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HABITUATION TO REPEATED RESTRAINT STRESS IS ASSOCIATED WITH LACK OF STRESS-INDUCED c-fos EXPRESSION IN PRIMARY SENSORY PROCESSING AREAS OF THE RAT BRAIN

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Abstract—Rats repeatedly exposed to restraint show a reduced hypothalamic–pituitary–adrenal axis response upon restraint re-exposure. This hypothalamic–pituitary–adrenal axis response habituation to restraint does not generalize to other novel stressors and is associated with a decrease in stress-induced c-fos expression in a number of stress-reactive brain regions. We examined whether habituation to repeated restraint is also associated with adaptation of immediate early gene expression in brain regions that process and relay primary sensory information. These brain regions may not be expected to show gene expression adaptation to repeated restraint because of their necessary role in experience discrimination. Rats were divided into a repeated restraint group (five 1-hour daily restraint sessions) and an unstressed group (restraint naïve). On the sixth day rats from each group were either killed with no additional stress experience or at 15, 30 or 60 min during restraint. Immediate early gene expression (corticotrophin-releasing hormone heteronuclear RNA, c-fos mRNA, zif268 mRNA) was determined by *in situ* hybridization. A reduction in stress-induced hypothalamic–pituitary–adrenal axis hormone secretion (plasma corticosterone and adrenocorticotropic hormone) and immediate early gene expression levels in the paraventricular nucleus of the hypothalamus, the lateral septum and the orbital cortex was observed in repeated restraint as compared with restraint naïve animals. This reduction was already evident at 15 min of restraint. Unexpectedly, we also found in repeated restraint rats a reduction in restraint-induced c-fos expression in primary sensory-processing brain areas (primary somatosensory cortex, and ventroposteriomedial and dorsolateral geniculate nuclei of thalamus). The overall levels of hippocampal mineralocorticoid receptor heteronuclear RNA or glucocorticoid receptor mRNA were not decreased by repeated restraint, as may occur in response to severe chronic stress. We propose that repeated restraint leads to a systems-level adaptation whereby re-exposure to restraint elicits a rapid inhibitory modulation of primary sensory processing (i.e. sensory gating), thereby producing a widespread attenuation of the neural response to restraint. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: *in situ* hybridization, HPA axis, immediate early genes.

Experiences that are characterized as stressful elicit a complex array of behavioral and physiological changes believed to contribute to optimal coping of the organism with the situation. These responses include the release of glucocorticoid hormones from the adrenal cortex, controlled by the hypothalamus–pituitary–adrenal system (HPA axis). Glucocorticoids produce a wide range of regulatory physiological effects such as the promotion of glycogen, muscle, and fat catabolism in order to mobilize energy required for the immediate adaptive responses to stress (Munck et al., 1984). An important feature of many real-life stressors is their recurrent or persistent nature; under these circumstances, chronic elevation of glucocorticoid levels can exacerbate a number of pathologies including immune disorders, neurodegeneration and major depression (McEwen and Stellar, 1993; Gold and Chrousos, 2002). Often repeated stress leads to a decrease in the HPA axis response (stress habituation), protecting the organism from the potentially damaging effects of hypercorticosteroidism (Armario et al., 2004). The mechanisms involved in the adaptation to daily stress, however, are poorly understood. Glucocorticoid negative feedback participates in the depression of the HPA response (Cole et al., 2000) and of corticotrophin-releasing hormone (CRH) gene expression (Pinnock and Herbert, 2001) during habituation to restraint stress. However, decreased HPA axis activity appears not to be the result of a generalized diminished responsivity of the axis, but rather, of changes in the neural activity of afferents involved in its control. This is evidenced by the fact that habituation is stimulus specific. Consequently, prior habituation to one stressor (homotypic stimulus) does not reduce the HPA response to a different stressor (heterotypic stimulus) (Armario et al., 1988; Hashimoto et al., 1988; Hauger et al., 1988; Terrazzino et al., 1995; Akana and Dallman, 1997; Ma et al., 1999; Pace et al., 2001; Fernandes et al., 2002). Thus, some degree of altered central neural control appears responsible for habituation of HPA responses to stress.

To assess whether stress response habituation is associated with a reduction in the stress-induced neural ac-

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Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; CRH, corticotrophin-releasing hormone; GR, glucocorticoid receptor; hnRNA, heteronuclear RNA; HPA, hypothalamus–pituitary–adrenal; LS, lateral septum; MR, mineralocorticoid receptor; N, restraint naïve; PVN, paraventricular nucleus of the hypothalamus; ROI, region of interest; RR, repeatedly restrained; SSC, standard saline citrate; VPM, ventroposteriomedial thalamic nucleus.

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tivation present in various brain regions, a number of studies have examined immediate early gene expression. These studies have looked at habituation to a variety of stressors (e.g. prone restraint, supine restraint, immobilization, noise, social defeat) with a special focus on response adaptation of the HPA axis and of c-fos mRNA/protein in primary stress-responsive areas in the forebrain and brain stem (Lachuer et al., 1994; Melia et al., 1994; Umemoto et al., 1994a; Watanabe et al., 1994; Chen and Herbert, 1995; Umemoto et al., 1997; Bonaz and Rivest, 1998; Martinez et al., 1998; Stamp and Herbert, 1999; Campeau et al., 2002). With some exceptions, probably due to use of more intense stressors (Makino et al., 1995; Umemoto et al., 1997), the majority of studies report a robust habituation of the HPA axis hormone response to repeated stress. The stress-induced expression of c-fos also reliably decreases with repeated stress exposure in the paraventricular nucleus of the hypothalamus (PVN) and in other stress-responsive cortical (e.g. medial prefrontal cortex), limbic (e.g. bed nucleus of the stria terminalis, various hypothalamic subregions and amygdala) and brainstem (e.g. locus ceruleus, raphe nuclei, central gray, nucleus of the solitary tract) structures. Two anatomical exceptions that have been highlighted are the orbital frontal cortex and the lateral septum (LS). Campeau and coworkers (2002) observed a sensitization rather than habituation of the c-fos mRNA response in the orbital frontal cortex to repeated noise stress. Also, a lack of habituation of Fos protein in the LS to repeated supine restraint has been noted, although the same group did see habituation of this brain region in subsequent studies (Chen and Herbert, 1995; Chung et al., 1999; Stamp and Herbert, 1999). Due to the habituation of stress-induced c-fos expression reported in many other brain regions, a failure of habituation in the orbital frontal cortex and LS may reflect a special role of these structures in the development and/or maintenance of habituation.

While a wealth of information has been provided from these previous studies, generalizations about the specific brain regions affected and underlying mechanisms of stress adaptation are lacking, partly due to the different stress protocols used and variety of brain regions examined. In order to further examine the extent to which habituation of an HPA axis response to repeated stress is associated with changes in central neural activity, we have studied habituation to repeated restraint (six daily 1 h sessions). Exposing rats to repeated restraint is a widely used model of psychological stress adaptation (Glavin et al., 1994). We have chosen to study habituation to a psychological stressor since understanding the adaptation processes to this type of stress may be especially informative to the neurobiology underlying stress-related disorders (Chrousos and Gold, 1992). For this study we have examined the expression of two different immediate early genes, c-fos and zif268. Both genes are rapidly induced in the rat brain by a wide range of experiences, including those characterized as stressors (Herdegen and Leah, 1998). The specific expression patterns of these two genes may differ, however, and their direct comparison may provide a more comprehensive reflection of neural activity (Cullinan et al., 1995; Kaplan et al., 1996). We have examined gene expression at the level of mRNA rather than protein because of the increased temporal resolution provided by the more rapid induction and transient nature of these mRNA levels. This has allowed us to examine the acute response time-course in order to see if habituation is expressed early or later during the response to restraint challenge. Finally, we have selected different brain regions to examine that may represent different stages in the neural processing of the restraint experience. As examined in previous studies, we have used the HPA axis response as an indicator of the activity of a functional stress response system—thus we have measured plasma corticosterone and adrenocorticotropic hormone (ACTH), and observed concurrent stress activation of the c-fos and zif268 genes in the PVN. Although other studies have shown that the majority of Fos induction in the PVN with restraint is localized to CRH neurons (Dayas et al., 1999), we have also examined CRH heteronuclear RNA (hnRNA) as a direct measure of activity within this cell population. In addition, in order to examine the extent of habituation throughout the brain we have selected two brain regions that have previously been reported to be stress reactive, but, as discussed above, do not necessarily show habituation of immediate early gene response to repeated homotypic stress (LS and orbital frontal cortex) (Chen and Herbert, 1995; Campeau et al., 2002). Most importantly, a unique aspect of this study is the examination of the immediate early gene response within primary sensory pathways. We have specifically examined the region of primary somatosensory cortex and associated thalamic relay nucleus that receives sensory input from the vibrissae. We expect considerable stimulation of the whiskers as they are deflected by the walls of the restrainer in rats challenged with restraint for the first and sixth time. Our assumption is that neural activity in primary sensory systems precedes central processing of the stressfulness of the experience, and therefore may not exhibit immediate early gene response habituation. As a final measure, we examined mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) gene expression in the hippocampus. This measure may provide an indicator of the severity of our repeated stress paradigm since other studies have found that chronic stress can lead to a downregulation of hippocampal mineralocorticoid and/or GR expression (Sapolsky et al., 1984; Makino et al., 1995; Lopez et al., 1998; Paskitti et al., 2000).

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) were allowed a two week acclimation period after arrival to the animal facilities at the University of Colorado before experimental use (10 weeks old, weight range 270–290 g at experimental onset). Animals were housed two per polycarbonate tub with wood shavings, and were allowed food (Purina Rat Chow; Ralston Purina, St. Louis, MO, USA) and tap water *ad libitum*. The colony room lights were regulated on a 12-h light/dark cycle, with lights on