

# Prolonged exposure to milk caseins in early postnatal life increases depressive-like behaviour

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Nutrition during the postnatal period can influence brain development and play an important role in the progression of neuropsychiatric disorders such as depression. Milk is usually the main source of nutrition for infants during early postnatal development. Casein, a major protein in milk, releases casomorphines with opioid activity upon digestion<sup>1</sup>. These casomorphines have been shown to cross the blood brain barrier<sup>2</sup> and therefore may influence the development of the opioid system. Indeed, it has been shown that weaning rat pups at postnatal day 21 (PND21) compared to weaning later (PND25) stimulates the developmental expression of delta opioid receptors (DOPr) in cortical brain regions. Furthermore, this has been shown to be dependent on the loss of dietary casein<sup>3</sup>. DOPr are known to play a major role in anxiety and depression<sup>4</sup>. It is therefore hypothesised that prolonged exposure to milk caseins, beyond the normal age of weaning may influence the development of DOPr, resulting in alterations to mood.

In the current study male rats were provided with either casein rich or casein free milk, from PND 21 (the normal age of weaning in rats) through to PND 25. On PND25, depressive-like behaviour was tested using the forced-swim test (FST) and brain and urine samples were collected for further analysis. Quantitative receptor autoradiography of DOPr and oxytocin receptors (OTR) were performed in the brains of the aforementioned rats, using 7nM [<sup>3</sup>H] Delt-1 and 50 pM [<sup>125</sup>I] OVTA respectively. Metabonomic analysis was performed in the urine samples collected from these animals.

On PND 25 the group of pups provided with casein rich milk displayed a behavioural phenotype consistent with depression as indicated by increased immobility times ( $p < 0.01$  Student's t-test  $n = 8$ ) (Figure 1). A significant difference in DOPr density in the deep layer of the somatosensory cortex was observed between the two groups (One-way ANNOVA  $p < 0.05$ ) (Figure 2). OTR autoradiographic binding identified a significant down regulation of OTR in casein rich animals. The most significant difference was observed in the basal lateral amygdala (One-way ANNOVA  $p < 0.01$ ) (Figure 3). Furthermore, metabonomic analysis of urine samples revealed significant difference in metabolites between the two groups (Figure 4).

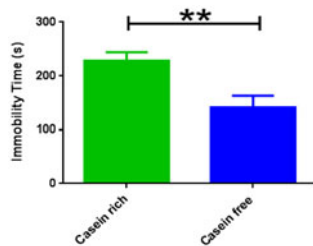


Figure 1: FST Total Immobility Time

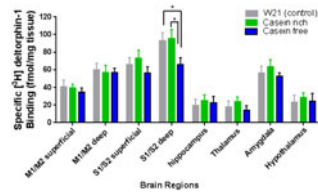


Figure 2: DOPr binding results

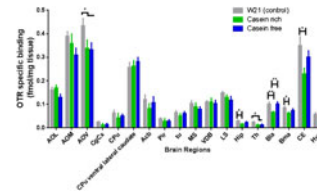


Figure 3: OTR binding results

Metabolite	Diet
Betaine	Casein free
Lactate	Casein free
Acetate	Casein free
Hippurate	Casein rich

Figure 4: metabolomics results

These data show first evidence that a casein rich diet consumed in early life results in alterations to receptors in the brain, which might be associated with the development of mood disorders. The differences in urinary metabolites indicate a possible gut-brain axis role in mediating the observed effects. The exact mechanisms underlying these observed effects still remain to be determined.

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2. Sun Z, and Cade J R (1999). "A Peptide Found in Schizophrenia and Autism Causes Behavioral Changes in Rats." *Autism*, **3**(1), 85–95.
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