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Research report

Understanding alterations in serotonin connectivity in a rat model of depression within the monoamine-deficiency and the hippocampal-neurogenesis frameworks

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HIGHLIGHTS

- Stress-induced changes in Raphe connectivity are connection-specific.
- Raphe connectivity with the basal ganglia and hippocampus is reduced in CMS rats.
- Raphe connectivity with the septum increases in CMS rats.
- Alterations in connectivity are asymmetrical and predominant in the right hemisphere.
- Direct and indirect mechanisms for raphe–hippocampus modulation are hypothesized.

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ABSTRACT

The monoamine-deficiency and the hippocampal-neurogenesis hypotheses of depression propose that alterations in the serotonin system and of hippocampal functionality are critical in the pathogenesis of depression. We measured the alterations in the connectivity level of the raphe nucleus in the chronic mild stress (CMS) rat model of depression using the manganese enhanced MRI method (MEMRI). Manganese ions were injected into the median raphe and their anterograde intracellular propagation was followed. Depression-like behavior was demonstrated using the sucrose preference tests.

We show that the raphe's connectivity is differentially altered through chronic stress. In line with the monoamine-deficiency hypothesis, the connectivity of the raphe with the basal ganglia (BG) output nuclei, the hippocampus, the habenula and the entorhinal and insular cortices was reduced in CMS rats, suggesting an overall reduction in raphe excitability. Connectivity reductions were predominantly found in the right hemisphere, strengthening previous evidence pointing at a-symmetric hemispheric involvement in depression. Despite the general reduction in raphe connectivity, enhanced connectivity was found between the raphe and the septum, suggesting that alterations are connection-specific.

On the basis of our results – while yet equivocal – we further discuss the possible coupling between the serotonergic and dopaminergic systems and two distinct mechanisms (direct and indirect) in which alterations in raphe connectivity may affect hippocampal dysfunction in chronic stress, thus linking the monoamine-deficiency and the hippocampal-neurogenesis hypotheses.

1. Introduction

1.1. Depression, monoamines and hippocampal neurogenesis

Depression is the most prevalent form of psychiatric disability and is estimated to become the leading cause of disability in the world by 2030 [1]. However, its pathophysiology remains poorly understood. Several hypotheses were suggested, among which the monoamine-deficiency and the hippocampal-neurogenesis hypotheses are perhaps the most well-established. While several monoamines, particularly serotonin, have been shown to be involved in the regulation of hippocampal neurogenesis [2], the nature of the relationship between serotonergic and hippocampal dysfunction in the context of depression remains unclear. In addition, it is generally accepted that depression is a multi-system disorder [3] involving several monoamine systems, yet it is not

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Table 1
The weekly schedule of stressors used in the CMS group over 5 weeks.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Morning Strobe light		White noise	Strobe light	Cage tilt	White noise	
Afternoon						White noise
Evening	Food deprivation	Soiled cages	Water deprivation	Strobe light	Lights on	Strobe light
Night						

sufficiently understood how these monoaminergic systems, primarily the dopaminergic and serotonergic systems, interact in the context of depression.

Reduced levels of dopamine were demonstrated in clinically depressed patients [4,5] and its involvement in the pathophysiology of depression was further suggested on the basis of clinical observations of Parkinson's disease (PD) patients presenting with depressive disorders [6,7]. The relationship between the serotonergic and the dopaminergic systems was demonstrated in several ways: serotonergic stimulation of the prefrontal cortex [8] or the striatum [9] has been shown to result in increased levels of dopamine release; a compensatory up-regulation of D2 receptor density in the basal ganglia and the cerebellum has been demonstrated in depressed patients [10]; and we have previously demonstrated that the coupling between the serotonergic and the dopaminergic systems is mediated by the habenula complex [11,12].

Various pieces of evidence point at the involvement of the hippocampus in the pathogenesis of depression, including the observation of a decrease in hippocampal volume in major depression (MD) patients [13]. The hippocampal-neurogenesis hypothesis of depression is based on the understanding that elevated stress levels may lead to increased hippocampal dendritic atrophy and suppressed neurogenesis in the dentate gyrus granule neurons [14]. While enriched environments, exercise and learning may lead to an up-regulation of neurogenesis [15], natural aging processes and chronic stress lead to a down-regulation of neurogenesis. Further evidence comes from the observation that antidepressant drugs can increase the turnover of hippocampal neurons by enhancing neurogenesis processes [16,17].

1.2. Measuring serotonergic connectivity in a rat mode of depression

The current view of brain functionality places critical importance on the detection of normal and altered communication between brain structures rather than on measuring the functionality of each structure in solitude. With this latter perspective in mind, we aimed to examine whether and how the serotonergic system relates to and interacts with the hippocampus and with additional monoaminergic systems, primarily the dopaminergic, in the context of chronic stress and depression-like behavior. Specifically, we measured alterations in the medial and dorsal raphe's anterograde connectivity under chronic stress. We implemented the well-established chronic-mild-stress (CMS) rat model of depression and used manganese-enhanced MRI (MEMRI) [18–22] to evaluate connectivity by directly injecting manganese ions into the medial raphe nucleus. This method enabled us to estimate the multi-synaptic, efferent connectivity of neurons in the injected site. Manganese ion accumulation causes signal enhancements in T1-weighted MRI due to reduction of the longitudinal relaxation time. Detecting an MEMRI signal in a region distant from the injection site requires sufficient accumulation of manganese ions in that region. For this to occur, not only must a structural connection between the region and the injection site be present, but the activity level of that connection must be strong enough to allow for sufficient manganese transference. This method is therefore most suitable for examining differences between the active raphe connectivity patterns between CMS and control rat groups.

2. Methods

2.1. Rat model

2.1.1. Animals

All experiments were approved by the Hebrew University Committee for Institutional Animal Care (approval number: OPRR-A01-5011) and conducted in accordance with the guidelines of the National Institutes of Health, USA. Male Sprague-Dawley rats weighing 260–300 g at the beginning of the experiment (Harlan, Biotec, Jerusalem) were housed in pairs, held in a 12 h light/dark schedule, and provided food and water ad libitum (when not under CMS stressor of food or water deprivation).

One 5-week CMS group and two control groups were used in the study. Manganese was injected directly into the median raphe in the CMS group ($n = 9$) and in the main control group not subjected to CMS ($n = 10$). An additional control group was used to control for possible effects of manganese diffusion and transference through the CSF by directly injecting manganese into the ventricle located above the medial raphe ($n = 3$).

2.1.2. Chronic mild stress

We used a modified version of the basic model described by Willner [23]. Rats were subjected to 1–2 of the following stressors daily: food deprivation overnight, water deprivation overnight, cage tilted to a 45° angle, stroboscopic light (4–8 Hz), white noise (70–80 dB), soiled cages (300 ml of water per cage), and lights on during dark-time phase. Stressors were designed in a weekly schedule (see Table 1) and administered for 5 weeks.

2.1.3. Behavior test

Sucrose preference and intake behavior tests were performed during the dark phase of the light cycle, in a dimly lit room at least 12 h after implementation of the last stressor. Statistic comparisons were performed using *t*-statistics. This experiment aimed to measure anhedonic behavior [24]. Rats were given two drinking bottles: one containing water and the other containing water sweetened with sucrose. Bottles were placed adjacent to each other on counterbalanced locations to rule out side preferences. During the three days prior to the baseline test, rats were given a bottle with sweetened water for several hours a day in order to accustom them to the sweet solution and reach a stable baseline. The drinking bottles were handed to the rats before the beginning of the dark phase and were left in the cages for two nights. Bottles were then weighed and the sweetened water vs. plain water consumption was compared. "Sucrose preference" was defined as sweetened water consumption divided by the total consumption (sweetened + plain). "Sucrose intake" was defined as the net amount of sweetened water consumption. A level of 0.3% sucrose was used after empirically being found to yield an approximate 75% sucrose preference in the baseline measurements.