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The Fibroblast Growth Factor System is Downregulated Following Social Defeat

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Abstract

The fibroblast growth factor (FGF) system has previously been found to be altered in post-mortem brains of individuals with major depressive disorder (MDD). The present study tested whether the FGF system is altered following acute social defeat. Rats were exposed to four consecutive days of either a social defeat paradigm or novel cages. Animals were sacrificed after the last social defeat session and gene expression was assessed in the hippocampus by mRNA *in situ* hybridization. Molecular components of the FGF system were significantly downregulated following social defeat. Specifically, FGF2 and FGFR1 mRNA expression was decreased in various subfields of the hippocampus. Decreased tone of the FGF system following an acute social stressor is congruent with human post-mortem results of FGF system downregulation in depression. These findings suggest that modulating the FGF system may have therapeutic value in the treatment of MDD.

Keywords

Social Defeat; FGF2; FGFR1; *in situ* hybridization; Depression

In relation to depressive disorders, an involvement of the FGF system is beginning to emerge from clinical and basic research studies. Post-mortem studies on individuals with major depressive disorder (MDD) revealed an extensive alteration of several components of the FGF family. While some FGF ligands were upregulated (e.g. FGF9) several other ligands were downregulated in cortical areas, including FGF1 and FGF2 [7]. Importantly, expression of FGF receptors, particularly FGFR2 and FGFR3 were consistently downregulated, suggesting an overall decrease in the final output of this system. These observations have been recently extended to the hippocampus and several other brain regions (Evans et al., personal communication). Taken together, these findings suggest that decreased cortical and hippocampal FGF tone is either an antecedent or consequent to MDD. This is also consistent with the fact that chronic administration of antidepressants to rodents increased the levels of neurotrophins in cortical and hippocampal structures including FGF2 levels [27,19].

Results from studies using animal models of learning and memory and anxiety-like behavior indicate a strong correlation between FGF2 levels in brain and expression of adaptive cognitive

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or emotional behavior. For example, learning of a spatial memory task was associated with increased FGF2 levels in the hippocampus [11]. Similarly, exposure to environmental complexity -a manipulation that improves learning and memory and decreases anxiety behavior- also increased FGF2 expression in several brain regions [6]. Finally, FGF2 has also been found to be increased in the hippocampus after treatment with anxiolytics [10].

Social defeat is an ethological stressor that induces several behavioral, physiological, endocrine and neural changes reminiscent of depressive-like behavior [15,16]. Many of its sequelae can be reversed by physiological or pharmacological antidepressant therapy [17,28,22]. Interestingly, brain-derived neurotrophic factor (BDNF) has previously been found to be altered in hippocampal regions following acute social defeat [1,30]. The aim of this study was to explore whether gene expression in the FGF system was altered following four defeat episodes. Based on human post-mortem findings in MDD, we hypothesized that exposure to social defeat might downregulate the FGF system in the hippocampus.

Male Sprague-Dawley rats (220–250g; Charles River Laboratories, Wilmington, MA.) were housed under a 12 hr light/dark cycle with food and water available *ad libitum*. All Sprague-Dawley rats were maintained at the University of Michigan animal facilities in accordance with University Committee Use and Care of Animals. The experiments were conducted according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. All efforts were made to minimize the number of animals used and their suffering. Resident Long-Evans rats were housed with an ovariectomized female rat for the social defeat paradigm.

Rats were subjected to either social defeat (n=9) or placed into novel cages as controls (n=12) on four consecutive days. The social stress paradigm consisted of four encounters with an aggressive Long-Evans male rat [14]. Briefly, intruder rats were exposed to resident males for 15 minutes. Intruders were considered defeated if they were attacked and exhibited supine, escape or upright behaviors. Rats were sacrificed 2h 30 min following the fourth session of either social defeat or exposure to novel cages.

For mRNA in situ hybridization of FGF2 and FGFR1, tissue was sectioned at -20°C at $10\mu\text{m}$ and sliced in series throughout the hippocampus, mounted on SuperFrost Plus slides (FisherScientific) and stored at -80°C until processed. *In situ* hybridization methodology and analysis has been previously described elsewhere [13]. Sections were taken every $200\mu\text{m}$. The sequences of rat mRNA used for generating probes of genes are complementary to the following RefSeq database nos. FGF2 (NM_019305, 716–994); FGFR1 (NM_024146, 320–977). All probes were synthesized in our laboratory. All cDNA segments were extracted (Qiaquick Gel Extraction Kit, Qiagen, Valencia, CA), subcloned in Bluescript SK (Stratagene, LA Jolla, CA) and confirmed by nucleotide sequencing. Exposure times were experimentally determined for each probe to maximize signal and are as follows: FGF2 (7 days) and FGFR1 (7 days). Data from multiple sections per animal were averaged resulting in a mean integrated optical density value for each animal and then averaged for each group. All studies were analyzed by a Student's t-test. Data are presented as mean $\pm$ SEM.

Following four days of social defeat, the defeated animals exhibited significantly lower levels of FGF2 than controls in CA1 ($t(19) = -6.44, p < 0.001$), CA2 ($t(19) = -4.12, p < 0.001$), CA3 ($t(19) = -6.12, p < 0.001$) and the dentate gyrus ($t(19) = -5.47, p < 0.001$), see Figure 1. The defeated animals also exhibited significantly lower levels of FGFR1 in CA1 ($t(19) = -5.13, p < 0.001$), CA3 ($t(19) = -2.14, p < 0.05$) and the dentate gyrus ($t(19) = -2.25, p < 0.05$) than controls, see Figure 2. This suggests that the FGF system is decreased in the hippocampus following four defeat episodes.

This is the first evidence of alterations in the FGF system after an acute social stressor. Following four days of social defeat, FGF2 and FGFR1 mRNA levels were decreased in the hippocampus. This finding is consistent with decreased levels of both ligands and receptors in the FGF system in post-mortem brains of individuals of major depressive disorder [7]. These findings are also of significance given that FGF2 mRNA was reported to be decreased in the hippocampus of MDD [9].

The experience of stress may play a role in the vulnerability to psychopathology, such as major depressive disorder [20]. Examining the role of the FGF system in relation to social stress may provide clues to the neurobiological underpinnings of depression. The present experiment examined this question following acute social stress. Several investigators have previously found acute social stress to decrease cell survival, but not cell proliferation [29]. This is in contrast to chronic social defeat where both cell survival and proliferation have been shown to be decreased in the hippocampus [4,5,31].

Antidepressants have previously been used to counteract the effects of chronic social defeat [4]. Antidepressant treatments are also known to increase neurogenesis, as well as FGF2 levels in the hippocampus [16,19,21]. Furthermore, the FGF system may play an important role in social defeat, as FGF2 signaling through FGFR1 is capable of inducing both proliferation and survival [18,24,32]. Perhaps the decrease in the FGF system is responsible for the decrease in neurogenesis observed following various social defeat paradigms. It would be interesting to determine whether treatment with FGF2 could reverse the behavioral and pathological changes induced by social defeat. Nevertheless, alterations in the FGF system may be relevant to psychiatric conditions modulated by stress, such as depression.

Not surprisingly, stress has previously been shown to alter the FGF system. Early life stress has been found to decrease FGF2 levels in adulthood [23]. Conversely, good maternal behavior results in offspring with higher levels of FGF2 in the hippocampus as adults [2]. Thus, we would hypothesize that high levels of FGF2 would be neuroprotective of stress and result in lower levels of depression-like behavior.

The functional relevance of the FGF system alterations in the different hippocampal regions is interesting to consider. The decreased FGF2 and FGFR1 mRNA expression following acute social defeat is in agreement with the decrease in another neurotrophic factor, brain-derived neurotrophic factor (BDNF), in the hippocampus following acute social defeat [25]. In terms of structure, acute social stress has been found to alter dendritic morphology in the hippocampus, but via a mechanism distinct from chronic social stress. Acute and chronic stress may also have differential effects on functional measures. As reviewed by Buwalda et al. [3], acute stress may regulate long-term potentiation, whereas chronic stress may regulate long-term depression. It would be interesting to determine the effects of chronic social defeat on gene expression in the FGF system.

The FGF system is very complex, with over 22 ligands and 4 receptors, many of which are expressed in the brain [26]. These ligands can have complex interactions with each other and produce compensatory changes. Furthermore, the receptors have complex patterns of expression, both in neurons and glia and have multiple splice variants [8,12]. Thus, a full characterization of alterations in this system following a behavioral manipulation such as social defeat is likely challenging. Nevertheless, in light of the above observations, we suggest that it will be worthwhile to determine the overall pattern of gene expression of the various FGF ligands and receptors and ascertain how closely they match the observations in human brains. Moreover, it would be important to determine whether administration of FGF receptor agonists can function as antidepressants and/or reverse some of the consequences of social defeat.

In Sum: Acute social defeat reveals downregulation in hippocampal gene expression of two key members of the FGF system, FGF2 and FGFR1. This suggests that the FGF system may represent an important target for modulation in human depression, and social defeat offers a useful rodent model to study the impact of its modulation.

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Figure 1. Decreased FGF2 gene expression in the hippocampus after social defeat
(A) Representative image of control (left) and social defeat (right) mRNA by *in situ* hybridization. Hippocampal FGF2 gene expression by *in situ* hybridization after social defeat.

Figure 2. Decreased FGFR1 gene expression in the hippocampus after social defeat
(A) Representative image of control (left) and social defeat (right) mRNA by *in situ* hybridization. (B) Hippocampal FGFR1 gene expression by *in situ* hybridization after social defeat.