Supplemental Materials

THE ROLE OF BETA- AND ALPHA-ADRENERGIC RECEPTORS ON ALCOHOL DRINKING

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Supp. Table 1: Pharmacological compounds, doses used and references.

Agent	Dose	References
Propranolol (i.p) Prazosin (i.p) Betaxolol (i.p) ICI 118,551 (i.p) Propranolol (i.c) Prazosin (i.c) Betaxolol (i.c)	2.5, 5 and 10mg/kg 0.25 mg/kg 2.5 and 5mg/kg 1mg/kg 0.5, 2,5,10 ug 0.3ug 307ng	Gilpin and Koob, 2010; Rasmussen *et al*., 2014 Forget *et al*., 2010 Mantsch *et al*., 2010; Rudoy and Van Bockstaele,2013 McReynolds *et al*., 2014, Mantsch *et al*., 2010 Funk *et al*., 1996; Rojas et al., 2015, Yamada *et al*., 2011 Vranjkovic *et al*., 2014 Vranjkovic *et al*., 2014

i.p intraperitoneal
i.c intracranial

Supp. Table 2: Cohorts used for each experiment and number of animals per cohort.

Experiment	Cohort(s)
Propranolol (i.p) 2.5mg/kg	A
Propranolol (i.p) 5mg/kg	A +B
Propranolol (i.p) 10mg/kg	A
Betaxolol (i.p) 2.5mg/kg	C*
Betaxolol (i.p) 5mg/kg	C
ICI (i.p) 1mg/kg	C
Prazosin (0.25mg/kg) + Propranolol (2.5 mg/kg)	G
Propranolol (aINS) 0.5 and 2ug	D
Propranolol (aINS) 5 and 10ug	E
Betaxolol (aINS) 307ng	E
Propranolol (mPFC) 0.5 and 5ug	F
Propranolol 10ug and Betaxolol 307ng (mPFC)	F **
Prazosin (mPFC) 0.3ug	F

Sample sizes: Cohort A=10; B=6; C=15; D=7; E=9; F=7; G=14
*Cohort C had 15 animals, but 1 animal died during the experiment with betaxolol 2.5 mg/kg

** Cohort F had 7 animals, but 1 animal lost the implant during the experiment with Propranolol 10ug and betaxolol 307ng

Supplemental Figures Legend

Suppl. Figure 1. Effect of propranolol (5mg/kg) on Cohort A and Cohort B separately. (A) Schematic of administration of 10 then 5 then 2.5 mg/kg propranolol in Cohort A. (B,C) Drinking data from (B) Cohort A (10 animals) and (C) Cohort B (6 animals, only tested with 5 mg/kg propranolol.

Suppl. Figure 2. Data for percent change in firing, calculated by 100*(drinking-during-drug/drinking-during-vehicle) shown for (A) CLAD and (B) AOD. Data for prazosin 0.75 and 1.5 mg/kg are from De Oliveira Sergio et al. (2021) (grey bars and red-orange outline). No differences across groups in change in drinking.

Suppl. Figure 3. Histology of placements. (A) aINS placements. (B) mPFC placements. Br: bregma.

Suppl. Figure 4. Percent change in firing, calculated by 100*(drinking-during-drug/drinking-during-vehicle) for intra-aINS injections. (A) Propranolol 0.5 or 2ug into aINS. (B) Propranolol 5 and 10ug into aINS (B). (C) Betaxolol 307ng into aINS. * $p<0.05$ paired t-test CLAD vehicle vs 2ug propranolol.

Suppl. Figure 5. Percent change in firing, calculated by 100*(drinking-during-drug/drinking-during-vehicle) for intra-mPFC injections. (A) Propranolol 0.5 or 2ug into mPFC. (B) Propranolol 10ug and betaxolol 307ng into mPFC. (C) Prazosin 0.3ug into mPFC.

Results and Discussion of Suppl.Fig.1:

Prior studies showed that the co-administration of prazosin and propranolol was able to decrease alcohol intake during alcohol withdrawal and after long imposed abstinence in P rats (Rasmussen et al., 2014) as well as decreased the compulsive-like behavior on marble burying test in mice (Lustberg et al., 2020). Interestingly, on both studies the effect of the co-administration of prazosin and propranolol was more effective than each compound alone.

Our findings here showed that the combination of ineffective doses of prazosin (0.25mg/kg) and propranolol (2.5 mg/kg) decreased AOD and CLAD (Fig.4). This information could be value for future clinical trials since that the combination of low doses of both compounds could reduce the chance of side effects.

For Suppl. Fig.1, we compare the percent change in drinking with drug (vs it's own vehicle) across a number of treatment conditions, including systemic injection of prazosin at the doses of 0.75 and 1.75 mg/kg which reduced AOD and CLAD (data from De Oliveira Sergio et al., 2021). Percent changes in drinking with prazosin + propranolol was not different other conditions (Fig.S1A: one-way ANOVA; $F(\text{treatment};3,55) = 0.288$, $p = 0.833$; Fig.S1B: one-way ANOVA; $F(\text{treatment};3,83) = 1.649$, $p = 0.156$].

Results and Discussion of Suppl.Fig.3:

The propranolol experiments started with 10 animals (Cohort A) first tested with the dose of 10 mg/kg of propranolol, and subsequently with 5 and 2.5mg/kg respectively. The results for the first 10 animals with the intermediate dose showed a treatment and drinking effect as well as the lowest correlation value for all doses tested [n=10; two-way ANOVA; $F_{(treatment;1,9)}$=6.196, p=0.035; $F_{(drinking\text{-}condition;1,9)}$=20.914, p=0.001; $F_{(interaction;1,9)}$=3.416, p= 0.098]. Thus, 6 additional animals (Cohort B) were tested with 5mg/kg of propranolol. The findings showed a treatment and drinking effect and no correlation, as observed before for the first 10 animals [n=6; two-way ANOVA; $F_{(treatment;1,5)}$=4.605, p=0.085; $F_{(drinking\text{-}condition;1,5)}$=6.098, p=0.057; $F_{(interaction;1,5)}$=0.417, p= 0.547].

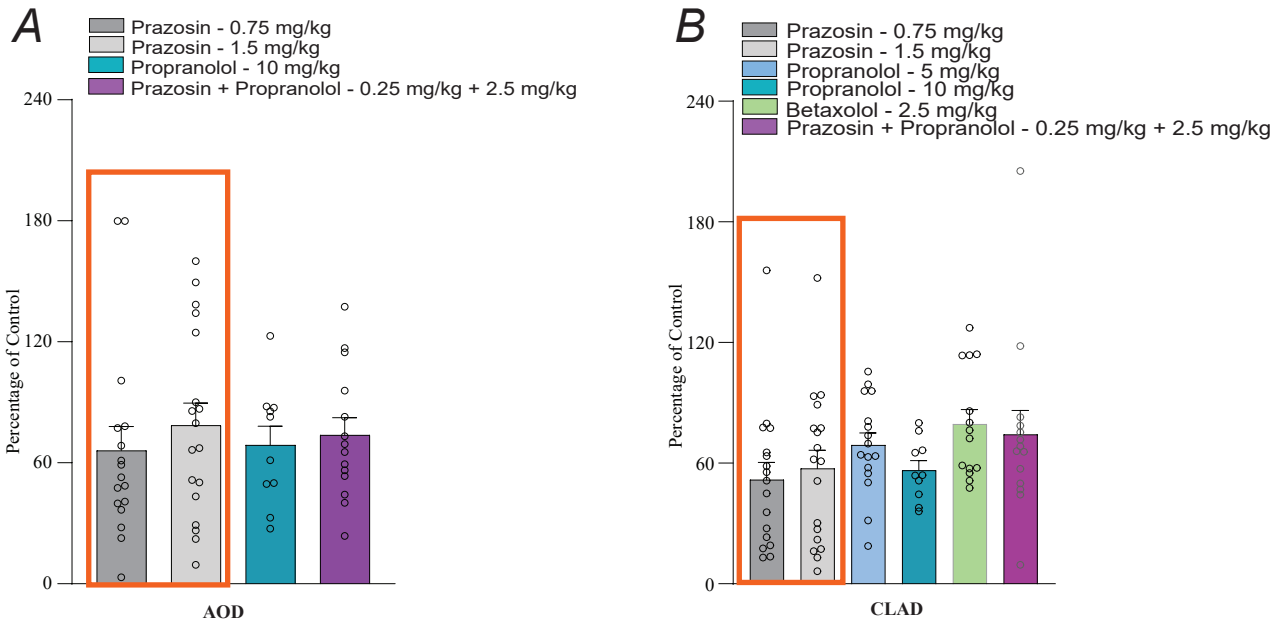
Results and Discussion of Suppl.Fig.4:

We analyzed the percent change in firing, calculated by 100*(drinking-during-drug/drinking-during-vehicle) for intra-aINS injections. We compared differences using a paired t-test. There were no AOD versus CLAD differences in drug/vehicle for the administration into aINS of propranolol 0.5ug (Fig.4A; Prop 0.5ug AOD vs CLAD - $t_{(6)}$=1.530, p=0.176) however, there was a difference for the administration of propranolol 2ug (Fig.4A;Prop 2 AOD vs CLAD - $t_{(6)}$=2.570, p=0.043) showing that the changes in drinking were significantly greater for AOD than CLAD. Moreover, there were no AOD versus CLAD differences in drug/vehicle for the administration into aINS of propranolol 5ug (FIG.4B;Prop 5ug AOD vs CLAD - $t_{(8)}$=0.350, p=0.734), also there was no difference for the administration of propranolol 10ug (Fig.4B; Prop 10 AOD vs CLAD - $t_{(8)}$=0.996, p=0.348) and there was a trend for AOD versus CLAD differences in drug/vehicle for the administration into aINS of betaxolol 307ng (Fig.4C; betaxolol 307ng AOD vs CLAD - $t_{(8)}$=2.229, p=0.056).

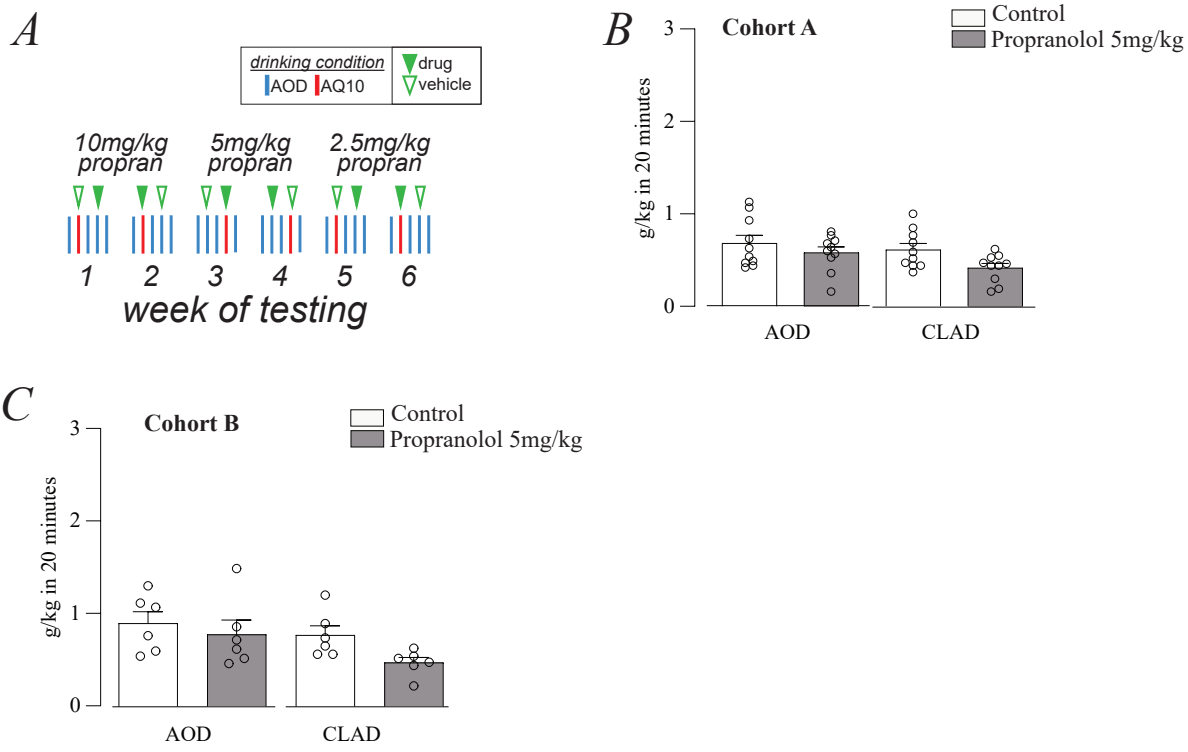
Results and Discussion of Suppl.Fig.5:

We analyzed the percent change in firing, calculated by 100*(drinking-during-drug/drinking-during-vehicle) for intra-mPFC injections. We compared differences using a paired t-test. There were no AOD versus CLAD differences in drug/vehicle for the administration into mPFC of propranolol 0.5ug (Fig.5A; Prop 0.5ug AOD vs CLAD - $t_{(8)}$=0.864, p=0.412), also for the administration of propranolol 5ug (Fig.5A; Prop 5 AOD vs CLAD - $t_{(8)}$=0.809, p=0.441). Moreover, there were no AOD versus CLAD differences in drug/vehicle for the administration into mPFC of propranolol 10ug (Fig.5B; Prop 10ug AOD vs CLAD - $t_{(7)}$=0.304, p=0.770), and no difference for the administration of betaxolol 307ng (Fig.5B; betaxolol AOD vs CLAD - $t_{(7)}$=0.052, p=0.959), as well as no difference for differences in drug/vehicle for the administration into mPFC of prazosin 0.3ug (Fig.5C; prazosin AOD vs CLAD - $t_{(8)}$=0.869, p=0.410).

De Olivera Sergio et al., Supp. Figure 1



De Olivera Sergio et al., Fig Supp. 2



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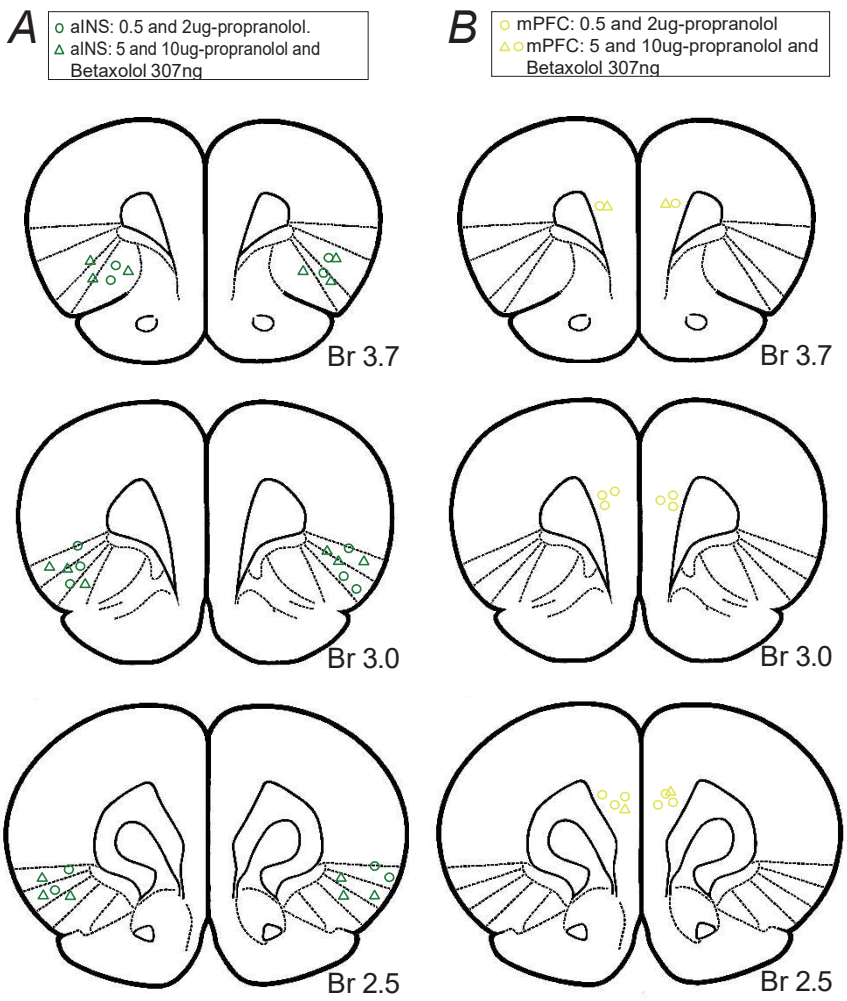

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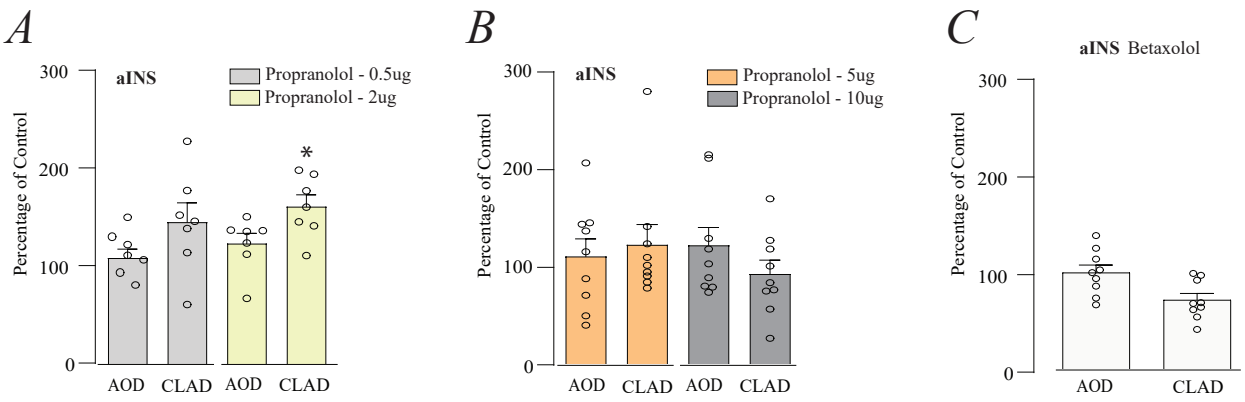

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