Effects of ketamine administration in the expression of Sox 2 in zebrafish CNS

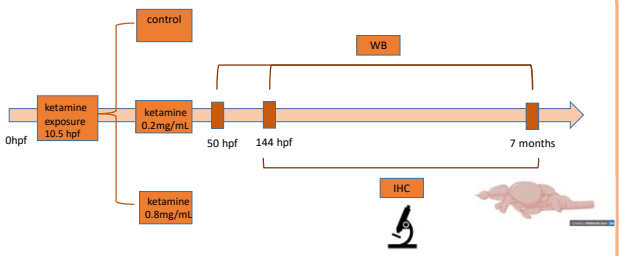
C. Santos [*], L. Félix [†], A.M. Valentim [‡,§], L. Antunes [†] and M.L. Pinto [¶,±]

[*]Centro de Investigação Vasco da Gama (CIVG), Escola Universitária Vasco da Gama (EUVG), Coimbra, PT, [†]Centro de Investigação e de Tecnologias Agroambientais e Biológicas (CITAB), [‡]Instituto de Investigação e Inovação em Saúde (i3S) and [§]Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, PT, [¶]Centro de Ciência Animal e Veterinária (CECAV) and [±]AL4AnimalS Laboratório Associado, Universidade de Trás-os-Montes and Alto Douro, Vila Real, PT

1. INTRODUCTION

Zebrafish is one of the most used animal species in research with increased significance in toxicology studies, due to well described biological advantages. Ketamine is a commonly used anaesthetic in humans the effects of which on development and stemness capability of the central nervous system (CNS) of zebrafish are poorly described. The transcription factor sex determining region Y-box 2 (Sox 2) has multiple roles, being a major pluripotency factor required in early neurogenesis. This study aimed to determine the Sox 2 expression and distribution patterns in the zebrafish brain at different timepoints following ketamine exposure.

2. MATERIALS AND METHODS



3. RESULTS

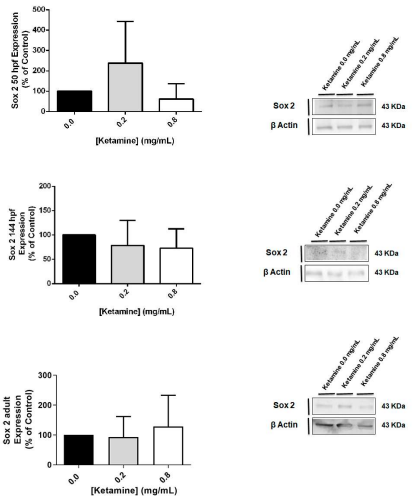


Figure 1. The levels of Sox 2 were quantified by WB in 50 hpf embryos, 144 hpf larvae and 7 months adults, after exposure to different concentrations of ketamine. Results were analyzed using Graphpad (version 8.01). Statistical analysis, was performed with one sample T-test, or one sample Wilcoxon signed rank test.

No significant alterations in Sox 2 levels were detected by WB (Fig. 1).

Sox 2 immunoexpression in CNS was found in the areas previously reported to be positive (olfactory bulb - OB and telencephalic ventricle), and in areas previously described as Sox 2 negative in adults, the cerebellum – Ce and vagal lobe. Expression in the control group (Fig. 2) was found in OB (B, C) *tectum opticum* – TeO (D), diencephalic ventricle and periventricular hypothalamus (E), Ce (F), rhombencephalic ventricle and vagal lobe (G).

Expression in the ketamine 0.8 mg/mL exposed animals (Fig. 3) was seen in OB A), telencephalon B), TeO C) and Ce D).

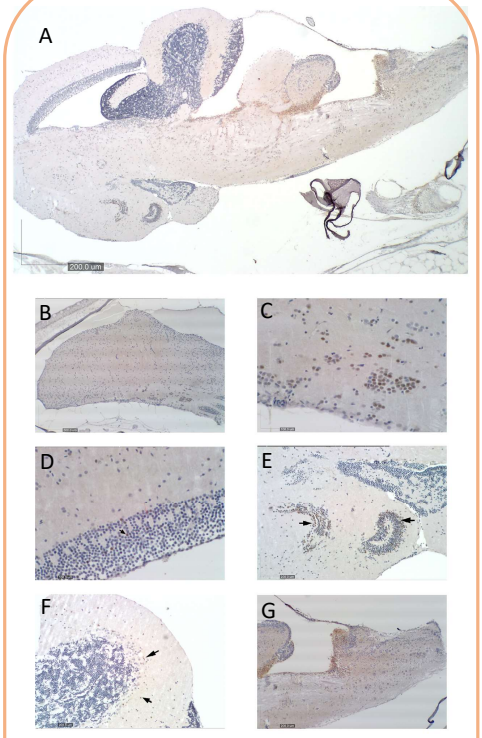


Figure 2. Representative images of Sox 2 mmunoexpression in CNS of 7 months adult zebrafish.
Control group animals. A general view, B and C OB, D TeO, E DiV and periventricular hypothalamus, F Ce and G RV and vagal lobe expression.

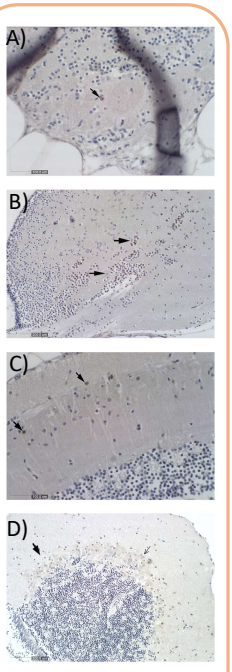


Figure 3. representative images of Sox 2 Immunoexpression in CNS, ketamine 0.8 mg/mL exposed animals.

4. CONCLUSION

Widespread Sox 2 expression in adult zebrafish CNS indicates that ketamine has no detrimental effects and that, in contrast to mammals, the stemness and pluripotency capabilities of Sox 2 are retained throughout the zebrafish life.

REFERENCES:

1. DeOliveira-Mello, L., Lara, J. M., Arevalo, R., Velasco, A., & Mack, A. F. (2019). Sox2 expression in the visual system of two teleost species. *Brain Research*, *1722*, 146350. https://doi.org/10.1016/j.brainres.2019.146350
2. Germanà, A., Montalbano, G., Guerrera, M. C., Amato, V., Laurà, R., Magnoli, D., Campo, S., Suarez-Fernandez, E., Ciriaco, E., & Vega, J. A. (2011). Developmental changes in the expression of Sox2 in the zebrafish brain. *Microscopy Research and Technique*, *74*(4), 347–354. https://doi.org/10.1002/jemt.20915
3. Grandel, H., Kaslin, J., Ganz, J., Wenzel, I., & Brand, M. (2006). Neural stem cells and neurogenesis in the adult zebrafish brain: Origin, proliferation dynamics, migration and cell fate. *Developmental Biology*, *295*(1), 263–277. https://doi.org/10.1016/j.ydbio.2006.03.040
4. Wullimann, M. F.; Rupp, B.; Reichert, H. (1996). 5 The brain of the zebrafish Danio rerio: a neuroanatomical atlas External view a b. In *Neuroanatomy of the Zebrafish Brain || The brain of the zebrafish Danio rerio: a neuroanatomical atlas.* (pp. 19–87). https://doi.org/10.1007/978-3-0348-8979-7_5

ACKNOWLEDGMENTS:
This work was funded by FEDER funds through the Operational Competitiveness Programme – COMPETE and by National Funds through FCT – Fundação para a Ciência e a Tecnologia under the project FCOMP-01-0124-FEDER-028683 (PTDC/CVT-WEL/4672/2012).
This work was supported by the projects **UIDB/00772/2020** and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT)..

Joint Congress of the European Society/College of Veterinary Pathology and Clinical Pathology
30th August - Residents' day
31st August, 1 & 2 September Joint Congress