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EFFECT OF DIAZEPAM AND YOHIMBINE ON NEURONAL ACTIVITY IN SHAM AND HEMIPARKINSONIAN RATS

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Abstract—The prefrontal cortex and the amygdala are critical for the emotional guidance of behavior and are believed to be a site of action for many anxiolytics and anxiogenics. Despite extensive studies examining how these drugs affect behavior, there is little information regarding their effects on neuronal activity. Additionally, with recent recognition of anxiety as a non-motor symptom of Parkinson's disease, it is unknown if activity in the cortex and the amygdala is altered. Previously, we reported that hemiparkinsonian rats had higher baseline anxiety-like behavior and diminished responsiveness to the acute anxiolytic, diazepam. In contrast, sham-lesioned rats exhibited anxiolytic behavior to diazepam. In this study, we monitored *in vivo* single-unit spiking activity simultaneously from the anterior cingulate cortex (ACC) and the basolateral amygdala (BLA) in anesthetized sham-lesioned and hemiparkinsonian rats to unmask neuro-circuits underpinning the difference in diazepam responsiveness. We found that baseline spiking activity in the ACC was the same in both sham and hemiparkinsonian rats. We also noted a similar phenomenon for baseline activity in the BLA between sham and hemiparkinsonian rats. However, neuronal spiking activity after diazepam administration (1.5 mg/kg, SubQ) was lower than in controls in the ACC of sham-lesioned rats whereas no difference was noted after diazepam treatment in hemiparkinsonian rats. BLA neuronal spiking activity was unaffected by diazepam administration in either animal group. On the other hand, yohimbine treatment (5 mg/kg, SubQ) coincided with lower neuronal spiking activity compared to controls in the BLA of sham-lesioned rats, but was unchanged from controls in hemiparkinsonian rats. Yohimbine did not affect ACC neuronal spiking activity in either group. Overall, the lack of ACC responsiveness to diazepam in hemiparkinsonian, but not sham-lesioned rats underscores a plausible fundamental difference in anxiety-related neural signaling between animal groups.

Key words: anxiety, Parkinson's disease, non-motor symptom, single-unit recordings, anterior cingulate cortex, basolateral amygdala, 6-OHDA.

INTRODUCTION

Clinically significant anxiety is a known major non-motor symptom in Parkinson's disease (PD) affecting around 25–43% of patients (Dissanayaka et al., 2016). Yet, clinical and preclinical research regarding the pathophysiology of anxiety disorders in PD remains insufficient and treatments for this complication of PD are ineffective in many patients. Two brain areas that may contribute to anxiety disorder in PD are the anterior cingulate cortex (ACC) and the basolateral amygdala (BLA). A better understanding of the pathophysiological underpinnings of anxiety in PD could lead to more effective pharmacological or surgical treatments.

The ACC is part of the medial prefrontal cortex (mPFC) that integrates emotion and cognition to facilitate communication between cortical and limbic regions in the brain. It is also crucial for the regulation of emotional behavior and extinction of fear responses (Bissiere et al., 2006). It is thought to provide top-down control of anxiety by reciprocally connecting with the amygdala to regulate emotional, autonomic, and neuroendocrine function. Dysfunction of the ACC is thought to underlie anxiety disorders in both human and rodent studies (Bremner, 2004; Singewald, 2007).

The amygdala is a key region involved in aversive learning, stress response, and memory acquisition, storage, and modulation of stressful or fearful experiences (Buffalari and Grace, 2009; Ehrlich et al., 2009). It is the focus of many studies investigating anxiety and fear (LeDoux, 2007) and is thought to assign emotional significance to specific sensory inputs (Singewald, 2007). The amygdala can be divided into two main subnuclei: the BLA complex, and the central amygdala (CeA). The lateral division receives the majority of sensory input from the thalamus and cortex, while the CeA serves as the primary output of the amygdala to downstream targets

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Abbreviations: 6-OHDA, 6-hydroxydopamine; ACC, anterior cingulate cortex; BLA, basolateral amygdala; CeA, central amygdala; CV, Cresyl Violet; GABA, gamma-aminobutyric acid; LAT, limb-use asymmetry test; MFB, medical forebrain bundle; mPFC, medial prefrontal cortex; NE, norepinephrine; PBS, phosphate-buffer saline; PD, Parkinson's disease; TH, tyrosine hydroxylase.

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such as the brainstem and hypothalamus (Diederich et al., 2016). There are also intercalated cell masses interspersed between these divisions which inhibit local circuits and gate interactions within the amygdala (Ehrlich et al., 2009). Hyperactivity of the amygdala has been shown in multiple studies of patients with anxiety disorders (Bremner, 2004) and inactivation of the BLA has anxiolytic effects in rodents (Bueno et al., 2005). The BLA has been proposed as a strong candidate to mediate interactions between the dopaminergic system and stress responses (Belujon and Grace, 2015).

Since the ACC and BLA are two brain areas involved in anxiety which receive dopaminergic innervation (Russo and Nestler, 2013) and these efferents degenerate in PD (Tadaiesky et al., 2008), it is plausible that this phenomenon could lead to aberrant activity underlying or contributing to anxiety disorders in PD (Espejo, 1997; Tessitore et al., 2002; Pillay et al., 2006). However, there is relatively sparse information regarding changes in neuronal activity in these anxiety-related brain areas or their responsiveness to anxiolytics in preclinical and clinical studies in PD. Yet, we recently found that hemiparkinsonian rats exhibited a higher prevalence of anxiety-like behavior than sham-lesioned rats. Furthermore, the former exhibited reduced responsiveness to an acute anxiolytic, diazepam, whereas the latter group showed reduced anxiety-like behavior in the elevated plus maze and open-field test (O'Connor et al., 2016). Although the data posited fundamental differences in diazepam neural modulation between animal groups, our study focused primarily on evaluating behavioral changes to anxiolytic treatment. Here, we record single-unit neuronal spiking activity in anesthetized hemiparkinsonian and sham rats to elucidate whether baseline differences in the ACC and BLA differ between these rat groups. Additionally, we assess how neurons in each brain area are affected by systemic injections of diazepam or the anxiogenic, yohimbine. This will help determine whether neuronal responsiveness to these drugs contributes to the varied baseline anxiety-like behavior and diazepam efficacy between sham-lesioned and hemiparkinsonian rats in our previous study.

EXPERIMENTAL PROCEDURES

Rat model of hemiparkinsonism

Animals and surgical procedure. Animal use was conducted in accordance with the Albany Medical College Institutional Animal Care and Use Committee consistent with the National Institutes of Health guidelines for the care and use of laboratory animals. In total, there were 59 rats used in this study (29 sham and 30 hemiparkinsonian animals) and all efforts were taken to minimize animal suffering and the number of animals used in the study. Methodology to produce hemiparkinsonian rats was previously described (O'Connor et al., 2016). In brief, 6-hydroxydopamine hydrobromide (6-OHDA) was injected into the right medical forebrain bundle (MFB) in adult male Sprague–Dawley rats at coordinates according to the rat atlas (Paxinos and Watson, 1998) at 7.5 mm ventral from dura, 1.2 mm lateral to the midline and 4.4 mm posterior from bregma. Animals weighing 200–250 g (Taconic Farms, Rensselaer, NY, USA) were anesthetized with 4% isoflurane which was reduced to 2% throughout the craniotomy using an inhalant system (Harvard Apparatus, Holliston, MA, USA). Desipramine (25 mg/kg) and pargyline (50 mg/kg) were injected intraperitoneally (IP) 30 min prior to surgery to prevent reuptake of 6-OHDA into noradrenergic neurons and inhibit monoamine oxidase B activity, respectively. Eye lubricant (Major Pharmaceuticals, Libonia, MI, USA) was applied to the animal's eyes to prevent dehydration, 2% lidocaine gel (Akorn Pharmaceutics, Lake Forest, IL, USA) was applied to ear bars to minimize discomfort and 0.5% bupivacaine (Hospira, Lake Forest, IL, USA) was injected subcutaneously at the incision site for analgesia. Rats were placed in a stereotactic frame (Harvard Apparatus, Holliston, MA, USA) and a burr hole was drilled in the skull to allow for a 10-μl Hamilton syringe (Hamilton Company, Reno, NV, USA) to be inserted into the right MFB and deliver 4.5 μl of 6-OHDA (3 μg/μl made in 0.1% ascorbic acid) at a rate of 0.5 μl/min. The needle was removed 10 min post-injection. In sham animals, 4.5-μl saline (0.9% NaCl) was injected instead of 6-OHDA. The incision site was closed with staples and antibacterial ointment (Actavis Mid Atlantic LLC, Lincolnton, NC, USA) was applied to the closure. Postoperative penicillin (80 μg/kg, subQ) was given and buprenorphine (0.12 g/kg) injected subcutaneously immediately following surgery and twice a day for 2 days post-surgery.

Assessment of forelimb akinesia. Two weeks following the craniotomy surgery, motor function was assessed to determine PD phenotype using the limb use asymmetry test (LAT). Animals were placed in a Plexiglas cylinder, 12 inches in diameter and 24 inches tall, for 5 min and allowed to move freely. Behavioral monitoring included counting the number of right and left paw touches on the side of the cylinder and recording the number of right paw touches compared to the total forepaw touches. Marked degeneration of dopaminergic neurons in the right striatum coincides with greater touches made with the animal's right, unimpaired paw (# right touches/ total # touches X 100) (Schallert et al., 2000) whereas sham rats touch with both forepaws equally.

Anesthetized recordings

In vivo *electrophysiological recordings in anesthetized animals.* At least two weeks after the 6-OHDA injection and craniotomy, animals were anesthetized with an IP Urethane injection (1.2–1.5 g/kg in saline). The craniotomy surgery was performed as mentioned above and burr holes were drilled in the skull so bundles of 3 to 5 tungsten microelectrodes with an impedance resistance of 500 kΩ, 77-μm shaft diameter and 1-μm tip diameter (Harvard Apparatus, MA, USA) could be inserted in the right ACC at 2.7 mm posterior to bregma, 0.6 mm lateral to midline and 1.8–3.2 mm ventral to