taurine did not produce any significant alterations of locomotor activity either when administered alone or in combination with ethanol, using the present paradigm, there is a significant contribution of taurine in combination with caffeine and ethanol. An interaction of caffeine and taurine on ethanol induced behavior is indirectly supported by previous studies demonstrating that systemic administration of “energy drink” reduced the depressant effects of ethanol on locomotion, and that a modest ethanol-induced behavioral sensitization was enhanced in mice following systemic administration of “energy drink” (Ferreira et al., 2004; Ferreira et al., 2013). As to the mechanisms underlying the potentiation observed we can at this stage only speculate. Since ethanol-induced locomotor stimulation has been linked to dopamine activation (Carlsson et al., 1974; Liljequist et al., 1981; Wise and Bozarth, 1987; Engel et al., 1988) it could be speculated that the combination of caffeine and taurine directly or indirectly facilitates pre- or post-synaptic mechanisms of dopamine neurotransmission related to ethanol action. Both drugs are known to facilitate the release of intracellular calcium (Nehlig et al., 1992; Schaffer et al., 2000), interact with $GABA_A$ and glycine receptors (Nehlig et al., 1992; Olive, 2002) and influence glutamatergic NMDA receptors (Quarta et al., 2004; Chan et al., 2014). Thus, there are a number of possible mechanisms by which caffeine and taurine could influence ethanol-induced neurotransmission and behavior.

In conclusion, the present study provides further evidence that caffeine has a pronounced influence on ethanol-induced locomotion. Although the effects of taurine by itself and in combination with ethanol in general appear modest the potentiating effect observed at a specific dose combination of taurine, caffeine and ethanol should not be neglected. As we and others previously have found taurine to be involved in ethanol-induced behavior and ethanol-induced dopamine elevation, which in turn has been linked to ethanol intake, taurine could, likely together with caffeine, have a role in the increased ethanol consumption observed in humans drinking alcohol mixed with energy drinks.

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Declaration of competing interest

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2019.172795.

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