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Research report

Omega-3 fatty acids improve behavioral coping to stress in multiparous rats

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HIGHLIGHTS

- Diets with and without omega-3 fatty acids were consumed by dams for two cycles of gestation and lactation.
- Consumption of omega-3 fatty acids decreased escape time in the shuttle box escape test, indicated improved learning in response to stress.
- Consumption of omega-3 fatty acids decreased novelty reactivity and habituation in the open field test.
- Despair-related behavior, fearfulness, anhedonia, and motor coordination were not affected by omega-3 fatty acid consumption.
- Consumption of a diet containing omega-3 fatty acids resulted in behavioral changes consistent with resistance to anxiety and depression.

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ABSTRACT

Behavioral coping refers to the ability to modify behavior to escape from stress, and is protective against the development of depressive disorders. Omega-3 fatty acid (n-3 FA) intake is inversely correlated with anxiety and depression in humans. The objective of this study was to determine if consumption of n-3 FAs promotes adaptive coping behaviors in a multiparous rat model. Twenty female rats were randomly assigned to diets with or without n-3 FA containing menhaden oil or sunflower oil as the fat source, respectively. Rats experienced two cycles of gestation and lactation. Behavioral testing began on the second day after the last parturition. Rats consuming n-3 FAs displayed improved escape learning in the shuttle box test. Specifically, rats consuming n-3 FAs escaped footshock more quickly and had a greater number of successful escapes in the shuttle box than rats not consuming n-3 FAs. Diet did not affect general activity in the open field, but rats consuming n-3 FAs showed less reactivity and habituation to novelty in the open field than rats not consuming n-3 FAs. Immobility and swimming in the forced swim test, risk-taking assessed by the light/dark test, sucrose drinking, and motor coordination were not significantly affected by diet. A diet enriched with n-3 FAs promoted behavioral escape changes consistent with increased adaptive coping to stressful events, suggesting that n-3 FAs may help prevent the development of stress-related depressive disorders.

1. Introduction

Adaptive behavioral coping after exposure to stressful events is protective against the development of depressive disorders in humans [1]. Alternatively, inability to escape from chronic stress increases the susceptibility to depression [2]. Pregnancy and motherhood are events that greatly increase the stress of everyday life and may be taxing enough to induce a helpless (depressive) state in some women [3–5]. Learned helplessness in rats is characterized by the failure to escape a stressful situation [6]. Escape learning tests help determine whether a rodent can learn to overcome stressful events, a phenomenon also known as adaptive behavioral coping. The shuttle box escape test evaluates the response of rodents to a stressful footshock and offers insight into their escape learning abilities when faced with acute stress. When studying adaptive coping in the context of nutritional interventions in multiparous rats, we began with the hypothesis that dietary omega-3 fatty acids (n-3 FA) may facilitate behavioral escape of rats undergoing pregnancy and lactation.

N-3 FAs play an important role in fetal development but their absence or supplementation during gestation may alter maternal postpartum behavior [5,7]. Eicosapentaenoic acid (EPA) and

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docosahexaenoic acid (DHA) are two of the most important n-3 FAs because they are integral components of the phospholipid bilayer in neuronal cell membranes, making them relevant to membrane physiology and neurotransmission [8]. Although the exact mechanism through which n-3 FAs act is unclear, evidence suggests that n-3 FAs have anti-inflammatory properties that ultimately protect neurons from the effects of stress, enhancing neuronal survival. When n-3 FAs are unavailable for incorporation into the phospholipid bilayer of neuronal cell membranes, omega-6 fatty acids (n-6 FAs) tend to be substituted [9–11]. N-6 FAs are the substrate for cytosolic phospholipase A_2. This reaction produces prostaglandin E_2 (PGE-2). PGE-2 subsequently stimulates the production of proinflammatory cytokines. N-3 FAs decrease PGE-2 [12] and proinflammatory cytokines as well as other mediators of inflammation (reviewed in [13]). In addition, consumption n-3 FAs results in lower serum concentrations of corticosterone [14–19] and proinflammatory molecules [18,19] following the exposure of rodents to a variety of stressors protecting, for example, glutaminergic neurons in the hippocampus from damage during stress (reviewed in [20]). N-3 FAs also increase brain derived neurotrophic factor (BDNF) levels [21,22]. Recently, n-3 FA consumption was shown to increase dendritic branching in the hippocampus [23], a phenomenon induced by BDNF [24,25]. Thus, n-3 FAs appear to both prevent the neuronal impairment associated with stress and inflammation as well as promote neuronal plasticity.

Epidemiological evidence indicates that diets lacking n-3 FAs during pregnancy may contribute to behavioral disturbances such as anxiety and difficulty concentrating and these symptoms make it difficult for a woman to adjust to life as a new mother [26]. Like anxiety, depression is seen in the postpartum time period and the two disorders are often comorbid [5,7]. The risk for these disorders increases with parity, closely-spaced pregnancies, a familial history of mood disorders, significant life stress and previous occurrence of mood disorders [3,5,27]. Without proper treatment, symptoms such as altered feeding patterns, feeling overwhelmed, sad or disinterested mood, and thoughts of harming the baby or one's self pose obvious threats. On the other hand, conventional pharmaceutical treatment is not ideal as medication can pass to the fetus and neonate via blood and breast milk; thus, there is a need for safe pregnancy and postpartum alternative interventions to improve coping behavior.

Because depletion of n-3 FA reserves and multiple, closely-spaced pregnancies may predispose women to anxiety and mood disorders in the postpartum period, consumption of diets lacking n-3 FAs and two cycles of gestation and lactation separated by 8–10 days has been used to model the postpartum human condition in rats (hereafter referred to as "multiparous") [19,28,29]. The majority of the research in multiparous rats that consume diets with and without n-3 FAs is focused on outcomes other than behavior. The concentration of n-3 FAs in brain phospholipids and red blood cells are higher in multiparous rodents that consume them [10,11,19,28]. Multiparous rodents lacking dietary n-3 FAs have higher corticosterone secretion in response to stress [19] and decreased activity of monoamine oxidase A (MAO-A), the enzyme that metabolizes neurotransmitters such as serotonin and dopamine [30], all indications that n-3 FAs may be necessary for the prevention of postpartum behavioral disorders. Behaviorally speaking, Levant and colleagues found an increased latency to the first bout of immobility but not reduced immobility in the forced swim test in multiparous rats that consumed n-3 FAs [19] when compared to rats deficient in n-3 FAs, while Chen and colleagues observed decreased anxiety in the elevated plus maze in rats that consumed n-3FAs as evidenced by more time spent in open arms as well as increased number of entries into open arms [30].

Dietary n-3 FA supplementation holds promise with respect to the prevention and treatment of postpartum behavioral disorders, yet there are virtually no studies evaluating the effect of dietary n-3 FA levels on multiple behaviors using the multiparous rodent model; thus, the objective of the present study was to determine whether multiparous rats consuming diets lacking or containing high levels of n-3 FAs would exhibit differences in escape learning and other behaviors that may be relevant to human postpartum stress-related disorders.

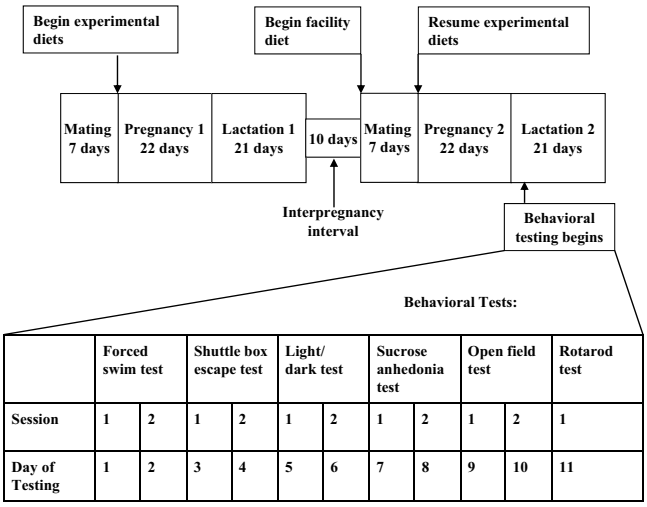

Fig. 1. Experimental timeline. Twenty female rats were mated two consecutive times over the course of twelve weeks and consumed either a diet containing or lacking n-3 fatty acids. Behavioral experiments commenced on day 2 after the second parturition. Behavioral tests were conducted in the order shown.

2. Materials and methods

2.1. Animals and diets

This study used a multiparous rat model similar to that repeatedly used by Levant et al. that was selected based on results showing that a diet lacking n-3 FAs reduces neuronal DHA content after just one pregnancy, and that this change in neuronal cell membrane composition is correlated with increased depression-related behavior [10,11,19,28,29]. Our experimental timeline is shown in Fig. 1. The sequence of behavioral testing was chosen in order to better detect differences in behavior as a result of treatment. Twenty female Long-Evans rats were group housed, handled three times per week, and consumed the standard University of Texas at Austin animal facility diet until 8 weeks of age. At 8 weeks of age, two female rats were housed with one unrelated male rat for a mating period of 7 days. Following mating, the female rats were randomly assigned to one of two diets, described below ($n = 10$ per group). Dams were pair-housed for the first 2 weeks of the two scheduled pregnancies. Dams were singly housed for the last week of each pregnancy through parturition and housed with their pups throughout weaning and for the duration of behavioral testing. Two days after parturition, each litter was culled to eight pups, consisting of four male and four female pups when possible. All animal procedures were approved by the Institutional Animal Care and Use Committees of the University of Texas at Austin and Texas State University.

Diets were based on the breeding diet, AIN-93G, and manufactured by Research Diets, Inc. (New Brunswick, NJ). The "without n-3 FA" group consumed AIN-93G, formulated with 7% sunflower oil, thereby lacking n-3 FAs. The "with n-3 FA" group consumed AIN-93G formulated with 7% Virginia Prime Gold™ menhaden oil (Omega Protein Inc., Houston, TX), which contained 24% n-3 FA volume/volume. The menhaden oil consisted of 14.2% EPA and 10.3%