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Stephanie Akosa
stephanie.akosa@uconn.edu

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Development of Physiological Markers for Tetrabenazine-Induced Motivational Dysfunctions Using Electroencephalography in Male and Female Rats

Stephanie Akosa
Dr. Salamone’s Lab

BS: Physiology and Neurobiology
Minor: Psychological Sciences

University of Connecticut, Class of 2021
Abstract

Depression is a mental illness that is increasingly rampant in our society. With its prevalence, various drugs have become commonplace for treatment. Many of these drugs are serotonin reuptake inhibitors, or SSRIs. These drugs are able to mitigate symptoms of depression such as rumination and anxiety. However, they are not very successful in treating the amotivation and anergia that are seen in these patients. In order to investigate which drugs will be the most successful in treating the symptoms of depression, it is important to develop an animal model that can accurately represent the motivational symptoms of depression in humans. The goal of the present study was to use female and male rats to develop electroencephalography (EEG) markers that can be readily translatable to the pathophysiology of female and male patients with depression. In this study, tetrabenazine (TBZ), a vesicular monoamine transporter-2 (VMAT-2) inhibitor, or a vehicle (VEH) control, was administered to a group of 8 female rats and 7 male rats prior to measuring EEG in the home cage. Recordings were taken from each treatment condition in the medial prefrontal cortex (mPFC), motor (M1/M2), and medial parietal (mParietal) cortices to investigate the effect of TBZ on these cortices and to determine whether they are comparable to the neurological markers associated with depression in humans. It was found that male rats demonstrated a predominant peak frequency at baseline in EEG activity across all three regions in the alpha/theta frequency range (4-12 Hz, with a sharp peak at 6-8.5 Hz frequency range). The sharp 6-8.5 Hz peak was suppressed when TBZ was administered in a 1.0 mg/kg dose. The EEG recordings of the female rats were not significantly affected. These findings can be used as a foundation to develop further EEG studies in behaving rats that can be translated to treatments for clinical depression within humans.
Introduction

Major depressive disorder affects a large portion of the population, with over 264 million people being diagnosed with this across the globe (WHO, 2020). As stated by the National Institutes of Health, depression is “a common but serious mood disorder. It causes severe symptoms that affect how you feel, think, and handle daily activities” (Depression 2018). As the number of individuals affected by depression has increased over the years, more people have had to grapple with the cognitive, emotional, and physical symptoms that come with this psychopathology (Stahl, 2006; Treadway and Zald, 2011; Fava et al. 2014; Salamone et al. 2016a).

One major cognitive impairment of depression is amotivation. Specifically, when given assessments for effort-related decision making, humans with depression are less likely to choose the option that requires more effort (Treadway et al. 2012a; Yang et al. 2014). In humans, this lack of motivation can severely reduce the quality of one’s life, affecting one’s ability to work, attend school, and maintain relationships (WHO 2020). To combat these symptoms and improve quality of life, it is important to develop an understanding of how depression works and determine which drug treatments are the most effective. This necessitates animal models for research, which allow for the use of controlled settings as well as tools and methods that could not be ethically used in humans (Hitzemann 2000). Through the use of animal models, neurochemical changes that lead to effort-related motivation dysfunctions have been well-established. For example, interference in the dopamine transmission of the ventral striatum lowers participation in high-effort instrumental activities elicited by conditioned stimuli, whereas low-effort instrumental activities are unaffected (Aberman and Salamone 1999; Salamone and Correa 2012). Within our laboratory, we have used the effort-based choice model on rats to study
these motivational dysfunctions in psychopathology. We have conducted investigations in which tetrabenazine (TBZ) was administered to rats. TBZ works by inhibiting the vesicular monoamine transporter 2 (VMAT-2) to deplete dopamine, altering effort-based choice behavior in rats. In comparison with administration of a vehicle control, TBZ reduced the number of lever presses, while the consumption of concurrently available laboratory chow increased (Randall et al. 2014; Yohn et al. 2016a). Thus, similarly to humans, rats demonstrate a low-effort bias when subjected to the pharmacological conditions that have also shown to produce a low-effort bias in humans (Salamone et al. 2016a,b,c; Yohn et al. 2016a,b,c). Moreover, administration of TBZ can induce depressive symptoms in people, including fatigue and amotivation (Salamone et al. 2016a).

In terms of the neural basis of depression, there is evidence demonstrating that mesolimbic DA circuits in the forebrain in conjunction with the ventral striatum, prefrontal cortex, and ventral pallidum play a significant role in the selection of high-effort activities (Salamone et al. 1991, 1994; 2007, 2016a,b, 2018; Walton et al. 2003; Floresco and Ghods-Sharifi 2007; Mingote et al. 2008; Farrar et al. 2008, 2010; Winstanley and Floresco 2016). Still, more research is needed to properly identify and describe the psychopathology of neural circuits in effort-related dysfunctions.

Recent studies have suggested that cortical electroencephalographic markers may differ between healthy and depressed individuals. There has been particular emphasis on the frontal cortex, which is primarily responsible for cognitive effort tasks, or CET performance (Winstanley and Floresco 2016). In a study of 37 depressed and 35 nondepressed individuals, the frontal alpha EEG asymmetry of the depressed individuals at rest was significantly increased (Gollan et al. 2014). Further, evidence also shows that depressed people have a reduction of EEG markers in this cortex that are present during the performance of motivated behaviors and in the
anticipation of reinforcement (Nelson et al. 2018; Gheza et al. 2019). Specifically, it has been determined that bilateral asymmetry of EEGs in the frontal cortex in humans is an important marker for effort-related motivation (Allen, Coan, & Nazarian, 2004; Coan and Allen 2004).

In addition to EEGs, imaging has also been utilized to investigate the neural basis of anergia, psychomotor retardation (Hickie et al. 1999; Capuron et al. 2007), and effort-related decision making (Wardle et al. 2011; Schouppe et al. 2014; Huang et al. 2016; Hogan et al. 2018; Aridan et al. 2019). The results have been consistent with animal studies in that the striatum and frontal cortex are important for effort-based decision making as well as approach motivation, or motivation which is widely prompted by positive stimuli (Winstanley and Floresco 2016; Harmon-Jones et al. 2013).

The purpose of the present experiments was to further investigate these findings, comparing the characteristics of the mPFC, M1/M2, and mParietal EEG readings in the left and right hemispheres of awake male and female rats in baseline and TBZ-induced, DA-depleted conditions. The mPFC was the focus of this study due to the abundance of studies drawing connections between executive function, effort-based choice, decision making, and the prefrontal cortex. Additionally, the mPFC is thought to reflect the effects of TBZ on the ventral striatum and striatopallidal circuits that are important for effort-based decision making (Rotolo Ph.D. Dissertation, 2020). EEGs from the mParietal and M1/M2 cortices were recorded to serve as a point of reference for the mPFC while simultaneously ascertaining whether TBZ affected other cortical areas. In summation, this investigation was done to work towards creating physiological markers for altered DA transmission and effort-related dysfunction in preclinical animal models that are translatable to human male and female studies.
Materials and Methods

Surgery

Rats were anesthetized with 100 mg/kg of ketamine and 10 mg/kg of xylazine prior to being placed within the stereotaxic device for electrode implantation surgery. Surgical implantation of 8 ¼” stainless-steel screw electrodes were performed on 8 untrained female rats and 7 untrained male rats (total n=15). The EEG electrodes were placed next to the mPFC, M1/M2, and mParietal in addition to two screws that served as ground and reference electrodes. Rats were monitored for pupil reflex, knee-jerk reflex, and breathing throughout the surgery. Recordings were carried out after a recovery period of 7 days.

Electrophysiological Recording Experiment

A Digital Lynx SX Electrophysiology System (Neuralynx) was used to record the EEG activity of female and male rats in the awake state. Wide-band activity (1-2000 Hz, 4006 samples/sec) was recorded using the Neuralynx system and was analyzed offline using MATLAB software (MathWorks Inc, Natick, MA). Approximately 1 minute of baseline EEG activity was recorded from each rat after a short period of reading stabilization following the connection of the rat to the Neuralynx system via cable. The rats were then injected intraperitoneally with either vehicle or TBZ (1.0 mg/kg). A lead time of 120 minutes elapsed before resuming recordings. The following week, each rat’s treatment was counterbalanced with either vehicle or TBZ.
**Data Preprocessing**

Signal processing and related procedures were carried out with the FieldTrip software package (Oostenveld et al. 2011; http://www.fieldtriptoolbox.org/) and custom scripts in MATLAB (The MathWorks, Natick, MA). The following steps were used to preprocess the EEG data: 1) data were mean subtracted; 2) data were divided into 1-s epochs for the purpose of removing the epochs that contained artifacts; 3) the first epoch and the last epoch were removed; 4) data was inspected to determine and remove the epochs that contained high-amplitude artifacts according to Z-score threshold criterion.

**Neurophysiological Analysis**

Power spectral analysis was carried out by first segmenting the preprocessed EEG data into 1-s epochs with 0.5-s overlap. We then used Welch’s overlapped averaged periodogram method (Welch, 1967) with a Hamming window on each epoch to estimate the power spectral density (PSD) over frequencies from 1 Hz to 100 Hz (0.5 Hz resolution).
Results

EEG recordings were taken on 8 untrained female rats and 7 untrained male rats from their mPFC, M1/M2, and mParietal cortices via implanted EEG electrodes. A PSD was used to determine the effect of 1.0 mg/kg TBZ versus VEH treatment on EEG activity in awake female (n=8) (Figure 1) and male (n=7) rats (Figure 2). During baseline home cage EEG recording from the mPFC, M1/M2, and mParietal cortex of both freely moving male and female groups, the predominant peak in EEG activity in the lower frequency ranges in all three brain cortices is distinctly in the alpha/theta frequency range (4-12 Hz, with a sharp peak at 6-8.5 Hz frequency range). In order to correct for the baseline values, power of pre- and post-injection was subtracted from the baseline. In other words, the change from pre-injection to post-injection of TBZ or VEH was determined. The values were then analyzed by analysis of variance (ANOVA) mixed effects model. Across all three brain cortices, there was a significant sex x drug (vehicle versus TBZ) effect interaction (F(1,317) = 4.74, p < 0.05) (see Table 1). There was a sharp 6-8.5 Hz peak that was not blunted in females (F(1,155) = 1.13, p = n.s.) (See Figure 1 and Table 2). However, the power of this peak was significantly affected by the drug in male rats (F(1,159) = 5.01, p < 0.05) (see Figure 2 and Table 3). Thus, the effect of the drug was different in males versus females. Specifically, this peak was significantly blunted relative to vehicles by administration of 1.0 mg/kg TBZ in male rats, but not in females.
Table 1. ANOVA marginal test for fixed effects of sex (male versus female), drug (VEH versus TBZ), region (mPFC, M1/M2, mParietal), (left versus right) lr, and sex:drug (interaction of sex and drug). The effects of sex and drug approached significance (p = 0.08 < 0.5 ; p = 0.06 < 0.5). The interaction between sex and drug was significant, meaning the effect of the drug is different in males than it is in females (p = 0.03 < 0.05).
Figure 1. The effects of the VMAT-2 inhibitor TBZ on bilateral EEG recordings in the mPFC, M1/M2, and mParietal cortices in female rats (mean ±SEM; n=8). All rats were administered intraperitoneal injections of VEH or 1.0 mg/kg TBZ on one week and counterbalanced with either VEH or TBZ the following week. Females did not have a statistically significant suppression of the 6-8.5 Hz peak (p < 0.5).

Table 2. ANOVA marginal test for female rats. Parameters are fixed effects of drug (VEH versus TBZ), region (mPFC, M1/M2, mParietal), and lr. The effect of the drug was not significant (p = n.s.)
Figure 2. The effects of the VMAT-2 inhibitor TBZ on bilateral EEG recordings in the mPFC, M1/M2, and mParietal cortices in male rats (mean ±SEM; n=7). All rats were administered intraperitoneal injections of VEH or 1.0 mg/kg TBZ on one week and counterbalanced with either VEH or TBZ the following week. Males had a statistically significant suppression of the 6-8.5 Hz peak (p < 0.5).

Table 3. ANOVA marginal test for male rats. Parameters are fixed effects of drug (VEH versus TBZ), region (mPFC, M1/M2, mParietal), and lr. The effect of the drug was significant (p < 0.05).
**Discussion**

Our lab conducted these studies to determine the neurophysiological effects of TBZ on untrained, freely moving male and female rats. This involved using EEG recordings that focused on power spectral density analyses of signals from the bilateral mPFC, M1/M2, and mParietal cortices. The use of animal models to study effort and motivation is valid as the data collected from previous animal studies correlates to what is seen in patients with major depressive disorder. This investigation is one the first in a series that will lead to the studies of the electrophysiology of trained animals carrying out effort-related choice tasks. Performing investigations in trained animals is important to allow for the discovery of more sensitive electrophysiological biomarkers in males and females (Rotolo Ph.D. Dissertation, 2020). Thus, the present studies are necessary in order to develop the initial EEG markers that are readily translatable to humans.

In this investigation, 8 female rats and 7 male rats were each surgically implanted with cortical EEG electrodes to record from the mPFC, M1/M2, and mParietal cortices. Based on recordings post-injection of TBZ compared to baseline along with a vehicle control, statistically significant results were found for male rats. The results demonstrated a predominant peak in EEG activity at baseline in all three brain cortices clearly in the alpha/theta frequency range (4-12 Hz, with a sharp peak at 6-8.5 Hz frequency range). Using the ANOVA mixed effects model, the data shows that collapsed across all three brain regions, there was a significant sex x drug interaction (F(1,317) = 4.74, p < 0.05) (see Table 1). This peak remained unaffected in female rats (F(1,155) = 1.13, p = n.s.) (See Figure 1 and Table 2). In male rats, there was a significant suppression of the sharp 6-8.5 Hz peak (F(1,159) = 5.01, p < 0.05) (See Figure 2 and Table 3). In conclusion, the effect of the drug on the observed EEG power spectra was different in males
versus females. Specifically, the peak frequency band was significantly blunted relative to vehicle by administration of 1.0 mg/kg TBZ in male rats, but not in females. This has potential implications that DA depletion seen in depressed individuals does not affect the male and female brain in the same manner. Additionally, a limitation of this investigation was that only one dosage amount (1.0 mg/kg TBZ) was used. For future studies, administering rats various dosages would allow for a deeper investigation of how the male and female brain are affected by different degrees of DA depletion.

The findings of this investigation will also serve as a good foundation for future research on how frontal EEG recordings change during the performance of effort-related tasks. During the PROG lever pressing task, rats must press a lever a certain number of times to deliver favorable, carbohydrate-rich pellets that act as reinforcers for this behavior. As time goes on, the ratio of presses to released pellets progressively increases. During this task, it has been shown that with an increase in the lever pressing ratio, rats reach a break point during which they stop lever pressing and switch to consumption of the readily-accessible, but less preferable lab chow (Cordony 2019). According to Pizzagalli et al. (2005), Nelson et al. (2018), and Gheza et al. (2019), it is hypothesized that there will be alterations in frontal EEG activity when rats reach their break point. It has also been hypothesized that the administration of TBZ will result in a decrease in lever pressing and an increase in chow intake, which will also lead to changes in frontal EEG activity. More knowledge of EEG activity and electrophysiological biomarkers would allow for further investigation of neural circuitry via anterograde and retrograde tracing. Given the anatomical connections between the ventral striatum and ventral pallidum via the thalamus to the mPFC, and the role of this circuitry in effort-related decision making, it is important to investigate how different neural pathways are affected by TBZ (Fareri et al. 2017).
Such findings would significantly deepen our understanding of the circuit mechanisms that are responsible for effort-based aspects of motivation.
References


