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Dietary n-3 polyunsaturated fatty acid deprivation together with early maternal separation increases anxiety and vulnerability to stress in adult rats ☆

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ABSTRACT

Low concentrations of n-3 polyunsaturated fatty acid (PUFA) and chronic stress are implicated in susceptibility to mood disorders. We have investigated the combined effects of chronic n-3 PUFA dietary deficiency and early maternal separation (MS) stress on the reactivity to stressful situations of rats as adults.

Pups fed a control or an n-3 PUFA deficient diet were daily separated for two weeks before weaning They were all tested at 3 month-old to determine their anxiety, and their ability to learn two aversive tasks differing in the control they could exert on the situation: auditory fear conditioning and brightness avoidance discrimination.

Neither the n-3 PUFA-deficient diet nor MS alone significantly affected behavior. But n-3 PUFA-deficient rats that had been separated were more anxious and fearful in inescapable situations, while their ability to cope with an aversive avoidance task remained unaffected.

These results support the notion that PUFA-unbalanced diet, together with stress, may be a determinant risk factor in emotional disorders.

1. Introduction

The imbalance between n-6 and n-3 polyunsaturated fatty acids (PUFA) in the diets of Western nations has increased because of a rise in the n-6 PUFA supply and a lower n-3 PUFA intake [1]. This imbalance has been involved in the increasing prevalence of many diseases, including cardio-vascular disorders, diabetes and obesity, notably by exacerbating the inflammatory processes involving n-6 PUFAs and their derivatives [2]. Because the concentration of the major n-3 long-chain PUFA, docosahexaenoic acid (DHA, 22:6n-3), is higher in the brain than in other tissues, questions have been raised about the influence of an inadequate n-3 PUFA dietary intake on cognition and neural disorders [3,4]. It has been suggested that abnormal brain PUFA content could lead to the development of mood disorders and several studies indicate that an altered n-3 PUFA status is a non-genetic risk factor in depression [5–7].

Rodents fed a diet low in n-3 PUFAs have a reduced brain DHA content and higher scores of anxiety, aggressive and depressive behavior [8,9]. Disruptions of learning and memory processes have also been reported [3] in a variety of tests, including olfactory discrimination tasks [10,11], passive-avoidance tasks [12] and spatial performance in the Morris water maze and the Barnes maze [13–15].

We have shown that a brain deprived of DHA due to a lack of dietary α-linolenic acid (18:3n-3, the DHA precursor) leads to deregulation of the meso-cortico-limbic dopaminergic pathway in rats, followed by hypodopaminergia in the fontal cortex and hyperdopaminergia in the nucleus accumbens [16–18]. In addition, we showed that these neurochemical alterations are associated with changes in emotional response revealed by increased consumption of sucrose and abnormal responses to novelty [19]. These results further indicated that, when associated with an early chronic stress of maternal separation (MS), the n-3

Abbreviations: CS, conditioned stimulus; DHA, docosahexaenoic acid; EPM, elevated plus maze; MS, maternal separation; PUFA, polyunsaturated fatty acid; PND, post-natal day, PTSD, post-traumatic stress disorder; US, unconditioned stimulus

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PUFA deficient diet exacerbates the changes in emotional responses, suggesting that a DHA deficiency and early chronic stress may act in synergy.

The present study was done to confirm this possibility on rats raised on a chronic n-3 PUFA-deficient diet and subjected to the early stress of MS, when their complementary behavioral responses were tested. Early MS which disrupts the normal maternal-infant interaction, mimicking human parental neglect in early life, is considered to be very stressful for rats [20]. We therefore compared the emotional and behavioral responses of four groups of adult rats. Rats were fed either a balanced diet or an n-3 PUFA-deficient diet. Half the rats from each dietary group were separated from their mothers each day for two weeks before weaning. At three months, the basal degree of anxiety was determined for all groups of rats using a reliable tool for measuring anxiety-like behavior, the elevated plus-maze [21]. Rats were then tested successively in two aversive situations, tone-fear conditioning and a brightness-discriminative avoidance task, that differed in the control they could exert over events, and thus in the induced stress. For tone fear conditioning, a neutral tone was paired with an electrical foot shock, eliciting fear responses, such as freezing behavior, by an associative process. During extinction of this conditioning, repeated exposure to the tone without an electrical shock results in a progressive decrease in fear. In addition, giving electrical foot shocks in a new context, after extinction, reinstates the fear of the tone which can be used to evaluate the strength of extinction [22]. The freezing response obtained during fear conditioning, extinction and reinstatement determines the level of fear developed by each group of rats when they are unable to exert any control over the events. In the avoidance task, rats were trained to reach the lighted arm of a Y-maze in order to escape and possibly avoid an electrical foot-shock. In this situation, rats can control the events and avoid the electrical foot shock by rapidly reaching the goal arm. We postulated that the effects of the stress of MS on the behavior of adult rats would be amplified by a chronic lack of n-3 PUFA.

2. Methods and materials

2.1. Animals and diets

We used our multigenerational deprivation model [19]. Two generations of female rats were fed a diet containing only n-6 PUFAs to produce an extremely low basal n-3 PUFA intake [n-3 PUFA-deficient diet] (see [23] for diet composition). Two weeks before mating, the second generation of deficient females was assigned to one of two dietary groups: one was fed the deficient diet, and the other was fed a diet with an adequate n-6 and n-3 PUFAs content [control diet]. Both diets contained 6 g lipid/100 g diet. The control diet contained a mixture of peanut and rapeseed oils and contained 1200 mg linoleic acid per 100 g diet and 300 mg α-linolenic acid per 100 g diet, corresponding to the recommended n-6:n-3 ratio [24]. The n-3 PUFA-deficient diet included peanut oil, which provided 1200 mg linoleic acid per 100 g diet and negligible α-linolenic acid (<0.1 g) (UPAE, Institut National de la Recherche Agronomique, INRA). Only male offspring were used in the study to avoid gender-induced differences. At weaning, the rats from each litter were housed two or three per cage until adulthood (2–3 months) with free access to the same diet as their mothers, and under controlled temperature (22 ± 1 °C), humidity ($50 \pm 10\%$) and light cycles (7 AM–7 PM). The experimental protocol complied with the European Community guidelines (directive 86/609/EEC) and was approved by the INRA Committee.

2.2. Maternal separation procedure

Maternal separated offspring were all removed from their dams for 6 h (10 AM–4 PM) each day from post-natal day (PND) 6 to PND 21 (weaning) as described by Matthew et al. [25]. They were placed in cages that contained shavings from their respective dam's cage and kept in a temperature (30–32 °C) and humidity-controlled incubator [19]. Each litter was returned to their dam's cage at the end of the separation period. Pups not subjected to MS were simply removed from their mothers for a few minutes each day. Pups were weaned from their mothers on PND 21 and housed in groups of two or three until they weighted 250–300 g (2–3 months of age). Four experimental groups were thus constituted: controls, n-3 PUFA deficient, maternal separated, n-3 PUFA deficient + maternal separated ($n=11$ for each group).

2.3. Behavioral procedure

2.3.1. Elevated plus maze (EPM)

The EPM test was performed first to determine the rats' basal anxiety and motor activity [26,27]. The EPM test was conducted in a dimly lit room (25 W lamp). The plus maze apparatus constructed of black PVC was 60 cm above the floor. Four arms (50 cm long and 11 cm wide) with two open arms and two closed arms with 50-cm high walls were arranged in a cross with two arms facing each other. Each animal was placed in the center of the EPM, facing a closed arm, for a 5 min period of free exploration. A video camera, placed above the center of the apparatus was relayed to a monitor in an adjoining room to score the behavior live. All four paws had to cross the entry of the open or the closed arm to be considered an entry. We measured two parameters: the time spent in the open arms relative to the time spent in both open and closed arms was used to estimate anxiety, while the total number of entries into the four arms of the maze was used to measure the motor activity developed by each group of rats. All the rats were returned to their home cages at the end of the 5-min test and the plus maze was cleaned with an ethanol solution.

2.3.2. Classical fear conditioning

Two chambers ($20 \times 24 \times 23$ cm^3) were used. The fear conditioning chamber was lighted with an external 40 W lamp and had an electrifiable grid floor, black walls, except for the front door, which was transparent Plexiglas to allow observation of the rat. An exhaust fan provided a continuous background noise (70 dB). The other chamber was only used to deliver two additional foot shocks before the reinstatement session and was identical to the first chamber but had black and white striped walls. The light was provided by a 15 W light and there was no fan noise. Each box was equipped with a loudspeaker in the center of the ceiling to deliver the tone. A video camera mounted facing the chambers and connected to a video recorder was used to monitor behavior, which was observed and scored live in the adjoining room. The tone (80 dB, 2 kHz, 20 s) was delivered by a tone-generator and the foot shock (0.8 mA, 0.5 s) by a scramble-foot shock generator (constructed in house). Both were controlled on-line by an IPC computer.

2.3.2.1. Behavioral parameters. Freezing behavior, defined as the absence of all movement, except for respiratory and slow pendulum movements, while the rat was in the stereotype crouching posture [28], was used as an index of fear. The freezing response time was measured blindly during the 30-s