

Introduction

Alcohol use disorder (AUD) is a complex neuropsychiatric condition that combines behavioral, neurobiological, and psychosocial alterations **1**. The development of AUD has been characterized as a three-stage cycle: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation **2**. We need behavioral models that capture AUD aspects, such as alcohol craving, seeking, compulsive intake, dependence, and relapse. These three stages can be more precisely modelled in animals with the assumption that they emulate the human AUD characteristics. **3**. Longitudinal neuroimaging analysis of structure may help us understand the neuroadaptive changes in the brain as a consequence of AUD.

Method

We implemented a per-clinical AUD model, the Intermittent-Access Ethanol 2-Bottle-Choice Drinking Paradigm (IA2BC) **4**. The model started in P45 with n=3 (2 female) Wistar rats with continuous-Access 10%-20% ethanol on a 12-hour reversed light/dark cycle (lights off at 7 am). Brain images were acquired using a Bruker 7T MRI scanner with a 2x2 surface array rat coil. T1-weighted images were acquired using a FLASH 3D sequence, TR/TE = 30.76/5 ms, isometric voxel = 160 μ m with two repetitions. First acquisition was before the IA2BC model as baseline, and the second MRI acquisition was after finishing the 20 sessions (45 days) of ethanol intake. This study was approved by the bioethics committee of the Institute of Neurobiology, Universidad Nacional Autónoma de México (protocol 113.A).

All images were converted from Bruker format to nifti using brkraw tool, and then preprocessed using an in-house pipeline based on MINC-toolkit-v2, ANTs and MRtrix V3 which performed the following steps: center image, denoising and N4 Bias Field Correction. We used deformation-based morphometry to create Jacobian maps per subject and time point using an in-house pipeline, Two Level DBM (https://github.com/CoBrALab/twolevel_ants_dbm) based on ANTsX/ANTs tools v2.3.1 5 based on Fisher atlas parcellation, with mixed model GLM and correction for multiple comparisons using an FDR 5% **6**. In order to analyse brain changes and addiction stages (binge/intoxication or preoccupation/anticipation), we built a logistic model with the brain regions from the two stages of the addiction cycle and the ethanol intake for each session.

Results

The main alcohol intake (g/kg/24hrs) was 3.64 ± 0.66 (mean/sd). (Fig. 1) We found local structural changes after 20 sessions of alcohol consumption in several brain regions (Figure 2). When evaluating the changes in deformation based morphometry in rats, in both logistic models we did not find any significant coefficients of brain changes related to a specific stage.

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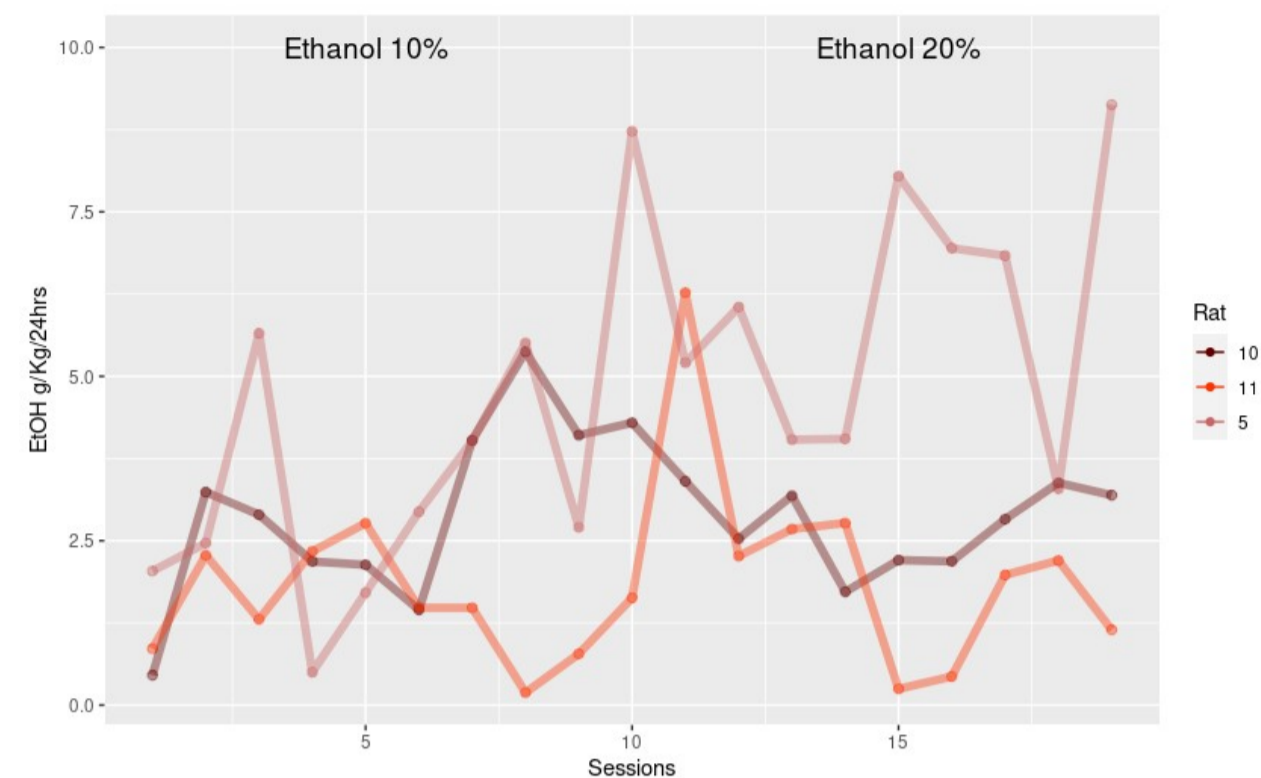


Figure 1. Main ethanol intake in Alcohol model 10%-20%

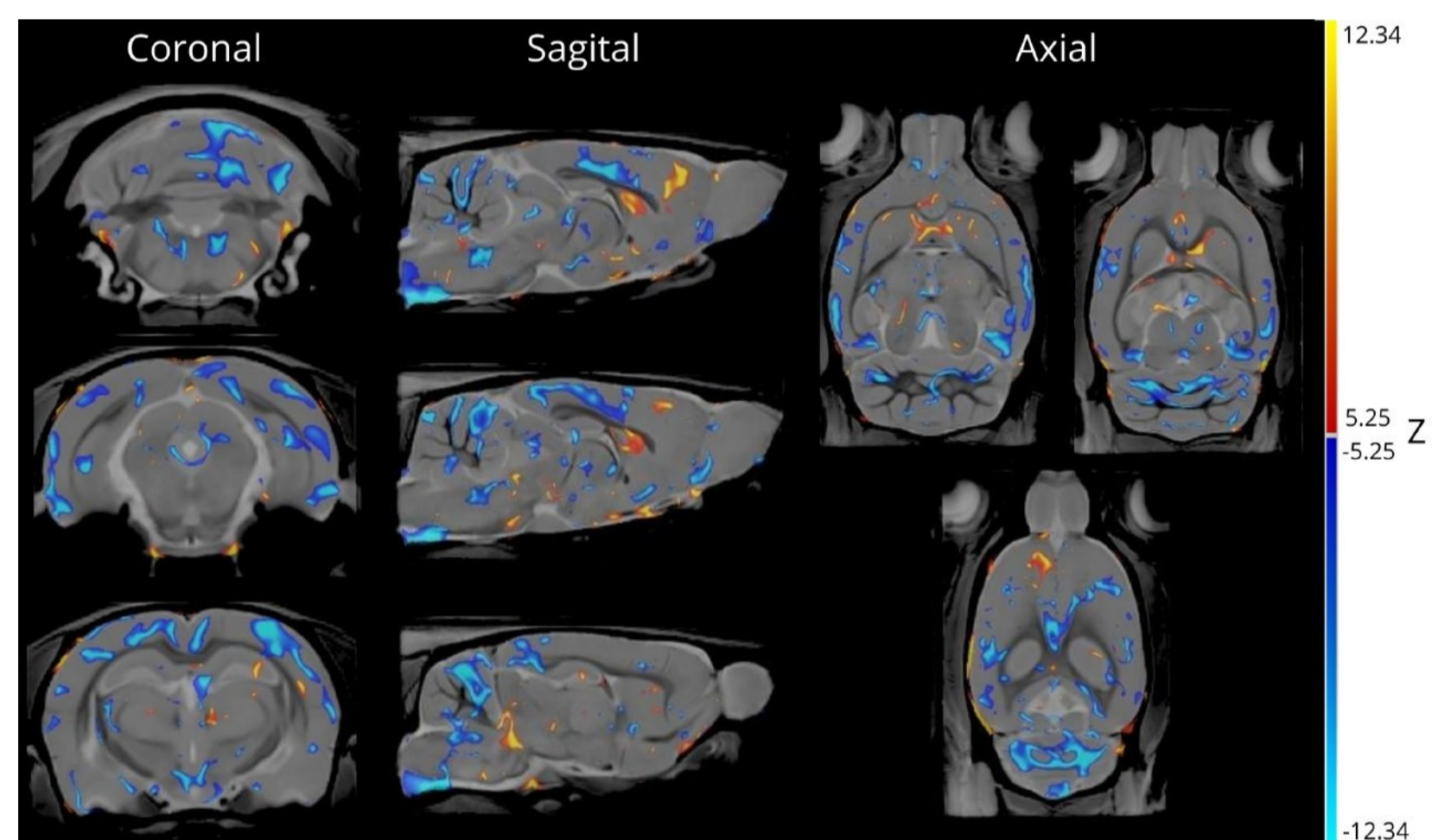


Figure 2. Jacobian map voxel to voxel

Conclusions

We found robust local neuroadaptive structural changes related to chronic voluntary alcohol use in a small sample of Wistar rats. Our future study will include a sample of 36 rats to the EtOH group and 12 rats to the healthy control as well as functional connectivity analysis and immunofluorescence techniques to understand the functional microstructural neuroadaptive changes related to AUD.

References

- 1- American Psychiatric Association. (2013). The diagnostic and statistical manual of mental disorders: DSM 5.
- 2- Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, 35(1), 217-238.
- 3- Goltseker, K., Hopf, F. W., & Barak, S. (2019). Advances in behavioral animal models of alcohol use disorder. *Alcohol*, 74, 73-82.
- 4- Simms, J. A., Steensland, P., Medina, B., Abernathy, K. E., Chandler, L. J., Wise, R., & Bartlett, S. E. (2008). Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcoholism: Clinical and Experimental Research*, 32(10), 1816-1823.
- 5- Avants, B. B., Tustison, N., & Song, G. (2009). Advanced normalization tools (ANTs). *Insight j*, 2(365), 1-35.
- 6- Goerzen, D., Fowler, C., Devenyi, G. A., Germann, J., Madularu, D., Chakravarty, M. M., & Near, J. (2020). An MRI-Derived Neuroanatomical Atlas of the Fischer 344 Rat Brain. *Scientific Reports*, 10(1), 1-13.
- 7- Zhao, Q., Fritz, M., Pfefferbaum, A., Sullivan, E. V., Pohl, K. M., & Zahr, N. M. (2018). Jacobian maps reveal under-reported brain regions sensitive to extreme binge ethanol intoxication in the rat. *Frontiers in neuroanatomy*, 12, 108.