# ENVIRONMENTAL ENRICHMENT EXERTS ANXIOLYTIC AND PANICOLYTIC-LIKE RESPONSES AND DECREASES THE ACTIVATION OF SEROTONERGIC NEURONS IN THE DORSAL RAPHE

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## BACKGROUND

Environmental enrichment (EE) is an animal management technique that seems to have several beneficial effects in the treatment of psychiatric disorders related to stress, such as anxiety and panic. It has been previously shown that one of the main neurochemical systems associated with stress and stress-related disorders is the serotonergic system that arises in the dorsal raphe nucleus (DR). Interestingly, previous evidence indicates that the DR is not a homogenous structure, but a region composed of distinct subpopulations of serotonergic and non-serotonergic neurons, both morphologically and functionally distinct. However, the benefits of EE on anxiety-related behaviors are not as clear as those induced by this procedure in other areas of the neurosciences, still requiring the need for additional research to further elucidate the processes by which the enriched environment exerts its behavioral effects, either as an anxiolytic agent or as an inoculated stressor.

## OBJECTIVE

To investigate the effects of a two-week exposure to EE vs. standard environment (control) in the avoidance and escape tasks measured in the elevated T-maze (ETM) model of anxiety in rats. These responses have been associated, respectively, to generalized anxiety disorder and panic disorder. The study also evaluated delta FosB-immunoreactivity (delta FosB-ir) in serotonergic cells of the different subnuclei of the dorsal raphe, which have been related to the modulation of anxiety and panic.

## METHODS

The study was approved by the Ethical Committee for Animal Research nº 9977150715/2015. Male Wistar rats were submitted or not to EE for a period of two weeks and tested in the ETM. Immediately after the behavioral tests, all animals were deeply anesthetized, their brains were post-fixed and collected to perform double-labeled immunohistochemistry (delta FosB and anti-serotonine) in the dorsal raphe subnuclei. The regions analyzed were: dorsal subnuclei, ventral subnuclei, lateral wings, caudal subnuclei, interfascicular subnuclei and median raphe. Statistical significance of $P<0.05$ was ascertained by two-way ANOVA for repeated measures and unpaired Student t-test, as appropriate.

**Fig. 1 –** 32 adult male Wistar rats.

**Fig. 2 –** 16 control rats remained in standard cages during 2 weeks

**Fig. 3 & 4** - 16 rats were submitted to EE during 2 weeks.

**Fig. 5 –** ETM avoidance was measured by recording the time taken by the rats to withdraw from the enclosed arm of the ETM in three consecutive trials at 30s intervals (baseline, avoidance 1 and 2).

**Fig. 6** Escape was measured by recording the time taken to leave the open arm of the ETM in three consecutive trials (escape 1 to 3), again with 30s inter-trial intervals.

•**Fig. 7**- Immediately after the ETM, animals were tested in the open field (5 min) for the number of lines crossed and number of rearings (locomotor activity).

**Fig. 8** – Immunohistochemistry for delta FosB, serotonin and double staining in the DR subnuclei and MR.

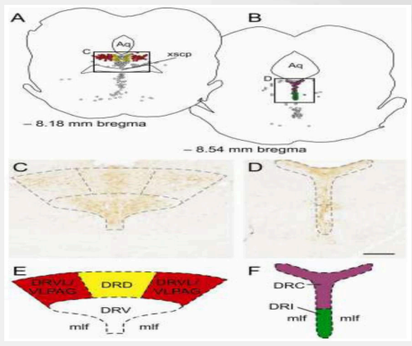
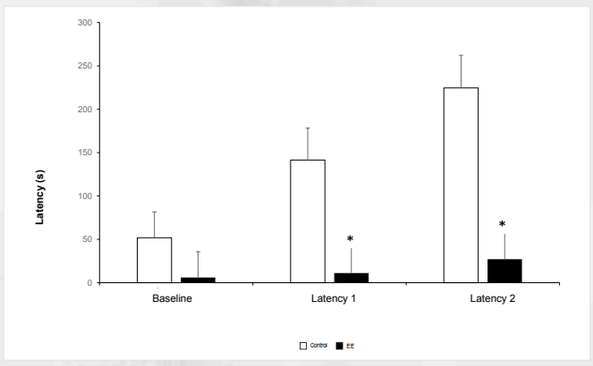
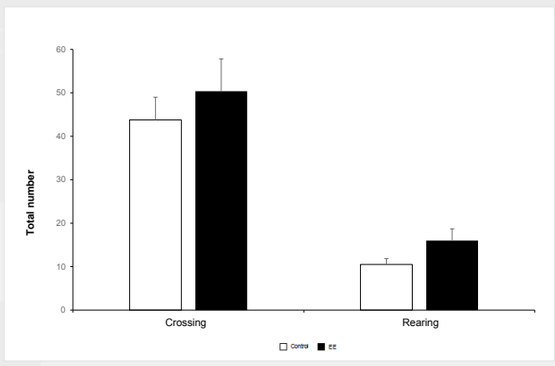



**Fig. 9 –** DR subnuclei analyzed, besides the MR.

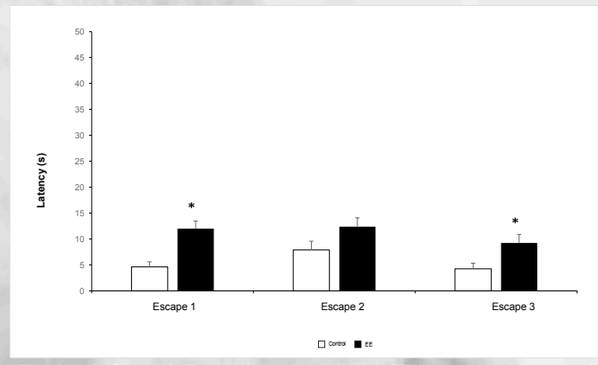
**Fig. 10** – Bright field illumination to quantify the number of *ir*-neurons, using the Image Pro-Plus program.

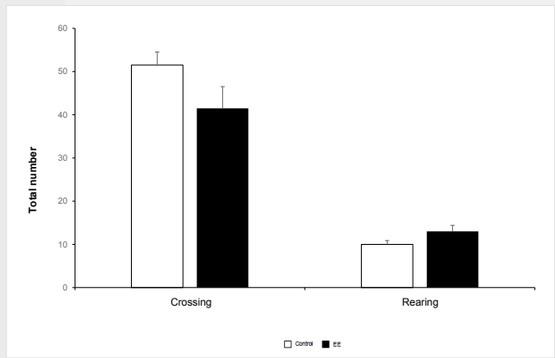
## RESULTS




•**Fig. 11 – Left panel**: Avoidance latencies (mean ±SEM) presented by control animals and animals submitted to environmental enrichment for two weeks in the ETM**. Right panel:** Number of crossings and rearings in the open field . *P < 0.05 with respect to control (ANOVA followed by unpaired Student t-test).




•**Fig. 12 – Left panel:** Escape latencies (mean ±SEM) presented by control animals and animals submitted to environmental enrichment for two weeks in the ETM**. Right panel:** Number of crossings and rearings in the open field . *P < 0.05 with respect to control (ANOVA followed by unpaired Student t-test).

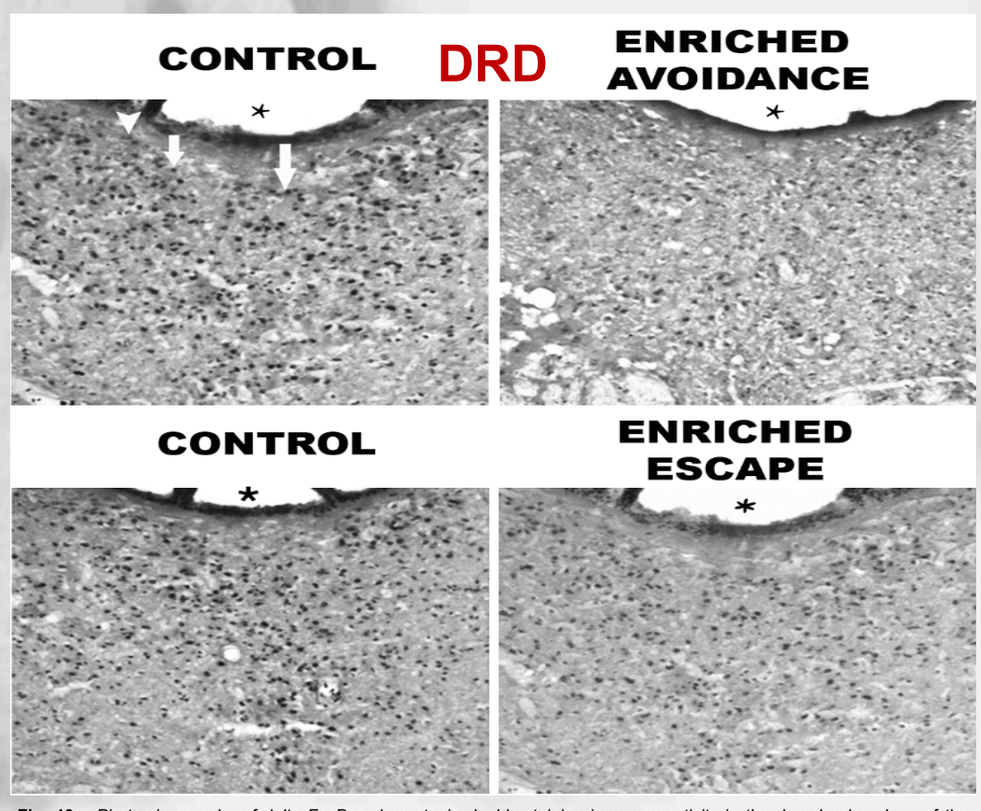



**Fig. 13 –** Photomicrography of delta FosB and serotonin double-staining immunoreactivity in the dorsal subnucleus of the dorsal raphe dorsal (DRD) in control animals and animals submitted to two weeks of EE, subjected to avoidance and escape tasks in the ETM. Magnification of 200 x, * cerebral aqueduct, arrow head = serotonin-ir, smaller arrow = Fos-ir, bigger arrow = double staining.

## CONCLUSIONS

**Exposure to EE for two weeks promotes anxiolytic and panicolytic-like effects**. It is the first report so far where EA exerts a panicolytic effect. It also **decreases the number of double-labeled neurons in the dorsal subnuclei of the dorsal raphe.** These results contribute to a better understanding of the effects of EE on anxiety and panic and its physiopathology. It also corroborates the idea that serotonin neurons of distinct subnuclei are differently activated.

## FINANCIAL SUPPORT

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