

## **Effects of maternal separation on brain stress systems: Modulation by voluntary exercise in male rats**

CAMPBELL EJ, JAMES MH, HODGSON DM, DAYAS CV.

School of Biomedical Sciences and Pharmacy, School of Psychology, University of Newcastle, Centre for Translational Neuroscience, Hunter Medical Research Institute, Newcastle, Australia.

---

### ABSTRACT

Early life stress (ELS) has been shown to predispose animals to anxiety- and depression-like behaviour in adulthood. Recent evidence suggests that repeated stress in adulthood dysregulates the hypothalamic *orexin/hypocretin* system. The current study examined the effects of maternal separation (MS), a well validated rodent model of ELS, on the expression of anxiety-like behaviour following the re-exposure to stress in adulthood. The pattern of Fos-expression in hypothalamic orexin neurons and stress sensitive brain regions was also characterised. Finally, this study examined whether the effects of this double-hit of stress could be reversed using a voluntary exercise intervention during early adulthood.

Male rat pups (n=25) were removed from dams for 3hrs on postnatal days (PND) 2-14 (MS). Controls (C; n=25) remained undisturbed during this period except for weekly weighing. On PND 75, animals were randomly allocated to either a 'stress' (30min restraint stress) or 'no stress' condition (S or NS). A subset of MS animals (n=6) was allowed access to exercise wheels for 1hr/day from PND 40-70. Following this, all animals were behaviourally tested in the open field apparatus for 10mins. Two hours after initiation of restraint, animals were perfused and brains were processed for Fos-protein immunohistochemistry and co-labelled for orexin or tyrosine-hydroxylase (TH). Counts of Fos-positive neurons were made in the hypothalamus, paraventricular nucleus (PVN), paraventricular thalamus (PVT) and ventral tegmental area (VTA).

MS-NS rats exhibited behaviour that was indistinguishable from C-NS rats. However, male MS-S rats exhibited decreased exploratory behaviour in the open field task compared to C-S rats. This was associated with a decrease in the percentage of Fos-positive orexin cells in the hypothalamus and reduced Fos-protein in the PVN, PVT and TH-positive VTA cells compared to C-S rats. Interestingly, the exercise intervention reversed the behavioural effects of MS following stress and normalized orexin cell and VTA-TH cell Fos-expression.

In conclusion, MS resulted in altered open field behaviour and hypoactivation of the orexin system in response to adult stress. The current study indicates that changes in orexin system function may involve altered activity in stress-sensitive brain regions such as the VTA, PVN and PVT. Importantly, the behavioural and neural changes observed were reversed by voluntary exercise in early adulthood. These findings highlight the importance of non-pharmacological interventions in the treatment of stress-related disorders.