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n-3 PUFAs have beneficial effects on anxiety and cognition in female rats: Effects of early life stress

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Abstract Stressful life events, especially those in early life, can exert long-lasting changes in the brain, increasing vulnerability to mental illness especially in females. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) play a critical role in the development and function of the central nervous system (CNS). Thus, we investigated the influence of an eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) (80% EPA, 20% DHA) n-3 PUFAs mixture on stress-related behavioural and neurobiological responses.

Sprague-Dawley female rats were subjected to an early-life stress, maternal separation (MS) procedure from postnatal days 2 to 12. Non-separated (NS) and MS rats were administered saline, EPA/DHA 0.4 g/kg/day or EPA/DHA 1 g/kg/day, respectively. In adulthood, EPA/DHA treated animals had a dose dependent reduction in anxiety in NS rats. Furthermore, cognitive performance in the novel object recognition task (NOR) was improved by EPA/DHA treatment in NS animals only. EPA/DHA 1 g/kg/day decreased behavioural despair in the forced swim test.

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Notably, EPA/DHA high dose increased the translocation of GRs into the nucleus of NS rat hippocampus. However, the levels of mBDNF remained unchanged in all the experimental groups. The corticosterone response to an acute stress was blunted in MS rats and this was further attenuated by pre-treatment with EPA/DHA. Immune response and monoamine neurotransmission were significantly altered by early-life stress. In conclusion, our study supports the view that n-3 PUFAs are beneficial in neurodevelopmentally normal animals but have little positive benefit in animals exposed to early life stress.

1. Introduction

Stressful life events, especially those in early life, can exert long-lasting changes in the brain increasing the likelihood of adverse health consequences (Cryan and Dinan, 2013; Lupien et al., 2009). The World Health Organization (WHO) predicts that by 2020, mental illness, including stress-related diseases, will be the second leading cause of disabilities globally (Lucassen et al., 2014). Women appear to have an increased risk of suffering from stressful life events and seem to be more susceptible to the development of major mental illnesses such as major depression including atypical depression (Dinan, 2005). However, it is worthy of note that currently, in contrast to men, there is a distinct paucity of information describing stress responses in women (Taylor et al., 2000). Thus, understanding the mechanisms behind the higher susceptibility of women to the pathophysiology of stress may open up novel strategies to improve the ability of women to cope with stressful life events.

Inadequate maternal care has been linked to developmental, emotional, and social deficits in human infants (Field, 1998) and in the rat (Caldji et al., 1998). In rodents, the maternal separation (MS) model is a well-known paradigm used to investigate the biological consequences of early-life stress (O'Mahony et al., 2011). MS alters response in several behavioural paradigms in adult rodents (O'Mahony et al., 2009) producing a phenotype which is comparable to depression in adult humans (Schmidt et al., 2011). Such stress also induces long-lasting alterations in many systems which impact cognition and emotional state (Maccari et al., 2014). For instance, we have found that MS increases proinflammatory cytokines in the periphery and results in exaggerated hypothalamic—pituitary adrenal (HPA) axis responses (O'Mahony et al., 2011). Moreover, the negative feedback inhibition of the HPA, regulated by hippocampal glucocorticoid receptors (GRs) as well as glucocorticoids secretion, is altered in adult rodents after early-life stress experience (Levine and Wiener, 1988; Lupien et al., 2009). This is of importance considering that dysregulation of the HPA, which controls the physiological response to stress, is implicated in the pathogenesis of depression (Holsboer, 2000; Julio-Pieper and Dinan, 2010).

It is well recognized that changes in diet are a viable strategy for enhancing cognitive abilities, protecting the brain from damage, counteracting the effects of ageing and warding off mental disorders (Logan, 2003). Polyunsaturated fatty acids (PUFAs) constitute 20% of dry weight brain and as constituents of the cellular membranes, they exert a profound impact on an organism's development, structure, and function (Yehuda et al., 1999). Growing evidence shows that n-3 PUFAs play an important role in the regulation of monoamine neurotransmission (Chalon, 2006). Rats deficient in n-3 PUFAs show dramatically reduced dopamine and serotonin levels in the frontal cortex and lower density of the synaptic vesicles in the CA1 region of the hippocampus (Chalon, 2006). This impairment can be translated to deficiencies related with learning performance as well as structural changes within the mesocorticolimbic pathway (Yoshida et al., 1997).

The effects of n-3 PUFAs on cognitive function, depressive- and anxiety-like symptoms have been linked to the action of brain-derived neurotrophic factor (BDNF) (Autry and Monteggia, 2012) which increases neuroplasticity and cell survival (Maitre, 1996). Several studies have reported BDNF as one of the primary targets for regulation by n-3 PUFAs (Rao et al., 2007). Moreover, recent findings have shown a connection between BDNF and glucocorticoid signalling that contribute to the regulation of the HPA, as well as to the modulation of stress response (Jeanneteau et al., 2012). Indeed, glucocorticoid receptors (GR), largely expressed in the hippocampus, have been shown to regulate the levels of BDNF through specific molecular signalling (Chen et al., 2012). Furthermore, the abundance and activity of GRs is linked with the functionality of the HPA, the dysregulation of which is implicated with maladaptive responses to the stress (Pariante, 2009). Supplementation of n-3 PUFAs has been shown to impede the disruption of normal HPA functionality associated with the development of neuropsychiatric disorders (Larrieu et al., 2014). Moreover, n-3 PUFAs, among their biological properties, have shown anti-inflammatory effects as a possible pathway through which they can be effective both in preventing or treating stress related disorders (Grosso et al., 2014).

In the light of these observations, we hypothesized that chronic intake of n-3 PUFAs would improve the performance of adult female rats exposed to early life stress. Furthermore, we predicted that these effects would be related to molecular changes at BDNF and GR levels as well as with changes in monoamine neurotransmission.

2. Materials and methods

2.1. Maternal separation

Maternal separation was performed as previously described (O'Connor et al., 2013; O'Mahony et al., 2008). Briefly, Sprague Dawley male and female rats were obtained from Harlan Laboratories UK (250—300 g) and mated in the local animal unit. Food and water was available *ad libitum* and