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Research report

Stress-induced differences in the limbic system Fos expression are more pronounced in rats differing in responsiveness to novelty than social position

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ABSTRACT

We determined the interaction between such individual behavioural profiles as locomotor response to novelty or social position and the activation (Fos expression) of the brain's limbic regions following chronic laboratory and social interaction stress. Male Wistar rats ($n = 45$), housed separately and handled for 2 weeks, were divided into high (HR) and low (LR) responders to novelty. Seven days later, 12 HRs and 12 LRs were subjected to a chronic 23 consecutive day social interaction test (Nov/SocI group), 5 HRs and 5 LRs were subjected to chronic laboratory stress: carrying from the vivarium to the laboratory for 23 consecutive days (Nov/Carr group) while the remaining rats stayed in the vivarium in their home cages (Nov/Home group). The highest limbic system activation was found 7 days later in the Nov/SocI rats. In comparison with the LRs, the HRs showed a higher number of Fos^+ cells in most of the limbic prosencephalic structures (24 areas) in the Nov/SocI group, and in 12 areas, especially in the amygdala and the hypothalamus, in the Nov/Carr group. There were no HR/LR differences in the limbic system's activity in the Nov/Home group. Within dominance/submission differences, a higher Fos expression was found in 6 structures, especially in the limbic cortex, in the dominant rather than the subordinate HRs. We conclude that chronic social and laboratory stress persistently activates the limbic system, with the largest effects in the brains of rats responding maximally to novelty. Social position was less predictive of Fos expression than was activity to novelty.

1. Introduction

Locomotor response to novelty (Deminiere et al., 1989; Piazza and Le Moal, 1996; Piazza et al., 1989) or social dominance/submission (Bartolomucci et al., 2005; Blanchard et al., 1993; Kollack-Walker et al., 1997) has been found to be a particularly useful measure of inter-individual behavioural variability. In a novelty test (Nov), some individuals show vigorous and long-lasting exploratory activity (high responders, HRs) whereas others become quiescent after a short period of exploration (low responders, LRs). The differences among HR/LR in locomotor response to novelty correlate with other physiological and behavioural measures, including an increased responsiveness to stress (Kabbaj et al., 2000) and a hyperdopaminergic and hypercholinergic state in the HRs (Hooks et al., 1992; Piazza et al., 1991; Rouge-Pont et al., 1993; Thiel et al., 1998, 1999), accompanied by decreased anxiety and enhanced sensation-seeking motivation (Dellu et al., 1996; Kabbaj and Akil, 2001; Kabbaj et al., 2000; Piazza et al., 1993). More recently, Kerman et al. (2011) found that selectively bred high novelty seeking (bHR) rats exhibited greater inter-male aggression compared to low novelty seeking (bLRs) animals when forcing home-cage intrusion.

Abbreviations: ACo, anterior cortical amygdaloid nucleus; AD, anterodorsal thalamic nucleus; AH, anterior hypothalamic area; AM, anteromedial thalamic nucleus; Arc, arcuate hypothalamic nucleus; AV, anteroventral thalamic nucleus; BLA, basolateral amygdaloid nucleus; BST, bed nucleus of the stria terminalis; CA1, area CA1 of the hippocampus; CA2, area CA2 of the hippocampus; CA3, area CA3 of the hippocampus; Ce, central amygdaloid nucleus; CG1, cingulate cortex, area 1; CG2, cingulate cortex, area 2; D, dominant; DG, dentate gyrus of the hippocampus; DMH, dorsomedial hypothalamic nucleus; Ent, entorhinal cortex; HDB, nucleus of the horizontal limb of the diagonal band; HR, high responder to novelty; LH, lateral hypothalamic area; LHb, lateral habenular nucleus; LPO, lateral preoptic area; LR, low responder to novelty; LS, lateral septal nucleus; MD, mediodorsal thalamic nucleus; Me, medial amygdaloid nucleus; MHb, medial habenular nucleus; MPO, medial preoptic area; MS, medial septal nucleus; Nov/SocI, rat subjected to novelty and chronic (23 days) social interaction test; Nov/Carr, rat subjected to novelty and carrying from the vivarium to the laboratory for 23 consecutive days; Nov/Home, rat subjected to novelty and staying in their home cages in the vivarium; PRh, perirhinal cortex; PVN, paraventricular hypothalamic nucleus; RSA, retrosplenial agranular cortex; RSG, retrosplenial granular cortex; S, subordinate; SO, supraoptic nucleus of the hypothalamus; VDB, nucleus of the vertical limb of the diagonal band; VMH, ventromedial hypothalamic nucleus.

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One of the most powerful environmental stressors derives from interaction with other specimens in animal societies. Experimentally, individual differences in social position can be detected using a variety of social-interaction (SocI) tests (e.g., Albonetti and Farabollini, 1994; Blanchard and Blanchard, 1977; Blanchard et al., 2001; Grant, 1963). Dominant and submissive or subordinate animals differ in a number of behavioural and neurochemical measures (for a review see Blanchard et al., 1993, 2001). Subordinates have been found to be less active, and less aggressive, showing enhanced anxiety-like behaviour, a higher basal level of corticosterone (Blanchard et al., 1995) and increased secretion of glucocorticoids during an agonistic encounter (Kollack-Walker et al., 1997, 1999). At the neurochemical level they show increased central serotonergic activity and a selective increase in tyrosine hydroxylase expression in the noradrenergic but not in the dopaminergic neurons (Blanchard et al., 2001). A review of the literature (Ambrosio et al., 1995; Blanchard et al., 1993, 2001; Deminiere et al., 1992; Dellu et al., 1996; Deroche et al., 1993; Elmer et al., 1995; Kabbaj and Akil, 2001; Kabbaj et al., 2000; Piazza et al., 1989, 1991, 1993; Thiel et al., 1999, 1998) shows that subordinate or socially defeated animals share some behavioural features with the HRs (the increased self-administration of psychoactive drugs, a higher glucocorticoid level, memory deficits) and some with LRs (lower motor activity, enhanced anxiety, a higher serotonin level). Recent findings demonstrate that selectively bred high novelty seeking (bHR) rats exhibited increased aggressive behaviour and diminished intrusion-induced *c-fos* expression in select serotonergic brainstem cell groups (Kerman et al., 2011).

Social interaction stress is associated with aversive emotional arousal or fear conditions (e.g. Grant, 1963; Kollack-Walker et al., 1999; Martinez et al., 2002; Tamashiro et al., 2005) and chronic social stress in rats could be useful to investigate the neuronal mechanisms of adaptation to chronic stress challenge. However, very few studies have investigated the influence of individual differences in behavioural response with respect to the long-term consequences on brain activity in animals exposed to the chronic social stress (Matsuda et al., 1996; Paul et al., 2011). The hypothesis that chronic social stress, repeated over many consecutive days, can evoke persistent or long-lasting changes in the limbic system's activation with different patterns of adaptation, depending on inter-individual behavioural variability, was tested in the present study. As the presence of Fos-containing neurons is usually used for identifying activated neurons in the brain (Martinez et al., 2002; Boucher et al., 2011; Morrison et al., 2012), we assessed Fos protein detection in the limbic cortical, septal, hippocampal, thalamic and hypothalamic structures to determine how differences in behavioural response to novelty and/or social position would influence brain activation and/or adaptation patterns at 7 days following a chronic social interaction stress in rats. To investigate the influence of chronic social stress on the pattern of Fos immunoreactivity in the limbic system, some of HRs and LRs were subjected to a chronic 23 consecutive day social interaction (SocI) test and were divided into dominants (D) and subordinates (S) (Nov/SocI group). As concerns the influence of such laboratory routines as the carrying (Carr) of the animals and a change of cages on limbic system activation, those rats subjected solely to the novelty test were carried from the vivarium to the laboratory for 23 consecutive days (Nov/Carr group) or else stayed in the vivarium in their home cages (Nov/Home group).

2. Methods

2.1. Animals

All of the animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experiment was conducted under the authority of the Local Ethical Committee for the Care and Use of Laboratory Animals of the Medical University of Gdansk, Poland. The number of animals used was the minimum judged necessary to obtain significant results, and all efforts were made to minimize the animals' discomfort.

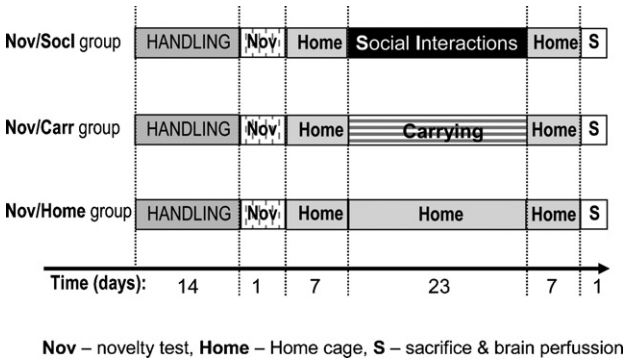

Fig. 1. Diagram of the experimental procedure. Nov/SocI group ($n = 24$) – rats subjected to novelty (Nov) and the 23 consecutive day social interaction test; Nov/Carr ($n = 10$) – rats subjected to novelty and carrying from the vivarium to the laboratory for 23 consecutive days; Nov/Home ($n = 10$) – rats subjected to novelty and staying in their home cages (Home). Nov – novelty test, Home – home cage, and S – sacrifice and brain perfusion.

Male Wistar rats ($n = 45$), weighing 250–300 g were used. They were housed individually in a light (6 a.m. on/6 p.m. off) and temperature (22 °C) controlled environment with food and water available ad libitum. The animals were handled daily for about two weeks before the beginning of the experiment so as to minimize any stress caused by the experimental procedures. All of the animals were subjected to a novelty test. Rat with median score of locomotor response to novelty was rejected from further experiments. One week following the novelty test, 12 randomly chosen HRs and 12 randomly chosen LRs were subjected to a social interaction test (Nov/SocI group). Every rat was exposed to social confrontations (with every other Nov/SocI rat), one per day, for 23 consecutive days. Ten of the 20 rats subjected to just the novelty test were carried from the vivarium to the laboratory for 23 consecutive days (Nov/Carr group, 5 HRs and 5 LRs) to ensure a similar level of stress connected with laboratory routines as that influencing the Nov/SocI rats while carrying them to perform the social interaction test. The Nov/Carr rats had no contact (visual, auditory or olfactory) with the Nov/SocI rats during their social interactions. After the novelty test, the 10 remaining rats stayed in their home cages so as to minimize environmental influence or else laboratory stress (Nov/Home group, 5 HRs and 5 LRs). Fig. 1 shows the diagram of the experimental procedure.

2.2. Behavioural screening

2.2.1. Novelty test (Nov)

The novelty test was performed according to a method that we have described previously (Wrona et al., 2004, 2003). All of the rats were placed in the actometer (Opto Varimex Minor – Columbus, USA) for 2 h (4–6 p.m. according to Piazza et al., 1989) while their locomotor activity was automatically recorded. For each animal the number of horizontal plane photocell counts accumulated over 2 h was used as an index of individual locomotor response to the new environment. According to Piazza et al. (1989), animals with an activity score above the group median were designated as high responders (HRs) while those with an activity score lower than the median were designated as low responders (LRs). Rat with the median activity score was rejected from further experiments.

2.2.2. Social interaction test (SocI)

Twenty-four randomly chosen rats (12 HRs and 12 LRs) were subjected to the social interaction test one week after the novelty test (Nov/SocI group). As with to our previous study (Wrona et al., 2005), the animals were divided into dominant (D) and subordinate (S) groups according to the procedure described by Albonetti and Farabollini (1994). Each experimental animal was put into a clean standard laboratory cage (similar to the home-cage) together with an unfamiliar rat. The pairs of animals were observed in terms of aggressive-defensive behaviours during the course of 20 min. They were video-taped and the social behaviour of the experimental subject and the opponent was then recorded on a PC with the aid of specialized software. The following behavioural parameters, based on Albonetti and Farabollini (1994) and our previous study (Wrona et al., 2005), were considered:

Offensive: attack, bite, posture On-the-Top, aggressive allo-grooming, lateral threat, upright offense.
Defensive: posture On-the-Back, freezing, defensive sideways posture, upright defence, retreat.

For each individual subject, the total defensive (DEF) and offensive (OFF) behaviours and then the OFF/DEF ratio were computed. Rats with a ratio of