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Brain Medicine

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A novel animal paradigm of long-term, stress-induced hippocampal atrophy

Brain Medicine; https://doi.org/10.61373/bm024l.0040

Long-term hippocampal atrophy is a key feature of major depression. In contrast, in rodents subjected to chronic stress there is reversibly decreased hippocampal volume. We show that exposure to seven days of restraint stress alone or with antidepressant treatment combined with a persistent high-fat diet environment lasting 165 days resulted in long-term, stress-induced hippocampal volume reduction in rats, better reflecting the hippocampal shrinkage that is well documented in patients with major depressive disorder.

Hippocampal volume reduction is one of the most reproduced imaging findings documented in patients with major depressive disorder (MDD) (1). Understanding the mechanisms by which this shrinkage happens is relevant, as the hippocampus is part of the limbic stress pathway and plays vital roles in learning, memory, and neuroendocrine regulation (2). The hippocampus has essential roles in "declarative memory" formation, which involves representations of facts and events that are subject to conscious recollection, verbal reflection, and explicit expression; it also has a key role in memory consolidation, a crucial process that converts short-term memory into long-lasting memory that is stored in the neocortex (3). Other recently published hippocampal functions and networks that may also be involved, such as appetitive processing (4).

Cognitive impairments have been reported in MDD (5). Those include explicit memory deficits that are negatively correlated with baseline plasma cortisol concentrations. The severity of cognitive impairment and hippocampal volume decrease are more significant in patients with multiple MDD episodes (1). A hippocampal volume decrease of up to 20% has been demonstrated after controlling for the total brain, amygdala, or temporal lobe volume. Significantly, this shrinkage (*i*) correlates with the total duration of major depression, (*ii*) may be detected decades after the last depressive episode has resolved (1), and (*iii*) can be prevented by antidepressant treatment. A damaged hippocampus affects the memory recall of traumatic events and leads to fragmentation of memory elements of personal history and impairment in learning capacity with clinical consequences, which leads to dissociative symptomatology, perceptual distortions, identity instability, decreased social performance, and inflexible and maladaptive behaviors.

Other lines of investigation have shown convincing evidence for a role of chronic stress in MDD, including the following findings: (*i*) antidepressants directly downregulate hypothalamic-pituitary-adrenal (HPA) axis function; (*ii*) antagonism of corticotropin-releasing hormone attenuates behavioral, neuroendocrine and autonomic responses to stress in primates, and (*iii*) increased noradrenaline concentration is elevated in the cerebrospinal fluid throughout 24 hours of the day, even during sleep, which suggests that dysregulation of a stress-related system is primary and not merely a reaction to depressed mood (6).

In contrast to humans, stress-induced hippocampal atrophy is reversible in rats. Here we present a novel rodent model of long-term, stress-induced hippocampal atrophy. In our studies, we have used chronic restraint stress (CRS) because (*i*) It has good predictive validity, as antidepressant treatment improves behavioral correlates of CRS. (*ii*) It has good face validity, as CRS increases plasma cortisol and immobility time in the forced swimming test. (*iii*) It decreases hippocampal neurogenesis. (*iv*) The construct validity of the CRS model is similar to that of other chronic stress models.

Data from our lab show that the CRS protocol, consisting of daily 6 h sessions of restraint stress for 2 weeks, induces, in male rats, what has been described as stress-induced behaviors (7). After CRS, rats displayed anhedonia (decreased sucrose preference, Fig. 1A), avoidance (increased feed latency in the novelty suppressed feeding, Fig. 1B), and increased number of marbles buried in the marble burying test, Fig. 1C) we well as weight loss (Fig. 1D).

We subsequently developed an animal paradigm that models exposure to stress and antidepressants in a high-fat environment (8), better mimicking what happens in humans. Our paradigm, lasting a total of 177 days, consists of chronic restraint stress for 7 days, antidepressant treatment for 7 days, and long-term high-fat diet (TD95217; Harlan) intake starting at day 12 and lasting throughout the recovery phase until day 177, the "stress-diet induced obesity" (SDIO) paradigm (8).

We show here that the SDIO paradigm results in decreased hippocampal weight that persists months after stress and antidepressant

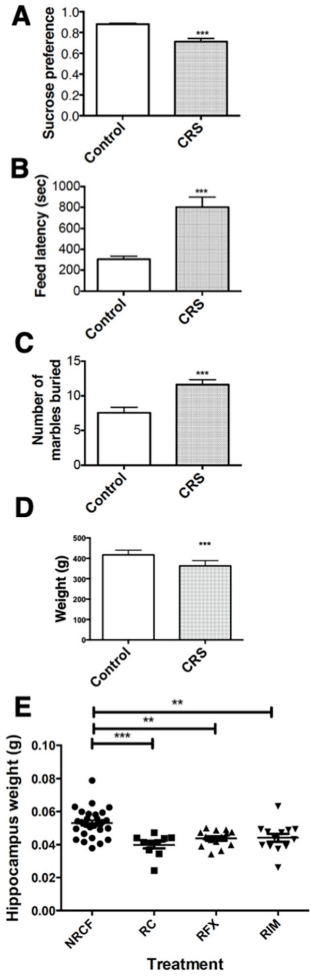

Figure 1. Short- and long-term effects of chronic restraint stress (CRS). Short-term effects ($n = 8$ per group): after 2 weeks of CRS, male rats displayed decreased sucrose preference (A), increased feed latency in the novelty-suppressed feeding test/assay (B), increased number of marbles buried in the marble burying test (C), and weight loss (D). Long-term effects: at experimental day 177, in comparison with non-restraint controls on fat diet (NRCF, $n = 13$) hippocampal weight was decreased in three restraint groups that were also on the same fat diet: RC, restraint with 0.5 ml of saline (0.9% NaCl; Hospira) and without antidepressant ($n = 13$); RFX, restraint treated with fluoxetine (Sigma-Aldrich, 10 mg kg^{-1}, $n = 13$); RIM, restraint treated with imipramine (Sigma-Aldrich, 10 mg kg^{-1}, $n = 13$) (E). ** = P < 0.01; *** = P < 0.001, Student's *t*-test (A-D) and ANOVA (E).

Received: 6 March 2024. Revised: 3 April 2024. Accepted: 19 April 2024.
Published online: 2 May 2024.

treatment have ended, at a point in which the animals no longer display avoidance or chronic stress-induced behaviors. Animals submitted to chronic stress, treated or non-treated with antidepressants, displayed decreased hippocampal weight in comparison with experimental control animals that were non-stressed and non-treated (Fig. 1E). Short-term antidepressant treatment had no significant effects on hippocampal weight. Please see the supporting online material for further experimental details.

In previously described animal models of chronic stress, stress-induced hippocampal alterations reverse with the cessation of stress. Therefore, the fact that we have found decreased hippocampal mass in animals submitted to CRS in our SDIO model several months after termination of stress supports the notion that our model constitutes a better approximation of the human condition, as clinical data support a prolonged nature of the volume loss seen in remitted MDD individuals (9). It should be noted that it is possible that chronically increased body weight could contribute to an enduring stress response that would perpetuate hippocampal atrophy. These features and time course make the SDIO model suitable for those interested in exploring the nature of chronic stress-induced hippocampal shrinkage. Future studies should test the hypothesis that environmental factors such as long-term high-fat diet (compared to regular diet), and antidepressant treatment contribute to long-term, stress-induced hippocampal shrinkage. Therefore, it may be relevant to monitor hippocampal volume in human patients with depression subjected to chronic physiological stress such as that propelled by high-fat diets. Moreover, this paradigm may inform future studies on hippocampal networks that are impacted by stress, including those related to long-term high-fat exposure, such as hypothalamic-hippocampal orexigenic subnetworks (4).

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Funding Sources
This work was supported in part by grants from the National Institutes of Health (NIH) R01MH127423, R21MH128726, and R21MH126405 to M.-L.W. and J.L.

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