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Research report

Antidepressant actions of lateral habenula deep brain stimulation differentially correlate with CaMKII/GSK3/AMPK signaling locally and in the infralimbic cortex

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HIGHLIGHTS

- Animals treated with LHb DBS displayed antidepressant-like response in the FST.
- Greater phoshorylation of CaMKII and GSK3 in the LHb correlated with reduced immobility.
- Reduced phoshorylation of AMPK in the IL correlated with reduced immobility.
- LHb and IL share an inverse relationship with CaMKII, GSK3 and AMPK expression and they contribute to possible antidepressant actions of LHb DBS.

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ABSTRACT

High frequency deep brain stimulation (DBS) of the lateral habenula (LHb) reduces symptoms of depression in severely treatment-resistant individuals. Despite the observed therapeutic effects, the molecular underpinnings of DBS are poorly understood. This study investigated the efficacy of high frequency LHb DBS (130 Hz; 200 μA; 90 μs) in an animal model of tricyclic antidepressant resistance. Further, we reported DBS mediated changes in Ca^{2+}/calmodulin-dependent protein kinase (CaMKIIα/β), glycogen synthase kinase 3 (GSK3α/β) and AMP-activated protein kinase (AMPK) both locally and in the infralimbic cortex (IL). Protein expressions were then correlated to immobility time during the forced swim test (FST). Antidepressant actions were quantified via FST. Treatment groups comprised of animals treated with adrenocorticotropic hormone alone (ACTH; 100 μg/day, 14 days, n = 7), ACTH with active DBS (n = 7), sham DBS (n = 8), surgery only (n = 8) or control (n = 8). Active DBS significantly reduced immobility in ACTH-treated animals ($p < 0.05$). For this group, western blot results demonstrated phosphorylation status of LHb CaMKIIα/β and GSK3α/β significantly correlated to immobility time in the FST. Concurrently, we observed phosphorylation status of CaMKIIα/β, GSK3α/β, and AMPK in the IL to be negatively correlated with antidepressant actions of DBS. These findings suggest that activity dependent phosphorylation of CaMKIIα/β, and GSK3α/β in the LHb together with the downregulation of CaMKIIα/β, GSK3α/β, and AMPK in the IL, contribute to the antidepressant actions of DBS.

1. Introduction

The lateral habenula (LHb) plays a critical role in mood, reward, incentive motivation and stress responses [1–5]. Patients suffering from severe depression [6] and animal depression models of alpha-methyl-*para*-tyrosine administration, amphetamine withdrawal, stress exposures, genetic manipulation or selective breeding [5,7–10], show elevated activity in this region. Local deactivation of the LHb via lesioning elicits antidepressant responses,

Abbreviations: TRD, Treatment resistant depression; HPA, Hypothalamic pituitary axis; LHb, Lateral habenula; IL, Infralimbic; DBS, Deep brain stimulation; CaMKII, Calcium/calmodulin dependent kinase type II; GSK3, Gycogen synthase kinase 3; AMPK, AMP-activated protein kinase.

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promoting escape behavior in the congenital learned helplessness (cLH) rats during inescapable paradigm [11]. These behavioral changes were mediated, in part, by the regulation of dorsal raphe serotonin levels and ventral tegmentum dopamine transmission [11–14]. Recently, deep brain stimulation (DBS) of the LHb has been trialed for treatment resistant depression (TRD), with therapeutic effects reported to coincide with periods of active stimulation [15]. Similarly, LHb DBS effectively achieved an antidepressant response in the cLH model of depression [16,17]. Nevertheless, the mechanisms through which LHb activity is up- or downregulated and whether it yields pro- or antidepressant actions, respectively, are not well understood.

Stress-induced molecular adaptations in the LHb region have important implications for modulating associative networks and depression-like behaviors [17–20]. In particular, recent attention has been given to the role calcium/calmodulin-dependent protein kinase type II (CaMKII) in learning and synaptic plasticity. Both exposure to stress and administration of the antidepressant escitalopram altered the expression of CaMKII in the LHb [19]. In cLH model, CaMKIIα and CaMKIIβ levels were shown to be significantly up-regulated in this region, prior to receiving effective antidepressant treatment [17]. Conversely, the down-regulation or blockade of CaMKIIβ activity reversed the depressive phenotype in these animals in standardized behavioral paradigms [17]. CaMKII is involved in many signaling cascades critical to cellular homeostasis, growth and plasticity, and functions together with glycogen synthase kinase 3 (GSK3) to regulate synaptic vesicle recycling in mature synapses [21,22]. This activity-dependent interaction poses a possible mechanistic link between high frequency DBS and local CaMKII/GSK3 modulation. GSK3 also serves as a mediator in cellular metabolism and plasticity. Upstream of this, AMP-activated protein kinase (AMPK), a cellular energy sensor, is activated when cellular energy reserves are low and coordinates intracellular functions mediating plasticity [23]. Stress-induced changes in energy metabolism and substrate utilization via this cell signaling system is implicated in the pathophysiology of depression [24,25]. Plasticity within this intracellular pathway may be an important contributor to the pathophysiological underpinnings of TRD.

Like the LHb, hypermetabolism of the subgenual cingulate gyrus (SCG) is well documented in the TRD population, and down regulation of this activity follows a successful antidepressant response, regardless of treatment method [26–30]. The LHb and SCG regulate affective and cognitive aspects of depressive symptoms [31–34]. The link between their hyperactivity and depressive states has been well-documented in patients [27,35,36]. Based on the existing literature, LHb function is important for alleviating depression symptomatology. Therefore, the present study further aims to validate this using LHb DBS in an animal model of antidepressant resistance, induced via chronic adrenocorticotropic hormone (ACTH) treatment [37–43]. In addition, we aimed to quantify antidepressant-like behavioral effects and its associated metabolic signaling in LHb and IL. DBS effects on CaMKIIα/β, GSK3α/β, and AMPK expressions locally as well as in the infralimbic cortex (IL; rodent homologue of SCG), will be examined in terms of the correlative relationship between behavior × protein signal.

2. Materials and methods

All experiments were approved by Mayo Clinic Institutional Animal Care and Use Committee and conducted in accordance with The Code of Ethics of the EU Directive 2010/63/EU for animal experiments. All animals were housed individually, with food and water available ad libitum.

2.1. Surgical procedures and post-operative care

Male Wistar rats (190–230 g) were anesthetized with isoflurane (1–3%) for stereotactic surgery. A 1.5–2 cm incision was made and small holes were broached in the skull at the site corresponding to the targets. Twisted bipolar platinum iridum electrodes (5.6 mm long, 0.075 mm in diameter; Plastics One, Virginia, USA) were bilaterally implanted into the LHb (from bregma: antero-posterior (AP) −3.7 mm, medial-lateral (ML), ±0.8 mm, dorsal-ventral (DV) −5.4 mm; Paxinos & Watson, 2007) of animals in active and sham DBS groups. Stimulating electrodes and screws were mounted with dental cement. Animals in the sham-surgery group were anesthetized and had four screws placed in the skull and secured with dental cement. Control animals did not undergo any surgical procedures.

2.2. Experimental procedure

After 3 days of acclimatization, rats received 14 days of either saline (0.9%) or ACTH (100 μg/day) treatment as described previously (38). On day 7, DBS electrodes were implanted as described above. Continuous high frequency DBS stimulation (130 Hz, 90 μS, 200 μA) was delivered for 3 days prior to the FST (Iso-Flex/Master-8; AMPI, Jerusalem, Israel).

2.3. Behavioral testing

Open field test (OFT) was used to quantify animals' general locomotor activity in a novel environment. A plexiglass box with a 622.3 mm x 622.3 mm base, and camera oriented with a top-down view was used for this test (CleverSys Inc., Virginia, USA). Animals were each placed in the central zone of the arena, and allowed to move freely for 6 min and behavior recorded by a video camera unit.

Porsolt forced swim test (FST) was used to quantify antidepressant response. Animals were placed into a narrow cylinder filled with water on the training day and consecutive test day. The apparatus consisted of clear plexiglass cylindrical tanks (60 cm height x 20 cm diameter; CleverSys Inc., Virginia, USA) filled with tap water (23–25 °C) to a depth of 25 cm. Animals were initially exposed to 15 min learning trial on day 14, conducted 2 h after the OFT. A 6 min test session was then conducted on the subsequent day. For this test, the animals' behavior was quantified to assess either active (swim, climb, escape) vs. passive (immobility, pass-dives) coping mechanisms. Behavioral data were analyzed using the cleversys topscan and forced swim software (CleverSys Inc., Virginia, USA) and validated by hand scoring.

2.4. Tissue collection and western blot analysis

Animals were injected with 100 mg/kg of Fatal Plus (Vortech, Michigan, USA) 30 min following the FST test. Brains were rapidly removed and frozen on dry ice. Tissue samples were dissected under 3.5× magnification using a ThermalTray system (Biocision, California, USA) together with dry ice to maintain stability of the frozen sample. A 1 mm coronal slice section was obtained for the IL and LHb regions, which were further dissected out using a 1 mm tissue biopsy punch. Electrode track and tip were visible in LHb slices and location was documented on correlating rodent brain atlas images (Fig. 1) [60] All tissue was then utilized for protein quantification.

1 mm^3 tissue samples from each region were homogenized in lysis RIPA buffer for protein quantification. Samples containing equivalent amounts of protein were applied to 15% acrylaminde denaturing gels and transferred to an Immobulin P for 1.5 h at 90 V. Each membrane was blocked for 15 min with 10 mL of tris-buffered