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Ventromedial prefrontal cortex regulates depressive-like behavior and rapid eye movement sleep in the rat

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ABSTRACT

Major depressive disorder (MDD) is a debilitating disease with symptoms like persistent depressed mood and sleep disturbances. The prefrontal cortex (PFC) has been implicated as an important structure in the neural circuitry of MDD, with pronounced abnormalities in blood flow and metabolic activity in PFC subregions, including the subgenual cingulate cortex (sgACC, or Brodmann area 25). In addition, deep brain stimulation in the sgACC has recently been shown to alleviate treatment-resistant depression. Depressed patients also show characteristic changes in sleep: insomnia, increased rapid-eye-movement (REM) sleep and shortened REM sleep latency. We hypothesized that sleep changes and depressive behavior may be a consequence of the abnormal PFC activity in MDD. The rat ventromedial PFC (vmPFC, prelimbic and infralimbic cortices) is considered to be the homolog of the human sgACC, so we examined the effect of excitotic lesions in the vmPFC on sleep–wake and depressive behavior. We also made lesions in the adjacent dorsal region (dmPFC) to compare the effect of this similar but distinct mPFC region. We found that both dmPFC and vmPFC lesions led to increased REM sleep, but only vmPFC-lesioned animals displayed increased sleep fragmentation, shortened REM latency and increased immobility in the forced swim test. Anatomic tracing suggests that the mPFC projects to the pontine REM-off neurons that interact with REM-on neurons in the dorsal pons. These results support our hypothesis that neuronal loss in the rat vmPFC resembles several characteristics of MDD and may be a critical area for modulating both mood and sleep.

1. Introduction

Major Depressive Disorder (MDD) is one of the leading causes of disability worldwide and continues to increase in prevalence (World Health Organization, 2002, 2012). MDD is diagnosed by the presence of at least five symptoms, which must include depressed mood or anhedonia (Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), 1994). Despite the prominence of MDD and other mood-related disorders, its etiology remains unclear.

MDD likely involves dysfunction in a network of structures in the brain. Based on human imaging studies, this network is believed to include regions of the limbic system: the prefrontal cortex, hippocampus, nucleus accumbens, and amygdala (Nestler et al., 2002). The medial prefrontal cortex (mPFC) has been of particular interest because subregions of the mPFC show marked changes in neural activity in depressed patients compared to healthy controls. For instance, the ventral mPFC (vmPFC) of MDD patients exhibits increased cerebral blood flow (Drevets et al., 1992) and metabolism (Mayberg, 1997), while the subgenual anterior cingulate cortex (sgACC) (Drevets et al., 1997; Jahn et al., 2010; Ongür et al., 1998; van Tol et al., 2010), shows increases in glucose metabolism in depression (Drevets et al., 1997, 2008) that normalize with successful antidepressant treatment, including deep-brain stimulation near this area (Mayberg et al., 2000, 2005). In addition, responders to acute sleep deprivation exhibited decreased activity in the ventral (including subgenual) region of the anterior cingulate cortex (Clark et al., 2006). The involvement of the mPFC in MDD suggests a key role for this structure in regulating the affective and behavioral aspects of depression.

Recent animal studies have also implicated the mPFC as a possible key site for depression etiology. Chronic stress protocols, which induce depression-like behaviors, led to decreased glial cell count (Banasr and Duman, 2008) and dendritic atrophy in this

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region (Brown, 2005). Glial cell ablations in the rat mPFC produced depression-like behaviors, including decreased sucrose preference and increased immobility in the forced swim test (Banasr and Duman, 2008). Excitotoxic neuronal lesions in the rat mPFC led to 'learned helplessness' upon exposure to inescapable footshocks (Klein et al., 2010). Interestingly, deep brain stimulation in the mPFC induced an antidepressant-like response in the forced swim test (Hamani et al., 2010), and optogenetic stimulation in the mPFC of mice susceptible to social defeat stress increased their sucrose preference (Covington et al., 2010), although 100 Hz stimulation may be mostly inhibitory.

These studies suggest that the mPFC may regulate a variety of depressotypic behaviors, including abnormal sleep. Depressed patients complain of sleep disturbances and have increases in REM sleep, along with decreased REM latency (the interval of time between sleep onset and REM sleep onset; Berger and Riemann, 1993; Reynolds and Kupfer, 1987; Steiger and Kimura, 2010), and many antidepressant drugs suppress REM sleep (Rijnbeek et al., 2003). Animals that undergo a chronic mild stress protocol demonstrate changes in sleep similar to those observed in depressed humans (Grønli et al., 2004). Furthermore, several longitudinal studies confirm that poor sleep quality and decreased (or significantly increased) quantity of sleep increases the risk of developing an affective disorder (Ford and Kamerow, 1989; Hohagen et al., 1993; Katz and McHorney, 1998), regardless of family history. Because the basic structure of sleep is similar in humans and rodents, and because the underlying circuitry regulating sleep is also thought to be similar, sleep disturbances represent an important marker for testing the role of neural circuits in depression. We hypothesized that prefrontal cortex dysfunction may be critical in producing depression-associated behaviors, including changes in sleep patterns. Previous studies have described conflicting roles of the rat mPFC subregions, the anterior cingulate (dmPFC) and the prelimbic/infralimbic (vmPFC) cortices (Bissiere et al., 2006; Hamani et al., 2010; Klein et al., 2010; Scopinho et al., 2010). As the vmPFC especially projects to limbic areas involved in the control of emotion (Sesack et al., 1989) and has thus been suggested to be the homolog of the human sgACC, we hypothesized that this region in particular may play a key role in depressotypic behavior and sleep changes in rodents. To test this hypothesis, we made neuronal lesions in the ventral and dorsal subdivisions of the medial prefrontal cortices of rats, measured their sleep behaviors, and tested the animals for depressive-like behavior in the forced swim test (FST) (Detke et al., 1995; Porsolt et al., 1977). We subsequently propose a model of sleep modulation via the prefrontal cortex.

2. General experimental design

In our experiments, adult male rats were surgically lesioned in two different regions of the medial prefrontal cortex. Saline-injected animals were used as controls. EEG and EMG electrodes were also surgically implanted, and sleep–wake behavior was observed for the experimental groups and controls to observe the effects the lesions had on the animals' sleep and REM sleep latency (a measure often used in human and rodent depression studies). In addition, the forced swim test was performed to investigate the rats' behavior with respect to a standard rodent depression model.

3. Methods and materials

3.1. Animals

All animals used were pathogen-free adult male Sprague–Dawley rats (350–400 g) purchased from Taconic (Hudson, NY). They were housed in individual cages (Allentown Inc, New Jersey) in rat-specific holding rooms controlled for temperature (22 ± 1 °C) and humidity. Food (Cat. No 5008, Formulab, USA) 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.

3.2. Animal surgery and EEG/EMG implantation

Prior to surgery, animals 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 (IBO, Tocris, Ellisville, MO), 0.9% saline (Med-Vet, Mettawa, IL) or neural tracer (BD, Molecular Probes, Grand Island, NY or 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 μm tip) connected to an air compression system. A series of 20–40 psi puffs of air were used to deliver the compounds into and with the following coordinates and volumes: dmPFC: AP +3.0–3.5 mm, DV-1.4 mm, RL ± 0.6 mm, 66 nL 1–5% IBO; vmPFC: AP +3.0–3.5 mm, DV-3.4 mm, RL ± 0.6 mm, 66–99 nL 5% IBO, 16.5 nL 1% CTB; vlPAG: AP −7.2 mm, DV-5.6 mm, RL-1.2 mm, 33 nL 1% CTB; SLD: AP −9.4 mm, DV-6.3 mm, RL-1.2 mm, 16.5 nL 1% CTB (Paxinos and Watson, 2007).

On the same day some of the animals received four EEG screw electrodes (Plastics One, Roanoke, VA) that were screwed into skull (Two front screws at AP = 0.0 mm, ML = 3.0 mm; two rear screws at AP = −2.0 mm, ML = 3.0 mm), 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. Upon completion of the procedure, animals were 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.

3.3. Sleep recordings and analysis

Rats typically resume visibly normal activity within 48 h post surgery. In our experiments animals were given at least a week of post-surgical recovery before being placed in isolated recording chambers in preparation for sleep recordings. 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 48 h using VitalRecorder (Kissei Comtec Co., Nagano, Japan). 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 12 s 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. Deep 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, and light sleep was identified by low EMG and sleep posture (video) as well as slow EEG. REM sleep was identified by theta waves (4–7 Hz) of consistent low amplitude on the EEG recording accompanied by very low EMG activity (Video recordings were used to confirm active/inactive states and are especially critical in identifying quiet wake and light sleep). REM sleep latency was defined as the interval of time between sleep onset and REM sleep onset averaged over 24 h. This is the standard definition used for rodents, in contrast to a single latency measure (as for humans), because rodents do not have as consolidated sleep as humans. 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 $p < 0.05$.

3.4. Forced swim test

The forced swim test procedure was conducted as described previously (Castagné et al., 2011; Detke et al., 1995). The test was conducted over two days using acrylic cylinders (20 cm × 40 cm; Northeast Plastics, Philadelphia, PA) that were filled 30 cm with 25 °C water. On the first day, animals were placed in the cylinder for 15 min while being video recorded using a computer running Ethovision (Noldus, Leesburg, VA). The animals were subsequently removed and gently handled and dried before being returned to their cages. On the second day, the swim test was repeated for 5 min. All animals were habituated to the room at 12:00 and the swim tests were completed between 13:00 and 15:00 to limit any circadian influences. Total amounts of immobility during the 5-min test session were scored using Ethovision and parameters validated for rats (EthoVision, 2004) (sampling rate 5 Hz; immobility threshold 11.5%).

3.5. Statistical analysis of forced swim test

FST immobility between groups were analyzed using unpaired *t*-test and adjusted using Bonferroni's correction, using a significance threshold $p < 0.05$. Both