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Brucine N-oxide reduces ethanol intake and preference in alcohol-preferring male Fawn-Hooded rats

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Abstract

Background: Alcohol use disorder places a heavy burden on global public health systems and thus is in urgent need of improved pharmacotherapies. Previously, our group has demonstrated that 30 mg/kg of the indole alkaloid brucine significantly attenuates alcohol drinking behavior; however, the high toxicity, poor water solubility, short half-life, and limited therapeutic window of brucine restrain its clinical application as an anti-alcoholism medication. We subsequently hypothesized that the oxide of brucine (brucine N-oxide) would produce a similar behavioral effect without the risk profile associated with brucine.

Methods: Male Fawn-Hooded rats with high innate alcohol preference underwent two-bottle choice procedures (Experiments 1-3). Experiment 1 examined the effects of 7 daily BNO injections of 0, 30, 50 or 70 mg/kg (s.c.) on voluntary alcohol consumption (n = 9/group). Experiment 2 evaluated the impact of a single dose of 0 or 70 mg/kg BNO on the

increased alcohol intake induced by a 4d alcohol deprivation (n = 8/group). Experiment 3 tested the effect of 7 daily BNO injections of 0 or 70 mg/kg (s.c.) on sucrose preference (n = 6/group). Experiment 4 measured the median lethal dose (LD50) values of BNO and brucine to compare their acute toxicity in rats. Experiment 5 tested whether BNO (0, 30, 50 and 70 mg/kg, s.c.) affected locomotor activity using an open-field paradigm (n= 8/group). Finally, Experiment 6 evaluated the possible conditioned rewarding effects of 0, 30, 50, and 70 mg/kg BNO using the conditioned place preference paradigm (n = 6/group).

Results: BNO administration dose-dependently attenuated alcohol consumption without affecting food intake, total fluid consumption or the natural preference for a sucrose solution, with 70 mg/kg BNO reducing consumption by 22.8%. A single dose of 70 mg/kg BNO significantly inhibited the alcohol deprivation effect. The LD50 values of BNO and brucine in rats were determined to be 1103.5 ± 177.0 mg/kg and 264.6 ± 17.7 mg/kg, respectively. Finally, BNO administration did not affect spontaneous locomotor activity or induce a place preference.

Conclusions: BNO may help to control excessive alcohol use and should be considered a treatment strategy for future study and development.

Keywords : brucine N-oxide; alcohol; alcohol use disorder; Fawn-Hooded (FH/Wjd) rat; glycine receptor

1. Introduction

People drink alcohol to socialize, relax and relieve pressure in contemporary society. However, some individuals can develop alcohol use disorders (AUD) after repeated alcohol consumption, which induces chronic and progressive alterations in the central nervous system (CNS). AUD inflicts economic and social losses on the individual as well as society at large and urgently requires the development of more effective therapies. A global survey on alcohol and health has revealed that harmful alcohol use results in 3,000,000 deaths and contributes to 5% of the burden of diseases worldwide annually (World Health Organization, 2018). Moreover, excessive alcohol consumption positively correlates with the development of liver, oesophagus, larynx, pharynx, oral cavity and female breast cancers (Bagnardi et al., 2001, 2013; Talamini et al., 2002; Znaor et al., 2003; Turati et al., 2013).

89 Clinically, naltrexone and acamprosate are prescribed to control the
90 symptoms of AUD; however, the side effects of naltrexone and the low
91 bioavailability of acamprosate may limit their therapeutic usage (Kaaber
92 et al., 1987; Croop et al., 1997; Jupp & Lawrence, 2010; Witkiewitz et
93 al., 2012). Consequently, it is necessary to develop medications with
94 higher efficacy and fewer side effects for better compliance from patients.

95
96 *Semen strychni* is a traditional Chinese medicine that has been used for
97 hundreds of years due to its significant analgesic and anti-inflammatory
98 properties (Yin et al., 2003). The major active components of this
99 compound are indole alkaloids, including brucine (Fig. 1a) and brucine
100 N-oxide (BNO; Fig. 1b). Previously, we found that brucine had an
101 inhibitory effect on alcohol drinking behavior in FH/Wjd rats, an innate
102 alcohol-preferring rat strain (Li et al., 2014). However, brucine is
103 classified as a toxic substance with a high elimination rate and a narrow
104 therapeutic window, which impedes its potential translation to clinical
105 settings (Yin et al., 2003; Li et al., 2014). Because oxidation is a common
106 pathway of detoxification and delay of the elimination of alkaloids
107 (Ziegler & Gold, 1971; Rose & Castagnoli, 1983), we hypothesized that
108 the use of BNO, an oxide of brucine, might result in a similar
109 anti-alcohol-drinking effect, but with negligible toxicity and a wide
110 therapeutic dose range.

In this study, we first investigated the effects of BNO administration on alcohol drinking phenotypes and sucrose preference using an oral drinking paradigm in rats. We further investigated the acute toxicity of brucine and BNO. Finally, we determined whether BNO alters locomotor activity or induces a place conditioning effect.

2. Materials and methods

2.1. Animals

FH/Wjd rats were generously provided by Prof. Andrew J. Lawrence at the Florey Institute of Neuroscience and Mental Health, University of Melbourne (Melbourne, Australia) and bred at the Department of Laboratory Animal Sciences, Peking University Health Science Center (License number: SCXK-(Jing) 2011-0012). Experimental rats were housed and raised in hyaline plastic boxes and had free access to water and food in a condition-controlled room (Relative humidity: 50% $\pm$ 10%; temperature: 22 °C $\pm$ 1 °C) on a 12-h light/darkness cycle (on 8:00 am, off 8:00 pm). We used male rats of approximately 10 weeks old and habituated them to the environment prior to experiments. The experimental procedures were approved by the Local Committee on Animal Care and Use. Animals were treated according to the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

BNO hydrate (Sigma-Aldrich, St. Louis, MO, USA) and morphine hydrochloride (Qinghai Pharmaceutical Plant, Qinghai, China) were prepared with saline and administered subcutaneously in a volume of 1 mL/kg. Ethanol (5%, 10%, v/v; Beijing Chemical Factory, Beijing, China) and sucrose (0.5%, w/v; Beijing Chemical Factory, Beijing, China) solutions were prepared in tap water.

2.3. Ethanol two-bottle choice drinking test

This test was performed according to the classic paradigm (Pandey et al., 2004; Li et al., 2014; Eisenhardt et al., 2015). FH/Wjd rats were housed separately in the home cage and given one bottle containing 5% ethanol solution and one water bottle continuously for 8 consecutive weeks as an adaptive phase to ensure a stable level of ethanol consumption prior to testing. A 5% solution was used because FH/Wjd rats have a higher preference for ethanol at this concentration (Li et al., 2014). Solutions were replaced and the positions of the bottles were switched daily. Thirty-six rats with high alcohol preference ($> 65\%$) were divided into 4 groups using a randomized block design ($n = 9/\text{group}$). BNO treatment at 0 mg/kg (saline), 30, 50 and 70 mg/kg was injected twice a day (at 8:00 am and 7:30 pm) for 7d. The procedure lasted for 13 days, which

included a 3d baseline period, 7d of drug treatment and a 3d post-treatment response measurement. Ethanol, water and food consumption were monitored every 24 h.

2.4. Alcohol deprivation test

The alcohol deprivation effect is characterized by a pronounced but transient elevation of ethanol consumption after a period of abstinence in rodents, which model alcohol relapse phenotypes (Spanagel et al., 1996; McBride et al., 2002; Rezvani et al., 2002; Li et al., 2014; Vengeliene et al., 2014). During this procedure, 24 alcohol-preferring FH/Wjd rats were given 10% ethanol and water for 8 weeks. The concentration of 10% was used based on higher daily alcohol consumption in FH/Wjd rats (Li et al., 2014). Rats were randomly divided into 3 groups: one baseline and two groups given a 4d alcohol deprivation ($n = 8/\text{group}$). At 7:30 pm of the last day, the deprivation groups were injected with 0 or 70 mg/kg BNO. The ethanol bottle was returned, and ethanol and water intake were recorded at 24 and 48 h.

2.5. Sucrose two-bottle choice drinking test

Here, a two-bottle choice paradigm was employed to assess whether BNO treatment affects the natural preference for a sucrose solution (Hu et al., 2011). FH/Wjd rats were provided with one bottle containing 0.5%

sucrose and one water bottle. Drinking solutions were replaced and the positions of the bottles were switched daily. Twelve rats (sucrose preference > 60%) were divided into 2 groups (n = 6/group) and administered 0 or 70 mg/kg twice daily (at 8:00 am and 7:30 pm), with sucrose preference monitored every 24 h.

2.6. Acute toxicity test

In this test, a modified Up-and-Down procedure was adopted to determine the acute toxicity of BNO and brucine (Meyer et al., 2005; Xu, 1991). Here, 20 FH/Wjd rats (10 male and 10 female) for each alkaloid were used to measure median lethal dose (LD50) values. Rats were fasted overnight prior to BNO or brucine administration. Rats remaining alive for 48 h after a single dose of BNO or brucine were classified using the term “survival”; otherwise, a classification of “death” was given. LD50 values were calculated based on doses, numbers of survivals and deaths.

2.7. Locomotor activity test

An open-field test was employed to assess the effect of BNO on general locomotor activity (Wen et al., 2012; Li et al., 2014). Thirty-two FH/Wjd rats were separated into 4 groups (n = 8/group). Following injections with 0, 30, 50 or 70 mg/kg BNO, rodents were placed into 4 identical sound-attenuating chambers (49 cm × 49 cm × 54 cm, without ceiling) to

measure locomotor activity for 4 h. Total horizontal distance was monitored and recorded with DigBehv spontaneous activity monitors, and then analyzed using DigBehv software Version 2.0 (Shanghai Jiliang Software Technology Co. Ltd., China). Locomotion was calculated as horizontal traveling distance in 10min bins.

2.8. Conditioned Place Preference (CPP)

CPP measures the ability of a previously novel environment to acquire conditioned reinforcing properties when paired with a putatively rewarding or aversive stimulus, such as a drug, via Pavlovian learning (Childs et al., 2019). Conditioned reward using CPP is oftentimes assessed by the difference in time spent between a drug-paired context and a non-drug context. Here, CPP procedures were utilized to evaluate the conditioned reinforcing profile of BNO. We used an unbiased device comprised of 3 different chambers (a middle chamber of 14 cm×23 cm×20 cm, L×W×H; 2 side chambers of 28 cm×23 cm×20 cm, L×W×H) separated by a retractable guillotine door (Liu et al., 2012; Zhang et al., 2012). The side chambers were defined as distinguishable conditioning rooms with 2 somatosensory cues (visual: 5 radially-arranged or 4 squarely-arranged low-power light bulbs; tactile: stainless steel rod or stainless steel mesh floor). Behavior was monitored by 3 infrared photocells (3 cm above the floor) in each chamber. One day prior to

experiments, rats were placed in the chambers to habituate. Our procedure then proceeded as follows over 10 consecutive days: a 15-min preconditioning session (day 1; drug free), eight 45-min conditioning sessions (day 2 to day 9; drug or saline treatment), and a 15-min test session (day 10; drug free). On day 1, rats were placed into the central compartment of the apparatus without the guillotine doors. The time spent in each compartment was determined to measure their natural place preference (exclusion criteria: time difference >120 s between the period in two side compartments). In total, 30 rats met the inclusion criteria and were separated into 5 groups (n = 6/group). On conditioning days, each rat was trained with alternative injections of saline (unpaired compartment) or drugs (0, 30, 50, and 70 mg/kg BNO or 6 mg/kg morphine; paired compartment) and then immediately placed into the proper compartments. Morphine at 6 mg/kg was used as a positive control to induce a CPP response (Zhang et al., 2012). On day 10, the rats were given access to the entire apparatus, and the ratio of time spent (s) in the drug-paired side to that in the saline-paired side was calculated as the CPP score.

2.8. Statistical Analysis

Data from two-bottle choice drinking paradigms and locomotor activity test were analyzed using repeated measures analysis of variance

(RM-ANOVA) (factors: treatment, day), followed by LSD post-hoc tests. For ADE and CPP tests, unpaired t tests and one-way ANOVA, respectively, were used to analyze the data. Data were expressed as the mean $\pm$ SEM. The level of significance was $p < 0.05$.

3. Results

3.1. BNO treatment reduce ethanol intake and preference

The ethanol two-bottle choice paradigm has been extensively employed to model human alcohol drinking (Rezvani et al., 2002). BNO treatment led to a significant reduction in ethanol intake [$F(3, 32) = 5.88, p < 0.01$] (Fig. 2A) and preference [$F(3, 32) = 3.57, p < 0.05$] (Fig. 2B) during the drug-treatment period. BNO injections at 70 mg/kg significantly inhibited daily voluntary alcohol intake and preference by 22.8% and 14.2%, respectively, during the 7d treatment period. The inhibitory effect of BNO on cumulative ethanol intake was also dose-dependent, as shown in Fig. 3 (50 mg/kg: $p < 0.05$; 70 mg/kg: $p < 0.001$). Total fluid consumption was unchanged [$F(3, 32) = 2.58$, not significant (NS)], while water intake showed a clear dose-dependent trend toward an increase, but no overall statistical significance [$F(3, 32) = 2.78$, NS] (Fig. 2C). Food intake [$F(3, 32) = 2.67$, NS] (Fig. 2D) did not differ between groups. Notably, there was a sustained inhibition of alcohol intake, which remained below the baseline for at least 3 days post BNO administration.

265

266 3.2. BNO treatment inhibits an alcohol deprivation effect

267 A marked enhancement in ethanol intake in alcohol-preferring animals
268 after a period of abstinence is referred to as an alcohol deprivation effect
269 (ADE) (Rezvani et al., 2002; Li et al., 2014). Here, we monitored the
270 drinking behavior in rats after ethanol was reintroduced. We found that
271 acute 70 mg/kg BNO administration inhibited ethanol consumption (Fig.
272 4A; d1: $t(14) = 5.388$, $p < 0.01$; d2: $t(14) = 3.743$, $p < 0.01$) and
273 preference (Fig. 4B; d1: $t(14) = 6.775$, $p < 0.01$; d2: $t(14) = 2.328$, $p <$
274 0.05) in alcohol-preferring FH/Wjd rats, without altering total fluid intake
275 (Fig. 4C; d1: $t(14) = 1.461$, NS; d2: $t(14) = 2.061$, NS), as compared to
276 the saline-treated group. In particular, ethanol consumption in the
277 BNO-treatment group was inhibited by 44.4% (7.17 ± 0.5 g/kg vs 4.0
278 ± 0.38 g/kg), while ethanol preference was attenuated by 34.1% (92.1%
279 $\pm 2.2\%$ vs $58\% \pm 4.9\%$), on the first ADE test (d1). To draw a robust
280 conclusion, the effectiveness of this paradigm was confirmed by the
281 comparisons in ethanol intake (Fig. 4A; d1: $t(14) = -2.548$, $p < 0.05$; d2:
282 $t(14) = -2.97$, $p < 0.05$) and preference (Fig. 4B; d1: $t(14) = -4.243$,
283 $p < 0.01$; d2: $t(14) = -3.922$, $p < 0.01$) between the baseline and
284 saline-treated groups.

285

286 3.3. BNO treatment does not alter preference for a natural reward

Here, we examined whether the effect of BNO treatment on alcohol drinking is specific or extends to natural rewards. BNO treatment did not result in an alteration of sucrose preference ($F(1, 10) = 0.0004$, NS) (Fig. 5), suggesting that the BNO effect on alcohol drinking is alcohol-specific and not a non-specific effect on rewarding stimuli.

3.4 BNO has lower acute toxicity than brucine

Using a modified Up-and-Down procedure, we measured the LD50 value of BNO in rats as 1103.5 ± 177.0 mg/kg, which is lower than that of brucine at 264.6 ± 17.7 mg/kg. These data indicate that BNO has a higher safety profile than brucine, and thus is more appropriate for future study and potential clinical development.

3.5. BNO treatment does not influence locomotor activity

There was no statistical difference among the groups in horizontal locomotor activity either in 10-min bins analyzed using a two-way RM-ANOVA [$F(3, 28) = 0.796$, NS] (Fig. 6) or across the 4 h test using a one-way ANOVA [$F(3, 28) = 0.796$, NS] (Fig.6 inset). These results show that general locomotor activity is not altered by BNO treatment at behaviorally-effective doses.

3.6. BNO does not induce side preference or aversion

Saline- and BNO-treated rats did not differ in CPP scores [$F(3, 20) = 1.401$, NS] (Fig.7). In contrast, as expected, rats administered 6 mg/kg morphine demonstrated a significant CPP ($t(10) = -2.8$, $p < 0.05$), confirming the effectiveness of this paradigm (Zhang et al., 2012). BNO treatment at 30, 50 and 70 mg/kg did not produce a place preference or aversion, suggesting that it has no conditioned rewarding or aversive properties.

4. Discussion

Harmful alcohol use has become a public health issue that urgently requires effective and safe interventions. In this study, we demonstrated that treatment with BNO exerted a significant inhibitory effect on ethanol intake and preference in alcohol-preferring FH/Wjd rats in a dose-dependent fashion. BNO injections at 70 mg/kg (twice daily for 7d) decreased daily ethanol consumption by 22.8% (3.90 ± 0.56 g/kg versus 3.01 ± 0.91 g/kg) and preference by 14.2% ($91.65\% \pm 9.51\%$ versus $77.45\% \pm 21.84\%$) during the drug-treatment period. The current data also indicate that BNO resulted in a specific amelioration of excessive drinking phenotypes, as repeated 70 mg/kg BNO treatment did not impair the natural preference of FH/Wjd rats for sucrose solution. Furthermore, both food and total fluid intake were unaffected by BNO. Importantly, we also did not observe the development of a tolerance effect to repeated

BNO injections during the 7d treatment period. This is in contrast to
acamprosate, which can result in tolerance if administered repeatedly
(Lidö et al., 2012). Our data preliminarily suggest that BNO can be
developed into an effective medication with high specificity and no
known tolerance.

The ADE is characterized by a pronounced but short-term increase in
ethanol consumption in laboratory animals, which is used to mimic
human relapse-like phenotypes with reliable face and predictive validity
(Spanagel et al., 1996; McBride et al., 2002; Rezvani et al., 2002). The
amplitude of ADE is highly correlated with the alcohol sensitivity of
animals, determined by genetic expression (Vengeliene et al., 2014).
Vengeliene et al proposed that rats rather than mice are a more
appropriate system to mimic alcohol relapse drinking and examine the
relapse-relieving properties of candidate compounds, finding that rats
showed stable ADEs after repeated alcohol deprivation, predictable
compulsive drinking phenotypes after long-term alcohol intoxication, and
reliable, not paradoxical, pharmacological effects after certain
interventions compared to mice (Vengeliene et al., 2014). Therefore,
FH/Wjd rats with high alcohol preference provide an appropriate model
to screen the therapeutic compounds for AUD treatment, and to clarify
the underlying neurobiology of relapse. In this study, alcoholic FH/Wjd

353 rats had 8-week alcohol access and stable intake prior to alcohol
354 deprivation. We demonstrated that a single 70 mg/kg BNO administration
355 resulted in a significant inhibition of ethanol relapse phenotypes
356 (consumption by 44.4% and preference by 34.1%) on d1 of re-exposure.
357 These data demonstrate that BNO may have anti-relapse effects, which is
358 critical in the development of pharmacotherapies for AUDs.

359
360 Based on our findings, we predict that BNO medications may have low
361 toxicity and few side effects. We determined that the LD50 of BNO was
362 significantly less than that of brucine, measured using a modified
363 Up-and-Down procedure (Xu, et al., 1991; Meyer et al., 2005).
364 Comparatively, the effective doses are approximately 70 mg/kg and 30
365 mg/kg, for BNO and brucine, respectively, in alcohol-preferring FH/Wjd
366 rats. Thus, BNO has much lower toxicity and a wider therapeutic window
367 compared to brucine (Chen et al., 2013; Li et al., 2014). In addition,
368 spontaneous locomotor activity in rats was not impaired by BNO at doses
369 effective in inhibiting alcohol drinking, demonstrating that the
370 suppression of alcohol drinking behavior cannot be ascribed to motor
371 function impairment or sedation (Li et al., 2014). Finally, the CPP
372 paradigm was used to evaluate the conditioned reinforcing effect of BNO
373 in rats (Liu et al., 2012; Li et al., 2014). We found that BNO treatment at
374 70 mg/kg, an effective dose as demonstrated above, did not result in

either preference or aversion for the BNO-paired compartment, preliminarily suggesting that the effects on alcohol drinking likely do not result from interoceptive properties of BNO.

The metabolism of BNO appears to be complicated, as it is reported that BNO and brucine can transform into each other in circulating blood. BNO as a tertiary amine can be enzymatically dealkylated and structurally decomposed to secondary amines, while a proportion of BNO can be metabolically reduced into brucine by the enzyme aldehyde oxidase. Retrogradely, brucine can also be oxidized into BNO, catalyzed by cytochrome P450 and the monooxygenase with flavin, which is a critical detoxification method of alkaloids (Takekawa et al., 1997, 2001). *Chen* et al discovered that 65.4% brucine could be readily metabolized into the main metabolite BNO in rat liver tissue in a 2h incubation period via an *in vitro* metabolism study (Chen et al., 2012). Pharmacologically, BNO rather than brucine should be the main existent form for both brucine and BNO in circulating blood, as BNO is highly hydrophilic and soluble, and has a longer elimination period (Bickel et al., 1971; Ziegler & Gold, 1971; Rose & Castagnoli, 1983). Our speculation is that both BNO and brucine are active ingredients in ameliorating alcohol drinking phenotypes, because the critical structure that plays an active part in pharmacological functions are identical, while the coordinated oxygen

atom in the chemical structure of BNO failed to modify pharmacological activity, but lowered toxicity and increased hydrophilicity.

The mechanism of BNO to relieve alcohol drinking phenotypes remains to be elucidated. Both BNO and brucine can pass the brain blood barrier, and thus it is likely that their effects are exerted in the CNS (Cai et al., 2009). We propose that the effect of BNO is exerted via inhibitory glycine receptors, a type of inhibitory receptors extensively distributed in spinal cord and brain stem. *Gallegos et al* demonstrated that glycine receptors in dopamine receptor D1 receptor (D1R) expressed in medium spiny neurons are sensitive to small quantities of ethanol and further modulate the activity of nucleus accumbens, even at 5 mM (Gallegos et al., 2019). Meanwhile, the structure Loop 2 of a glycine receptor is considered a key structure and determines sensitivity to ethanol (Eggers & Berger, 2004; Crawford et al., 2007; Perkins et al., 2012). In addition, homomeric $\alpha 1$ or $\alpha 2$ GlyRs in *Xenopus* oocytes may be potentiated, while the activities of GlyR can be enhanced through prolonging the burst duration by ethanol (Mascia et al., 1996; Welsh et al., 2009; San Martin et al., 2016). Furthermore, BNO and brucine antagonize the $\alpha 1$ and $\alpha 1\beta$ glycine receptors with K_i values of 1.4 and 1.7 $\mu\text{mol/L}$, respectively, in human embryonic kidney 293 cells (Jensen et al., 2006). It is postulated that BNO and the metabolite brucine can competitively antagonize the

glycine receptors to reduce ethanol-induced dopamine release in the nucleus accumbens, and normalize the maladaptive signaling in reward circuitry (Li et al., 2014). It has been found that the elimination half-life ($t_{1/2}$) of BNO and brucine in the blood are 5.7 and 1.04 h, respectively, in rats (Xu et al., 2003; Chen et al., 2009; Chen et al., 2011). Obviously, the post-treatment effect is attributed to the reparation of damaged circuits or the normalization of the neurotransmitter system by the BNO and brucine (Fig.2). However, the underlying neurobiology seems much more complex and awaits a deeper exploration, as some present findings appear in part contradictory to the proposed mechanism, i.e. that bilateral accumbal microinfusion of glycine reduced alcohol acquisition in Wistar rats (Molander et al, 2005). This result likely reflects the intense activation of NMDA receptors by overwhelmingly enhanced concentrations of glycine in the mesolimbic dopamine system, as glycine is a prerequisite co-agonist for the activation of NMDA receptors, which have a much higher density than glycine receptors (Irimia et al, 2017).

In summary, BNO selectively reduces alcohol drinking behavior in alcohol-preferring FH/Wjd rats, and is a promising candidate medication with higher safety, greater water solubility, longer elimination half-life and a larger therapeutic window. The homologue class of brucine may be a potential family for alcoholism therapy.

441

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448 **References**

449 Bagnardi V, Blangiardo M, La Vecchia C, Corrao G (2001) A
450 meta-analysis of alcohol drinking and cancer risk. *Brit J Cancer*
451 85:1700-1705.

452 Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, Scotti
453 L, Jenab M, Turati F, Pasquali E, Pelucchi C, Bellocco R, Negri E,
454 Corrao G, Rehm J, Boffetta P, La Vecchia C (2013) Light alcohol
455 drinking and cancer: a meta-analysis. *Ann Oncol* 24:301-308.

456 Bali A, Randhawa PK, Jaggi AS (2015) Stress and opioids: role of
457 opioids in modulating stress-related behavior and effect of stress on
458 morphine conditioned place preference. *Neurosci Biobehav Rev*
459 51:138-50.

460 Bickel MH, Willi P, Gigon PL (1971) Species and substrate differences
461 of liver microsomal N-dealkylation and N-oxidation of tertiary
462 amines and N-dealkylation of N-oxides. *Xenobiotica* 1(4):533-4.

- Cai BC, Xu XY, Pan Y, Wang TS, Li YC (2009) Tissue distribution of strychnos nux-vomica alkaloid in rats. *Chin Pharmacol Bull* 20(4):421-424.
- Chen J, Hu W, Cai BC, Lou CH, Wang LJ, Wang W (2009). Pharmacokinetics of Brucine in rats. *Chin Pharm J* 44(10):778-780.
- Chen J, Hou T, Fang Y, Chen ZP, Liu X, Cai H, Lu T, Yan GJ, Cai BC (2011) HPLC determination of strychnine and brucine in rat tissues and the distribution study of processed semen strychni. *Yakugaku zasshi* 131:721-729.
- Chen J, Hu W, Qu YQ, Dong J, Gu W, Gao Y, Fang Y, Fang F, Chen ZP, Cai BC (2013) Evaluation of the pharmacodynamics and pharmacokinetics of brucine following transdermal administration. *Fitoterapia* 86:193-201.
- Chen X, Lai Y, Cai Z (2012) Simultaneous analysis of strychnine and brucine and their major metabolites by liquid chromatography-electrospray ion trap mass spectrometry. *J Anal Toxicol* 36(3):171–176.
- Childs JE, K Driskill CM, Hsiu E, Kroener S (2019) Vagus nerve stimulation during extinction learning reduces conditioned place preference and context-induced reinstatement of cocaine seeking. *Brain Stimul* 12(6):1448–1455.
- Crawford DK, Trudell JR, Bertaccini EJ, Li K, Davies DL, Alkana RL

(2007) Evidence that ethanol acts on a target in Loop 2 of the extracellular domain of alpha1 glycine receptors. J Neurochem 102:2097-2109.

Croop R, Faulkner E, Labriola D (1997) The safety profile of naltrexone in the treatment of alcoholism. Results from a multicenter usage study. The Naltrexone Usage Study Group. Arch Gen Psychiatry 54:1130-1135.

Eggers ED, Berger AJ (2004) Mechanisms for the modulation of native glycine receptor channels by ethanol. J Neurophysiol 91:2685-2695.

Eisenhardt M, Leixner S, Luján R, Spanagel R, Bilbao A (2015) Glutamate Receptors within the Mesolimbic Dopamine System Mediate Alcohol Relapse Behavior. J Neurosci 35:15523-38.

Gallegos S, Muñoz B, Araya A, Aguayo LG (2019) High ethanol sensitive glycine receptors regulate firing in D1 medium spiny neurons in the nucleus accumbens. Neuropharmacol 160:107773.

Hu W, Lu T, Chen A, Huang Y, Hansen R, Chandler LJ, Zhang HT (2011) Inhibition of phosphodiesterase-4 decreases ethanol intake in mice. Psychopharmacol 218:331-339.

Irimia C, Buczynski MW, Natividad LA, Laredo SA, Avalos N, Parsons LH (2017) Dysregulated Glycine Signaling Contributes to Increased Impulsivity during Protracted Alcohol Abstinence. J Neurosci 37(7):1853–1861.

507 Jensen AA, Gharagozloo P, Birdsall NJ, Zlotos DP (2006)
508 Pharmacological characterisation of strychnine and brucine analogues
509 at glycine and $\alpha 7$ nicotinic acetylcholine receptors. *Eur J*
510 *Pharmacol* 539:27–33.

511 Jupp B, Lawrence AJ (2010) New horizons for therapeutics in drug and
512 alcohol abuse. *Pharmacol Ther* 125:138-168.

513 Kaaber K, Menne T, Veien NK, Baadsgaard O (1987) Some adverse
514 effects of disulfiram in the treatment of nickel-allergic patients. *Derm*
515 *Beruf Umwelt* 35(6):209–211.

516 Li Y, Liu Q, Gong Q, Li JX, Wei SP, Wang YT, Liang H, Zhang M, Jing
517 L, Yong Z, Lawrence AJ, Liang JH (2014) Brucine suppresses
518 ethanol intake and preference in alcohol-preferring Fawn-Hooded
519 rats. *Acta Pharmacol Sin* 35:853-861.

520 Lidö HH, Marston H, Söderpalm B (2012) The Glycine Reuptake
521 Inhibitor Org24598 and Acamprosate Reduce Ethanol Intake in the
522 Rat; Tolerance Development to Acamprosate but Not to Org24598.
523 *Addict Biol* 17(5):897-907.

524 Liu Q, Zhang M, Qin WJ, Wang YT, Li YL, Jing L, Li JX, Lawrence AJ,
525 Liang JH (2012). Septal nuclei critically mediate the development of
526 behavioral sensitization to a single morphine injection in rats. *Brain*
527 *Res* 1454:90-99.

528 Mascia MP, Machu TK, Harris RA (1996) Enhancement of homomeric

glycine receptor function by long-chain alcohols and anaesthetics. *Br J Pharmacol* 119(7):1331–1336.

McBride WJ, Le AD, Noronha A (2002) Central nervous system mechanisms in alcohol relapse. *Alcohol Clin Exp Res* 26:280-286.

Meyer SA, Marchand AJ, Hight JL, Roberts GH, Escalon LB, Inouye LS, MacMillan DK (2005) Up-and-down procedure (UDP) determinations of acute oral toxicity of nitroso degradation products of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *J Appl Toxicol* 25:427-434.

Molander A, Löf E, Stomberg R, Ericson M, Söderpalm B (2005) Involvement of accumbal glycine receptors in the regulation of voluntary ethanol intake in the rat. *Alcohol Clin Exp Res* 29:38–45.

Pandey SC, Roy A, Zhang H, Xu T (2004) Partial deletion of the cAMP response element-binding protein gene promotes alcohol-drinking behaviors. *J Neurosci* 24:5022-5030.

Perkins DL, Trudell JR, Asatryan L, Davies DL, Alkana RL (2012) Charge and geometry of residues in the loop 2 beta hairpin differentially affect agonist and ethanol sensitivity in glycine receptors. *J Pharmacol Exp Ther* 341:543-551.

Rezvani AH, Parsian A, Overstreet DH (2002) The Fawn-Hooded (FH/Wjd) rat: a genetic animal model of comorbid depression and alcoholism. *Psychiatric genetics* 12:1-16.

- 551 Rose J, Castagnoli N Jr (1983) The metabolism of tertiary amines. Med
552 Res Rev 3(1):73-88.
- 553 San Martin L, Cerda F, Jin C, Jimenez V, Yevenes GE, Hernandez T,
554 Nova D, Fuentealba J, Aguayo LG, Guzman L (2016) Reversal of
555 Ethanol-induced Intoxication by a Novel Modulator of G β γ Protein
556 Potentiation of the Glycine Receptor. J Biol Chem,
557 91(36):18791-18798.
- 558 Spanagel R, Holter SM, Allingham K, Landgraf R, Zieglgansberger W
559 (1996) Acamprosate and alcohol: I. Effects on alcohol intake
560 following alcohol deprivation in the rat. Eur J Pharmacol 305:39-44.
- 561 Takekawa K, Sugihara K, Kitamura S, Ohta S (2001) Enzymatic and
562 non-enzymatic reduction of brucine N-oxide by aldehyde oxidase and
563 catalase. Xenobiotica 31:769-782.
- 564 Takekawa K, Sugihara K, Kitamura S, Tatsumi K (1997) Nonenzymatic
565 reduction of brucine N-oxide by the heme group of cytochrome P450.
566 Biochem Mol Biol Int 42:977-981.
- 567 Talamini R, Bosetti C, La Vecchia C, Dal Maso L, Levi F, Bidoli E,
568 Negri E, Pasche C, Vaccarella S, Barzan L, Franceschi S (2002)
569 Combined effect of tobacco and alcohol on laryngeal cancer risk: a
570 case-control study. Cancer Causes Control 13:957-964.
- 571 Turati F, Garavello W, Tramacere I, Pelucchi C, Galeone C, Bagnardi V,
572 Corrao G, Islami F, Fedirko V, Boffetta P, La Vecchia C, Negri E

(2013) A meta-analysis of alcohol drinking and oral and pharyngeal cancers: results from subgroup analyses. *Alcohol Alcoholism* 48:107-118.

Vengeliene V, Bilbao A, Spanagel R (2014) The alcohol deprivation effect model for studying relapse behavior: a comparison between rats and mice. *Alcohol* 48:313-20.

Welsh BT, Goldstein BE, Mihic SJ. (2009) Single-channel analysis of ethanol enhancement of glycine receptor function. *J Pharmacol Exp Ther* 330(1):198–205.

Wen RT, Zhang M, Qin WJ, Liu Q, Wang WP, Lawrence AJ, Zhang HT, Liang JH (2012) The phosphodiesterase-4 (PDE4) inhibitor rolipram decreases ethanol seeking and consumption in alcohol-preferring Fawn-Hooded rats. *Alcohol Clin Exp Res* 36:2157-2167.

Witkiewitz K, Saville K, Hamreus K (2012) Acamprosate for treatment of alcohol dependence: mechanisms, efficacy, and clinical utility. *Ther Clin Risk Manag* 8:45-53.

Williams CS, Woodcock KR (2000) Do ethanol and metronidazole interact to produce a disulfiram-like reaction? *Ann Pharmacother* 34(2):255-7.

World Health Organization (2018) Global status report on alcohol and health. Geneva: World Health Organization.

Xu SY, Bian RL, Chen X (1991) Experimental Methodology of

Pharmacology. 2nd ed. People's Medical Publishing House, Beijing.

Xu XY, Cai BC, Pan Y., Wang TS (2003) Pharmacokinetics of the alkaloids from the processed seeds of *Strychnos nux-vomica* in rats. *Acta Pharmacol Sin* 38: 458-461.

Yin W, Wang TS, Yin FZ, Cai BC (2003) Analgesic and Anti-Inflammatory Properties of Brucine and Brucine N-oxide Extracted From Seeds of *Strychnos Nux-Vomica*. *J Ethnopharmacol* 88 (2-3): 205-14.

Zhang M, Jing L, Liu Q, Wen RT, Li JX, Li YL, Gong Q, Liang JH (2012) Tramadol induces conditioned place preference in rats: interactions with morphine and buprenorphine. *Neurosci Lett* 520: 87-91.

Ziegler DM, Gold S (1971) Oxidative Metabolism of Tertiary Amine Drugs by Human Liver Tissue. *Xenobiotica* 1: 4-5, 325-326

Znaor A, Brennan P, Gajalakshmi V, Mathew A, Shanta V, Varghese C, Boffetta P (2003) Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. *Int J Cancer* 105: 681-686.

Figure Legends

Figure 1 Chemical structures of brucine (a) and brucine N-oxide (b).

Figure 2 The effects of BNO treatment at 0, 30, 50 and 70 mg/kg (7 d, s.c., bid) on ethanol consumption, preference, water and food intake in alcohol-preferring FH/Wjd rats. (A and B) During the period of BNO injections, ethanol consumption and preference were significantly inhibited by BNO injections in rats ($n = 9$). (C and D) The increase in water intake was almost significant, though there was no significant overall effect, while food intake during BNO treatment did not differ among the groups. Test phases shown from left to right in the figure: the saline phase (day 1-3), the drug-treatment phase (day 4 - 10) and the post-treatment phase (day 11 - 13). * $p < 0.05$, ** $p < 0.01$ versus the saline group.

Figure 3 The effects of BNO treatment on the cumulative ethanol intake during the 7d injections in alcohol-preferring FH/Wjd rats. BNO treatment inhibited the cumulative ethanol consumption in rats ($n = 9$). * $p < 0.05$, *** $p < 0.001$ versus the saline group.

Figure 4 The effect of BNO treatment at 70 mg/kg (s.c.) on deprivation-induced ethanol consumption, preference and total fluid intake in alcohol-preferring FH/Wjd rats. Ethanol and water intake were monitored on d1 and d2 after the re-introduction. (A) 70 mg/kg BNO reduced ethanol intake on d1 and d2 compared to the saline group, while there was a significant ADE after a period of 4d deprivation in saline-treated rats ($n = 8$). (B) 70 mg/kg BNO

significantly reduced alcohol preference on d1 and d2 in rats. (C) The total amount of fluid intake did not differ among the groups. * $p < 0.05$ and ** $p < 0.01$ versus the saline group.

Figure 5 The effect of 70 mg/kg BNO (7 d, s.c., bid) on sucrose preference in FH/Wjd rats. BNO treatments did not change sucrose preference in rats ($n = 6$). Test phases shown from left to right in the figure: the saline phase (day 1- 3), the drug-treatment phase (day 4 - 10) and the post-treatment phase (day 11 - 13).

Figure 6 The effect of BNO treatment at 0, 30, 50 and 70 mg/kg (s.c.) on locomotor activity in FH/Wjd rats. BNO treatment did not alter spontaneous locomotor activity in rats ($n = 8$).

Figure 7 The effect of BNO treatment at 0, 30, 50 and 70 mg/kg (s.c.) on the CPP scores in FH/Wjd rats. BNO injections did not induce rewarding or aversive effects in rats ($n = 6$). Saline and morphine groups were used as control groups. ** $p < 0.01$ versus the saline group.

a

b

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