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Decline of hippocampal stress reactivity and neuronal ensemble coherence in a mouse model of depression

Jade Law[a,1], Yadira Ibarguen-Vargas[b,1], Catherine Belzung[c,d], Alexandre Surget[a,*]

[a] Institute of Neuroscience and Psychology, University of Glasgow, Glasgow, UK
[b] Kavli Institute for Systems Neuroscience, NTNU, Trondheim, Norway
[c] "Imaging and Brain" U930, Inserm, Tours, France
[d] UFR Sciences et Techniques, Université François Rabelais, Tours, France

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ABSTRACT

Dysregulations of stress systems, especially the hypothalamo–pituitary–adrenal (HPA) axis, have been commonly reported in major depression. Consistent results emphasized the role of the hippocampus in regulating stress systems and restoring an operative control on HPA axis following antidepressant treatments. However, very little is known about how the hippocampus integrates stress-related information and reacts to stressors beforehand. We therefore aimed to assess activations of hippocampal neuronal ensembles during stress-related experiences and evaluated the effects of a mouse model of depression, the Unpredictable Chronic Mild Stress (UCMS), and an antidepressant treatment (fluoxetine, 20 mg kg^{-1} day^{-1}, ip) in BALB/cByJ mice. The UCMS induced a depression-like syndrome characterized by a reduced weight gain, a progressive deterioration of the coat, an altered stress-coping strategy in behavioural tests and HPA axis dysregulations. Chronic fluoxetine had no effect in control non-stressed mice per se but reversed the syndrome induced by the UCMS, including an improvement of the HPA-system alterations. Neuronal activation was then assessed by immediate early-gene (c-fos) expression in different subfields of the CA3 and dentate gyrus (DG) along the dorso–ventral axis of the hippocampus, as they can support different computational functions. Our results showed that the hippocampus reacts to stressors by adjusting activations of cell ensembles. A pre-treatment with dexamethasone (DEX), a glucocorticoid receptor (GR) agonist that produced a delayed inhibition of the HPA axis activity, reduced novelty-related activations in the proximal CA3 (CA3c) and the DG of the dorsal hippocampus. All these effects were compromised by the UCMS, particularly by altering activation coherences within the dorsal CA3–DG network, but were rescued by chronic fluoxetine. Our study indicates therefore that variations of CA3–DG cell ensemble activation may contribute to stress integration in the hippocampus and that dysfunctions of this process may foster HPA-system dysregulations and depression-related states. It suggests that pharmacological interventions aiming to consolidate CA3–DG neural network might improve stress reactivity and possibly benefit to patients with major depression.

1. Introduction

Major depression currently sits at the top of the causes of disability worldwide, responsible for more burdens for today's societies than any other conditions (Ledford, 2014). Despite these outcomes, the neurobiological underpinnings of depression remain poorly understood and their identification has become a foremost priority for mental health research. Past research has been able however to associate a number of altered functions with major depression (Willner et al., 2013). One consistently reported dysfunction is the dysregulation of the hypothalamo–pituitary–adrenal (HPA) axis, the canonical stress hormone system which culminates with the release of glucocorticoids by the adrenal glands (de Kloet et al., 2005; Lucassen et al., 2014). HPA abnormalities are observed in a significant proportion of depressed patients, ranging from 35 to 65%, and are essentially characterized by exaggerated glucocorticoid releases, adrenal hypertrophy and/or increased expression of CRF, the ACTH secretagogue. These dysfunctions are thought to be primarily underlain by disruptions of the inhibitory control exerted by glucocorticoids themselves over the HPA axis (i.e. negative feed-

* Corresponding author at: Institute of Neuroscience and Psychology, University of Glasgow, West Medical Building, University Avenue, Glasgow G12 8QQ, United Kingdom.
E-mail addresses: alexander.surget@glasgow.ac.uk, alex.surget@gmail.com (A. Surget).
[1] These authors contributed equally to this work.

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back), since a large proportion of these patients do not decrease glucocorticoid secretions in response to dexamethasone (DEX), a glucocorticoid receptor (GR) agonist. Glucocorticoids can initiate the negative feedback at multiple levels: directly on the HPA axis via receptors in the hypothalamus and the pituitary, and indirectly via corticolimbic structures like the hippocampus (Ulrich-Lai and Herman, 2009). Remarkably, rodent models of depression identified the weakening of the inhibitory control mediated by the hippocampus as a major driver for the reported decline of the HPA axis negative feedback (Mizoguchi et al., 2003; Surget et al., 2011; Zhu et al., 2014).

Hippocampal involvement in depression has indeed been suggested by diverse morphological and functional changes. For instance, neuroimaging studies revealed an association between depression duration and smaller hippocampal volumes (Koolschijn et al., 2009). Animal models of depression also helped to identify a large range of molecular, cellular and functional alterations in the hippocampus, such as impaired synaptic plasticity, reduced spine density, dendritic shrinkage, or altered gene expression (Willner et al., 2013). More specifically, the dentate gyrus (DG), a hippocampal field that hosts one of the two neurogenic niches of the adult brain, has been particularly scrutinized since the demonstration that hippocampal neurogenesis is critically implicated in antidepressant effects, promoting their behavioural effects in animal models and restoring an operative hippocampal control on HPA axis (Santarelli et al., 2003; Surget et al., 2008, 2011). It is noteworthy that hippocampal contribution to the stress response not only includes HPA axis regulation but also encompasses behavioural and cognitive components to which DG is also critically involved.

The unique DG's extrinsic projections, the mossy fibres, are intrahippocampal and target CA3 pyramidal cells (Witter, 2010). Hence, contributions of the DG to stress response must inevitably pass through its effects on CA3. Interestingly, the intrinsic properties of hippocampal networks imply that DG and CA3 act synergistically and support different but complementary computational roles along the transverse axis of the hippocampus (Hunsaker et al., 2008; Rolls, 2013). Moreover, the hippocampus is not a homogeneous structure, and functional differences have been highlighted along the longitudinal axis too (O'Leary and Cryan, 2014; Strange et al., 2014). The dorsal part of the rodent hippocampus has been shown to be preferentially involved in spatial navigation and episodic memory while its ventral part has been associated with emotional reactivity, anxiety-related behaviours and HPA axis regulation. Accordingly, it has been suggested that the dorsal hippocampus controls the encoding of contextual and cognitive features of the stress response while emotional and physiological aspects depend more on the ventral part (Tanti and Belzung, 2013). However, despite a growing understanding about the role of the hippocampus in the stress response, much less is known about how it integrates stress-related information beforehand and reacts to stressors at cell population levels.

We therefore aimed to assess how the hippocampus integrates stress-related information and reacts to stressors in normal and depression-related conditions along its longitudinal and transverse axes. For this purpose, we determined activations of hippocampal neuronal ensembles during stress-related experiences (DEX injection and/or novelty stress based on forced exposure to a bright environment) and evaluated the effects of a mouse model of depression, the Unpredictable Chronic Mild Stress (UCMS), and a chronic antidepressant treatment (fluoxetine). In a first experiment, we examined physical variables (coat state, body weight), behaviours (stress coping strategy in the Novelty-Suppressing Feeding—NSF-test and the Splash test; exploratory drive in the Open-Field—OF-test and the Light/Dark box), as well as the HPA axis activity and regulation (corticosterone levels, reactivity to novelty stress and DEX suppression test, DST). In another cohort of mice, neuronal activations and coherence were examined by the expression of the immediate early-gene (IEG) c-Fos (Fos): in the proximal CA3c (the portion encapsulated by the blades of the DG), the more distal CA3b (the portion between the curve of CA3 and the lateral end of the DG), the suprapyramidal DG (DGs) and the infrapyramidal DG (DGi) along the transverse axis; in the dorsal (septal) and the ventral (temporal) portions of the hippocampus along the longitudinal axis.

2. Methods

2.1. Animals

Experiments were conducted on adult male BALB/cByJ mice aged 3 months at the beginning of the UCMS. Prior to the experiments, mice were maintained under standard laboratory conditions: air-conditioned room, 12/12 h light–dark cycle (lights on at 20:00 h), 22 ± 1 °C, group-housed (4–5 mice per cage), food and water ad libitum. All animal experiments were conducted in accordance with the guidelines of the European Commission (EC) Council Directive of 22 September 2010 (2010/63/EU). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Experimental procedures

The first experiment aimed to assess the physical, behavioural and corticotropic effects of UCMS and fluoxetine treatment (Fig. 1a). Mice were subjected to an 8-week UCMS procedure. Vehicle (0.9% NaCl) or fluoxetine (20 mg kg^{-1} day^{-1}) treatments were administered ip once a day from the 3rd week onward. Behavioural tests and the first HPA axis measurements were performed on the 8th week (day 1: OF, blood sampling; day 2: NSF test; day 3: Light/Dark box; day 4: Splash test) while the DST was carried out after 8 weeks. This experiment included four groups of mice depending on the environment (Control/UCMS) and the treatment (Vehicle/Fluoxetine), consisting of 8 mice per group for all the measures (except for the DST where each group was divided in 2 subgroups).

Another cohort of mice was used for the second experiment, which examined the effects of UCMS and chronic fluoxetine on hippocampal CA3/DG reactivity to stress (Fig. 1b). Mice were subjected to an 8-week UCMS procedure and to vehicle or fluoxetine (20 mg kg^{-1} day^{-1}, ip) from the 3rd week onward. After 8 weeks of UCMS, hippocampal CA3/DG reactivity to stress was assessed by Fos immunolabelling following exposure to novelty stress and pre-treatment with vehicle or DEX. This experiment included three groups of mice (Control-Veh, UCMS-Veh, UCMS-Flx), consisting of 12–14 mice per group.

All measures, behavioural tests and analyses were performed by experimenters blind to the experimental groups.

2.3. UCMS

The stress regimen used was previously described (Ibarguen-Vargas et al., 2009; Surget et al., 2009). Mice were repeatedly subjected to various mild socio-environmental stressors at any time of the day or the night according to an unpredictable schedule for a total period of 8 weeks (Fig. 1a). UCMS-exposed mice were maintained under the same standard laboratory conditions but were isolated in smaller individual cages (24 × 11 × 12 cm) while non-stressed control mice were also isolated but in larger cages (42 × 28 × 18 cm) with plastic tunnels and shelter. The stressors were based on periods of altered bedding (sawdust change, removal, or damp; substitution of sawdust with 21 °C water, rat, or cat faeces); cage tilting (45°); cage exchange (mice positioned in