Social stress: the good, the bad, and the neurotrophic factor
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DOI:
10.33612/diss.98795800

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 8

Summary
In modern society, people are living under constant pressure. On the one hand, mild “healthy” stressors can be a motivator, leading to increased individual productivity and creativity. On the other hand, however, constant, excessive stress over a long period of time can impair not only the mind but the overall well-being of a person. The burden of aforementioned societal pressure is shown by the increasing number of stress-associated health issues around the globe. Mood- and psychiatric disorders are just a small part of the wide range of diseases related to stress, according to literature.

One particularly debilitating example of a stress-related health issue is depression. Depression is considered a major global health issue, affecting every cultural, economic and age group. It is very likely that the one reading this thesis knows someone diagnosed with clinical depression, and other two or three that show some symptoms of it. Moreover, these numbers are expected to increase in the future, since society as a whole will likely not change. Estimates for the future are not pleasant, as 15% of the population is projected to develop some symptoms of depression. This would entail a significant burden to healthcare, as patients with depression spend a long time in treatment, and several of these treatments fail or only partially treat the disease. Depression is also a major economic issue, as patients with depression show low motivation and productivity, leading to substantial economic losses. Even more concerning are the comorbidities associated with depressive episodes and, in moderate to severe cases, suicidal tendencies.

Biologically, the brain is the region that is most affected by highly stressful situations. Neurotransmitter signaling, neuroendocrine function, and neuronal signaling are lowered during periods of chronic stress, and if left unchecked, these neurologic changes are amongst the causes of depressive symptoms and the first step towards clinical depression. Alteration of brain-derived neurotrophic factor (BDNF) concentration is one of many biochemical changes associated with depression. It is also observed in other brain conditions. Chapter 2 shows an overview of the behavior of BDNF in several psychiatric and neurodegenerative disorders. Additionally, we further evaluated the behavior of BDNF both in healthy and pathological conditions, and how BDNF could influence how therapy for these diseases is performed. In this regard we show consistency on BDNF to decrease in diseased states with a few exceptions. Therefore, although this neurotrophin itself is not a specific disease biomarker, BDNF can be associated with a disease pattern – or how (un)healthy the brain is at a specific moment.

In humans, BDNF in the serum has been shown to be decreased in psychiatric and neurodegenerative diseases, and this decrease is mostly correlated with cognitive impairment and behavioral changes. In animal models of disease, similar results have been found in specific brain regions, such as the
hippocampus and frontal cortex. However, only a few studies tried to associate peripheral and brain BDNF, and most of these studies attempted to do so mostly as a secondary goal. In Chapter 3 we decided to modulate BDNF concentration in rats by using two different factors that are known to have an effect on this neurotrophin: environmental conditions (environmental enrichment, EE; impoverished enrichment, IE; and standard enrichment, SE) and aging (6 months and 17 months, representing middle-aged and elderly in human standards, respectively). After 10 weeks in environmental conditions, we observed that isolated animals show an increased anxiety-like behavior when compared with animals that were submitted to environmental enrichment at both ages. EE animals had increased performance in the novel object recognition test. 2 weeks after behavioral assessment animals were terminated for post-mortem analysis. We found a significant effect of environment, with IE animals showing an overall decreased concentration of mature BDNF (mBDNF); its precursor, proBDNF; and Synaptophysin in the hippocampus when compared with EE, and EE showing significantly higher levels in proBDNF and Synaptophysin, but not BDNF, when compared with SE. Interestingly, none of the significant differences in concentration of mBDNF in the hippocampus were observed in the serum of these animals, showing that mBDNF concentration in the brain is not related to its concentration in the serum. These findings point towards the conclusion that different social environments are able to modify central mBDNF concentration regardless of age, either increasing or decreasing it, depending on the stimuli given, and that cerebral concentration of this neurotrophic is reflected on cognitive performance. However, these changes were not observed in the serum of these animals, which could imply that mBDNF might not be applicable as a serum biomarker for brain changes.

Depression is a multifaceted disorder, with many different factors contributing to its development and progression. One of the main hypotheses is that unresolved chronic stress can trigger depressive-like behavior by deregulation of the HPA-axis, inducing a modification in the cortisol response (usually increasing cortisol concentration in the blood) and impaired negative feedback of the HPA-axis. In Chapter 4 we used the repeated social defeat (RSD), a model of social stress that is able to induce depressive-like behavior and neuroinflammation in rats, to assess if social stress is modulated by inhibition of stress response (by adrenalectomy - ADX), and how ADX and RSD affect behavior and neuroinflammatory processes. Animals had bilateral ADX or Sham and submitted to a five-day RSD protocol or control seven days after surgery. One day after last RSD animals were tested for anxiety, locomotor (open field) and social behavior (social interaction). 1, 7 and 14 days after RSD animals were tested for anhedonic behavior (sucrose preference test). Two weeks after last RSD, animals were scanned for microgliosis using [11C] PBR28. There were no differences in the open field and no surgery, RSD or time effects in the sucrose preference test. Animals under RSD showed lower
social interaction in the social interaction test, which was interestingly not observed in animals both adrenalectomized and defeated. There was no difference in neuroinflammation process between any of the groups. The results show a somewhat expected effect of RSD in the social behavior, but HPA-axis disruption by ADX appears to block this fear-response of the animals towards others, likely by impairment of fight-or-flight response. Interestingly, there were no differences in microgliosis visible on $[^{11}\text{C}]$ PBR28, suggesting that, if RSD caused any neuroinflammation, it was already normalized after two weeks. Thus we assume that stronger modulators of depression might be needed in order to induce chronic depressive-like symptoms and neuroinflammation.

In Chapter 5 we repeated the RSD protocol modifying it to apply a stronger, more reactive interaction between resident and intruder. Additionally, we administered antidepressant and anti-inflammatory alkaloid harmine, or vehicle, intraperitoneally for 14 days. Animals had their weight observed daily, and locomotion and anxiety-like behavior were assessed by open field 1d and 9d after the last RSD. Anhedonia was measured 1d before, 1d and 10d after last RSD. Memory was assessed 10d after last RSD. Neuroinflammation was assessed 11d after last RSD, and hippocampus and frontal cortex were collected for BDNF concentration analysis. RSD had a significant impact on behavior and anhedonia, as shown by an increased anxiety parameter in the open field, and decreased preference for water with sucrose one day after RSD. There was no effect of harmine on these parameters, but harmine did show a significant lowering effect on weight and locomotor behavior. These effects lasted until the end of the experiment, as shown by the decreased locomotion and lower weight of harmine-treated animals. On the other hand, anxiety-like behavior found right after the last RSD was normalized in the long-term. RSD generated a fluctuation on a short-term (here observed by the behavioral outcome of the first OF and the SPT after RSD), but not a long-term effect, as seen by the lack of difference in uptake of $[^{11}\text{C}]$ PBR28 between RSD and control groups, together with unaffected long-term behavioral alterations on anxiety and depressive-like behavior. The changes of RSD or harmine did not alter BDNF concentration in frontal cortex or hippocampus, regions that are key for stress regulation and further brain homeostasis. To better assess the influence of harmine in depression and its effect as an antidepressant, there is a need for further studies using different stressors or longer time RSD protocols in order to induce a chronic stress response in the animals, thus allowing a more efficient analysis of how harmine could act under such conditions.

To understand how RSD works, it is also important to observe the effects this experimental setup has on the aggressors. In Chapter 6 we evaluated how the dopaminergic – more specifically, the $D_2$ receptors – are affected by social success, and if it might be related to a pathological increase in aggression (violence). Animals were tested for aggression in order to participate in the RSD
experimental setup, and those who failed to show aggressive behavior (average attack latency of 60 seconds after 5 trials) were considered non-aggressive. Animals that were screened as aggressive were submitted to several trials of RSD. We used $[^{11}\text{C}]$ Raclopride to assess D$_2$ receptors in the striatum (Caudate and Putamen) and nucleus accumbens (core and shell) and scanned at different time points: one day after last RSD (acute aggressors) and 14 days after last RSD (no-acute aggressors), and compared these animals to controls. Animals that had 14 days between last RSD and $[^{11}\text{C}]$ Raclopride scan showed higher aggressive behavior, with decreased attack latency over time, when compared with other groups. This effect was also observed in the striatal $[^{11}\text{C}]$ Raclopride binding potential, with an almost two-fold increase when compared with the other groups. However, in the nucleus accumbens, there was no difference between groups. The observed increase in D$_2$ receptor availability in non-acuteley exposed animals is in line with the literature. An increase in D$_2$ radiotracer binding in the accumbens was also observed by other studies in animal models of aggression. Lack of differences in D$_2$ binding on the experiments may be explained by the heterogeneity of the attack latency found in our results. This can be an explanation for the differences found between the acute and non-acute exposure of the aggressive animals to intruders, since the attack latency of the acute exposed animals did not show an association with the trial number, suggesting that their level of aggression may not be enough to have a significant effect on the binding potential of $[^{11}\text{C}]$ raclopride.

In conclusion, social stimuli had an impact in the brain, as shown by differences in behavior, neurotrophin, and synaptic plasticity markers, both in positive and negative social environments. It appears that the longer the stimulation, and the shorter the analysis period, the more pronounced is the behavioral change. It appears that longer stress protocols are needed in order to achieve a chronic state of anxiety- and depressive-like behaviors in an animal model of stress, allied with possibly a shorter interval between stressor and analysis. This was especially true for the social defeat model, as we were able to observe a short-term effect on behavior of animals submitted to social stress, although this effect was normalized in the longer-term. Neuroinflammatory and neurotrophic markers might follow the same pattern, as shown in the literature. Future studies using social defeat protocols, or resident-intruder models, might also need to take into consideration the possibility of intruders not developing depressive-like behavior, while other intruders do. Additionally, it is possible that residents need to be better screened for their aggressive behavior, with possibly other parameters together with attack latency. In our study setup, residents seem to develop a habituation state after being repeatedly introduced to an aggression protocol, as shown by the unchanged attack latency.