Baclofen and naltrexone, but not N-acetylcysteine, affect voluntary alcohol drinking in rats regardless of individual levels of alcohol intake

A. Maryse Minnaard\textsuperscript{a}, Geert M.J. Ramakers\textsuperscript{b}, Louk J.M.J. Vanderschuren\textsuperscript{a} and Heidi M.B. Lesscher\textsuperscript{a}

In humans, there is profound individual variation in the risk of alcohol use disorder (AUD). Because GABA, opioid and glutamate neurotransmission have been implicated in AUD, functional differences in these neural systems may underlie the individual vulnerability to AUD. We therefore determined the effects of drugs affecting GABA, opioid and glutamatergic neurotransmission on alcohol consumption in rats that differed in baseline alcohol intake. Subgroups of low-, medium- and high-alcohol-drinking rats were selected on the basis of alcohol consumption using an intermittent alcohol access procedure. The subgroups were treated with the GABA\textsubscript{B} receptor agonist baclofen, the opioid receptor antagonist naltrexone and the cysteine precursor N-acetylcysteine, and the effects on alcohol intake and preference were determined. Both baclofen and naltrexone reduced alcohol consumption, but N-acetylcysteine did not. These effects were comparable for low-, medium- and high-alcohol-drinking rats. However, there was a substantial degree of individual variation in the responsivity to baclofen and naltrexone, across the subgroups. Taken together, these results suggest that variation in alcohol consumption does not predict the responsivity to baclofen and naltrexone. This implies that individual variability in alcohol consumption on the one hand and sensitivity to treatment with these drugs on the other hand represent separate processes that likely involve distinct biological mechanisms. Behavioural Pharmacology 32: 251–257

Introduction

Alcohol use disorder (AUD) is a chronic relapsing disorder with major medical and socioeconomic consequences (Rehm \textit{et al.}, 2009; Connor \textit{et al.}, 2016). Treatment options for AUD include cognitive behavioural therapy, pharmacological treatment and social support strategies. However, treatment efficacies vary and short-term relapse after treatment is common (Miller \textit{et al.}, 2001; Moos and Moos, 2006). The quantity and pattern of alcohol intake varies widely between individuals, whereby only a minority develops an AUD. To study the neural processes that underlie individual differences in alcohol intake and AUD, animal models that capture the individual variability in alcohol consumption can be employed, such as intermittent alcohol access (IAA) in rodents (e.g. Simms \textit{et al.}, 2008; Momeni and Roman, 2014). We have previously described profound individual differences in alcohol intake and motivation in outbred rats after 2 months of IAA, whereby subgroups of low-, medium- and high-alcohol-drinking rats could be distinguished (Spoelder \textit{et al.}, 2015). Importantly, high-alcohol drinking rats display a typical AUD-like phenotype as they showed more motivation to obtain alcohol and more punishment-resistant alcohol-directed behaviour than low-alcohol-drinking rats (Spoelder \textit{et al.}, 2015, 2017).

Alcohol affects multiple neurotransmitter systems involved in emotion, cognition and motivation. Alcohol increases GABAergic activity in the brain (Roberto \textit{et al.}, 2003) and induces the release of endogenous opioids (Olive \textit{et al.}, 2001). Furthermore, alcohol can inhibit glutamatergic neurotransmission through \textit{N}-methyl-d-aspartate (NMDA) and mGlur5 metabotropic glutamate receptors (Tsai \textit{et al.}, 1995; Gonzales and Jaworski, 1997). In line with alcohol’s mechanisms of action, compounds acting on GABAergic, opioid or glutamate systems, such as the GABA\textsubscript{B} receptor agonist baclofen, the opioid receptor antagonist naltrexone and the cysteine

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.behaviouralpharm.com.

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

0955-8810 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.

DOI: 10.1097/FBP.0000000000000615

Copyright © 2020 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.
respectively, would differentially affect alcohol consumption, baclofen, naltrexone or signalling contribute to individual variation in alcohol consumption under IAA conditions in rats. To that aim, the effects of these drugs on voluntary alcohol consumption in relation to individual differences in alcohol consumption under IAA conditions in rats. We hypothesised that, the effects of these drugs on voluntary alcohol consumption were assessed in subpopulations of low-, medium- and high-alcohol-drinking rats. We hypothesised that, if variations in GABAergic, opioid or glutamatergic neurotransmission, have received attention as possible treatment options for AUD (van den Brink, 2012). Preclinical studies have shown that baclofen, naltrexone and N-acetylcysteine reduce alcohol consumption and motivation for alcohol, supporting their potential for the treatment of AUD (e.g. Colombo et al., 2000; Coonfield et al., 2004; Simms et al., 2008; Lebourgeois et al., 2018, 2019). However, clinical studies show large individual variation in the treatment response to these compounds in AUD patients (e.g. Addolorato et al., 2007; Kiefer et al., 2008; Garbutt et al., 2010; Müller et al., 2015; Beraha et al., 2016).

The aim of this study was to determine the effects of baclofen, naltrexone and N-acetylcysteine on alcohol drinking in relation to individual differences in alcohol consumption under IAA conditions in rats. To that aim, the effects of these drugs on voluntary alcohol consumption were assessed in subpopulations of low-, medium- and high-alcohol-drinking rats. We hypothesised that, if variations in GABAergic, opioid or glutamatergic signalling contribute to individual variation in alcohol consumption, baclofen, naltrexone or N-acetylcysteine, respectively, would differentially affect alcohol consumption in low-, medium- and high-alcohol-drinking rats.

Methods

Subjects

Fifty adult male Lister Hooded rats (Charles River, Germany) were used, weighing 200–250 g at the study onset. For details on housing conditions, see Spoelder et al. (2015, 2016, 2017). Experimental procedures were approved by the Central Authority for Scientific Procedures on Animals and were conducted in accordance with Dutch (Wet op de Dierproeven, 2014) and European legislation (Guideline 86/609/EEC; Directive 2010/63/EU).

Alcohol consumption

IAA procedures were used as previously described (e.g. Spoelder et al., 2015). In the first 4 weeks of IAA, alcohol exposure sessions commenced 2–3h into the dark cycle and lasted for 7h. Sessions were subsequently extended to 24h in the following months. Alcohol intake (g/kg) and alcohol preference (%) were calculated per rat per session. After 2 months, rats were ranked on the basis of the animals’ average alcohol intake (g/kg) per week and were assigned ranking scores. A total ranking score was computed, as the sum of the weekly ranking scores. Rats within the lower and upper 24% of the total ranking score ranges were selected as low- and high-alcohol-drinking rats (low drinking, n = 12; high drinking, n = 12), respectively. From the remaining group, rats that listed within the median 24% of the total population at least three times out of the eight weekly ranking scores were selected as medium-alcohol-drinking rats (medium drinking, n = 16) (Supplementary Figure 1, Supplemental digital content 1, http://links.lww.com/BPHARM/A62).

Drugs

Alcohol (99.5%; Klinipath, The Netherlands) was diluted to 20% (v/v) in tap water once per week. Baclofen (RS)-4-amino-3-(4-chlorophenyl)butanoic acid; Tocris Bioscience, UK) was administered intraperitoneally (i.p. 0, 0.3, 1 and 3 mg/kg) (based on, e.g., Colombo et al., 2003), 30 min before the alcohol-drinking sessions. Naltrexone hydrochloride [(5x)-17-(cyclopentylmethyl)-4,5-epoxy-3,14-dihydromorphan-6-one hydrochloride, Abcam, UK] was administered subcutaneously (s.c. 0, 0.3 and 1 mg/kg) (based on, e.g., Simms et al., 2008; Daoura and Nylander, 2011), 30 min before the alcohol-drinking sessions. N-Acetylcysteine [N-acetyl-L-cysteine (2R)-2-(acyethylamino)-3-sulfanylpropionic acid); Sigma-Aldrich, The Netherlands] was administered i.p. after adjustment to pH 7.4 at 0, 25, 50 and 100 mg/kg (based on, e.g., Lebourgeois et al., 2018, 60 min before the alcohol-drinking sessions. All drugs were dissolved in sterile saline (0.9% NaCl) and injected at 1 ml/kg.

Experimental procedure

All rats received a saline injection 1 week before drug testing started to habituate them to the injection procedure. All rats received all doses according to a within-subject Latin square design per drug. The rats were first treated with baclofen, followed by naltrexone and N-acetylcysteine. Alcohol and water bottles were weighed before each session and 2, 7 and 24h after session onset. Each treatment session was always followed by at least 1 day without alcohol access and at least one drug-free 24h alcohol consumption session between treatment sessions for the same drug. There were at least three drug-free 24h alcohol consumption sessions between different drugs. Naltrexone was administered with a 1-week washout period between each injection to circumvent carry-over effects (Daoura and Nylander, 2011).

Data analysis and statistics

The treatment effects on alcohol consumption were analysed using three-way repeated measures analysis of variance tests with dose and time (2, 7 and 24h) as within-subject variables and group (low-, medium- and high-alcohol-drinking rats) as the between-subject variable. When appropriate, post hoc analyses were performed using pairwise comparisons with a Bonferroni correction. Mauchly’s test of sphericity was used to test whether variances of the differences between levels were equal. If the assumption of sphericity was violated, degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity or Huynh–Feldt estimates of sphericity when the Greenhouse–Geisser estimate was >0.75.

Data were analysed and visualised using Microsoft Excel, GraphPad Prism (version 8.3.0, GraphPad Software Inc.,
San Diego, California, USA) and SPSS for Windows (version 25.0.0.1, IBM Corp., Armonk, New York, USA). Results are presented as mean±SEM unless otherwise stated and a significance criterion of \( P<0.05 \), two-tailed, was used.

**Results**

**Alcohol consumption after treatment with baclofen, naltrexone and \( N \)-acetylcysteine**

Baclofen decreased alcohol intake (\( F_{(3,105)}^{\text{dose}} = 16.812; \ P<0.001 \), dependent on the time in the session (\( F_{(4,134)}^{\text{dose} \times \text{time}} = 3.747; \ P=0.007 \)) (Fig. 1a) and with a near-significant group \( \times \) baclofen interaction (\( F_{(6,105)}^{\text{dose} \times \text{group}} = 2.127; \ P=0.056 \)). Post hoc analyses revealed that alcohol intake was reduced after treatment with the highest dose of baclofen (3.0 mg/kg) at all three timepoints (\( P<0.001 \)). There was a trend toward a reduction in alcohol preference upon baclofen treatment (\( F_{(3,111)}^{\text{dose}} = 0.079; \ P=0.754; \)) (Fig. 1c). There were also no effects of \( N \)-acetylcysteine on alcohol preference (\( F_{(3,111)}^{\text{dose}} = 0.079; \ P=0.971; \)) (Fig. 1c). There were also no effects of \( N \)-acetylcysteine on alcohol preference (\( F_{(3,111)}^{\text{dose}} = 0.079; \ P=0.971; \)) (Fig. 1c).

Naltrexone caused an overall decrease in alcohol intake (\( F_{(2,74)}^{\text{dose}} = 3.773; \ P=0.028 \)) (Fig. 1b). Post hoc analyses showed that alcohol intake was reduced after treatment with 1.0 mg/kg naltrexone (\( P=0.038 \)). The effects of naltrexone on alcohol intake were independent of the time in the session (\( F_{(3,99)}^{\text{dose} \times \text{time}} = 1.488; \ P=0.236 \)) and independent of group (\( F_{(6,105)}^{\text{dose} \times \text{group}} = 0.853; \ P=0.496; \)) (\( F_{(5,99)}^{\text{dose} \times \text{group}} = 0.900; \ P=0.490 \)). Naltrexone did not alter alcohol preference in any of the groups at any of the timepoints tested (\( F_{(2,74)}^{\text{dose}} = 0.488; \ P=0.616; \)) (\( F_{(3,96)}^{\text{dose}} = 0.438; \ P=0.697; \)) (\( F_{(4,74)}^{\text{dose}} = 0.297; \ P=0.879; \)) (\( F_{(5,96)}^{\text{dose} \times \text{group}} = 1.181; \ P=0.324 \)) (Supplementary Figure 2b, Supplemental digital content 1, [http://links.lww.com/BPHARM/A62](http://links.lww.com/BPHARM/A62)).

\( N \)-Acetylcysteine did not affect alcohol intake in any of the groups at any of the timepoints tested (\( F_{(3,111)}^{\text{dose}} = 0.399; \ P=0.754; \)) (\( F_{(4,143)}^{\text{dose} \times \text{time}} = 0.335; \ P=0.848; \)) (\( F_{(6,111)}^{\text{dose} \times \text{group}} = 1.488; \ P=0.189; \)) (\( F_{(8,143)}^{\text{dose} \times \text{group}} = 0.961; \ P=0.468 \)) (Fig. 1c). There were also no effects of \( N \)-acetylcysteine on alcohol preference (\( F_{(3,111)}^{\text{dose}} = 0.079; \ P=0.971; \)) (\( F_{(4,156)}^{\text{dose} \times \text{time}} = 0.228; \ P=0.930; \)) (\( F_{(6,141)}^{\text{dose} \times \text{group}} = 1.803; \ P=0.105; \)) (\( F_{(8,156)}^{\text{dose} \times \text{group}} = 0.966; \ P=0.467 \)) (Supplementary Figure 2c, Supplemental digital content 1, [http://links.lww.com/BPHARM/A62](http://links.lww.com/BPHARM/A62)).

Similar responsivities to baclofen, naltrexone and \( N \)-acetylcysteine for low-, medium- and high-alcohol-drinking rats do not preclude individual variation in the effects of these compounds on alcohol consumption. Therefore, we performed an additional analysis on the difference in alcohol intake between vehicle and the highest dose for each drug per individual rat (Fig. 2, 2 h data). These individual data revealed...
substantial variation in the degree to which baclofen and naltrexone reduced alcohol intake. Analysis of the variance between the groups confirmed equal variances across the groups for the effects of baclofen \((P = 0.202)\), naltrexone \((P = 0.417)\) and \(N\)-acetylcysteine \((P = 0.079)\).

**Discussion**

We compared the effects of the GABA\(_B\) receptor agonist baclofen, the opioid receptor antagonist naltrexone and the cysteine precursor \(N\)-acetylcysteine on alcohol consumption, in subgroups of rats that consumed low, medium, or high levels of alcohol. Treatment with baclofen and naltrexone, but not \(N\)-acetylcysteine, reduced alcohol intake. The effects of baclofen and naltrexone on alcohol consumption were comparable in low-, medium- and high-alcohol-drinking rats, indicating that individual differences in alcohol intake are not associated with differences in sensitivity to these drugs. However, there was substantial variation in the level of response to baclofen and naltrexone between individual animals. Together, these data imply that the individual variation in responsivity to these compounds and the level of alcohol consumption represent independent processes.
Baclofen

The GABA<sub>B</sub> receptor agonist baclofen reduced alcohol intake by ~25% and caused a trend towards a reduction in alcohol preference. These findings are in line with other preclinical studies that reported baclofen-induced reductions in alcohol consumption (Daoust et al., 1987; Colombo et al., 2000; Walker & Koob, 2007) and reinforcement in rats (Anstrom et al., 2003; Janak and Gill, 2003). Open-label clinical studies support the potential of baclofen to reduce alcohol craving and intake in alcohol-dependent individuals (Addolorato et al., 2000; Flannery et al., 2004). However, results from randomised clinical trials are inconsistent (Addolorato et al., 2002; Garbutt et al., 2010; Müller et al., 2015; Beraha et al., 2016) and variability in the effects of baclofen on alcohol consumption has also been reported across rat strains (Maccioni et al., 2012). Here, the suppressing effect of baclofen on alcohol intake was similar across subpopulations of low-, medium- and high-alcohol-consuming rats, suggesting that GABA<sub>B</sub>-mediated neurotransmission is not likely to contribute to individual differences in alcohol intake. Alcohol-prefering and nonprefering were previously shown to display differences in GABA<sub>B</sub> receptor function (Castelli et al., 2005), suggesting that GABA<sub>B</sub> receptors may contribute to variation in alcohol intake. However, in this latter study, the differences in GABA<sub>B</sub> receptor function disappeared after 1 month of alcohol consumption. Therefore, it is conceivable that initial differences in the GABA<sub>B</sub> receptor function might contribute to the emergence but not the maintenance of individual differences in alcohol intake.

Naltrexone

The opioid receptor antagonist naltrexone reduced alcohol intake (on average by 33%), without affecting alcohol preference. These findings are in line with previous reports for a variety of rat strains (Coonfield et al., 2004; Simms et al., 2008; Momeni et al., 2015). Moreover, clinical trials showed naltrexone-induced reductions in alcohol drinking, craving and relapse rates (O'Malley et al., 1992; Volpicelli et al., 1992; Heinälä et al., 2001). However, there is substantial individual variation in the effects of naltrexone in AUD patients (e.g. Kiefer et al., 2008), which has been related to variations in the µ-opioid receptor gene (Barr et al., 2010; Valderrama et al., 2010; Bilbao et al., 2015; Henderson-Redmond et al., 2018). Indeed, alcohol-prefering rats express µ-opioid receptors at higher levels in the VTA, nucleus accumbens and prefrontal cortex when compared to alcohol nonpreferring rats (de Waele et al., 1995; McBride et al., 1998; Marinelli et al., 2000). However, the similarity in the response to naltrexone across subpopulations of low-, medium- and high-alcohol-drinking rats suggests that µ-opioid receptors do not contribute to individual differences in alcohol intake.

N-Acetylcysteine

The cysteine precursor N-acetylcysteine did not affect alcohol intake and preference in the current study. This is in contrast to reports that show reduced alcohol self-administration upon treatment with N-acetylcysteine in rats (Quintanilla et al., 2016; Lebourgeois et al., 2018, 2019) and reduced alcohol-related behaviours in clinical samples (Back et al., 2016; Squeglio et al., 2018), although negative findings of N-acetylcysteine on alcohol use in humans have also been reported (Stoops et al., 2020). The discrepancy between our findings and other (pre)clinical reports may be related to methodological differences, in particular voluntary home-cage consumption versus operant self-administration.

Limitations

During the course of this study, all subgroups gradually increased alcohol intake and alcohol preference. A block design in which the three compounds were tested consecutively (first baclofen, then naltrexone and finally N-Acetylcysteine) was applied to limit the impact of increasing levels of alcohol intake on the variability in the data within each treatment block. N-Acetylcysteine was shown to suppress alcohol intake, also after prolonged alcohol exposure (Lebourgeois et al., 2019). It is, therefore, unlikely that the history of alcohol exposure affected the N-acetylcysteine data. Importantly, the differences in alcohol intake and preference between the subgroups remained significant throughout the study (data not shown), despite the shift in baseline alcohol intake levels.

High-alcohol-drinking rats develop AUD-like traits after 2 months of alcohol intake (Spoelder et al., 2015; 2017), but the focus of this study was merely on alcohol consumption. Therefore, the modest effects on alcohol intake do not preclude the potential value of baclofen, naltrexone and N-Acetylcysteine for the treatment of other aspects of AUD-like behaviour.

Conclusion

Together with an earlier study (Spoelder et al., 2016), our findings suggest that individual differences in alcohol intake are not associated with differences in sensitivity to GABA<sub>B</sub>, opioid, glutamate and dopamine modulators. Although the subgroups of low-, medium- and high-alcohol-drinking rats did not differ in their response to these compounds, we did observe substantial variation in the responsivity to baclofen and naltrexone across the subpopulations. The variation between individuals in their response to baclofen and to naltrexone parallels human reports of variation in efficacy of these compounds in the treatment of AUD (Kiefer et al., 2008; Pierce et al., 2018). Taken together, these findings suggest that individual differences in alcohol consumption and in responsivity to baclofen and naltrexone treatment are orthogonal processes, which likely involve differential biological mechanisms.

Acknowledgements

We thank Igor Magaraggia, Annemarie Baars, José Lozeman-van’t Klooster and Lisa Drost for their practical assistance. This work was supported by the Netherlands Organisation for Health Research and Development.
(ZonMw) under project number 912.14.093 (Shining light on loss of control).

Conflicts of interest
There are no conflicts of interest.

References
Addolorato G, Caputo F, Capristo E, Colombo G, Gessa GL, Gasbarrini G (2000). Ability of baclofen in reducing alcohol craving and intake: II—Clinical and preclinical evidence. Alcohol Clin Exp Res 24:67–71.
Addolorato G, Caputo F, Capristo E, Domenicali M, Bernardi M, Janini L, et al. (2002). Baclofen efficacy in reducing alcohol craving and intake: a preliminary double-blind randomized controlled study. Alcohol Alcohol 37:504–508.
Addolorato G, Leggio L, Ferrulli A, Cardone S, Vonglia L, Mirjello A, et al. (2007). Effectiveness and safety of baclofen for maintenance of alcohol abstinence in alcohol-dependent patients with liver cirrhosis: randomised, double-blind controlled study. Lancet 370:1915–1922.
Anstrom KK, Cromwell HC, and Markowski T, Woodward DJ (2003). Effect of baclofen on alcohol and sucrose self-administration in rats. Alcohol Clin Exp Res 27:900–908.
Back SE, McCauley JL, Korte KJ, Grof DS, Leavitt V, Gray KM, et al. (2016). A Double-blind, randomized, controlled pilot trial of N-acetylcysteine in veterans with posttraumatic stress disorder and substance use disorders. J Clin Psychiatry 77:e1439–e1446.
Barr CS, Chen SA, Schwandt ML, Lindell SG, Sun H, Suomi SJ, Heilig M (2010). Suppression of alcohol preference by naltrexone in the rhesus macaque: a critical role of genetic variation at the micro-opioid receptor gene locus. Biol Psychiatry 67:78–80.
Beraha EM, Salwenik E, Goudriaan AE, Bakker A, de Jong D, Smits N, et al. (2016). Efficacy and safety of high-dose baclofen for the treatment of alcohol dependence: a multicentre, randomised, double-blind controlled trial. Eur Neuropsychopharmacol 26:1950–1959.
Bilbao A, Robinson JE, Heilig M, Malanga CJ, Spanagel R, Sommer WH, Thorsell A (2015). A pharmacogenetic determinant of mu-opioid receptor antagonist effects on alcohol reward and consumption: evidence from randomized mice. Biol Psychiatry 78:850–858.
Castelli MP, Pibiri F, Pias AP, Carboni G, Orrù A, Gessa GL, et al. (2005). Differential G-protein coupling to GABAB receptor in limbic areas of alcohol-prefering and -nonpreferring rats. J Eur Pharmacol 523:67–70.
Colombo G, Gabrio R, Carai MA, Lobina C, Pari M, Reali R, et al. (2000). Ability of baclofen in reducing alcohol intake and withdrawal severity: I—Pharmacological. Alcohol Clin Exp Res 24:58–66.
Colombo G, Serra S, Brunetti G, Vaccà G, Carai MA, Gessa GL (2003). Suppression by baclofen of alcohol deprivation effect in Sardinian alcohol-prefering (pA) rats. Drug Alcohol Depend 70:105–108.
Connor JP, Haber PS, Hall WD (2016). Alcohol use disorders. Lancet 387:988–993.
Coomlin DL, Kiefer SW, Ferraro, FM 3rd, Sinclair JD (2004). Ethanol transmission, but not benzodiazepine receptor stimulation, modulates ethanol intake by rats. Alcohol 4:469–472.
de Waal JP, Kiarman A, Gionanouci C (1995). Distribution of the mu and delta opioid binding sites in the brain of the alcohol-prefering AA and alcohol-avoiding ANA lines of rats. J Pharmacol Exp Ther 275:518–527.
Flannery BA, Garbutt JC, Cody MW, Renn W, Grace K, Osborne M, et al. (2004). Baclofen for alcohol dependence: a preliminary open-label study. Alcohol Clin Exp Res 28:1517–1523.
Garbutt, JC, Kampov-Poleyev AB, Gallup R, Kalka-Juhl L, Flannery BA (2010). Efficacy and safety of baclofen for alcohol dependence: a randomized, double-blind, placebo-controlled trial. Alcohol Clin Exp Res 34:1849–1857.
Gonzales RA, Jaworski JN (1997). Alcohol and glutamate. Alcohol Health Res World 21:120–127.
Heinilä P, Alho H, Kiarman A, Lönngqvist J, Kuoppasalmi K, Sinclair JD (2001). Targeted use of naltrexone without prior detoxification in the treatment of alcohol dependence: a factorial double-blind, placebo-controlled trial. J Clin Psychopharmacol 21:287–292.
Henderson-Redmond AN, Lowe TE, Tian KB, Morgan DJ (2018). Increased ethanol drinking in “humanized” mice expressing the mu opioid receptor A118G polymorphism are mediated through sex-specific mechanisms. Brain Res Bull 138:12–19.
James PH, Michael Gill T (2003). Comparison of the effects of allopregnanolone with direct GABAergic agonists on ethanol self-administration with and without concurrently available sucrose. Alcohol 30:1–7.
Kiefer F, Jiménez-Arriero MA, Klein O, Diehl A, Rubio G (2008). Cloninger’s typology and treatment outcome in alcohol-dependent subjects during pharmacotherapy with naltrexone. Addict Biol 13:124–129.
Lebourgeois S, González-Marin MC, Antil J, Naassila M, Vipoux C (2015). Evaluation of N-acetylcysteine on ethanol self-administration in ethanol-dependent rats. Neuropharmacology 150:112–120.
Lebourgeois S, González-Marin MC, Jeanblanc J, Naassila M, Vipoux C (2018). Effect of N-acetylcysteine on motivation, seeking and relapse to ethanol self-administration. Addict Biol 23:643–652.
Maclean P, Zaru A, Loi B, Lobina C, Carai MA, Gessa GL, et al. (2012). Comparison of the effect of the GABAB receptor agonist, baclofen, and the positive allosteric modulator of the GABAB receptor, GS39783, on alcohol self-administration in 3 different lines of alcohol-prefering rats. Alcohol Clin Exp Res 36:1748–1766.
Marrinelli PW, Kiarman A, Naassialais K (2000). Opioid propeptide mRNA content and receptor density in the brains of AA and ANA rats. Life Sci 66:1915–1927.
McBride WJ, C churnet E, McKinzie DL, Lumeng L, Li TK (1998). Quantitative autoradiography of mu-opioid receptor in the CNS of alcohol-naive alcohol-prefering P and -nonprefering NP rats. Alcohol 18:317–323.
Miller WR, Walters ST, Botvin ME (2001). How effective is alcoholism treatment in the United States? J Stud Alcohol 62:211–220.
Momeni S, Roman E (2014). Subgroup-dependent effects of voluntary alcohol intake on behavioral profiles in outbred Wistar rats. Behav Brain Res 275:288–296.
Momeni S, Segerström L, Roman E (2015). Supplier-dependent differences in intermittent voluntary alcohol intake and response to naltrexone in Wistar rats. Front Neurosci 9:424.
Moos RH, Moos BS (2006). Rates and predictors of relapse after natural and treated remission from alcohol use disorders. Addiction 101:212–222.
Müller CA, Geisel O, Pelz P, Higl V, Krüger J, Stöckel A, et al. (2015). High-dose baclofen for the treatment of alcohol dependence (BACLAD study): a randomized, placebo-controlled trial. Eur Neuropsychopharmacol 25:1167–1177.
O’Malley SS, Jaffe AJ, Chang G, Schottenfeld RS, Meyer RE, Rounsaville B (1992). Naltrexone and coping skills therapy for alcohol dependence. A controlled study. Arch Gen Psychiatry 49:881–887.
Olve MF, Koenig HN, Nannini MA, Hodge CW (2001). Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine, and amphetamine. J Neurosci 21:RC184.
Pierce M, Sutherland A, Beraha EM, Morley K, van den Brink W (2018). Efficacy, tolerability, and safety of low-dose and high-dose baclofen in the treatment of alcohol dependence: a systematic review and meta-analysis. Eur Neuropsychopharmacol 28:795–806.
Quintanilla ME, Rivera-Meza M, Benicio-Cárdeno P, Salinas-Lupyaert C, Herrera-Marschitz M, Israel Y (2016). Beyond the “first hit”: marked inhibition by N-acetyl cysteine of chronic ethanol intake but not of early ethanol intake. Parallel effects on ethanol-induced saccharin motivation. Alcohol Clin Exp Res 40:1044–1051.
Relman LS, Mathers C, Popova S, Thavorncharoenpas M, Teerawattananon Y, Patra J (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-related disorders. Lancet 373:2223–2233.
Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR (2003). Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. Proc Natl Acad Sci USA 100:2053–2058.
Simms JA, Steensland P, Medina B, Abernathy KE, Chandler LJ, Wise R, Bartlett SE (2008). Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. Alcohol Clin Exp Res 32:1816–1823.
Spoelder M, Baars AM, Rotte MD, Vandenschuren LJ, Lesscher HM (2010). Dopamine receptor agonists modulate voluntary alcohol intake independently of individual levels of alcohol intake in rats. Psychopharmacology (Berl) 223:7275–7285.
Spoelder M, Pol S, Janssen BSG, Baars AM, Vandenschuren, LJM, Lesscher, HMB (2017). Loss of control over alcohol seeking in rats depends on individual vulnerability and duration of alcohol consumption experience. Behav Pharmacol 28:334–344.
Spoelder M, Hesselings P, Baars AM, Loeman-van ́t Klooster JG, Rotte MD, Vandenschuren LJ, Lesscher HM (2015). Individual variation in alcohol intake predicts reinforcement, motivation, and compulsive alcohol use in rats. Alcohol Clin Exp Res 39:2427–2437.
Squeglia LM, Tomko RL, Baker NL, McClure EA, Book GA, Gray KM (2018). The effect of N-acetylcysteine on alcohol use during a cannabis cessation trial. Drug Alcohol Depend 185:17–22.

Stoops WW, Strickland JC, Hays LR, Rayapati AO, Lile JA, Rush CR (2020). Influence of n-acetylcysteine maintenance on the pharmacodynamic effects of oral ethanol. Pharmacol Biochem Behav 198:173037.

Tsai G, Gastfriend DR, Coyle JT (1995). The glutamergic basis of human alcoholism. Ann J Psychiatry 152:332–340.

Vallender EJ, Rüedi-Bettischen D, Miller GM, Platt DM (2010). A pharmacogenetic model of naltrexone-induced attenuation of alcohol consumption in rhesus monkeys. Drug Alcohol Depend 109:252–256.

van den Brink W (2012). Evidence-based pharmacological treatment of substance use disorders and pathological gambling. Curr Drug Abuse Rev 5:3–31.

Volpicelli JR, Alterman AI, Hayashida M, O’Brien CP (1992). Naltrexone in the treatment of alcohol dependence. Arch Gen Psychiatry 49:876–880.

Walker BM, Koob GF (2007). The gamma-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. Alcohol Clin Exp Res 31:11–18.