Omega-3 fatty acids influence the rate of adult neurogenesis in the lobster, *Homarus americanus*

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Long chain polyunsaturated fatty acids (LC-PUFAs), which make up 20% of the brain's dry weight, are critical for healthy brain development and function because of their roles in membrane structure and cytokine regulation. Animals cannot synthesize these molecules in adequate quantities, and so they must be derived from dietary sources. Research suggests that abnormalities in fatty acid and membrane phospholipid metabolism may play a part in a wide range of neurodevelopmental (e.g., autism, Rett's syndrome) and psychiatric disorders (e.g., schizophrenia, depression, borderline personality disorder)[5,7-8]. Epidemiological studies also support a connection between seafood consumption, a rich dietary source of PUFAs, and a lower prevalence of depressive illnesses[7].

One of our primary interests is the possible relationship between depressive disorders and deficiencies in life-long neurogenesis, a link originally proposed by Jacobs et al.[6] and which is now supported by a host of animal studies. With this in mind, an interaction between omega-3 fatty acids in the diet and neurogenesis could at least partly explain the connection between depressive illness and the finding of low levels of fatty acids in afflicted individuals. This potential link has not, to our knowledge, been explored.

Within this category of molecules, specific omega-3 fatty acids (e.g., eicosapentaenoic [EPA, 20:5ω3] and docosahexaenoic [DHA, 22:6ω3]) appear to have particularly beneficial effects on human health using a variety of measures. The ratio of omega-6 and omega-3 fatty acids also seems to be an important health factor, with a recommended dietary ratio of 2:1 omega 6:omega 3 fatty acids[10]. In contrast to this recommendation, however, an average diet in the United States/western world is estimated to be closer to 20:1[7].

A review of the scientific literature on LC-PUFAs reveals that there is little understanding of how specific fatty acid levels and omega-6:omega-3 ratios impact cellular processes (e.g. the cell cycle, neurogenesis). *In vivo* cellular studies in humans that could answer some of these questions are extremely challenging for both scientific and ethical reasons. An alternative is to use animal models. The nervous systems of non-vertebrates offer significant advantages over mammalian systems, and at the cellular level the physiology and chemistry of neurons is basically the same as in vertebrates. We are therefore able to use the brain of the lobster, *Homarus americanus*, as a model system for examining the influence of environment on adult neurogenesis[1]. The lobster is long-lived, has an accessible and relatively simple nervous system, and quantifiable behaviors. As an economically important species, it has been the subject of intense research on all aspects of its biology, including (but not limited to) life style, nutrition, development and nervous system function[2-3]. This organism therefore offers many advantages for examining the influence of LC-PUFAs on neurogenesis.

In our study, groups of juvenile lobsters were maintained for 4 weeks on one of three diets: (1) brine shrimp (*Artemia franciscana*; a standard diet used for mariculture purposes), (2) omega-3

enriched *A. franciscana*, or 3) *Spirulina*-enriched *A. franciscana* (the current diet used at the lobster rearing facility at the New England Aquarium). These diets specifically vary in EPA levels (0.12, 2.6, and 2.3 mg/g dry weight respectively; M.F. Tlusty, unpublished data), and none of them contain DHA. The ratios of total omega-6:omega-3 PUFAs in these diets are approximately 1:2, 1:1 and 2:1 respectively. These diets were not intended to replicate the natural diet of young lobsters in the wild, which is composed of plankton rich in EPA (12.8 mg/g dry weight) and DHA (22.3 mg/g dry weight; M.F. Tlusty unpublished data). Rather, these diets were designed to provide a first glimpse of how controlled manipulation of LC-PUFA levels might influence the cell cycle, and specifically neurogenesis.

At the end of the period on these special diets, lobsters were incubated in bromodeoxyuridine (BrdU; 2mg/ml sea water) for 4 hours. Brains were then dissected from the lobsters and fixed for 12 hours in 4% paraformaldehyde in 0.1M phosphate buffer. Our standard immunocytochemical protocol for detection of bromodeoxyuridine[11] was used to fluorescently label BrdU. Brains were then viewed with a confocal laser microscope, and labeled cells in cell cluster 10 in the lobster midbrain[1,9] were counted in each of the three nutritional groups.

The results of this study show that neurogenesis in the lobster brain is indeed sensitive to diet. Lobsters fed the omega-3-enriched *or Spirulina*-enriched *Artemia* had the greatest numbers of newborn cells, while those fed unenriched *Artemia* had the fewest. The enriched diets resulted in a robust 50% increase in the numbers of cells born over a 4-hour period ($p=0.008$ two-tailed t-test, unenriched *Artemia* vs. omega-3 enriched *Artemia*; $p=0.01$ two-tailed t-test, unenriched *Artemia* vs. *Spirulina*-enriched *Artemia*). We do not know the fate of these additional labeled cells, and whether they survive or are eliminated by apoptosis. We know from work in related species that the BrdU-labeled cells born in this cell cluster normally mature and differentiate into neurons that will innervate two distinct synaptic areas in the midbrain[11-12]. It will be important to know whether the additional cells born as a result of dietary manipulations also differentiate along these same lines. While this study suggests a general trend that an increased level of EPA promotes neurogenesis, it did not control for omega-6:omega-3 and EPA:DHA ratios. Additional experiments controlling these nutritional aspects are currently underway.

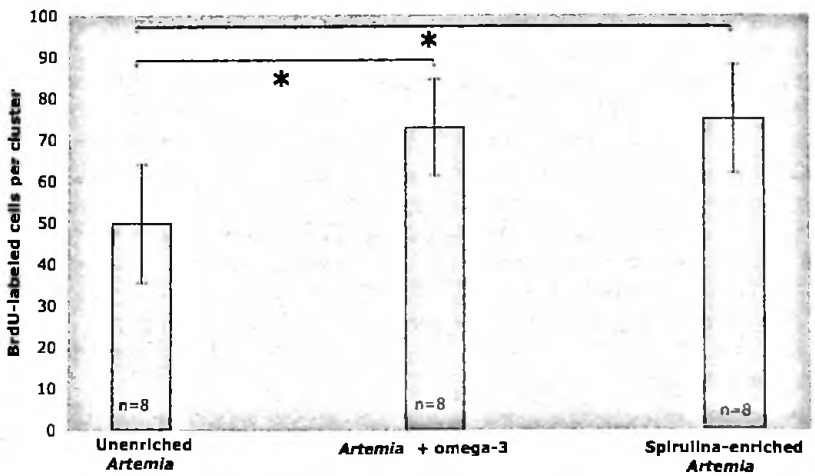

Figure 1. Levels of neurogenesis in cluster 10 in the brains of lobsters fed on three different diets. The numbers of new cells produced during 4 hours of bromodeoxyuridine incubation at dusk (16:30-20:30) were fluorescently labeled and counted. Two-tailed t-tests show significant differences between the BrdU counts in the brains of animals fed the unenriched vs. omega-3 enriched *Artemia* diets ($p= 0.008$), and between the unenriched vs. *Spirulina*-enriched *Artemia* diets ($p= 0.01$).

In experiments completed since returning to Wellesley College in August, we have shown that the circadian pattern in the rate of neurogenesis is also affected by diet. The original study documenting this phenomenon[4], which showed a marked increase in neurogenesis at dusk, used juvenile lobsters fed the unenriched *Artemia* diet which is deficient in LC-PUFAs. However, when animals are fed *Spirulina*-enriched *Artemia*, the circadian pattern disappears (B.S. Beltz, J.L. Benton, D.C. Sandeman and M.F. Tlusty, unpublished data). This is due to an increase in the lowest levels of neurogenesis throughout the daytime, which masks the rise in neurogenesis normally seen at dusk in the *Artemia*-fed animals. These data confirm an increase in the numbers of BrdU-labeled neurons with the LC-PUFA-enriched diets, and show that this is due to an overall increase in basal levels of neurogenesis as well as to a small increase in the maximal rate.

These experiments demonstrate that dietary intake of LC-PUFAs has a powerful influence over the rate of neurogenesis in the lobster brain. Future studies will manipulate levels of DHA, ratios of EPA:DHA, and overall omega-6:omega-3 ratios in this model system. The influence of fatty acid nutrition on life-long neurogenesis is expected to be equally important in vertebrates, because other environmental and endogenous factors regulating neurogenesis are highly conserved across a phylogenetically diverse range of species.

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