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Stress-induced alterations in large-scale functional networks of the rodent brain

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ABSTRACT

Stress-related psychopathology is associated with altered functioning of large-scale brain networks. Animal research into chronic stress, one of the most prominent environmental risk factors for development of psychopathology, has revealed molecular and cellular mechanisms potentially contributing to human mental disease. However, so far, these studies have not addressed the system-level changes in extended brain networks, thought to critically contribute to mental disorders. We here tested the effects of chronic stress exposure (10 days immobilization) on the structural integrity and functional connectivity patterns in the brain, using high-resolution structural MRI, diffusion kurtosis imaging, and resting-state functional MRI, while confirming the expected changes in neuronal dendritic morphology using Golgi-staining. Stress effectiveness was confirmed by a significantly lower body weight and increased adrenal weight. In line with previous research, stressed animals displayed neuronal dendritic hypertrophy in the amygdala and hypotrophy in the hippocampal and medial prefrontal cortex. Using independent component analysis of resting-state fMRI data, we identified ten functional connectivity networks in the rodent brain. Chronic stress appeared to increase connectivity within the somatosensory, visual, and default mode networks. Moreover, chronic stress exposure was associated with an increased volume and diffusivity of the lateral ventricles, whereas no other volumetric changes were observed. This study shows that chronic stress exposure in rodents induces alterations in functional network connectivity strength which partly resemble those observed in stress-related psychopathology. Moreover, these functional consequences of stress seem to be more prominent than the effects on gross volumetric change, indicating their significance for future research.

Introduction

Stress has a major impact on brain functioning. Whereas the stress response first and foremost constitutes a highly adaptive mechanism that enables an organism to respond optimally to potential threats, dysregulation or prolonged exposure to stress can cumulate in psychopathology, such as post-traumatic stress disorder or depression (de Kloet et al., 2005).

Implementing a top–down approach, neuroimaging studies in humans have investigated the functional and structural abnormalities observed in the mentally diseased brain. Patient studies revealed functional impairments and volumetric reductions of the hippocampus and prefrontal cortex, and a hyperactive amygdala (Drevets et al., 2008; Pitman et al., 2012). However, recent advances in the field have elicited a shift away from such region-of-interest-based approaches towards network based approaches, in which the brain is regarded as a set of functional networks, each representing a unique brain function. Importantly, these analyses revealed that stress-related psychopathology is also characterized by alterations in structural integrity and functional connectivity patterns throughout the brain, which might in fact distinguish the healthy from the diseased individual (Whitfield-Gabrieli and Ford, 2012; Admon et al., 2013; Patel et al., 2012).

To elucidate the potential neural underpinnings of stress-related illnesses, animal research has investigated the effects of prolonged (i.e., chronic) stress exposure on neuronal function and structure, using a bottom–up approach. Chronic stress was shown to affect both brain function and structure in a region-specific manner. Higher-order cognitive function, involving the hippocampus and medial prefrontal cortex, was shown to be deteriorated following chronic stress (McEwen, 2001; Pavlides et al., 2002; Liston et al., 2006; Cerqueira et al., 2007), which was accompanied by a reduction in hippocampal volume (Lee

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et al., 2009), and dendritic hypotrophy (reduced dendritic length and number of branch points) in hippocampal and medial prefrontal neurons (Woolley et al., 1990; Watanabe et al., 1992; Cook and Wellman, 2004; Radley et al., 2004). Conversely, amygdala neurons were shown to display dendritic hypertrophy, and an increased anxiety phenotype (Vyas et al., 2002). Remarkably, similar to the amygdala, chronic stress was shown to enhance plasticity of the most ventral part of the hippocampus, contrary to its dorsal part (Suvrathan et al., 2013; Maggio and Segal, 2007).

Despite the detailed knowledge about regional effects of chronic stress, it is currently entirely unknown whether these microscopic effects observed in animals translate to altered large-scale connectivity as observed in the diseased human brain. We here set out to investigate the effects of chronic stress exposure on the large-scale functional connectivity patterns and structural integrity of the rodent brain. Implementing a controlled design, we exposed male rats to 10 days of chronic immobilization stress, and tested its effects on functional connectivity networks as identified by independent component analysis (ICA) of resting-state functional MRI (rs-fMRI). Additionally, we performed *post-mortem* high-resolution structural MRI and diffusion kurtosis imaging (DKI) to assess structural changes resulting from stress exposure. To confirm the presence of the expected chronic stress-induced changes in dendritic morphology in the hippocampus, amygdala, and prefrontal cortex, a subset of rats was used for Golgi staining.

Materials & methods

Stress manipulation

Thirty-six male Wistar rats (RccHan™, Harlan) were housed in groups of three animals per cage with ad libitum access to food and water. Animals were kept in a temperature-controlled room (22–24 °C), with a light/dark cycle of 12 h (lights on at 7:00 A.M.). At the beginning of the experiments, animals were approximately 3 months old and weighed 325–400 g. The rats were randomly assigned to one of two experimental groups, entering either a chronic immobilization stress (CIS) or control protocol for 10 consecutive days. CIS consisted of complete immobilization (2 h/day, 10 A.M.–noon) in rodent immobilization bags without access to food or water (Vyas et al., 2002). Control animals were deprived of food and water for the same period of time (2 h/day, 10 A.M.–noon), but were otherwise left undisturbed in their home cage. All animals within one cage were assigned to the same protocol. To monitor the overall effects of the stress paradigm, animals were weighed daily, and the adrenal glands were removed and weighed after completion of the experiment. The animal experimental protocol was approved by the Utrecht University Ethical Committee on Animal Experiments, and the experiments were carried out in accordance with the guidelines of the European Communities Council Directive.

Morphological cell analysis

Protocol

Sixteen animals ($n_{stress} = 8$, $n_{control} = 8$) were sacrificed in the late morning on day 11 (one day after the end of the chronic stress/control procedure) and used for Golgi staining to confirm the expected changes in dendritic morphology due to chronic stress exposure (Magariños and McEwen, 1995; Vyas et al., 2002; Cook and Wellman, 2004; Radley et al., 2004; Liston et al., 2006). After decapitation, the brain was removed quickly, and coronally cut at approximately Bregma 0 mm to process the medial prefrontal cortex (mPFC) and the hippocampus–amygdala separately. The blocks of tissue were processed for rapid Golgi staining technique as described earlier (Castano et al., 1995; Gibb and Kolb, 1998; Shankaranarayana Rao et al., 2001). mPFC tissue was impregnated for 12 days, while impregnation of the hippocampus and amygdala was restricted to 8 days. After completion of the staining protocol, one hemisphere of each brain was used for preparing transverse sections from the dorsal hippocampus, and the other hemisphere was used for obtaining coronal sections from the amygdala. The mPFC was also coronally cut. For all regions, 150-μm-thick sections were obtained using a vibratome, and sections were collected serially, dehydrated in absolute alcohol, cleared in xylene, and coverslipped. Slides were coded before quantitative analysis, and the code was broken only after the analysis was completed. To be selected for analysis, Golgi-impregnated neurons had to satisfy the following criteria: 1) presence of untruncated primary or apical dendrites; 2) consistent and dark impregnation along the entire extent of all of the dendrites; and 3) relative isolation from neighboring impregnated neurons to avoid interfering with analysis. For morphological quantification of hippocampal and mPFC neurons, 5–10 pyramidal neurons from each animal in each group were analyzed from the dorsal CA3 region and prelimbic cortex (PrL) layer II/III, respectively. For the analysis of amygdalar morphology, 5–9 cells were selected from the basolateral complex of the amygdala (BLA) (between Bregma −2.0 mm and −3.2 mm). Based on morphological criteria described in the literature (McDonald, 1982, 1992), only pyramidal and stellate neurons were selected for analysis. Images were obtained (63× for CA3, and 40× for BLA and PrL) from the selected neurons using Zen 2011 (Carl Zeiss) in combination with an automated stage and focus control connected to the microscope. Image stacks of 1 μm thickness were automatically acquired and combined. Next, neurons were traced using NeuroLucida software (MicroBrightField, Inc. Colchester, VT, USA), to obtain a 3D representation of each cell. Numerical analysis and graphical processing were performed with NeuroExplorer (MicroBrightFields). Sholl plots (Sholl, 1953) were constructed by plotting the dendritic length as a function of radial distance from the soma center, which was automatically set to zero. The length of the dendrites within each subsequent radial bin at 30 μm increments was summed. Besides the Sholl analysis, results were expressed in terms of total (apical) dendritic length, total number of branch points, and total number of branch tips.

Statistical analysis

For the statistical analysis of the morphological data, cells displaying characteristics that deviated >3 standard deviations from the mean were considered outliers, and were removed from subsequent analysis. In total, 63 control and 60 stress BLA neurons were included into the analysis of BLA morphology. For the hippocampal CA3 region, we analyzed 57 control and 57 stress cells. For the PrL, a total of 50 layer II/III neurons of control and 48 neurons of stressed animals were included in the analysis. Since earlier studies reported that chronic stress mostly affects the apical and not basal dendritic tree (Magariños et al., 1996; Vyas et al., 2002; Cook and Wellman, 2004; Radley et al., 2004), we confined our analyses to the former. Statistical testing was performed using a mixed factors (within and between) ANOVA, and values were represented as the mean ± SEM. The Sholl analysis was tested using a repeated measures ANOVA, followed by a mixed factors ANOVA to test the group differences in dendritic length at a specific distance from the soma. Differences were considered statistically significant at $p < 0.05$.

Functional MRI

Protocol

The remaining animals ($n_{stress} = 10$, $n_{control} = 10$) were subjected to fMRI scanning on day 11 (one day after the end of the chronic stress/control procedure). Although the non-invasive nature of neuroimaging would have allowed for repeated testing (and thus a within-group comparison, potentially increasing power), animals were only scanned once at the end of the experiment. Repeated testing (i.e., before and after stress manipulation) would still require a control group to correct for potential differences merely caused by repeated testing (e.g., due to repeated use of anesthesia, or an age difference between the first and second sessions). Repeated testing of the control animals, which serve as