Sirtuin Activity in Dentate Gyrus Contributes to Chronic Stress-Induced Behavior and Extracellular Signal-Regulated Protein Kinases 1 and 2 Cascade Changes in the Hippocampus

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Background: Exposure to chronic stress produces negative effects on mood and hippocampus-dependent memory formation. Alterations in signaling cascades and histone acetylation present a mechanism of modulation of transcription that may underlie stress-dependent processes in the hippocampus critical to learning and memory and development of depressive behaviors.

Methods: The rat model of chronic variable stress (CVS) was used to investigate the role of changes in protein acetylation and other molecular components of hippocampus-dependent memory formation and anhedonic behavior in response to CVS.

Results: Chronic variable stress treatment decreased both extracellular signal-regulated protein kinases 1 and 2 activation and Bcl-2 expression in all three regions of the hippocampus that corresponded behaviorally with a decrease in memory for the novel object location task and increased anhedonia. Extracellular signal-regulated protein kinases 1 and 2 activation was not significantly affected in the amygdala and increased in the medial prefrontal cortex by CVS. Chronic variable stress had no significant effect on activation of Akt in the hippocampus. We investigated molecular and behavioral effects of infusion of the sirtuin inhibitor, sirtinol, into the dentate gyrus (DG). Sirtinol infusion into the DG prevented the CVS-mediated decrease in extracellular signal-regulated protein kinases 1 and 2 activity and Bcl-2 expression, as well as histone acetylation in the DG previously observed following CVS. This corresponded to enhanced performance on the novel object location memory task, as well as reduced anhedonic behavior.

Conclusions: These results suggest that changes in sirtuin activity contribute to changes in molecular cascades and histone acetylation within the hippocampus observed following CVS and may represent a novel therapeutic target for stress-induced depression.

Key Words: Anhedonia, Bcl-2, epigenetics, hippocampus, histone acetylation, spatial memory

While many studies have shown that chronic stress has the ability to exert negative influences on hippocampus function (1–4), the molecular mechanisms responsible for this effect are poorly understood. We previously demonstrated that the nicotinamide adenine dinucleotide-dependent deacetylase, SIRT1, is activated in the hippocampus during chronic stress (5). SIRT1 is part of the sirtuin family of class III histone deacetylases (SIRT1-7) that is highly expressed throughout the hippocampus (6) and is localized to both the cytoplasm and the cell nucleus, where it can deacetylate histones as well as stress response factors (7). The function of this increased SIRT1 activity in chronic stress is unknown.

Sirtuins play a role in cognitive function and mood regulation, but their role in memory formation is not well understood. Recent studies suggest that SIRT1 expression is essential for hippocampus-dependent memory formation (8,9), but another study showed that overexpression of SIRT1 inhibits memory formation in young and old animals (10). In humans, variations in the *SIRT1* gene are associated with anxiety (11), and *SIRT1* expression is correlated with prevalence of depression (12). Brain-specific *Sirt1* knockout mice showed reduced anxiety, while *Sirt1* overexpressing mice exhibited enhanced anxiety. Furthermore, socially defeated wild-type mice demonstrated reduced sucrose preference, while brain-specific *Sirt1* knockout mice did not (11), suggesting SIRT1 function mediates a susceptibility to stress-induced anhedonia. Additionally, SIRT1 in the nucleus accumbens is associated with chronic cocaine use in rodent models (13). This contrasting evidence suggests that sirtuin activity is important for both mood regulation and memory formation and its activity must be tightly regulated for proper hippocampus function. We hypothesized that hyperactivation of sirtuins during chronic stress modulates molecular and behavioral effects of chronic stress. We specifically investigated molecular cascades in the hippocampus, hippocampus-dependent memory, and anhedonic behavior.

Methods and Materials

Rats

Male Wistar rats (42 days of age; Harlan Inc., Indianapolis, Indiana) were housed in pairs on a 12:12 light/dark cycle (lights on at 0700 hours) and received food and water ad libitum. Rats were given 14 days to acclimate before experimental manipulation. All experimental procedures were conducted during the

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Received Jan 4, 2013; revised Jul 5, 2013; accepted Jul 12, 2013.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2013.07.029

light cycle and were approved by the Tulane University Institutional Animal Care and Use Committee.

Chronic Variable Stress

Rats (56 days of age) were randomly assigned to control and chronic variable stress (CVS) groups. Chronic variable stress was conducted using a modified method previously reported (5,14) (Supplemental Methods and Materials in Supplement 1) and consisted of twice-daily exposure to randomly assigned stressors applied over 14 days. Sirtinol infusion techniques are described in Supplemental Methods & Materials in Supplement 1. Physiological markers of stress are reported in Supplemental Results in Supplement 1.

Sucrose Preference Test

A subset of rats was tested on day 14 of CVS protocol for sucrose preference. Rats were given 12 hours (0700 to 1900 hours) to habituate to a free choice between two bottles, both containing tap water. At 1900 hours, one bottle was replaced with 3% sucrose. The other bottle remained as tap water. Rats were given free choice between sucrose and tap water for 12 hours (1900 to 0700 hours). To prevent possible effects of side preference, the position of the sucrose bottle was randomly distributed among the cages. Consumption was calculated as the percentage of sucrose fluid volume consumed to total fluid volume consumed.

Corticosterone Assay

Trunk blood was allowed to coagulate at room temperature for 90 minutes. Samples were centrifuged at 2000 *g* for 15 minutes, serum was collected, and samples were stored at −20°C. Samples were sent to the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core for corticosterone measurements by radioimmunoassay.

SIRT1 Activity Assay

SIRT1 activity was assessed in extracts from hippocampal preparations via a commercially available kit (Sirtuin 1 Activity Assay Kit; Calbiochem, San Diego, California) (Supplemental Methods and Materials in Supplement 1).

Tissue Processing

Molecular analysis was performed on a separate group of rats that did not receive memory or sucrose preference testing to avoid the effects of behavioral testing on molecular changes as previously described (5) (Supplemental Methods and Materials in Supplement 1).

Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were performed as previously described (15).

Messenger RNA Extraction and Reverse Transcriptase Polymerase Chain Reaction

Samples were processed using the RNAeasy Mini Kit (Qiagen, Alameda, California) according to the manufacturer's instructions. The RNA was purified with RNAeasy microcolumns and reverse-transcribed using iScript cDNA Synthesis Kit (Biorad, Hercules, California). Complementary DNA was quantified by quantitative polymerase chain reaction using iQ SYBR Green (BioRad). Each reaction was run in triplicate and analyzed using the ΔΔCt method. Real-time polymerase chain reaction assays were tested to determine and compare the efficiencies of the target and control gene amplifications to ensure high (90% to 100%) and similar efficiency. A single peak on the melt curve confirmed the presence of specific amplification products. The primer sequences used for the reverse transcriptase polymerase chain reactions are described in Supplement 1.

Results

Impaired Object Location Following Chronic Variable Stress

We first investigated the performance of control and CVS-treated rats in the object location task. On the day of training and testing (morning of day 15), rats received an initial sample trial and were tested for object location memory 30 minutes later (Figure 1A). No differences in total object investigation independent of location were detected between control rats and rats exposed to chronic variable stress during the sample trial ($t_{10} = \pm.86$, $p > .1$) or during the retention trial ($t_{10} = \pm.83$, $p > .1$). A significant difference in object location memory between the groups was revealed ($t_{10} = \pm3.32$, $p < .01$), in which rats subjected to chronic variable stress spent significantly less time investigating the object in the novel location during the retention trial than control rats (Figure 1B). Furthermore, control rats ($t_5 = \pm4.60$, $p < .01$), but not rats exposed to chronic variable stress ($t_5 = \pm.06$, $p > .1$), investigated the object in the novel location at a level greater than chance during the retention trial (Figure 1B). These results show that CVS has a significant negative effect on object location memory in adult male rats.

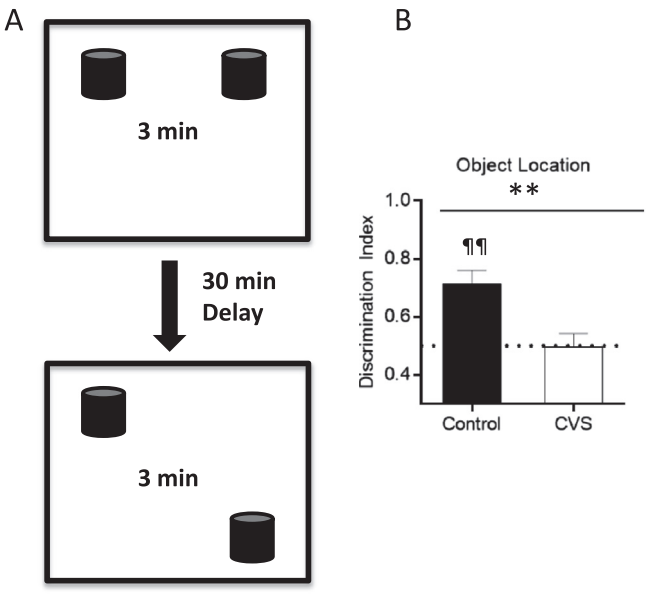

Figure 1. Chronic variable stress (CVS) decreases memory formation in the hippocampal-dependent task, novel object location. **(A)** Schematic representation of the behavioral protocol used for novel object location in a black Plexiglas open arena. During the sample phase, rats were exposed to two identical objects and allowed to freely explore for 3 minutes. Following a 30-minute delay period in their home cages, rats were returned to the open arena for the 3-minute retention trial in which one of the objects had been moved to a novel location. The discrimination index was defined as the ratio of time spent investigating the object in the novel location relative to total time investigating the objects during the first minute of the retention trial. **(B)** Chronic variable stress treated animals spent significantly less time investigating the object in the novel location than control rats. In addition, control rats only investigated the object in the novel location at a level greater than chance. The dotted line represents chance level (.5). Data are shown as means ± SE; $^{**}p < .01$ versus control, $^{¶¶}p < .01$ versus chance (.5); ($n = 6$ animals per group).