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Effect of antidepressant drugs on the vmPFC-limbic circuitry

Celene H. Chang, Michael C. Chen, Jun Lu*

Department of Neurology and Division of Sleep Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02115, USA

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ABSTRACT

Our recent study indicates that the lesions of the prefrontal cortex in rats result in depressive-like behavior in forced swim test and REM sleep alterations, two well-established biomarkers of depression disorder. We hypothesized that antidepressants may target the PFC to reverse depression. Systemic injections of antidepressants: the tricyclic antidepressant desipramine (DMI), the selective serotonin reuptake inhibitor fluoxetine, and the NMDA-antagonist ketamine selectively increased cFos expression (a marker of neuronal activity) in the deep layers of the ventromedial PFC (vmPFC) in rats. Of the vmPFC's limbic system targets, only the nucleus accumbens (NAc) was also activated by DMI. Using a retrograde tracer and a neuronal toxin, we also found that DMI-activated vmPFC neurons project to the NAc and that NAc activation by DMI was lost following vmPFC lesion. These results suggest that the vmPFC may be an essential target of antidepressant drugs, its projections to the NAc may be a key circuit regulating antidepressant action, and dysfunction of this pathway may contribute to depression.

1. Introduction

Monoamine oxidase inhibitors, tricyclic antidepressants (TCAs), and selective serotonin reuptake inhibitors (SSRIs) have all been prescribed over the past several decades to treat Major Depressive Disorder (MDD), yet we lack a complete understanding of the neural mechanisms of these drugs. Although these antidepressant medications are taken systemically, they have different effects on different regions of the brain (Conti et al., 2006). It is unclear, however, if these different classes of antidepressants share a common site of action in the brain and, if so, where that site is.

MDD likely involves multiple brain regions, but there is mounting evidence supporting the notion that the prefrontal cortex (PFC) plays an important role. Volume decreases have been reported in the orbitofrontal and subgenual cingulate cortices of depressed patients (Bremner et al., 2002; Drevets, 1998; Drevets et al., 1992; Ongür et al., 1998), and post-mortem analyses of the brains of depressed individuals show a significantly reduced number of glial cells (Drevets, 1998; Rajkowska et al., 1999) as well as neuronal size reduction (Chana et al., 2003). Depressed patients demonstrate increased activity per volume in the subgenual and ventrolateral PFC compared to non-depressed individuals (Drevets et al., 1992, 1997), and these changes are reversed in patients in remission (Drevets et al., 1992; Mayberg et al., 2000). In addition, there is evidence that early-life stress diminishes the ability of the vmPFC to sustain activity in response to repeated stressors (Wang et al., 2013).

Animal models of depression also suggest a role for the PFC in depression and its amelioration by antidepressants. Chronic restraint stress leads to dendritic atrophy in the rat medial PFC (mPFC), including reduced length and total branch numbers of apical dendrites in pyramidal cells (Brown, 2005; Cook and Wellman, 2004; Izquierdo et al., 2006; Radley et al., 2004), while antidepressant treatment increases markers of neuroplasticity (Sairanen et al., 2007) and changes in cell morphology in the PFC (Benes and Vincent, 1991; Li et al., 2010). Our recent study shows that the lesioning the vmPFC reduces REM sleep latency, increases REM sleep amounts, and increases immobile time in forced swim test (FST) in rats (Chang et al., 2014). These changes of REM sleep and hopelessness are consistent in behaviors of unipolar and bipolar depression in human. Ketamine is an NMDA-antagonist typically used as an anesthetic agent but has recently been found to rapidly alleviate depression in humans and rodents models of depression (Berman et al., 2000; Engin et al., 2009; Garcia et al., 2008; Li et al., 2010). Infusion of rapamycin – which blocks an important signaling pathway involved in cell growth and survival – into the rat mPFC prevents this antidepressant response (Li et al., 2010). Therefore, the PFC may be an important region for investigating both the underlying pathophysiology of MDD and the mechanism of antidepressants.

* Corresponding author. CLS 709, 3 Blackfan Circle, Boston, MA 02115, USA.
E-mail address: jlu@bidmc.harvard.edu (J. Lu).

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The evidence for a relationship between sleep, depression, and antidepressant medications was motivation to investigate how the medications may be acting on the sleep circuitries. We thus analyzed sleep-wake behavior of animals that were administered an antidepressant agent. In addition, relatively few studies have investigated the effects these drugs have on the PFC and its downstream targets. The rat mPFC, like the human PFC, projects to many limbic areas implicated in the control of affect, including the lateral septum, basolateral amygdala, insular cortex, and nucleus accumbens (Chiba et al., 2001; Uylings et al., 2003; Uylings and Van Eden, 1990). We hypothesized that antidepressants would directly alter neuronal activity in the mPFC and its circuitry. To test this hypothesis, we acutely administered the TCA desipramine, the SSRI fluoxetine, and ketamine to rats and examined patterns of the neuronal activity marker cFos in the mPFC and its downstream limbic targets. Furthermore, to verify the pathway of downstream neural activation, we repeated a subset of the experiments with animals that had neuronal lesions in the ventromedial PFC.

2. Materials and methods

2.1. Animals

All animals used were pathogen-free adult male Sprague–Dawley rats (300–350 g) purchased from Taconic (Hudson, NY). They were housed in rat-specific holding rooms controlled for temperature (22 ± 1 °C) and humidity in pairs until surgery, and subsequently separated into individual cages (7–9 days before sleep tests were performed). Food and water were available ad libitum, and lights were automatically switched on and off according to a 12:12 L:D cycle (lights on 8:00am–8:00pm). The animals were cared for in accordance with National Institutes of Health standards, and all procedures were pre-approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

2.2. Animal lesion surgery

Prior to surgery, animals ($n = 37$) were anesthetized with ketamine-xylazine (i.p. 800 mg/kg ketamine, 80 mg/kg xylazine, Med-Vet, Mettawa, IL) and then placed in a stereotaxic frame so that their head was fixed. Injections of ibotenic acid ($n = 11$, IBO, Tocris, Ellisville, MO), 0.9% saline ($n = 26$, Med-Vet, Mettawa, IL) or cholera toxin subunit B ($n = 4$, CTB, List Biological, Campbell, CA) were administered directly into the brain using a fine glass pipette (1 mm glass stock, tapering slowly to a 10–20 um tip) connected to an air compression system. A series of 20–40 psi puffs of air were used to deliver the compounds into the brain at the following coordinates and volumes: vmPFC: AP+3.0 mm DV-3.4 mm RL ± 0.6 mm, 66–99 nL 5% IBO, 16.5 nL 1.0% CTB; NAc: AP+2.0 mm DV-6.8 mm RL ± 1.0 mm, 23.1 nL 1% C-+-TB (Paxinos and Watson, 2007). Incisions were closed with wound clips. Upon completion of the procedure, the animal was given a subcutaneous injection of the analgesic meloxicam (1.0 mg/kg, Med-Vet, Mettawa, IL) and allowed to recover on a warm plate until awakened from anesthesia.

On the same day six animals received four EEG screw electrodes (Plastics One, Roanoke, VA) that were screwed into skull, and two flexible EMG wire electrodes were also placed on the left and right nuchal muscles. The free ends of the leads were placed in a plastic electrode pedestal that was cemented onto the skull using Jet Denture Repair Powder and Jet Liquid (Henry Schein, Melville, NY). Any animals that did not receive electrodes had their incision closed with wound clips.

2.3. Sleep recordings and analysis

After at least a week of post-surgical recovery, animals undergoing sleep recordings ($n = 6$) were placed in isolated recording chambers. Flexible cables that were mounted to fixed commutators were attached to the electrode pedestals, and the cages were placed such that the animals could move freely. As before, food and water were available ad libitum, ambient temperature was controlled, and the light:dark cycle was 12:12. Video cameras were placed to capture movement in the entire cage, and the animals were habituated without disturbance for at least two days and then recorded for 72 h using VitalRecorder (Kissei Comtec Co., Nagano, Japan). Animals were injected 2 h after lights on (9:00am) with drug ($n = 3$) (or saline, $n = 3$) on the second recording day, and with saline (or drug) on the third day. Upon completion of the recordings, animals were detached from the cables and returned to the holding room.

The EEG/EMG recordings were analyzed using SleepSign (Kissei Comtec Co., Nagano, Japan). The recordings were divided into 12s epochs and each epoch scored manually as wake, REM, or NREM sleep. Wake was identified by high frequency, desynchronized EEG accompanied by frequent EMG activity and observed behaviors on the video playback. NREM sleep was identified by the dominant presence of high-amplitude, low frequency (<4 Hz) EEG activity and little muscle tone on the EMG recording. REM sleep was identified by theta waves (4–7 Hz) of consistent low amplitude on the EEG recording accompanied by very low EMG activity. REM sleep latency was defined as the interval of time between sleep onset and REM sleep onset averaged over 24 h. Sleep-wake percentages, bout numbers, bout durations and REM latency were analyzed using unpaired t-test and adjusted using Bonferroni's correction, using a significance threshold $p < 0.05$.

2.4. Antidepressant drug treatments

To study neuronal activation patterns upon acute drug treatment, and the effect of prefrontal cortical lesions on the activation patterns, lesioned ($n = 11$) and sham-lesioned ($n = 22$) animals were placed in isolated chambers for at least two days. At 10am after habituation, animals were gently handled and weighed. At 10am the following day, animals were injected i.p. with desipramine hydrochloride ($n = 11$, 10 mg/kg in saline, Sigma, St. Louis, MO), fluoxetine hydrochloride ($n = 7$, Sigma, St. Louis, MO; 20 mg/kg in saline), ketamine ($n = 5$, 10 mg/kg in saline, Med-Vet, Mettawa, IL), or sterile saline ($n = 10$, 0.9%, Fisher Scientific, Pittsburgh, PA). This dose of desipramine has been shown to be effective in previous rodent models of depression (Borsini et al., 1981; Detke et al., 1995; Pulvirenti and Samanin, 1986), and the doses of fluoxetine and ketamine were chosen based on their effectiveness in decreasing immobility in the forced swim test rodent model of depression (Detke et al., 1995; Li et al., 2010) while not causing arousal. We believe this latter feature was key to minimize confounding effects on neuronal activation due to wakefulness. The animals were then placed back in their cages and in their chambers for two hours, after which they were sacrificed via perfusion and fixation (see below) and their brains stained for cFos immunohistochemistry. We chose these conditions (including performing injections in the morning) to maximize the likelihood that the animals would fall sleep following the injection.

We chose to implement a single, acute injection of antidepressant drugs because we wanted to first understand how and where the drugs are acting in the brain. Although antidepressant drugs generally improve symptoms after several weeks of regular ingestion, we believe it is important to understand what a single dose does as we attempt to unwrap its chronic effects. Therefore in this work we begin with acute injections to discover what areas of the brain are the drugs' main targets. In addition, for these reasons we did not test a wide range of drug concentrations. The purpose of our investigation was to discover the action of the antidepressant drugs at their biologically relevant concentrations.

2.5. Perfusion and fixation

Animals were anesthetized with 7% chloral hydrate (i.p. 500 mg/kg, Sigma, St. Louis, MO). The body cavity was opened using surgical scissors and a 16G needle was inserted into the left ventricle of the heart. The top of the right atrium was cut to allow blood to be drained. About 100 mL of saline was flushed through the vascular system using an intravenous line, followed by 500 mL of 10% buffered formalin (Fisher Scientific, Pittsburgh, PA). Upon fixation of the tissue the brain was removed from the skull and stored in 10% formalin for 4–5 h. The brains were then moved to 20% sucrose and 0.02% azide solution overnight.

2.6. Histology and immunohistochemistry

Brains were sliced into four series of 40um sections using a freezing microtome. The sections were stored in PBS-0.02% azide in 20 °C. For immunohistochemical staining, tissue sections were rinsed in PBS three times, 3–5 min each, then incubated for 30 min in 0.3% H_2O_2 (Sigma, St. Louis, MO) in PBT (phosphate buffer with Triton X-100; Sigma, St. Louis, MO) to oxidize any remaining blood. The sections were again rinsed in PBS and then incubated in primary antibody diluted in PBT-Azide for 1-2 nights, depending on the antibody (cFos Ab-5, PC38, rabbit polyclonal, 1:30,000, Calbiochem, Billerica, MA; Chemicon, Billerica, MA; CTB, 127H4810, goat polyclonal, 1:50,000, Sigma, St. Louis, MO). Tissue were then rinsed in PBS three times, and incubated in secondary antibody (1:1000, biotin SP-conjugated against appropriate species IgG, Jackson ImmunoResearch Laboratories, West Grove, PA) for 60–90 min. Sections were again rinsed in PBS and placed in ABC solution (1:1000 each Vectastain solutions A and B, Vector Laboratories, Burlingame, CA) for 60–90 min. Sections were rinsed in PBS and stained for 5 min in a solution consisting of: 1% DAB, 0.3% H_2O_2 (and 0.01% Ni, 0.005% $CoCl_2$ if desired a black stain). Tissue were then rinsed and mounted on microscope slides in gelatin.

Sections were Nissl stained by placing microscope slides of mounted tissue in ddH_2O for 5 min, followed by 10–30 s in 0.1% thionin staining solution (Sigma, St. Louis, MO). The slides were dehydrated step-wise by incubating in 50%, 70%, 95%, and 100% EtOH for 2 min each. Slides were then placed in xylene for at least three hours before covering with glass coverslips. The delineation of the vmPFC and dmPFC was based on Paxinos and Watson's Rat Atlas (Paxinos and Watson, 2007) and the cytoarchitectural characteristics as described in Hurley et al. (Hurley et al., 1991).

2.7. Cell counting

All cFos-stained cells were counted manually using a guide grid at 25× magnification. (While automated cell counting was attempted, variance in background staining – not unexpected despite identical treatment of the sections – warranted more careful analyses.) For the ACC and PLC, three sections were counted