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

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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 $GABA_B$ 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**

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Introduction

Alcohol use disorder (AUD) is a chronic relapsing disorder with major medical and socioeconomic consequences (Rehm *et al.*, 2009; Connor *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 *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 *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 *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 et al., 2015, 2017).

Alcohol affects multiple neurotransmitter systems involved in emotion, cognition and motivation. Alcohol increases GABAergic activity in the brain (Roberto *et al.*, 2003) and induces the release of endogenous opioids (Olive *et al.*, 2001). Furthermore, alcohol can inhibit glutamatergic neurotransmission through *N*-methyl-D-aspartate (NMDA) and mGlu5 metabotropic glutamate receptors (Tsai *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 $GABA_B$ receptor agonist baclofen, the opioid receptor antagonist naltrexone and the cysteine

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precursor *N*-acetylcysteine that modulates 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 2–3 h into the dark cycle and lasted for 7 h. Sessions were subsequently extended to 24 h 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 [(5α)-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydromorphinan-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-(acetylamino)-3-sulfanylpropanoic 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 24 h after session onset. Each treatment session was always followed by at least 1 day without alcohol access and at least one drug-free 24 h alcohol consumption session between treatment sessions for the same drug. There were at least three drug-free 24 h 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 24 h) 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

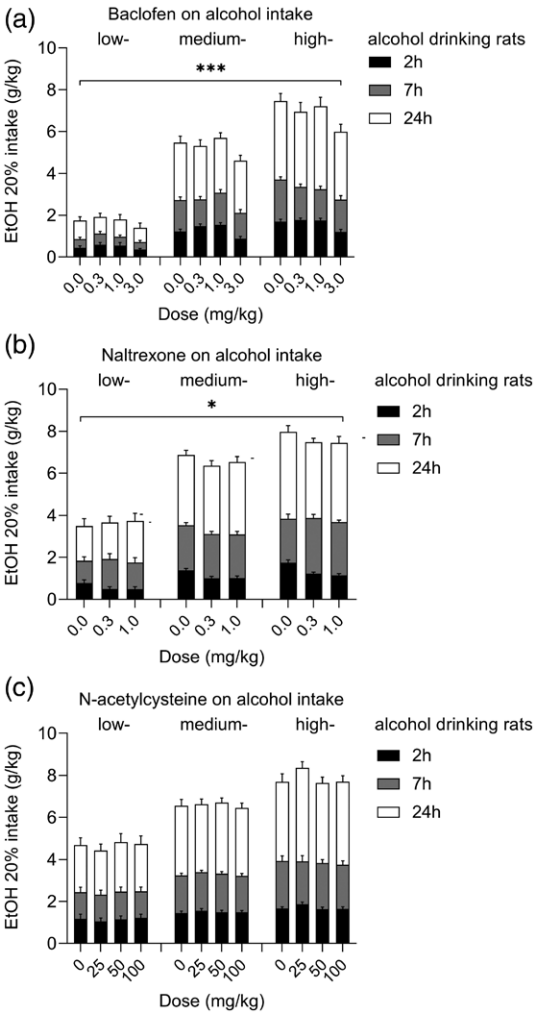
Baclofen decreased alcohol intake ($F_{(3,105)\text{dose}}=16.812$; $P<0.001$), dependent on the time in the session ($F_{(4,134)\text{dosextime}}=3.747$; $P=0.007$) (Fig. 1a) and with a near-significant group × baclofen interaction ($F_{(6,105)\text{dosexgroup}}=2.127$; $P=0.056$). Post hoc analyses 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 towards a reduction in alcohol preference upon baclofen treatment ($F_{(3,105)\text{dose}}=2.618$; $P=0.055$) (Supplementary Figure 2a, Supplemental digital content 1, *http://links.lww.com/BPHARM/A62*), independent of the time in the session ($F_{(4,127)\text{dosextime}}=2.034$; $P=0.100$) and group ($F_{(6,105)\text{dosexgroup}}=0.825$; $P=0.553$; $F_{(7,127)\text{dosextimexgroup}}=0.641$; $P=0.727$).

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 ($F_{(3,99)\text{dosextime}}=1.448$; $P=0.236$) and independent of group ($F_{(4,74)\text{dosexgroup}}=0.853$; $P=0.496$; $F_{(5,99)\text{dosextimexgroup}}=0.900$; $P=0.490$). Naltrexone did not alter alcohol preference in any of the groups at any of the timepoints tested ($F_{(2,74)\text{dose}}=0.488$; $P=0.616$; $F_{(3,96)\text{dosextime}}=0.438$; $P=0.697$; $F_{(4,74)\text{dosexgroup}}=0.297$; $P=0.879$; $F_{(5,96)\text{dosextimexgroup}}=1.181$; $P=0.324$) (Supplementary Figure 2b, Supplemental digital content 1, *http://links.lww.com/BPHARM/A62*).

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

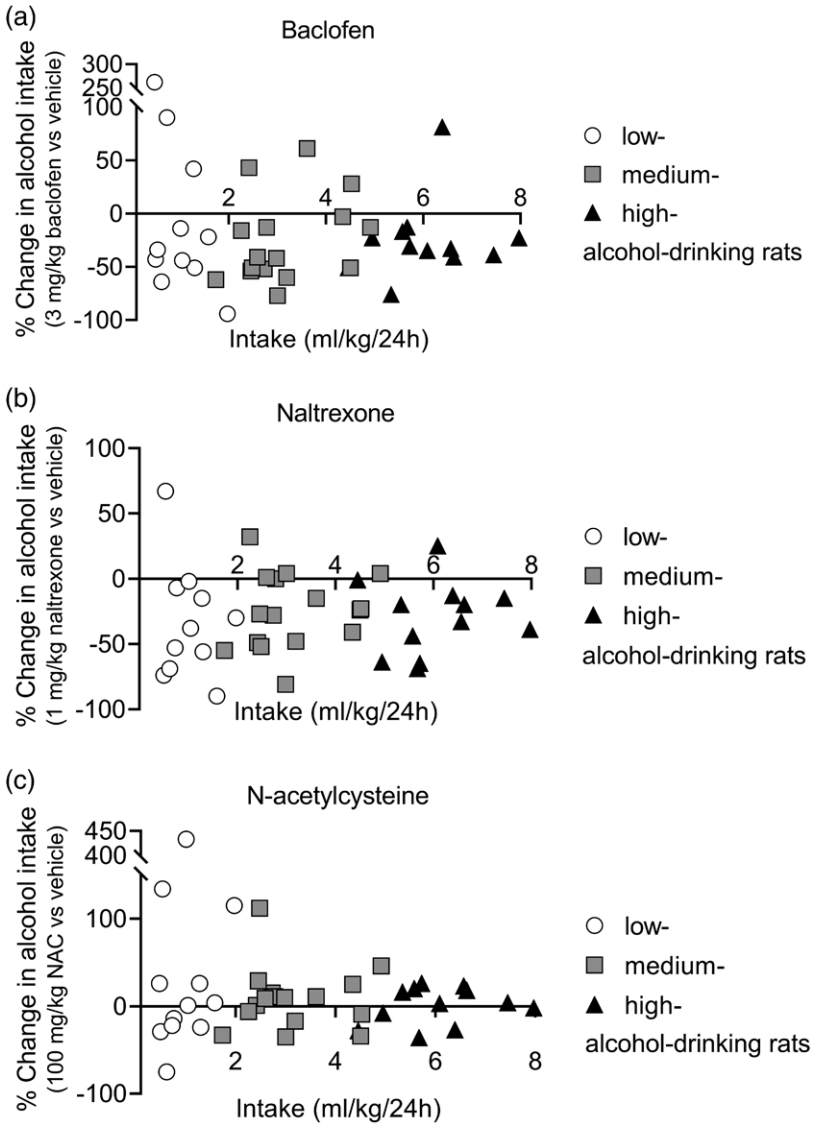
Similar responsivities to baclofen, naltrexone and *N*-acetylcysteine for low-, medium- and high-alcohol-drinking rats do not preclude individual variation in the effects of these compounds on alcohol consumption. Therefore, we performed an additional analysis on the difference in alcohol intake between vehicle and the highest dose for each drug per individual rat (Fig. 2, 2 h data). These individual data revealed

Fig. 1



The effects of baclofen, naltrexone and *N*-acetylcysteine on alcohol intake. The figure shows the cumulative levels of alcohol intake after treatment with (a) baclofen, (b) naltrexone and (c) *N*-acetylcysteine for the different doses. The stacked bars represent the different intervals, for example 0–2, 2–7 and 7–24 h for each session. Baclofen decreased alcohol intake to a similar extent in low- ($n=10$), medium- ($n=16$) and high-alcohol-drinking rats ($n=12$). Post hoc analyses revealed an overall reduction in alcohol intake after treatment with 3.0 mg/kg baclofen compared to vehicle. Naltrexone decreased alcohol intake and this effect was independent of group: low- ($n=12$); medium- ($n=16$); and high-alcohol-drinking rats ($n=12$). Post hoc analyses revealed an overall reduction in alcohol intake after treatment with 1.0 mg/kg naltrexone compared to vehicle. *N*-Acetylcysteine did not affect alcohol intake in any of the groups: low- ($n=12$); medium- ($n=16$); and high-alcohol-drinking rats ($n=12$) at any of the timepoints. Data are presented as the mean + SEM. *** significantly different from vehicle at all three timepoints, $P<0.001$; * significant overall difference from vehicle across session and groups, $P<0.05$.

Fig. 2



The change in alcohol intake upon treatment with baclofen, naltrexone and *N*-acetylcysteine for individual animals. The figure shows the changes in alcohol intake (in %) after treatment with (a) baclofen (3 mg/kg), (b) naltrexone (1 mg/kg) and (c) *N*-acetylcysteine (100 mg/kg) for individual rats, plotted against their baseline level of alcohol intake during the last 4 weeks prior to pharmacological experiments commenced. The datapoints are marked by group (low-=○, medium-=■, high-alcohol-drinking rats=▲). These data show the degree in individual variation in the response to the different compounds.

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 $GABA_B$ 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-drinking rats, suggesting that $GABA_B$-mediated neurotransmission is not likely to contribute to individual differences in alcohol intake. Alcohol-preferring and nonpreferring were previously shown to display differences in $GABA_B$ receptor function (Castelli *et al.*, 2005), suggesting that $GABA_B$ receptors may contribute to variation in alcohol intake. However, in this latter study, the differences in $GABA_B$ receptor function disappeared after 1 month of alcohol consumption. Therefore, it is conceivable that initial differences in the $GABA_B$ 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; Vallender *et al.*, 2010; Bilbao *et al.*, 2015; Henderson-Redmond *et al.*, 2018). Indeed, alcohol-preferring 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; Squeglia *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 $GABA_B$, 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.

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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–Preliminary clinical evidence. *Alcohol Clin Exp Res* **24**:67–71.

Addolorato G, Caputo F, Capristo E, Domenicali M, Bernardi M, Janiri 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, Vonghia L, Mirijello 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, 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, Gros DF, 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, Salemink 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 humanized mice. *Biol Psychiatry* **77**:850–858.

Castelli MP, Pibiri F, Piras AP, Carboni G, Orrù A, Gessa GL, *et al.* (2005). Differential G-protein coupling to GABAB receptor in limbic areas of alcohol-preferring and -nonpreferring rats. *Eur J Pharmacol* **523**:67–70.

Colombo G, Agabio R, Carai MA, Lobina C, Pani M, Reali R, *et al.* (2000). Ability of baclofen in reducing alcohol intake and withdrawal severity: I–Preclinical evidence. *Alcohol Clin Exp Res* **24**:58–66.

Colombo G, Serra S, Brunetti G, Vacca G, Carai MA, Gessa GL (2003). Suppression by baclofen of alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats. *Drug Alcohol Depend* **70**:105–108.

Connor JP, Haber PS, Hall WD (2016). Alcohol use disorders. *Lancet* **387**:988–998.

Coonfield DL, Kiefer SW, Ferraro, FM 3rd, Sinclair JD (2004). Ethanol palatability and consumption by high ethanol-drinking rats: manipulation of the opioid system with naltrexone. *Behav Neurosci* **118**:1089–1096.

Daoura L, Nylander I (2011). The response to naltrexone in ethanol-drinking rats depends on early environmental experiences. *Pharmacol Biochem Behav* **99**:626–633.

Daoust M, Saligaut C, Lhuintre JP, Moore N, Flipo JL, Boismare F (1987). GABA transmission, but not benzodiazepine receptor stimulation, modulates ethanol intake by rats. *Alcohol* **4**:469–472.

de Waele JP, Kiianmaa K, Gianoulakis C (1995). Distribution of the mu and delta opioid binding sites in the brain of the alcohol-preferring 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-Polevoy AB, Gallop 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.

Heinälä P, Alho H, Kiianmaa K, Lönnqvist 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 XB, 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.

Janak 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-Marín MC, Antol J, Naassila M, Vilpoux C (2019). Evaluation of N-acetylcysteine on ethanol self-administration in ethanol-dependent rats. *Neuropharmacology* **150**:112–120.

Lebourgeois S, González-Marín MC, Jeanblanc J, Naassila M, Vilpoux C (2018). Effect of N-acetylcysteine on motivation, seeking and relapse to ethanol self-administration. *Addict Biol* **23**:643–652.

Maccioni 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-preferring rats. *Alcohol Clin Exp Res* **36**:1748–1766.

Marinelli PW, Kiianmaa K, Gianoulakis C (2000). Opioid propeptide mRNA content and receptor density in the brains of AA and ANA rats. *Life Sci* **66**:1915–1927.

McBride WJ, Chernet E, McKinzie DL, Lumeng L, Li TK (1998). Quantitative autoradiography of mu-opioid receptors in the CNS of alcohol-naive alcohol-preferring P and -nonpreferring NP rats. *Alcohol* **16**:317–323.

Miller WR, Walters ST, Bennett 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, Stickel 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.

Olive 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, Sutterland 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, Berríos-Cárcamo P, Salinas-Luypaert 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.

Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use 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, Vanderschuren LJ, Lesscher HM (2016). Dopamine receptor agonists modulate voluntary alcohol intake independently of individual levels of alcohol intake in rats. *Psychopharmacology (Berl)* **233**:2715–2725.

Spoelder M, Pol S, Janssen BSG, Baars AM, Vanderschuren, LJMJ, 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, Hesseling P, Baars AM, Lozeman-van ‘t Klooster JG, Rotte MD, Vanderschuren 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 glutamatergic basis of human alcoholism. *Am J Psychiatry* **152**:332–340.

Vallender EJ, Rüedi-Bettschen 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.