DYSREGULATION OF STRUCTURAL PLASTICITY IN AN EXPERIMENTAL MODEL OF ANOREXIA NERVOSA: FOCUS ON THE MEDIAL PREFRONTAL CORTEX

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Introduction: Anorexia nervosa (AN) is a complex mental illness characterized by restricted eating, an intense fear of gaining weight, body weight below 85% of expected body mass index and strenuous exercise regimens. The first onset of AN is primarily concentrated at puberty, with 90–95% of the cases among females. AN begins with a restrictive diet and weight loss and progresses to an out-of-control spiral. At the neurobiological level, the imbalance between cognitive and reward networks likely interferes with motivation for treatment and ability to learn from experience, unabling patients to stop the vicious cycle of the disease. Despite an incidence rate of 1-2% in adolescent females and its high mortality rate, little is known about its aetiology or predictive factors.
In order to identify structural and molecular signature of AN history in the brain, we evaluated if the combination of food restriction and intense exercise drives weight loss seeking during adolescence through enduring morphological changes in the developing brain, altering the cytoskeleton of the synapses in the medial prefrontal cortex (mPFC).

Materials and methods: We employed the activity-based anorexia (ABA) rat model to evaluate both spine morphology and the expression of markers of the glutamate synapse in the mPFC. Female adolescent Sprague-Dawley rats at postnatal day (P) 35 were individually housed and divided in two groups: controls (CTRL, food ad libitum–sedentary) and ABA (food restricted and free access to an activity wheel). On P38, food access for the ABA group was limited to 2 h per day but unlimited in amount, at the beginning of the dark cycle, till P42, when all ABA rats reached the anorexic phenotype. At the scheduled time, i.e. on P42, during the acute phase of the pathology, animals were sacrificed and the mPFC was dissected. Western blot analyses were run on protein extracts. In order to have a comprehensive picture of dendritic spine dynamics in the adolescent brain, eight more animals (4 CTRL and 4 ABA) were deeply anesthetized and perfused with 1.5% paraformaldehyde in 0.1 M phosphate buffer. Morphological analyses of dendritic spines in mPFC were performed using a fluorescent dyolistic labeling technique.

Results: After 24 hours of AN induction, ABA rats reduced body weight and constantly increased wheel activity over days, as expected. At the structural level, in the mPFC of ABA rats we found a reduction in the density of dendritic spines coupled with a reduction of stubby-shaped spines and an increase of filopodia, i.e. the immature dendritic protrusions. In addition, morphological analyses revealed that ABA exposure did not alter dendritic spine length but, rather, it significantly reduced the head of the active protrusions, an effect that appears to be located in the mushroom-, stubby- and thin-shaped dendritic spines. Such impairment is strengthened by reduced expression in the post-synaptic density of F-actin, n-cadherin and reelin, structural markers of the dendritic spines.
Discussion and conclusions: Our data show that a lower number of dendritic spines characterizes the mPFC of ABA rats in the acute phase of the pathology, suggesting that induction of anorexic phenotype interfered with dendritic spine formation by influencing their maturation in a critical period of brain development. Moreover, this finding, in combination with the reduction of molecular markers of cytoskeletal stability, indicates that alterations of dendritic spines may represent one of the multiple mechanisms underlying central nervous system deficit in an experimental model of anorexia nervosa.

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