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Stress activates the nucleus incertus and modulates plasticity in the hippocampo-medial prefrontal cortical pathway

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ABSTRACT

The nucleus incertus (NI) is a small cluster of brainstem neurons presumed to play a role in stress responses. We show that swim stress (normal water: 30 min and cold water: 20 min) and elevation stress robustly induced c-Fos expression in the NI and significantly suppressed long-term potentiation (LTP) in the hippocampo-medial prefrontal cortical (HP-mPFC) pathway. To examine whether activation of CRF_1 receptors in the NI plays a role in the suppression of HP-mPFC LTP, antalarmin, a specific CRF_1 receptor antagonist, was infused directly into the NI either before presentation of (1) elevation stress or (2) high frequency stimulation. As predicted, the intra-NI infusion of antalarmin reversed the elevation stress-induced suppression of LTP in the HP-mPFC pathway. This report suggests that the CRF_1 receptor in the NI contributes to stress-related impairment in plasticity of the HP-mPFC pathway. The findings suggest that the NI-HP-mPFC is a stress responsive circuit in the rodent brain.

1. Introduction

The nucleus incertus (NI) is a group of neurons in the caudal part of mammalian brain stem. Corticotropin releasing factor type 1 (CRF_1) receptor expression in the NI not only defines the precise localisation of this neuronal group, but also hints at its role in stress responses (Rivest et al., 1995; Tanaka et al., 2005). The NI, apart from being the principal source of relaxin-3 and the epicentre of the brain relaxinergic system, is GABAergic (Ma et al., 2007) in nature and expresses various neurotransmitters and peptides [for review see (Ryan et al., 2011)]. NI neurons respond to stressors such as immobilisation or forced swimming as is evident from elevated c-Fos (Tanaka et al., 2005) and relaxin-3 mRNA (Banerjee et al., 2010) expression, respectively. Recently, we have shown that CRF_1 receptor positive NI neurons project to the medial prefrontal cortex (mPFC) and that infusion of CRF peptide (into the NI) or electrical stimulation of the NI inhibits neuronal firing in the medial prefrontal cortex (mPFC) and impairs long-term potentiation (LTP) in the hippocampo-medial prefrontal cortical (HP-mPFC) pathway (Farooq et al., 2013). Furthermore, elevation stress is known to impair LTP in the HP-mPFC pathway (Rocher et al., 2004). Here we investigated the effects of stressors – cold water swim (20 min), swim (30 min), and elevation (30 min) – on c-Fos expression in the NI and LTP in the HP-mPFC pathway. In addition, the effect of infusion of antalarmin, a selective CRF_1 receptor antagonist (Webster et al., 1996), into the NI (prior to presentation of stress or high frequency stimulation) on the elevation stress-induced impairment of HP-mPFC LTP was studied in order to elucidate the contribution of the NI in stress-induced impairment of HP-mPFC LTP.

2. Experimental procedures

2.1. Animals

All procedures on animals were approved by the Institutional Animal Care and Use Committee (IACUC), National University of Singapore, and were carried out in accordance with the Guidelines on the Care and Use of Animals for Scientific Purposes

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developed by the National Advisory Committee For Laboratory Animal Research, Singapore; the European Communities Council Directive of 24 November 1986 (86/609/EEC); and the Guidelines laid down by the NIH in the US regarding the care and use of animals for experimental procedures. Adult male Sprague-Dawley rats (290–350 g) were obtained from the Centre for Animal Resources (CARE), National University of Singapore. Pairs of rats were housed in individually ventilated cages in a housing room that was temperature-controlled (23 ± 1 °C) and maintained on a 12-h light–dark cycle (07:00–19:00 h) with free access to food and water. They were acclimatised for at least 3 days before the initiation of experiments.

2.2. Drugs and chemicals

Ketamine (Parnell Manufacturing Pty Ltd; Alexandria, NSW, Australia), xylazine (Ilium Xylazil, Troy Laboratories Pty Ltd; Glendenning, NSW, Australia), enrofloxacin (Baytril 5%, Bayer Health Care; Seoul, Korea) and carprofen (Carprieve, Norbrook Laboratories (GB), Ltd; Carlisle, UK) were freshly prepared in sterile isotonic saline before use. Antalarmin hydrochloride (Tocris, UK) was dissolved in sterile 0.9% saline by sonication at 60 °C for 10–15 min. Pentobarbital (Valabarb) was purchased from Jurox Pty Ltd, Australia.

2.3. Stress protocols

Rats were subjected to three kinds of stress protocols published (Rocher et al., 2004; Tan et al., 2004) elsewhere, and adopted with slight modifications as described below. In the cold swim stress, rats were individually forced to swim for a duration of 20 min in a semitransparent cylindrical plastic container (Diameter: 32 cm and height: 42 cm) filled with water (18 °C) to a depth of 30 cm. The swim stress procedure was similar to the cold swim stress condition except for the duration (30 min) of stress and the temperature (24–25 °C) of water. After swim stress (either variants), the rats were dried with a towel and returned to their home cages. In the elevation stress protocol, the rats were placed on an unsteady elevated (1 m above ground level) transparent Perspex glass platform (20 cm × 20 cm) for 30 min with two powerful lamps (90 cm apart) focusing on the rats. Non-stressed control rats were kept in their home cages for 30 min before subsequent procedures. The stressed (and non-stressed) rats were subjected to electrophysiological procedures or sacrificed after 2 h for the c-Fos study (Fig. 1A and B) as described below.

2.4. Surgery

One cohort of rats underwent cannula implantation surgery to facilitate intra-NI infusion antalarmin prior to stress procedures. Briefly, the rats were anaesthetised with the anaesthetic combination, ketamine (75 mg/kg) and xylazine (10 mg/kg), mounted on a stereotaxic frame and homeothermically maintained throughout the aseptic procedure. Following a midline sagittal incision, burr holes were drilled above the NI (AP: −9.7 mm and ML: 0). A guide cannula with a dummy stylette was lowered to a DV coordinate of 7.5 mm. The cannula was held in place with dental cement and anchoring skull screws. Rats were sutured, returned to the homecage and they received analgesic and antibiotic injections for the first five days of the 7-day rehabilitation period. During the experimentation, the dummy cannula was replaced by infusion cannula (with 1 mm projection from the guide cannula) that was connected to the microsyringe to facilitate administration of antalarmin hydrochloride or 0.9% saline.

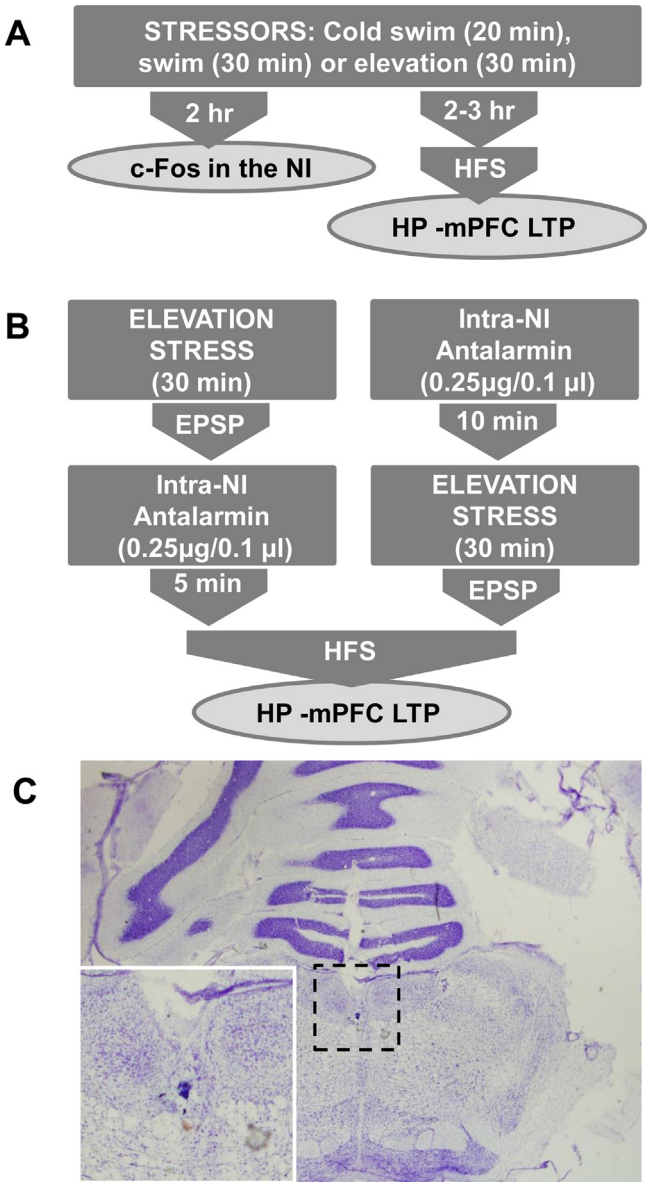

Fig. 1. Schematic representation of the study design. (A) Effects of different stressors on c-Fos expression in the NI and HP-mPFC LTP were studied in separate groups of rats. (B) Effects of intra-NI infusion of antalarmin were investigated prior to elevation stress or prior to HFS (post elevation stress) on the HP-mPFC LTP. (C) Representative micrograph of a brainstem section showing a Pontamine sky blue dye spot in the NI confirming the cannula position. The inset shows a digitally zoomed version of the boxed brainstem region.

2.5. Electrophysiology

Control and stressed rats were anaesthetised with an intraperitoneal injection of chloral hydrate (420 mg/kg) and mounted on a stereotaxic frame. The lateral tail was cannulated to facilitate administration of maintenance doses of chloral hydrate. Burr holes were drilled to target the mPFC (AP: 3.3 mm, ML: ±0.8 mm and DV: −4.2–4.7 mm from the skull) and CA1/ventral subiculum (VS) of the hippocampal formation (AP: −6.3, ML: ±5.5 and DV: −4–7.2 mm from the skull). A stainless steel monopolar electrode (SNE-300; Kopf Instruments) with a recording tip of length 250 μm and diameter of 100 μm was lowered into the mPFC, and a bipolar stainless steel stimulating electrode (SNE-100; Kopf Instruments, Tujunga, CA, USA) with diameters of 100 and 250 μm (central and outer, respectively) and a tip separation of 500 μm was lowered into