DEVELOPMENT AND APPLICATION OF A NOVEL LOW DOSE ETHANOL ASSAY FOR USE IN TRANSGENIC MICE

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INTRODUCTION

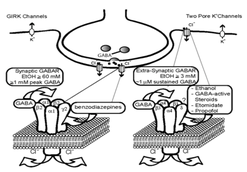
In humans, low dose ethanol has been found to attenuate explicit learning and memory processes, such as episodic encoding, but not implicit processes, such as priming. A similar pattern has been found in animal studies where low to moderate dose ethanol disrupts performance in hippocampus dependent tasks, such as the submerged platform version of the Morris water maze, without affecting performance in hippocampus independent tasks, such as the visible platform version.

The mechanisms by which ethanol mediates these effects are still largely unknown and highly controversial, but the γ-aminobutyric acid receptor ($GABA_AR$) has been implicated in ethanol's acute, chronic and withdrawal effects. Ethanol has been proposed to act on the $GABA_AR$ both directly through receptor binding and activity and indirectly through the modulatory activity of neurosteroids.

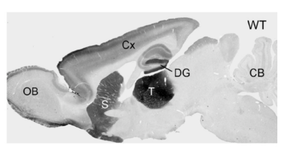
Ro15-4513, a $GABA_AR$ partial inverse agonist, has been shown to antagonize both ethanol-induced $GABA_AR$ 36Cl- flux and ethanol-mediated behavior. Electrophysiological studies on recombinant receptors showed that the α4βδ containing $GABA_AR$, primarily expressed extrasynaptically and important in the maintenance of the tonic current, was necessary for ethanol effects in the 3-30 mM range. These effects were reversed by Ro15-4513. Furthermore, this reversal was inhibited by ligands that prevent behavioral alcohol antagonism of Ro15-4513.

The goal of the present study was to develop a behavioral task in mice that is sensitive to low dose ethanol and then apply this task in transgenic mice to test specific hypothesis regarding the underlying mechanisms of ethanol action. We developed a task based on the context pre-exposure rescue of the immediate shock deficit. Animals exposed to a conditioning chamber and immediately shocked show no fear of the context. This has been termed the immediate shock deficit and contrasts with the robust level of freezing that occurs if the shock is delivered after a delay of at least one minute. If animals are pre-exposed to the chamber 24 hours prior to immediate shock they are able acquire fear of the context, this is referred to as the context pre-exposure rescue of the immediate shock deficit. The pre-exposure allows time for the formation of a unified contextual representation of the multi-modal cues in the conditioning chamber. This representation can then be retrieved and associated with the shock the following day and thus support the expression of conditional fear. Numerous studies have suggested that the formation of this contextual representation requires an intact and properly functioning hippocampus. Utilizing this immediate shock procedure drugs can be administered just during the brief pre-exposure, in the absence of shock. The level of fear produced by the immediate shock 24 hours later can then be used to determine the drug's effect on hippocampus dependent context learning. This study analyzed the effects of ethanol, the neurosteroid allopregnanalone (ALLO) and Ro15-4513 in C57Bl6 mice. Preliminary studies were also conducted in α4 $GABA_AR$ KO mice.

α4 $GABA_AR$ subunit expression



Wallner et al, 2003



Chandra et al, 2006

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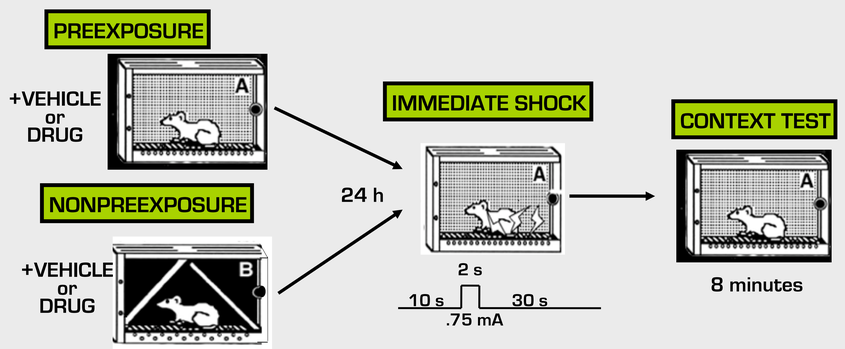
METHODS

On Day 1, subjects were given drug or vehicle by i.p. injection and returned to their homecages for 10 min. Animals preexposed (PRE) to the training context were placed in Context A for 10 min. Non-preexposed (NONPRE) animals were handled and injected in the same fashion as the PRE group, but placed in Context B. 24 hours later (Day 2), subjects were placed in Context A for 10 s before footshock (2 sec, 0.75 mA). They remained in the chamber for 30 s (42 s total) and then returned to their homecages. On Day 3, subjects were brought by cart to a holding room where they were left untouched in their homecages for 30 minutes before being placed in Context A for an 8 min context test.



RESULTS

Ethanol Disrupts the Context Pre-exposure Rescue of the Immediate Shock Deficit

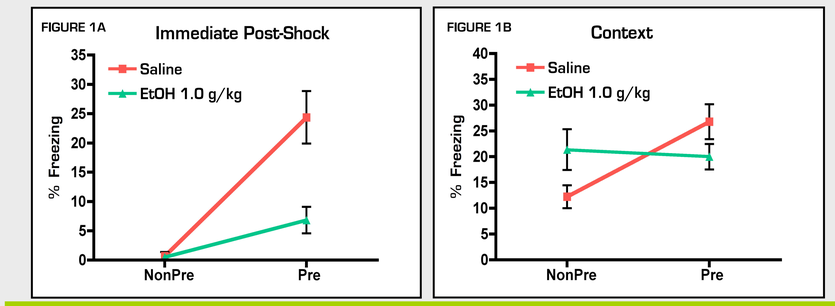


FIGURE 1:Freezing data following the immediate shock procedure. [A] Mean (± SEM) percent freezing in mice during the 30 second post-shock interval 24 hours after being treated with Saline or 1.0 g/kg Ethanol and Preexposure (Pre) to either the training context or a different context (NonPre) **[B]** Percent freezing in mice during the context test 24 hours after immediate shock training. The Preexposure rescue in the Saline groups is larger in magnitude in the post-shock interval relative to the Context Test. This is due to the increase in freezing in the NonPre group from 0 % Post-shock to approximately 12 % on the context test. Subsequent analysis focused on the Post-shock interval due to the greater sensitivity to Preexposure manipulations.

RESULTS

Ethanol does not cause withdrawal-induced changes in pain sensitivity

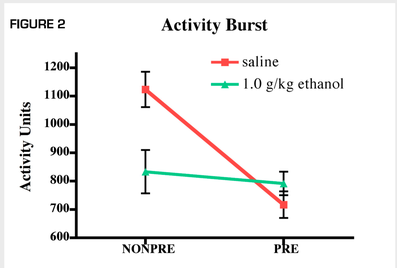


FIGURE 2: Mean (± SEM) activity burst response to the shock during training as grouped by ethanol dose and pre-exposure condition. There was no evidence for withdrawal induced hyperalgesia in ethanol treated mice. Saline treated mice show an enhanced activity burst in the Non-Preexposed relative to the Preexposed groups. This was difference was absent in the ethanol treated mice.

The ethanol-mediated impairment is mimicked by allopregnanolone. Ro15-4513 blocks the effect of ethanol but not allopregnanolone.

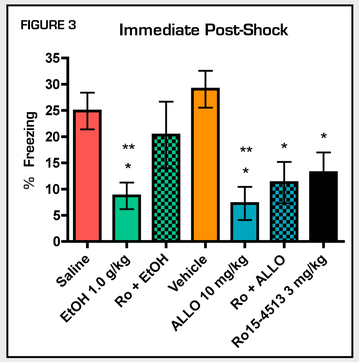


FIGURE 3: Freezing data following the immediate shock procedure: Effects of Ro15-4513 and Allopregnanolone. Mean (± SEM) percent freezing of PRE mice with saline, 1.0 g/kg EtOH, 1.0 g/kg coadministered with 3 mg/kg Ro15-4513 (Ro + EtOH), vehicle, ALLO 10 mg/kg, 3 mg/kg Ro15-4513 coadministered with 10 mg/kg ALLO (Ro + ALLO) and 3 mg/kg Ro15-4513 during the 30 s post-shock interval. Asterisks (*) and (**) represent a significant group difference from saline and vehicle and from Ro + EtOH, respectively.

The ethanol-mediated impairments are not due to indirect effects on locomotor activity

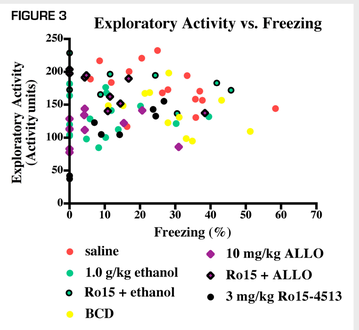


FIGURE 4: Post-Shock Freezing (X-axis) plotted against exploratory activity during the pre-exposure (Y-axis). No significant correlation was observed.

The ethanol-mediated impairment is present in α4 $GABA_AR$ KO mice

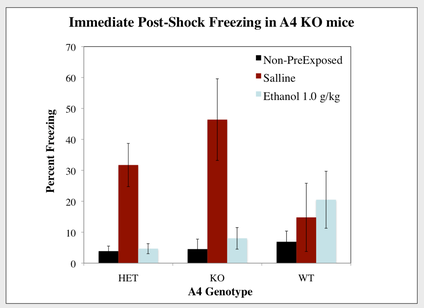


FIGURE 5: Freezing data following the immediate shock procedure in Alpha4 $GABA_AR$ KO mice, Preliminary data showing mean (± SEM) percent freezing during the post-shock interval in α4 WT, HET and KO mice. α4 KO mice show an intact ethanol-mediated disruption. Data from WT's is confounded by a small sample size (n = 3, 4 and 6, respectively

CONCLUSION

- A low of dose ethanol blocks the context pre-exposure rescue of the immediate shock deficit, indicating that it impairs the formation of hippocampus-dependent contextual representations. This effect is abolished by co-administration of the $GABA_AR$ partial inverse agonist Ro15-4513. A low dose of ALLO produces a similar impairment as ethanol, but this not reversible by Ro154513.
- The specificity of the Ro15-4513 antagonism supports the model that ethanol and Ro15-4513 compete for the same binding pocket on the $GABA_AR$. It also indicates that the ethanol impairment is not mediated indirectly via increased ALLO synthesis.
- The lack of a correlation between locomotor activity and freezing suggests that the ethanol-mediated impairment is not due to an indirect effect of decreased locomotion in ethanol treated mice. Analysis of the activity burst response to the shock suggest that 1.0 g/kg ethanol did not produce withdrawal induced hyperalgesia but did prevent the increased activity burst seen in the Saline Non-preexposed groups.
- Preliminary data suggests that α4 $GABA_AR$ KO mice do not show resistance to the ethanol-mediated impairment in this task.
- Conclusion: Ethanol, at doses relevant to human consumption, specifically disrupts hippocampal function and likely does so through direct action at the $GABA_AR$

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