ABSTRACT

Acute Agomelatine Administration Does Not Attenuate Deficits in Vocalizations, Inflammation, or Excitation in Kainic Acid Treated Pups

Matthew S. Binder, Ph.D.

Mentor: Joaquin N. Lugo, Ph.D.

Seizures during early-development result in an increase in proinflammatory cytokine production and impact communication behavior in murine models. Our study investigated the efficacy of the anti-inflammatory pharmaceutical agomelatine on neuroinflammatory processes and ultrasonic vocalizations (USVs) in mice. On postnatal day (PD) 10 male and female C57 BL6/J mice were administered kainic acid (KA) to induce status epilepticus (SE). The mice then received either agomelatine, DMSO (vehicle), or saline either 1-hour post SE or 24 hours post SE. The early life communication behavior ultrasonic vocalizations was evaluated on PD 11 and 12 and western blotting was conducted on PD 15. While KA administration lead to an increase in vocalizations overall on PD 11 at the 1-hour timepoint, agomelatine was unable to attenuate this deficit. No main effects of seizures were observed on PD 12 1-hour post treatment or on PD 11 or PD 12 in the 24-hour post treatment groups. When the quantity of USVs emitted per each call type was assessed, agomelatine was similarly unable to attenuate the increased quantity of frequency steps, upward, downward, chevron, and

composite call types observed throughout the 1-hour and 24-hour treatment groups. Similarly, the KA induced alterations in the average duration, peak frequency, fundamental frequency, and amplitude of the call types were also not altered by agomelatine administration. When markers of excitation and inflammation were assessed via western blotting, KA was found to increase GFAP, Iba1, and GluR1 relative to controls, with no significant difference present in the expression of mGluR1/5. Agomelatine did not significantly alter this upregulation. Overall, our study suggests that despite its high theoretical promise, agomelatine displays minimal efficacy to treat aberrant vocalizations, excitation, and neuroinflammation in neonates administered KA.

Acute Agomelatine Administration Does Not Attenuate Deficits in Vocalizations, Inflammation, or Excitation in Kainic Acid Treated Pups

by

Matthew S. Binder, B.A., B.S., M.A.

A Dissertation

Approved by the Department of Psychology and Neuroscience

_____

Charles A. Weaver III, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of

Doctor of Philosophy

Approved by the Dissertation Committee

_____

Joaquin N. Lugo, Ph.D., Chairperson

_____

Annie T. Ginty, Ph.D.

_____

N. Bradley Keele, Ph.D.

_____

Bessie W. Kebaara, Ph.D.

Accepted by the Graduate School

May 2020

_____

J. Larry Lyon, Ph.D., Dean

Page bearing signatures is kept on file in the Graduate School.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

I would first like to thank my mentor, Dr. Joaquin Lugo. You have played a significant and instrumental role in my growth as both a scientist and a person and have helped me to develop the habits that I need in order to be successful throughout my career. In short, it was a privilege to work with you and I could not have asked for a better mentor. I would also like to acknowledge my labmate and good friend, Samantha Hodges for all of your insight, camaraderie, and research acumen that you have proved over the years. I feel truly blessed to have worked alongside, your prowess in the lab is only rivaled by your levity and kindness. I feel truly fortunate to also have found mentors and aspirational figures in Dr. Bradley Keele, Dr. Annie Ginty, Dr. Melanie Sekeres and Dr. Bessie Kebaara. Your benevolence and insight on my various projects over the years, as well as on professional development, have been indispensable and have helped me in more ways than I can state. Put succinctly, I greatly appreciate each of you and will always value our conversations. I would like to thank my fellow Lugo lab members Suzanne Nolan, Paige Womble, David Narvaiz, and Zach Pranske. Each of you have helped me in innumerable ways over the years. I will always reflect back warmly on my time spent with you, and am fortunate to have had the opportunity to work with friends. Lastly, I would like to thank my parents, Jon and Karen, for their unwavering support, love, guidance, and belief in me. None of my accomplishments in life would have been possible without you. I would also like to thank my dog Cooper and my friends Shohn and Brian for your camaraderie and for always being there for me.

CHAPTER ONE

Introduction

Early life seizures (ELS) adversely impact the developing brain and predispose neonates to a variety of cognitive and behavioral comorbidities (Aaberg et al., 2016; Holmes & Ben-Ari, 2001). Comorbid conditions are a serious concern, as they have implications for medical costs, family care, treatment regimens, and the infants' overall health (Aaberg et al., 2016; Jafarpour, Stredny, Piantino, & Chapman, 2018; Roy et al., 2011). While there are various comorbidities that can arise following ELS, one of the most prevalent is autism spectrum disorder (ASD). ASD affects 30% of children that experience ELS, with one of the core behavioral features of ASD being deficits in communication (Clarke et al., 2005; DSM-5, 2013). Neonatal communicative deficits impact the relationship between caregiver and infant and impede the neonate's healthy social development, thereby having far reaching implications for the infant's quality of life (DeMyer et al., 1973; Esposito & Venuti, 2008; Kasari & Sigman, 1997). Although less severe comorbidities lead to a better long-term trajectory for seizure prone infants, there are few treatment options that address the communication deficits associated with ELS (Goodwin, Lambrinos, Ferro, Sabaz, & Speechley, 2015).

The efficacy of potential treatments to address seizure-induced deficits in communication can be assessed by analyzing ultrasonic vocalizations (USVs) in murine models. USVs are a communicative behavior that encompasses whistle like sounds which are emitted between 30-90 kHz, occurring when a pup is isolated from its dam (Branchi,

Santucci, & Alleva, 2001). The vocalizations made by the isolated pup can be recorded and the quantity, duration, amplitude (volume), peak frequency (the highest point of the call, one component of the pitch), and fundamental frequency (the lowest point of the call, another component of the pitch) can then be evaluated with recording software. Studies have shown that acquired models of ELS, such as mice treated with the chemoconvulsants pilocarpine and kainic acid, display significant quantitative and qualitative deficits in communicative behaviors (López-Meraz et al., 2014; Reynolds, Nolan, Huebschman, Hodges, & Lugo, 2017; Tsai et al., 2012). Therefore, due to the conservation of communication deficits following early life seizures, murine models of ELS can be used to assess the efficacy of promising treatments.

One potential therapeutic mechanism that may underlie the seizure-induced deficits in early life communicative behaviors is inflammation. In humans, increased proinflammatory cytokine production contributes to the pathophysiology following ELS and is also implicated in the onset of ASD (Mazarati, Lewis, & Pittman, 2017; Siniscalco, Schultz, Brigida, & Antonucci, 2018; Vezzani, French, Bartfai, & Baram, 2011). Infants with ASD have increased inflammation and exhibit a deficit in communication, thereby indicating that inflammation may be a contributing factor in seizure induced communication deficits (Siniscalco et al., 2018). In mice, the chemoconvulsant kainic acid has been shown to significantly increase microglia and astrocyte activity, facilitating proinflammatory cytokine production in addition to producing communication deficits (Chung & Han, 2003; Reynolds et al., 2017; Zhang & Zhu, 2011). Therefore, inflammation has been associated with altered communicative behaviors following ELS in humans and in murine seizure models, constituting a promising therapeutic target.

Agomelatine is a recently developed pharmaceutical that counters proinflammatory cytokine production while facilitating neuroprotection (Guardiola-Lemaitre et al., 2014). Agomelatine decreases aberrant inflammation by functioning as an agonist for melatonin receptors, which can inhibit and restrain microglia reactivity, thereby decreasing the expression of proinflammatory cytokines in pathologically inflamed models (Ding et al., 2014; Wu et al., 2011). Additionally, agomelatine has been shown to significantly reduce astrogliosis in inflammatory models in addition to decreasing excitation and conferring neuroprotection (Dagyte et al., 2010; Gressens et al., 2008; Tchekalarova, Atanasova, Kortenska, Atanasova, & Lazarov, 2018).

Due to agomelatine's anti-inflammatory capabilities and its large therapeutic index, its theoretical potential to treat the communication deficits that accompany early life seizures is high. However, agomelatine has not previously been assessed in ELS models, making its efficacy unknown. Therefore, the current study examined agomelatine's efficacy to attenuate deficits in ultrasonic vocalizations when administered both 1-hour and 24- hours post seizure induction. Furthermore, GluR1, mGluR1/5, microglia (Iba1), and astrocyte (GFAP) expression were assessed to evaluate agomelatine's anti-inflammatory and neuroprotective effects.

CHAPTER TWO

Review of Literature

Epilepsy

Epilepsy is a neurological disorder that predisposes an individual to recurrent unprovoked seizures (Stafstrom & Carmant, 2015). It is diagnosed when two or more unprovoked seizures have occurred greater than 24 hours apart. A seizure is aberrant electrical activity in the brain that is fundamentally an imbalance between excitation and inhibition (Ben-Ari & Dudek, 2010). There are two primary categories of seizures, focal and generalized. Focal seizures occur when the electrical surge is contained to only one hemisphere of the brain and may or may not result in a loss of awareness. Furthermore, focal seizures can turn into generalized seizures according to the seizures' location, duration, and magnitude (Stafstrom & Carmant, 2015). Generalized seizures occur when there is widespread seizure activity in both the right and the left hemispheres. These range from absence seizures, which display an abrupt onset and end, causing attentional lapses, to tonic-clonic seizures, wherein an individual losses consciousness and displays stiffened muscles and jerking movements in the extremities (Stafstrom & Carmant, 2015).

Typically, seizures are brief events lasting from several seconds to around 2 minutes. However, a state called status epilepticus (SE) can occur if a seizure lasts for more than 5 minutes or if an individual experiences 2 or more seizures without regaining consciousness in between (DeLorenzo, Pellock, Towne, & Boggs, 1995). SE is a serious

condition that has a high mortality and is present in as many as 16% of individuals with epilepsy (Cherian & Thomas, 2009). While SE can occur at all ages, its highest incidence is before the age of 2, as this is a period of rapid brain development, SE can increase the risk of post seizure sequel, thus contributing to epilepsy's lifelong effects (Roy et al., 2011).

Seizures and epilepsy can be produced from a myriad of factors but are broadly classified as either genetic or acquired. Genetic epilepsies consist of familial types of epilepsy and are caused by mutations to specific genes that are passed on within families, such as Tuberous sclerosis complex or Cowden syndrome (Millichap, 2013; Nabbout et al., 2018). Genetic epilepsy's exhibit a rapid onset, predominately leading to early life seizures in infants and young children (Steinlein, 2008). Conversely, acquired epilepsies can occur throughout the lifespan and are due to brain insult (Bradley, 2004). Factors such as such as brain trauma, stroke, infection, hypoxia, fever, or brain tumors can all lead to acquired epilepsy (Delorenzo, Sun, & Deshpande, 2005). In part, due to its broad etiology, epilepsy is quite common, with worldwide prevalence estimates ranging from 50 million to more than 65 million (Ngugi, Bottomley, Kleinschmidt, Sander, & Newton, 2010; WHO, 2017). Therefore, at least 1 in 26 people will be diagnosed with epilepsy at some point in their life, making it the fourth most common neurological disorder behind migraines, stroke, and Alzheimer's disease (England, Liverman, Schultz, & Strawbridge, 2012; Hesdorffer et al., 2011).

Unlike many neurological conditions, epilepsy displays a distinctive pattern of onset, exhibiting a bimodal distribution (Figure 1). Specifically, the prevalence of seizures increases after birth, reaching a peak within the first year of life (Berg, Jallon, &

Preux, 2013; Camfield & Camfield, 2015). After age 1 there is a steady decline in seizure prevalence, which plateaus around age 10 (Camfield & Camfield, 2015). From age 10 to age 55 there is a low but constant rate of seizures that persists until age 55 (Beghi & Giussani, 2018). After age 55 there is a steady increase in seizure prevalence as ischemic events, dementia, and cardiovascular insults become more common (Liu, Yu, & Lü, 2016). When assessing seizure prevalence, infants comprise the second most at risk group behind only the elderly, as one in 1 in every 75 neonates is diagnosed (Panayiotopoulos, 2005). Due to the vulnerability of the developing brain, seizures in neonates can lead to chronic deficits that persist throughout the lifespan.

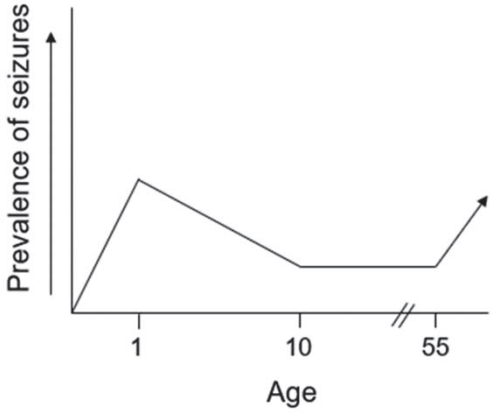


Figure 1. Seizure prevalence across the lifespan.

Despite the widespread prevalence of seizures, there is unfortunately no cure, although numerous treatments have been developed that can help mitigate seizure activity. Seizure treatments typically function by blocking sodium or calcium channels or by facilitating GABA activity in an attempt to normalize the excitatory to inhibitory balance in the brain (Treiman, 2001). Therefore, the current anti-seizure pharmaceuticals function by addressing the principal problem in epilepsy, the seizure. However, current

treatments do little to offset the accompanying comorbidities of seizures: the neurological aftermath that follows excessive excitation (Seidenberg, Pulsipher, & Hermann, 2009). These comorbidities constitute a significant and prevalent threat to epileptic individual's everyday functioning and quality of life (Srinivas & Shah, 2017). Furthermore, anti-seizure medications have severe side effects that can make any existing comorbidities worse (Lee et al., 2013; Seidenberg et al., 2009). Therefore, in terms of quality of life, the seizures that define the epileptic condition are only one part of the problem, with the associated comorbidities of seizures posing a significant problem of near equal value to stopping the seizures themselves.

Autism Spectrum Disorder and Communication Deficits

Autism Spectrum Disorder

The comorbidities of seizures are diverse, ranging from cognitive impairments to psychiatric conditions, to behavioral alterations (Seidenberg et al., 2009). However, one of the most common comorbidities of seizures is autism spectrum disorder (ASD), which has a 30% comorbidity with early life seizures. ASD is characterized by repetitive behaviors and deficits in social interaction and communication (Clarke et al., 2005; DSM-5). Autism is common, affecting one in every 42 males and 1 in every 182 females (Baio, 2014). It is diagnosed most often in childhood between the ages of 2 and 5 (Mandell, Novak, & Zubritsky, 2005; Moulton, Barton, Robins, Abrams, & Fein, 2016). Additionally, unlike other neurological conditions, ASD's diagnosis is behaviorally based, as there is currently no definitive biological test used to ascertain whether or not a child has autism (DSM-5).

One of the most common aberrant behaviors present in infants with autism are deficits in early life communication. Early life communication refers to making and maintaining eye contact with a caregiver, orienting behaviors, such as gaze or attentional fixation on a novel object or caregivers face, and crying behaviors (McDuffie, Yoder, & Stone, 2005; Schultz, 2005; Soltis, 2005). Due to the connection between ASD and communication, infants that will later be diagnosed with autism have marked deficiencies in each of these behaviors. Specifically, autistic infants make significantly less eye contact than neurotypical infants and also spend less time focusing their attention on faces in their environment (McDuffie et al., 2005; Schultz, 2005). However, the most robust of these deficits, and perhaps the one with the greatest predicative validity, are the deficits in early life crying behaviors (Esposito & Venuti, 2009).

Crying Behavior

Crying can be conceptualized as an automatic reaction to signal a biological need. Studies have demonstrated that the pitch, duration, and amplitude (loudness) of the cries are the most important aspects that facilitate adult recognition of an infant's cry (Zeskind & Marshall, 1988). Specifically, there are three styles of crying that are commonly observed in infants and that are distinguishable to care givers: the anger cry, which is a loud and prolonged vocalization, a hunger cry, which is a rhythmic and repetitive vocalization, and a cry of pain, which is has an abrupt onset and is an initial long cry with extended breath holding (Esposito, Hiroi, & Scattoni, 2017; Esposito & Venuti, 2008; Green, Whitney, & Potegal, 2011; Sheinkopf, Iverson, Rinaldi, & Lester, 2012). These crying patterns remain constant across cultures suggesting a high degree of

conservation (Barr, Konner, Bakeman, & Adamson, 1991; Esposito, Nakazawa, Venuti, & Bornstein, 2012; Wolff, 1969).

When crying behaviors have been longitudinally assessed, it has been shown that initially cries are purely physiologically based, emitted in response to a primary need. However, over time the crying behaviors become more social and can be influenced by environmental changes, such as a parent leaving the perceptual field, or if the caregiver stops singing or talking to the infant (Frodi, 1985). By 8 months of age the infants are more responsive to the effects their cries produce, that is to say the recumbent behavioral changes that the caregivers make in order to address the infants' need (Esposito & Venuti, 2008). At 12 months of age, the crying behavior is furthered honed and becomes an efficient early life communicative routine that will help to guide the development of language, while serving as a longstanding communicative approach in its own right (Rothgänger, 2003).

Crying Behaviors in Autism

When the crying of autistic infants has been assessed, numerous deficits have been reported. Specifically, infants with autism are less likely to cry when the parent leaves the immediate environment, and will cry without a known cause for their crying (Esposito & Venuti, 2008). Additionally, caregivers reported feeling more negative emotions and uneasy states after listening to autistic infant's crying as opposed to typically developing infants. This effect was observed in both male and female parents (Esposito et al., 2017; Esposito, Valenzi, Islam, & Bornstein, 2015). Lastly, infants with autism have been reported to cry at a higher pitch and to display shorter crying bouts while exhibiting irregular loudness of their cries relative to neurotypical infants

(Sheinkopf et al., 2012). Altogether, these studies indicate that autistic infants display crying episodes that are quantitatively and qualitatively different from typically developing infants.

Implications of Early Life Communication Deficits

Deficits in crying behaviors can have a pronounced effect on an autistic child's quality of life. Crying is among the first communicative behaviors that infants exhibit and is one of the few early life communicative behaviors that is socially predicated, thereby playing an integral role in establishing a bond between infant and caregiver. Any deficit in crying behaviors, or any aversive feelings induced in the caregiver from autistic infants' cries, can negatively affect this relationship, serving to further socially isolate the child (Esposito et al., 2017; Esposito & Venuti, 2008). Additionally, the neurodevelopmental period encompasses a time of remarkable vulnerability, therefore, any early life deficit in communication can persist throughout the lifespan and impede the development of other communicative behaviors, decreasing quality of life (DeMyer et al., 1973; Kasari & Sigman, 1997). Since, communication is integral to the human experience, deficits in communication will have far reaching consequences for an individual's well-being and societal immersion.

Despite the potential ramifications of early life communicative deficits on an infants' quality of life, there are currently extremely few effective treatments available, this lack is especially poignant since studies have shown that one of the best predictors of long-term outcome in infants with early life seizures and ASD is the severity of the comorbidity, with a less severe comorbidity being associated with a better prognosis (Goodwin et al., 2015).

Ultrasonic Vocalizations

Communicative behaviors in murine models can be assessed by analyzing ultrasonic vocalizations (USVs). USVs are an innate form of communication that are classified as "whistle like sounds" with frequencies ranging from 30 to 90 kHz (Branchi et al., 2001). While vocalizations can be emitted from adults as well as pups, they are most abundant throughout the first two weeks of life. They have been shown to increase on postnatal (PD) 5 reaching their peak on PD 7-9 and decrease after day 14 (Bowers, Perez-Pouchoulen, Edwards, & McCarthy, 2013; Branchi et al., 2001; Elwood & Keeling, 1982). Neonatal vocalizations are most commonly emitted when the pup is separated from its dam, a procedure known as the maternal isolation paradigm (Branchi, Santucci, Vitale, & Alleva, 1998). Studies have demonstrated that pups vocalize in this condition to induce a retrieval response in the mother that results in the pup being returned to the safety of the nest (Bowers et al., 2013). Therefore, pup vocalizations are an innate, automatic reaction to a biological need that produces a corresponding behavioral response in the dam. Thus, USVs can be conceptualized as being analogous to infant crying behaviors in humans.

Murine vocalizations are unique among neonatal behaviors due to the amount of information that can be gleaned from a single recording session. In addition to detecting the total quantity of vocalizations emitted, various spectral characteristics such as the amplitude (loudness), peak and fundamental frequency (pitch) and the duration of the USVs can also be determined. Furthermore, USV analysis programs render a 2-dimensional representation of each call, allowing for the calls to be placed into different categories based on their overall shape and pitch changes, providing qualitative

information. Therefore, assessing USVs provides a non-invasive, comprehensive measure of murine communicative behaviors and most importantly allows for the same parameters of interest assessed in infant crying studies to also be assessed in mouse vocalization studies.

Ultrasonic vocalizations are unique in that they provide a window into early development that may not otherwise be garnered. Additionally, models of genetic, as well as acquired, early life seizures present with deficits in vocalizations similar to what is observed in humans. For instance, TSC heterogeneous mice are a murine model of Tuberous sclerosis complex (TSC), a condition that leads to early life seizures due to a mutation in the TSC1/2 protein dimer (Nabbout et al., 2018). In addition to being a condition that results in early life seizures, TSC also shares a high comorbidity with autism (Vignoli et al., 2015). When ultrasonic vocalizations have been assessed in this model, *Tsc1* heterogeneous mice emit significantly different quantities of vocalizations relative to controls (Tsai et al., 2012). Neuronal subset specific (NS)-*Pten* knockout (KO) mice are a model of Cowden syndrome, which is characterized by spontaneous seizures and is one of the single largest genetic contributors to autism (Varga, Pastore, Prior, Herman, & McBride, 2009). NS-*Pten* KO mice have similarly been shown to emit significantly fewer vocalizations than wildtype (WT) mice, in addition to displaying an increased pitch and decreased duration and amplitude of USVs (Binder & Lugo, 2017).

Meanwhile, models of acquired seizures use a chemoconvulsant to induce seizures. Murine animals administered pilocarpine, a muscarinic agonist that results in a prolonged seizure state (status epilepticus (SE)), have been shown to display an increase in the quantity of vocalizations emitted as well as a decreased duration of the calls

(López-Meraz et al., 2014). Conversely, mice receiving kainic acid, a kainic acid receptor agonist that also induces status epilepticus, emit fewer vocalizations than controls and display qualitative differences in the call types produced (Reynolds et al., 2017). Therefore, while the specific increase or decrease in USVs may vary according to the time point assessed or model used, in both genetic and chemically induced models of early life seizures (ELS) there are pronounced deficits in communicative behaviors that resemble the autistic comorbidity that accompanies ELS in humans. Altogether, the literature indicates that not only are early life communicative behaviors conserved across species, but so are the deficits in communication that accompany seizures and an autistic-like phenotype.

Murine Models of Early Life Seizures

Induced Models of ELS

Induced models of early life seizures display a more specific and targeted mechanism of action than systemic or localized genetic mutant models. Therefore, acquired models of seizures may be of more benefit when assessing potential treatment options for comorbidities associated with seizures, as there is less underlying neural and biological noise to filter out. However, the relative selectiveness of each chemoconvulsant varies drastically from one model to the next.

Picrotoxin

Picrotoxin is a chemoconvulsant that is a noncompetitive GABA A receptor antagonist, a receptor that selectively conducts chloride which causes an inhibitory effect (Olsen, 2006). The administration of picrotoxin results in the onset of tonic-clonic

seizures, modeling generalized seizures. Specifically, aberrant electrical activity is widely distributed throughout the brain, initiating 30-40 minutes post injection, however, status epilepticus is not achieved (Coppola & Moshé, 2012; Hamani & Mello, 1997; Young & Dragunow, 1994). The primary deficits observed in this model are those in memory, attention, and locomotion (Bast, Pezze, & McGarrity, 2017; Mogenson, Wu, & Jones, 1980).

Pentylenetetrazol (PTZ)

Pentylenetetrazol (PTZ) also functions as a noncompetitive GABA A receptor antagonist, impairing GABA mediated inhibition (Samokhina & Samokhin, 2018). Therefore, PTZ produces generalized tonic clonic seizures similar to picrotoxin. PTZ administration also produces significant deficits in hippocampal dependent memory (Mishra & Goel, 2012). Due to the ubiquitous expression of GABA throughout the brain, both Picrotoxin and PTZ initiate a cascade of non-selective damage. Furthermore, picrotoxin and PTZ are typically used as kindling models and thus require repeated administrations over the course of several days in order to induce seizures (Olsen, 2006; Samokhina & Samokhin, 2018). Therefore, each model may be associated with more stressors than other chemoconvulsant models require and their location of onset may vary.

Pilocarpine

One of the most widely used chemoconvulsants is pilocarpine. Pilocarpine is a cholinergic muscarinic agonist that leads to excitotoxic lesions predominately concentrated in the limbic system (Curia, Longo, Biagini, Jones, & Avoli, 2008).

Seizures induced by a systemic administration of pilocarpine are persistent and long lasting, leading to significant neuronal damage and status epilepticus (Curia et al., 2008). Memory deficits, decreased locomotion, and alterations in anxiety and depressive behaviors are commonly reported (Müller, Gröticke, Bankstahl, & Löscher, 2009). A decrease in USV quantity and duration has also been reported in neonates injected with pilocarpine. Although pilocarpine is a model of temporal lobe epilepsy, specifically, displaying focal seizures, systemic injection often leads to widespread lesions in the thalamus, substantia nigra, cerebral cortex, olfactory cortices and the amygdala due to the broad expression of muscarinic receptors in the CNS (Rose Priel, dos Santos, & Cavalheiro, 1996). Furthermore, pilocarpine's function in neonates has been shown to be variable, with one study reporting no epileptic activity nor hippocampal damage following a single injection of pilocarpine, with 3 consecutive pilocarpine injections required to induce an epileptic phenotype (Curia et al., 2008).

Kainic Acid

Kainic acid (KA) is another widely used chemoconvulsant model. KA is an analog of L glutamate that is a kainic acid receptor agonist (Wang, Yu, Simonyi, Sun, & Sun, 2005). Kainic acid receptors are ionotropic glutamate reports that have a selective expression in the brain and are predominately found in the hippocampus, although they have also been located in smaller quantities in the amygdala, basal ganglia, and cerebellum (Garthwaite & Wilkin, 1982; Jin & Smith, 2011; Zheng, Zhang, Luo, & Zhu, 2011). Due to the relative concentrations of kainic acid receptors, there is a clear progression following its systematic administration, with the excitotoxicity beginning in the CA3 region of the hippocampus. The wave of excitation is then propagated via

GluR1, GluR2, and mGluR1/5 glutamate receptors, spreading to the CA1 region before continuing to the rest of the limbic system, leading to status epilepticus (Blumcke et al., 2000; Robinson & Deadwyler, 1981; Sommer, Roth, & Kiessling, 2001; Sperk et al., 1983; Zheng et al., 2011). Kainic acid in adult mice causes deficits in memory, hyperlocomotion, and increased impulsivity (Bardgett, Jackson, Taylor, & Csernansky, 1998; Park et al., 2012; Sarkisian et al., 1997). Meanwhile, in pups, kainic acid causes a decrease in ultrasonic vocalizations and an increase in complex call types relative to control mice (Reynolds et al., 2017). KA is a model of focal seizures and importantly, causes more selective damage and is more localized than other chemoconvulsant models, indicating that it may be an ideal model to assess the relationship between early life seizures, autism, and communication deficits (Zheng et al., 2011).

The Immune System

Innate and Adaptive Immune Responses

A primary contributing factor to both the onset of a seizure and the severity of any accompanying comorbidity is an increase in the immune response. The body's immune response can either be adaptive or innate. Innate immunity is a nonspecific host response that is the first line of protection against antigens. This system is rapidly activated, mobilizing immediately in response to a microorganism. Immune molecules such as neutrophils, leukocytes, natural killer cells, dendritic cells, and mast cells all play a crucial role in the activation of innate immunity and help to identify, target, and destroy any potentially harmful microorganisms (Iwasaki & Medzhitov, 2015; Turvey & Broide, 2010). Adaptive immunity is activated in response to innate immunity and forms a

targeted approach to antigens. Adaptive immunity is a slower process, but it allows the host to identify pathogens and then produce specific antibodies that counteract them. These cell-mediated immune responses are facilitated through the activation of B and T lymphocytes (Iwasaki & Medzhitov, 2010, 2015; Medzhitov & Janeway, 1997).

Both the innate and adaptive immune responses are regulated by cytokines. Cytokines are soluble proteins that are produced in response to an antigen, forming chemical messengers (J.-M. Zhang & An, 2007). The role of cytokines is to induce either a pro or an anti-inflammatory response in the target cell. Pro-inflammatory cytokines increase inflammation; mobilizing resources and increasing blood flow to the afflicted region, assisting in the healing process. The counterpart to pro-inflammatory cytokine secretion is anti-inflammatory cytokine secretion, which is essential in regulating the amount of inflammation present in the target tissue, diminishing inflammation when it is no longer beneficial (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). Together, these two mechanisms orchestrate the immune response.

Neuroimmune Response

While there are broad similarities between the body's immune response and the brain's immune response, the two systems are distinct from one another, serving the same function via different mechanisms. This drastic partition in mechanism is due to the brain being immune privileged, an organ that has its own unique immune response that is separate from that of the body (Forrester, McMenamin, & Dando, 2018). The unique immune response of the CNS is primarily due to the presence of the blood brain barrier, which keeps circulating molecules of both the body's innate and adaptive immune responses out of the brain, requiring the production of specialized immune cells not found

outside the CNS (Banks & Erickson, 2010; Ransohoff, Kivisäkk, & Kidd, 2003) The two primary components of the brain that function as immune cells are microglia and astrocytes.

Microglia

Microglia are the brain's resident macrophage, the principal immune component of the central nervous system. Microglia are derived from yolk sac progenitors which translate into the brain as early as embryonic day 8 and begin rapidly proliferating (Nayak, Roth, & McGavern, 2014). By the second postnatal week, 95% of microglia are born, making up approximately 10% of the adult brain cell population (Alliot, Godin, & Pessac, 1999).

Microglia are a form of innate immunity and exist in one of three states: ameboid, ramified, and activated. Ameboid microglia freely move throughout the brain and preform a scavenger role, using phagocytosis to break down cellular debris and removing unnecessary neurons or synapses. Ameboid microglia are particularly prominent in development and are vital in plasticity but have no inflammatory properties (Ling & Wong, 1993). Ramified microglia are quiescent, serving to maintain a stable cellular environment within the brain and to identify immune threats. They have a small cellular body and long projections suited towards cellular surveying, but lack inflammatory properties and phagocytosis. Upon immune insult, ramified microglia can transform into the third form of microglia, activated microglia (Davis, Salinas-Navarro, Cordeiro, Moons, & De Groef, 2017; Nayak et al., 2014). Activated microglia travel to the site of injury and engulf the target area. They have phagocytic properties and secrete either pro or anti-inflammatory cytokines, driving the immune response in the brain. Activated

microglia fall into two categories: M1 microglia and M2 microglia. M1 microglia respond to damage by secreting pro-inflammatory cytokines and reactive oxygen species which are designed to kill intracellular pathogens. M2 microglia are designed to deactivate, repair, or protect the body from inflammation, uptaking apoptotic cells and releasing anti-inflammatory cytokines (Tang & Le, 2016; Varin & Gordon, 2009). Therefore, M1 and M2 microglia have a complementary relationship, up regulating and down regulating the inflammatory response as needed in order to maintain an adaptive and healthy neural environment.

Astrocytes

Astrocytes are the most abundant glial cell population, accounting for 30% of the cells in the CNS. They perform a variety of functions ranging from synaptic and structural support, to axon guidance, to controlling the blood brain barrier, to transporting nutrients into and throughout the brain. Astrocytes are also an important contributor to the innate immune response in the brain (Sofroniew & Vinters, 2010). Specifically, after an immune insult, astrocytes in the surrounding area become reactive, a process known as astrogliosis. Astrogliosis can be conceptualized as the astrocytic response to CNS insult, complementing the action of microglia (Zhang, Hu, Qian, O'Callaghan, & Hong, 2010). In astrogliosis, astrocytes undergo numerous changes that allow them to secrete pro and anti-inflammatory cytokines and neurotoxins, while exhibiting phagocytic properties (Sofroniew, 2014). Overall, the purpose of astrogliosis is twofold, firstly it helps to address the injury by mobilizing resources and counteracting pathogens and secondly, it helps to isolate the damage caused by both the CNS insult as well as by the pro-inflammatory cytokines and neurotoxins being secreted (Eng & Ghirnikar, 1994).

Containing these responses is accomplished by gliosis or glial scarring. Gliosis is the process of filling the empty space generated by dead or dying neuronal cells and creating a barrier or seal around the injured area. This seal has the effect of quarantining both the initial damage as well as the damage done by the immune response, playing a vital role in reestablishing the integrity of the region (Burda & Sofroniew, 2014).

Inflammation and Seizures

It is important to note that while the basic function of the immune system is to play a protective role by eliminating potentially dangerous pathogens and microbes that would disrupt homeostasis, under certain conditions increased pro-inflammatory cytokine production by microglia and astrocytes can be pathological and perpetuate cell death, worsening CNS insults (Ramani et al., 2015). This maladaptive increase in inflammation is precisely what occurs after seizures. Studies have shown that seizures result in marked increases in both activated microglia and astrocytes, leading to high levels of pro-inflammatory cytokine production (Coulter & Steinhäuser, 2015; de Lanerolle, Lee, & Spencer, 2010; Hiragi, Ikegaya, & Koyama, 2018). This pro-inflammatory cytokine production has in turn been associated with the pathophysiology following ELS.

Autoimmune diseases such as systemic lupus, vasculitis, multiple sclerosis, and paraneoplastic syndromes can exacerbate seizure occurrence and severity while increasing neuronal damage (Devinsky, Schein, & Najjar, 2013; Serafini et al., 2016). There is also ample clinical evidence to suggest that this effect is not specific to autoimmune diseases. Studies have shown that a high fever, which is associated with increases in inflammation, can similarly contribute to and worsen, a seizure phenotype (Dubé, Brewster, Richichi, Zha, & Baram, 2007). Therefore, seizures exhibit a positive

feedback loop with inflammation, wherein there are marked increases in inflammation following seizures, which in turn leads to cell death that can contribute to future sensitization of the region and excitotoxicity (Vezzani et al., 2011). Notably, studies have found that in conditions where there are high amounts of circulating cytokines, treatment with anti-inflammatory agents is often more successful than the standard anti-epileptic drug regimen at mitigating deficits, identifying inflammation as a potential therapeutic target (Rana & Musto, 2018; Vezzani, 2015; Vezzani et al., 2011).

Clinical findings illustrating a connection between inflammation and seizure severity have also been corroborated in murine models. The induction of recurrent seizures, or of status epilepticus, in mice and rats has been shown to lead to increases in microglia activation and astrogliosis, resulting in increased levels of pro-inflammatory cytokines in the brain regions associated with seizure onset (Hiragi et al., 2018; Vargas-Sánchez et al., 2018). Furthermore, a systemic injection of lipopolysaccharide, an agent that directly increases pro-inflammatory cytokines, has been shown to lower seizure threshold both acutely and chronically (Auvin, Mazarati, Shin, & Sankar, 2010; Auvin et al., 2009; Galic et al., 2008; Heida & Pittman, 2005; Sayyah, Javad-Pour, & Ghazi-Khansari, 2003). This effect was even more pronounced in pups, as a single injection of lipopolysaccharide on postnatal day 7 or 14 resulted in alterations in neuronal excitability that persisted throughout the lifespan (Lewis, Kesler, Candy, Rho, & Pittman, 2018; Rana & Musto, 2018; Vezzani et al., 2011). Altogether the literature in both human and murine populations clearly implicate inflammation as playing a significant role in seizure occurrence and pathology.

Inflammation and Comorbid Conditions

In addition to directly contributing to the severity of seizures, inflammation has also been shown to play a role in the onset of conditions that are comorbid with seizures. For instance, depression is one of the most prevalent comorbidities associated with epilepsy and is thought to in part result from increased pro-inflammatory cytokine production (Felger, 2019; Kanner, 2006). Epilepsy associated depression can be treated with anti-inflammatory agents, illustrating the potential effectiveness of counteracting inflammation when treating conditions that accompany seizures (Köhler et al., 2014).

Inflammation is also a key feature in individuals with autism spectrum disorder. In patients with ASD, microglia are upregulated and cytokine levels are increased in the cerebrospinal fluid (Rodriguez & Kern, 2011). Other studies have supported this finding, demonstrating that ASD results in a dysregulation of the immune system that leads to an overall pro-inflammatory phenotype (Gladysz, Krzywdzinska, & Hozyasz, 2018; Jyonouchi, Sun, & Le, 2001; Siniscalco et al., 2018). Since individuals with ASD exhibit a trademark deficit in communication, there is evidence to suggest that an underlying factor that may contribute to communicative deficits is increased inflammation (DSM-5).

Murine models support this conclusion, as a variety of models that present with increased inflammation and an autistic-like phenotype also display deficits in ultrasonic vocalizations. One commonly used mouse model of autism is the BTBR model. mice. BTBR mice display deficits in sociability, communication, and exhibit repetitive behaviors in addition to displaying cognitive deficits commiserate with the ASD phenotype (McFarlane et al., 2008; McTighe, Neal, Lin, Hughes, & Smith, 2013; Moy et al., 2007). These mice also innately display an inflammatory immune profile and have

been shown to emit a decreased quantity of USVs relative to controls (Nadeem et al., 2018; Scattoni, Gandhy, Ricceri, & Crawley, 2008). Additionally, in the maternal immune activation model of ASD, wherein the pregnant dam's immune system is stimulated by lipopolysaccharide (LPS) or Polyinosinic:polycytidylic acid (poly I:C), leading to the birth of autistic-like pups, there is similarly a decrease in pup vocalization production (Malkova, Yu, Hsiao, Moore, & Patterson, 2012; Reisinger et al., 2015; Simões et al., 2018). Early-life seizure models that are induced by the injection of chemoconvulsants also exhibit communicative deficits and increased inflammation, with pilocarpine treated pups displaying a decreased latency to vocalize as well as producing USVs of a significantly shorter duration compared to saline injected pups (López-Meraz et al., 2014). Both C57BL/6J and 129SvEvTac pups treated with kainic acid display significant decreases in the quantity of vocalizations emitted, as well as differences in the types of calls used (Reynolds et al., 2017; Reynolds, Smith, Jefferson, & Lugo, 2016). Therefore, inflammation has been associated with altered communicative behaviors following early life seizures in humans and in murine seizure models, constituting a promising therapeutic target.

Agomelatine

Overview

An anti-inflammatory agent that is ideally suited to treat the communication deficits following early life seizures is the pharmaceutical agomelatine. Agomelatine is a MT1 and MT2 melatonin receptor agonist and a 5-hydroxytryptamine 2C (5-HT2C) serotonin receptor antagonist that counters pro-inflammatory cytokine production while

facilitating neuroprotection (Guardiola-Lemaitre et al., 2014). It has a high therapeutic index, and was originally developed as an anti-depressant. However, agomelatine's mechanism suggests that it may be able to effectively mitigate the communication deficits that accompany early life seizures (Komaram, Nukala, Palla, Nambaru, & Kasturi, 2015; Stein et al., 2017).

Melatonin

Agomelatine's principal effects are to function as a melatonin agonist. Melatonin (*N*-acetyl-5-methoxytryptamine) is an endogenous indolmine that is a ubiquitous lipid soluble molecule, present throughout both the brain and the body (Yu, Dickson, Jung, Koh, & Hille, 2016). Melatonin regulates the circadian rhythm and serves as a potent neuroprotective agent. These diverse roles are mediated through melatonin's 2 receptors, MT1 and MT2, both of which are g-protein coupled and when activated, inhibit adenylyl cyclase, leading to a reduction in the formation of cyclic adenosine monophosphate (cAMP) (Esposito & Cuzzocrea, 2010; Liu, Clough, et al., 2016; Slanar, Pelisek, & Vanecek, 2000). Decreased cAMP in turn leads to decreased activity of protein kinase A (PKA) and the phosphorylation of the cAMP-responsive element binding protein (CREB), which phosphorylates numerous cellular substrates (Zanassi et al., 2001). Additionally, both MT1 and MT2 receptors can also couple to phospholipase C pathways (Liu, Clough, et al., 2016). This is especially pertinent in seizures as phospholipase C activation in part regulates mGluR1/5 activity, which has been shown to protect against excitotoxicity (Domin et al., 2010). Therefore, while melatonin is typically associated with the circadian rhythm it has a plurality functions due to its broad

mechanisms of action and may be ideally suited to counteract prominent comorbidities of ELS.

Melatonin Circadian Rthymn

Melatonins capacity to synchronize the circadian rhythm is due to its secretion from the pineal gland, which is in turn regulated by light cues (Xie et al., 2017). Specifically, incoming light wavelengths activate glutamate, stimulating the glutamate-containing retinohypothalamic tract that projects to the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN then either inhibits or disinhibits the pineal gland, leading to the production or diminishment of melatonin. (Ebadi & Govitrapong, 1986; Emet et al., 2016; Huang, Lee, & Yang, 2005). Melatonin production is inverse to the presence of light, in such a way so that circulating melatonin is inhibited during daylight hours, maintaining a low but constant level, with the onset of evening significantly increasing the amount of melatonin in the blood, peaking later in the evening (Burgess & Fogg, 2008; Grivas & Savvidou, 2007). The result of these actions is feeling drowsy once the sun goes down and relaxed at night, preparing the body for sleep (Zisapel, 2018).

Melatonin's Protective Effects

Melatonin's protective effects encompass a range of adaptive changes, as melatonin can be classified as an anti-aging molecule, is a powerful antioxidant, and a potent anti-inflammatory agent (Esposito & Cuzzocrea, 2010; Jenwitheesuk, Nopparat, Mukda, Wongchitrat, & Govitrapong, 2014; Reiter, Calvo, Karbownik, Qi, & Tan, 2000). The protective effects of melatonin are accomplished through its ability to

modulate intracellular signal transduction and to enhance or suppress the responses of different cells to incoming signals (Carbajo-Pescador et al., 2011; Jockers, Maurice, Boutin, & Delagrange, 2008). These effects are further potentiated by the wide distribution of melatonin receptors and melatonin's ability to be produced in a variety cells separate from the pineal gland. The extra pineal synthesis of melatonin encompasses the skin, retina, gastrointestinal system, liver, kidney, thyroid, pancreas, thymus, spleen, carotid body, reproductive tract, endothelial cells, mitochondria, and immunocompetent cells, including astrocytes (Acuna-Castroviejo et al., 2014; Bubenik, Brown, & Grota, 1977; Chen, Fichna, Bashashati, Li, & Storr, 2011; Liu et al., 2007; Reiter et al., 2016; Tan, Manchester, Qin, & Reiter, 2016). Therefore, the ubiquitous expression of melatonin makes it particularly well suited to serve as a protectant capable of mitigating immune insults and cellular damage.

Antioxidant Effects

In the case of oxidative stress, the aberrant production of reactive oxidative species that lead to cell damage and death, melatonin can directly detoxify reactive oxygen and nitrogen species, serving as a potent free radical scavenger capable of protecting cells from oxidative damage (Reiter et al., 2003). Its effectiveness has been shown to be equal to, if not greater than, strong antioxidants such as vitamins E and C, displaying a large therapeutic efficacy (Reiter et al., 2016). Moreover, the metabolites of melatonin, c3OHM, acetyl-N-formyl-5-methoxykynuramine (AFMK), and acetyl-N-5-methoxykynuramine (AMK) also display anti-oxidant capabilities (Galano, Tan, & Reiter, 2014; Ressmeyer et al., 2003; Tan, Hardeland, Manchester, Galano, & Reiter, 2014; Tan et al., 2000; Tan et al., 2002). Therefore, melatonin has been shown to initiate

an anti-oxidant cascade that allows it to neutralize 10 radical products as opposed to other free radical scavengers that only detoxify a single oxidizing molecule (Reiter et al., 2016). In addition to melatonin's direct effects, it also facilitates the antioxidant enzymes glutathione and superoxide dismutase, while suppressing pro-oxidant enzymes (Aydogan, Yerer, & Goktas, 2006; Okatani, Wakatsuki, & Kaneda, 2000). Taken together, melatonin significantly decreases oxidative stress through a variety of processes.

Anti-inflammatory Effects

In the case of inflammation, melatonin exerts its protective effects through receptor mediated and receptor independent mechanisms, displaying enormous functional diversity and versatility (Esposito & Cuzzocrea, 2010). The ubiquitous distribution of melatonin receptors throughout both the CNS and PNS, as well as its high lipid solubility, further complement its action and ensure that melatonin's effects are not constrained to only one region or cell type (Hardeland, 2010). Neurologically, melatonin inhibits and restrains M1 microglia, keeping them in an ameboid or ramified state and preventing them from becoming active (Hu et al., 2019; Nabavi et al., 2019). Crucially, melatonin does not hinder M2 microglia's immunosuppressive properties and can enhance their effect via stimulation of TREM2, a marker of microglia mediated protection (Azedi et al., 2019; Nabavi et al., 2019). These direct effects on microglia result in a decreased expression of pro-inflammatory cytokines in pathologically inflamed models (Ding et al., 2014; Wu et al., 2011). Melatonin has also been shown to inhibit COX2 and reduce prostaglandin, both of which activate microglia (Cuzzocrea, Costantino, Mazzon, & Caputi, 1999; Deng, Tang, Tseng, & Wu, 2006; Gimeno, Landa, Sterin-Speziale, Cardinali, & Gimeno, 1980; Vijitruth et al., 2006). Additionally, melatonin significantly

reduces astrogliosis in both neonatal and adult inflammatory models (Babaee et al., 2015; Hu et al., 2017). The culmination of these properties have been shown to reverse both acute and chronic inflammation, in addition to displaying an efficacy in attenuating the severe inflammation and endothelial disruption present in bacterial and viral infections that cause sepsis (Esteban-Zubero et al., 2016; Galley et al., 2014; Ortiz et al., 2014; Reiter et al., 2016; Srinivasan, Mohamed, & Kato, 2012). Moreover, melatonin is well tolerated and is not associated with the side effects of other anti-inflammatory agents. Specifically, unlike steroids, melatonin does not suppress the immune system but rather enhances it. Melatonin is also better tolerated than nonsteroidal anti-inflammatory drugs (NSAIDs), since it inhibits COX-2 without altering COX1, thereby avoiding one of the major disadvantages of NSAIDs (Esposito & Cuzzocrea, 2010; Gilad et al., 1998). Altogether, melatonin comprises a significant mediator in the immune system capable of counteracting pathological pro-inflammatory cytokine secretion.

While melatonin's neuroprotective effects are a function of both anti-inflammatory and antioxidant properties, its effect on cell survivability and protection are not solely contingent upon these two mechanisms. Melatonin has been shown to directly prevent neuronal apoptosis in neurodegenerative conditions as well as in cells following an immune insult (Sainz et al., 2003; Wang, 2009). It also works on benzodiazepine receptors, serving to increase GABA while potentiating its effects, thus displaying potential in the treatment of excitotoxic conditions and their accompanying comorbidities (Niles, 1991; Niles, Pickering, & Arciszewski, 1987). Additionally, melatonin increases brain derived neurotropic factor (BDNF) which is integral in establishing synaptic plasticity following an insult and has also been shown to exert therapeutic and protective

effects in models of neurological damage (Falcicchia et al., 2018). Overall, melatonin promotes neuroprotection through countering pathological pro-inflammatory cytokine production, scavenging free radicals, decreasing excitation, inhibiting apoptosis, and increasing BDNF.

Melatonin, Seizures, and Comorbidities

When melatonin has been investigated in the context of seizures, it has been shown to confer numerous beneficial effects due to its anti-inflammatory and antioxidant properties. Specifically, melatonin is decreased in children with intractable epilepsy but displays a 3-fold increase following seizures. This has been interpreted as the body's endogenous response to mitigate seizure damage and preserve functioning (Bazil, Short, Crispin, & Zheng, 2000). Furthermore, the addition of melatonin agonists in patients with intractable epilepsy was shown to significantly decrease the onset of diurnal seizures (Goldberg-Stern, Oren, Peled, & Garty, 2012). Melatonin agonists have also been shown to improve sleep efficiency in individuals with ELS and most importantly, improve behavior and communication abilities in children with epilepsy, suggesting that increases in melatonin levels may be able to attenuate communicative deficits early in life (Peled, Shorer, Peled, & Pillar, 2001). Furthermore, melatonin supplements reduce prominent aspects of epilepsy's autistic comorbidity such as anxiety, behavioral problems, gastrointestinal dysfunction, social withdrawal, stereotyped behaviors, and once again communication deficits (Gagnon & Godbout, 2018; Malow et al., 2012; Tordjman et al., 2013; Wright et al., 2011). Therefore, increases in melatonin have shown a striking ability to mitigate both the aberrant electricity activity and the behavioral abnormalities associated with epilepsy and its autistic comorbidity.

When melatonin agonists have been assessed in murine models of early life seizures they display a similar therapeutic affinity to that seen in humans. Specifically, melatonin agonists have been shown to inhibit audiogenic and electrical seizures, in addition to reducing the convulsant activity induced via pentetrazole, pilocarpine, L-cysteine, and kainite administration (Banach, Gurdziel, Jedrych, & Borowicz, 2011; Costa-Lotufo et al., 2002; Savina, Balashova, & Shchipakina, 2006; Yahyavi-Firouz-Abadi, Tahsili-Fahadan, Riazi, Ghahremani, & Dehpour, 2007). Additionally, melatonin improves social behavior in valproic acid treated rats while facilitating long term potentiation, further implicating the hormone as playing a pivotal role in the deficits associated with seizures and autism (Tian et al., 2014). Altogether, melatonin displays a remarkable affinity for treatment in both humans and murine models of ELS and ASD, constituting a compelling therapeutic target.

5-HT2C

Agomelatine's secondary effects are to antagonize the 5-HT2C receptor. 5-HT2C is a G protein coupled receptor that is activated by endogenous serotonin. Its activation leads to an increase in phospholipase C (PLC), which in turn upregulates diaminoglycerol (DAG) and inositol-1,4,5-triphosphate (IP3) from membrane-localized phosphoinositides, culminating in the phosphorylation of numerous cellular substrates (de Bodinat et al., 2010). These actions facilitate calcium release, predominately playing a stimulatory role, with the 5-HT2C receptor mediating excitatory transmission throughout the hippocampus, amygdala, geniculate nuclei, portions of the frontal cortex, and the striatum (Clemett, Punhani, Duxon, Blackburn, & Fone, 2000; Herrick-Davis et al., 2015; Sheldon & Aghajanian, 1991; Tavares,

Baptista-de-Souza, & Canto-de-Souza, 2018). 5-HT2C has also been shown to contribute to the pathophysiology following seizures. Specifically, there is enhanced 5-HT2C receptor binding in the hippocampus observed in animal models of epilepsy, suggesting that the receptor may perpetuate excitotoxic damage (Krishnakumar, Nandhu, & Paulose, 2009). Further evidence has implicated increased 5-HT2C activity in playing a role in the memory deficits and the depressive comorbidity that commonly accompanies seizures (Khaliq, Haider, Saleem, Memon, & Haleem, 2012; Millan, 2005; Yohn, Gergues, & Samuels, 2017).

Antagonizing 5-HT2C leads to anxiolytic and anti-depressant effects in addition to decreasing excitation, offsetting the increased 5-HT2C receptor binding found in epileptic hippocampi (Krishnakumar et al., 2009; Opal et al., 2013). One possible mechanism by which 5-HT2C reduces electrical activity in the hippocampus is via its inhibition of phospholipase C and by proxy, its modulatory effects on mGluR1/5 (de Bodinat et al., 2010; Domin et al., 2010). mGluR1/5 antagonism has been shown to confer neuroprotection in the hippocampus of kainite treated animals, and may therefore be one way in which 5-HT2C antagonism mitigates excitotoxic damage (Domin et al., 2010). Additionally, 5-HTC antagonism has been shown to facilitate BDNF expression, further fostering neuroprotection and neurogenesis (Hill et al., 2011; Opal et al., 2013). Moreover, 5-HT2C antagonism potentiates the effects of melatonin, as agomelatine's MT1/MT2 and 5-HT2C properties exhibit a complimentary and synergistic relationship (de Bodinat et al., 2010). Therefore, agomelatine administration leads to adaptive changes that far exceed what each receptor is able to accomplish alone (Racagni et al., 2011). Furthermore, in spite of the widespread changes induced by agomelatine, there are

minimal pervasive side effects, making it a safe, yet effective potential treatment option (Guaiana et al., 2013).

Agomelatine in Excitotoxic Models

When agomelatine has been used in seizure and excitotoxic models it yields promising results. In kainic acid treated animals agomelatine prevented comorbid depression by suppressing inflammatory signaling (Tchekalarova et al., 2018). Specifically, agomelatine reduced pro-inflammatory cytokine levels while preventing microgliosis and astrogliosis, decreasing inflammation (Tchekalarova et al., 2018). Agomelatine has also been shown to confer neuroprotection in the basolateral amygdala, dentate gyrus, and the CA1, CA2, and CA3 regions of the hippocampus in kainic acid treated mice in addition to displaying antioxidant effects (Tchekalarova et al., 2017). Specifically, agomelatine has also been shown to modulate glutamate levels in the hippocampus, conferring protective effects (Milanese et al., 2013; Tardito et al., 2010) In pups, agomelatine also displays a strong therapeutic potential. In a neonatal excitotoxic mouse model, agomelatine promoted secondary lesion repair and served as a neuroprotectant (Gressens et al., 2008). Moreover, agomelatine was well-tolerated and showed efficacious effects both when administered immediately after the excitotoxic insult and when administered 8 hours later. By comparison, the melatonin agonist that was also assessed was only efficacious when administered immediately after the insult, indicating that agomelatine has a larger therapeutic window than other treatments (Gressens et al., 2008). Furthermore, agomelatine exerts disease state dependent affects in early life seizure models and thereby has not been shown to significantly impair the behavior of control mice (Morley-Fletcher et al., 2011). Altogether, the literature strongly

suggests that agomelatine is ideally suited to counteract the deficits in communication that accompany early life seizures.

Significance

Seizure induced deficits in early life communication can have devastating effects on an infant's quality of life. Furthermore, there are few treatment options available that address this comorbid condition, despite its prevalence and severity. While the literature suggests that increased pro-inflammatory cytokine secretion may be one mechanism underlying the communication deficits present following ELS, few studies have assessed the therapeutic potential of anti-inflammatory pharmaceuticals toward this end. Agomelatine is well-tolerated and has strong anti-inflammatory and neuroprotective properties, yet its efficacy to address the communication deficits present in neonates after ELS is unknown. Thus, while the theoretical potential of agomelatine is high, its pragmatic utility remains less defined.

Previous Studies Directly Related to this Research

Both preliminary and published work from our lab has shown that an injection of KA in 129SvEvTac pups on postnatal day 10 leads to significant changes in early life communicative behaviors. When the pups were assessed via the maternal isolation paradigm on PD 12, male KA treated pups emitted significantly fewer ultrasonic vocalizations at 50 kHz than the control group (Figure 2a). Additionally, male pups in the seizure condition vocalized for a significantly shorter duration at 50 kHz and in total than control pups ($p < .05$) (Figure 2 a,b) (Reynolds et al., 2016).

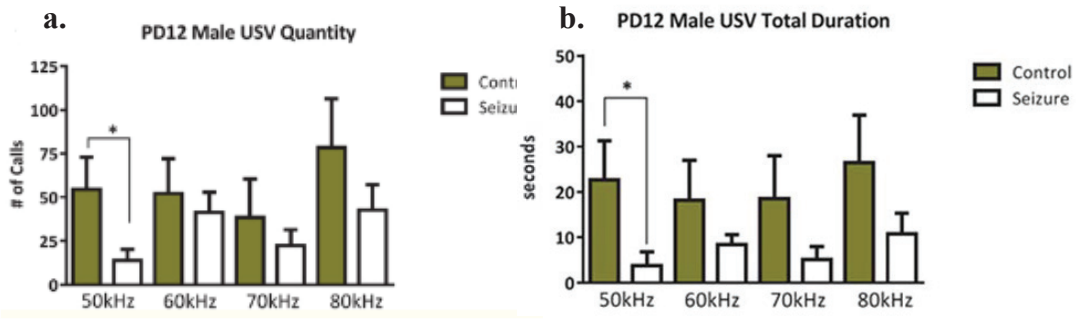


Figure 2. a. The quantity of USVs emitted in 129 SvEvTac seizure and control pups. KA treated males emitted fewer USVs at 50 kHz than control pups on PD 12. b. Total duration of calls. KA treated pups had calls of a significantly shorter duration for 50 kHz calls. Figures taken from Reynolds et al., 2016.

We have also demonstrated that an injection of KA in C57 BL/6J pups on postnatal day 10 similarly leads to significant deficits in early life communicative behaviors. Specifically, when ultrasonic vocalizations were assessed two days following status epilepticus, on PD 12, the total quantity of USVs emitted was significantly decreased in seizure mice when compared to controls ($p = .001$) (Figure 3a). Furthermore, ELS resulted in pups emitting comparatively louder calls and vocalizing for a shorter duration when compared to control pups ($p < .05$). The ELS group also demonstrated qualitative differences in the vocalizations, exhibiting a significantly different distribution of vocalization call types ($p < .05$) (Figure 3b) (Reynolds et al., 2017).

Pilot testing in our lab revealed that when C57 BL/6J pups are administered saline, 30% DMSO (vehicle), or 25 mg/kg of agomelatine on PD 10, there is no significant difference in the quantity of vocalizations emitted on PD 11 between groups ($p >.05$) (Figure 4). Therefore, agomelatine and its vehicle do not significantly affect USV production relative to controls. Pilot testing also determined that a safe dose of agomelatine in PD 10 pups is a single 25 mg/kg dose.

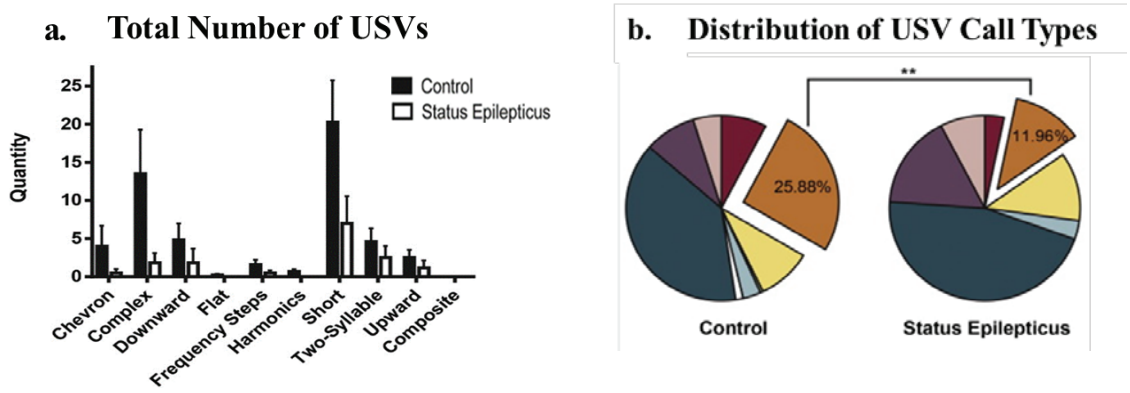


Figure 3. a. The quantity of USVs emitted in seizure and control pups. Seizure pups displayed a significant decrease in the total number of USVs across all call types $p = .001$. b. Call types utilized. Pups in the seizure condition produced significantly fewer complex calls compared to controls ($p < .05$). Figures taken from Reynolds et al., 2017.

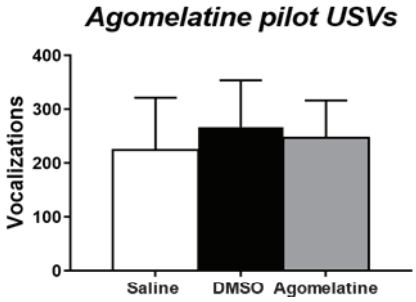


Figure 4. Neonatal agomelatine pilot data. There are no significant differences between USVs in pups receiving saline, DMSO, or Agomelatine ($n = 4$ per group, $p > .05$).

Study Aims

The first aim of this project was to determine agomelatine's efficacy to attenuate early life communicative deficits when administered 1-hour post seizure. The second aim was to assess the therapeutic window of agomelatine by administering the treatment 24 hours post seizure and evaluating its effects on kainic acid induced communication deficits. The third and final aim was to determine agomelatine's anti-inflammatory and excitomodulatory effects in kainic acid treated animals by assessing microglia (Iba1), and astrocyte reactivity (GFAP), in addition to assessing the excitatory markers GluR1, and mGluR1/5 via western blotting.

CHAPTER THREE

Methods

Subjects

Male and female C57BL/6 J (BL/6) were originally purchased from Jackson Labs then were bred for more than 10 generations at Baylor University. The pups used in this experiment were housed in individual cages with their parents and littermates. A total of 240 pups, 10 per group per timepoint were used, as determined by an *a priori* power analysis. Specifically, there were 6 male and 6 female treatment groups for both the 1-hour and 24-hour timepoints: KA/agomelatine, KA/DMSO, KA/saline, saline/agomelatine, saline/DMSO, and saline/saline. All behavioral procedures took place between 12 and 4 p.m. Animals were housed in a facility kept at 22 °C on a 12-hr light/dark cycle. Mice had *ad libitum* access to food and water. All procedures performed were in accordance with Baylor University Institutional Animal Care and Use Committee, as well as the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Seizure and Pharmacological Treatment

On PD 10 the pups were randomly assigned and administered a single intraperitoneal (i.p.) injection of kainic acid (1.5 mg/kg) or 0.9% physiological saline, per established protocol (Reynolds et al., 2017). After KA injection, the pups were observed until they entered status epilepticus (SE) and the seizures stopped. SE was assessed, and the seizure was scored, via the Racine scale (Racine, 1972). The treatment groups

received an injection of agomelatine (25 mg/kg) and the control groups received the vehicle (30% DMSO) or saline 1-hour or 24 hours post SE (Figures 5 A,B). All doses were either based on the literature or established via pilot studies before the study (Blevins, Stanley, & Reidelberger, 2002; Reynolds et al., 2017).

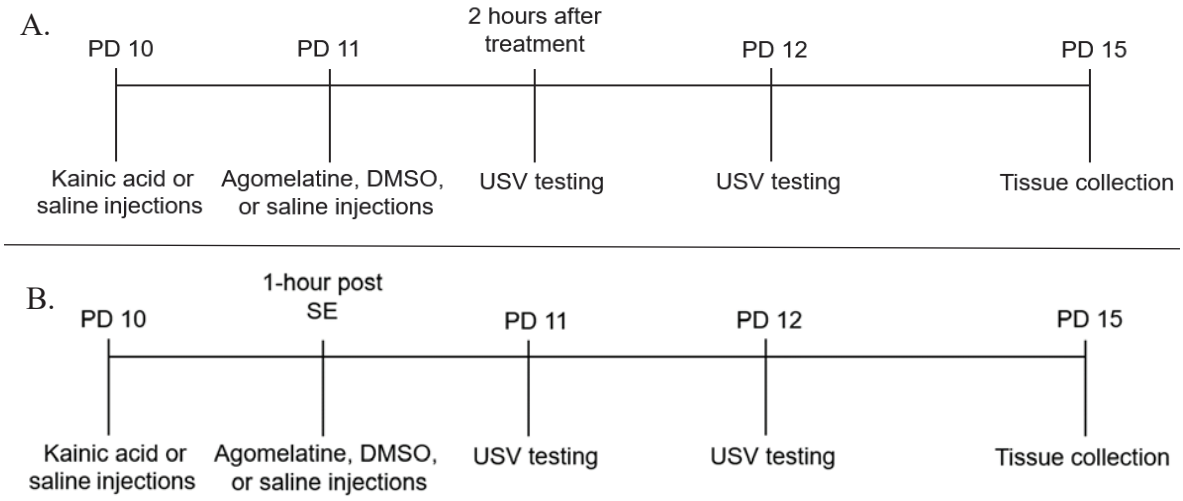


Figure 5. A. The timeline of experiments for the 1-hour post SE timepoint. B. The timeline of experiments for the 24-hour post SE timepoint.

Ultrasonic Vocalizations (USVs)

Ultrasonic vocalizations were elicited via the maternal isolation paradigm which has been shown to consistently elicit USVs in pups (Shair, 2007). Mice were tested on postnatal days (PD) 11 and 12 to determine the effect of agomelatine on early life communicative behaviors 24 hours and 48 hours post treatment, in addition to aligning with other studies' time points (Binder & Lugo, 2017; Reynolds et al., 2017; Reynolds et al., 2016). Before testing, pups were weighed and allowed to habituate in the testing room for 30 minutes. Next, they were removed from their dam and placed into a clean, preheated (22°C) cage. Each pup was then individually tested by removing the mouse from the warmed cage and placing it into another room temperature cage contained

within a 40 cm x 40 cm x 30 cm sound-attenuated chamber, as detailed in numerous other studies (Scattoni et al., 2008; Shair, 2007; Tsai et al., 2012). The vocalizations were recorded for 2 minutes using an ultrasonic microphone (CM16/CMPA, Avisoft Bioacoustics, Germany, part #40011) and an ultrasound recording program (UltraSoundGate 116Hb, Avisoft Bioacoustics, part # 41161/41162). Following testing, the pups were placed back in the pre-warmed cage, returning to their dam once testing had concluded.

Ultrasonic Vocalization Analysis

Ultrasonic vocalizations were analyzed with the Avisoft SASLab Pro software (Avisoft SASLabPro, RRID:SCR_014438). The parameters of measure consisted of: a fast Fourier transformation (FTT) length of 1024, a time window overlap of 75%, a 100% hamming window, a frequency resolution of 488 Hz, a time resolution of 1 ms, and a sampling frequency of 22,050, in accordance with prior studies (Binder & Lugo, 2017; Scattoni et al., 2008). Qualitative aspects of the vocalizations were determined by visually identifying each call using an established call type taxonomy in addition to grouping the calls based on their internal pitch changes, length, and shape (Scattoni et al., 2008). The predefined call type categories were complex, harmonic, two syllable, upward, downward, flat, chevron, short, composite, and frequency steps call types (Scattoni et al., 2008). The scorers were blind to the condition of the animal at the time of scoring.

Western Blotting

Western blotting was used to examine potential changes in excitability and neuroinflammation. Hippocampal tissue was taken from the neonatal cohorts on PD 15, as microglia levels peak on this day (Kim et al., 2015). Immediately following the rapid dissection, the structures were rinsed in 1X PBS on ice and then placed in dry ice before being stored at −80°C. Tissue processing consists of homogenizing the tissue in a homogenizing buffer (0.32 M sucrose, 1 mM EDTA, 5 mM Hepes) containing protease inhibitor cocktail (Sigma, St. Louis, MO) and were processed as previously described (Lugo et al., 2008). This process produces synaptosomes and total homogenate samples. The samples were then given a 1-minute spin at 1000 g at 4°C. A portion of the supernatant was removed then retained for total homogenate. The remaining supernatant underwent another spin cycle at 800 g at 4°C for 10 minutes. After the 10-minute spin, the supernatant was transferred to a third tube which was spun at 7200 g at 4°C for 15 minutes. The supernatant was next removed and the pellet was used to create synaptosome samples. Samples were prepared then diluted in Laemmli loading buffer (4X: 0.25 M Tris, pH 6.8, 6% SDS, 40% Glycerol, 0.04% Bromophenol Blue, 200 mM Dithiothreitol).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to suspend the total homogenate and crude synaptosome samples. Following the SDS-PAGE, proteins were transferred to Hybond-P polyvinyl difluoride membranes (GE Healthcare, Piscataway, NJ, USA). Membranes were incubated for 1-hour at room temperature in a blocking solution (5% non-fat milk) diluted in 1X Tris Buffered Saline (50 mM Tris-HCl, pH 7.4, 150 mM NaCl) with 0.1% Tween (1X TTBS) and 1mM

Na_3VO_4]. The membranes were then incubated overnight at 4°C with primary antibodies in 5% milk in TTBS. For this study we examined Iba1, GFAP, mGluR1/5, and GluR1 (1:250 Iba1, 1:1000 GFAP, mGluR1/5, GluR1; Cell Signaling Technology PhosphoSolutions®, USA). Following the overnight incubation, membranes were washed in 1X TTBS (3 x 5 minutes). Membranes were then incubated with horseradish peroxidase labeled secondary antibody, anti-rabbit IgG (1:20,000; Cell Signaling Technology, Boston, MA, USA), or anti-mouse IgG (1:20,000). The membranes were again washed in 1X TTBS (3x5 minutes) then were incubated with GE ECL Prime (GE Healthcare, Piscataway, NJ, USA). Immunoreactive bands were captured by a ProteinSimple western blot imaging system (ProteinSimple, Santa Clara, CA, USA). The ProteinSimple AlphaView software was used to measure the optical density of the resulting immunoreactive bands. Measurements obtained from all bands of interest were normalized to actin levels within the same lane. Also, all groups were normalized to the average of the control group per blot.

Statistical Analysis

All analyses were conducted using GraphPad Prism 7 software (La Jolla, CA) or SPSS 25.0 (IBM, USA). To detect significant differences in the total quantity of calls on PD 11 and 12 a multivariate analysis of variance (MANOVA) was performed with seizure, sex, and treatment as the fixed factors and call quantity as the dependent variable. Any interactions in this study were clarified by splitting the files according to sex then creating a grouping variable and running Tukey's HSD *post hoc* for multiple comparisons. In addition to assessing the total amount of vocalizations emitted per each group, we also assessed the quantity of USVs emitted per the complex, downward,

frequency steps, upward, chevron, and composite call types. This was done to garner a more comprehensive profile of USVs following ELS and also to align with a prior study investigating USVs following KA administration (Reynolds et al., 2017). For this analysis, a MANOVA that had seizure, sex, and treatment as the between subjects factors and the quantity of calls emitted per complex, downward, frequency steps, upward, chevron, and composite call types as the dependent factors was used. Similarly, to detect any significant differences in the duration, peak frequency, fundamental frequency, or the peak amplitude of complex, downward, frequency steps, upward, chevron, and composite call types, a MANOVA was run with the file being split by sex to clarify interactions. Western blot analysis was conducted via a MANOVA that was split according to sex with seizure and treatment as the fixed factors and the expression of Iba1, GFAP, GluR1, and mGluR1/5 as the dependent variables. A value of $p < .05$ was considered significant for each statistical test, with figures depicting the mean ± standard error of the mean (SEM).

CHAPTER FOUR

Results

Total Quantity of USVs 1-hour Post SE Treatment

PD 11: 1-hour Timepoint

Univariate ANOVA's were run for each day for the 1-hour treatment timepoint with seizure, sex, and treatment as the fixed factors and the number of calls as the dependent variable. When assessing total USVs on PD 11 in mice that received treatment one hour after SE there was a main effect of seizure ($F(1,122) = 4.10$, $p = .05$), but no main effect of sex ($F(1,122) = 2.22$, $p = .14$) or treatment ($F(2,122) = .181$, $p = .84$). Kainic acid treated mice emitted a greater quantity of vocalizations than control mice but there were no significant differences per sex or per treatment (Figure 6 A). Additionally, there were no interactions found between seizure and treatment ($F(2,122) = .638$, $p = .53$), seizure and sex ($F(1,122) = .373$, $p = .54$), sex and treatment ($F(2,122) = 3.00$, $p = .06$) treatment and sex, and seizure, sex, and treatment ($F(2,122) = .20$, $p = .82$).

PD 12: 1-hour Timepoint

When the total USVs were assessed on PD 12 in mice receiving treatment 1-hour after SE there was a main effect of sex ($F(1,122) = 4.46$, $p = .04$) but no main effect of seizure ($F(1,122) = .12$, $p = .73$), or treatment ($F(2,122) = .43$, $p = .65$). Female mice were shown to emit fewer vocalizations than male mice but there were no significant differences per treatment or per seizure (Figure 6 B). Additionally, there were no

interactions between seizure and treatment ($F(2,122) = 1.89$, $p = .16$), seizure and sex ($F(1,122) = 2.40$, $p = .13$), sex and treatment ($F(2,122) = .21$, $p = .81$) or seizure, sex, and treatment ($F(2,122) = .81$, $p = .45$).

Total Quantity of USVs 24 Hours Post SE Treatment

PD 11: 24-hour Timepoint

Univariate ANOVA's were run for each day for the 24-hour treatment timepoint with seizure, sex, and treatment as the fixed factors and the number of calls as the dependent variable. Analysis revealed a main effect of treatment ($F(2,122) = 3.40$, $p = .003$), but no main effect of seizure ($F(1,122) = 3.55$, $p = .78$), or sex ($F(1,122) = .78$, $p = .09$). Mice administered DMSO emitted the greatest quantity of USVs followed by saline, with agomelatine treated mice emitting the fewest quantity of vocalizations (Figure 6 C). There was an interaction between sex and treatment ($F(2,122) = .40$, $p = .01$) but no interactions were present between seizure and treatment ($F(2,122) = .84$, $p = .43$), seizure and sex ($F(1,122) = .55$, $p = .95$), or seizure, sex, and treatment ($F(2,122) = .20$, $p = .53$). A Tukey's post hoc analysis was used to assess the interaction between sex and treatment, however, no significant differences were found, $p > .05$.

PD 12: 24-hour Timepoint

When the total USVs were assessed on PD 12 in mice receiving treatment 24-hours after SE no main effect for seizure ($F(1,122) = .03$, $p = .87$), sex ($F(1,122) = 1.84$, $p = .18$), or treatment ($F(2,122) = .35$, $p = .71$) were found. There were also no interactions between seizure and treatment ($F(2,122) = .76$, $p = .47$), seizure and sex

($F(1,122) = .13$, $p = .72$), sex and treatment ($F(2,122) = 1.55$, $p = .22$) or seizure, sex, and treatment ($F(2,122) = .05$, $p = .95$) (Figure 6 D).

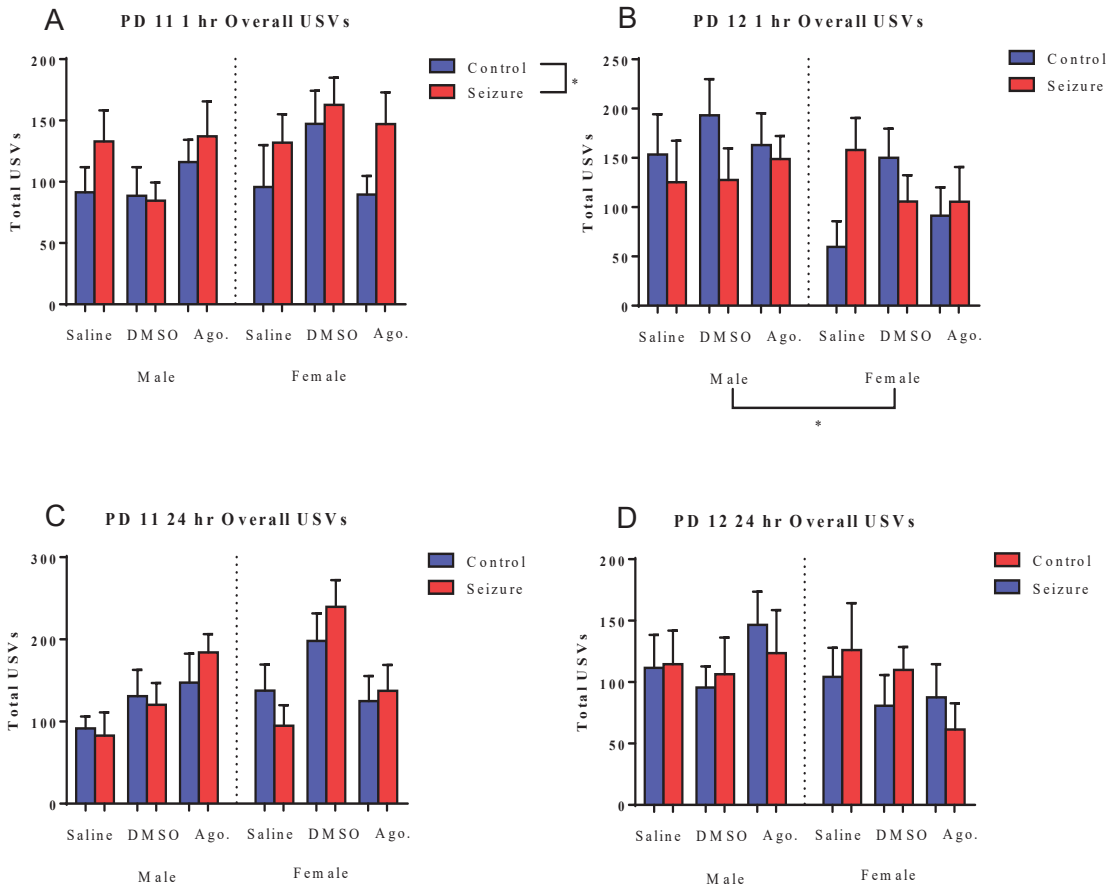


Figure 6. The overall quantity of calls emitted. A. On PD 11 for the 1-hour timepoint seizure animals emitted more USVs than control treated animals but no differences were found per treatment or sex. B On PD 12 1-hour post treatment No difference was found between seizure and control mice, however, females emitted fewer USVs than males. C. On PD 11 at the 24-hour timepoint no differences were found between green groups. D. On PD 12 at the 24-hour timepoint no differences were found between groups. Bars represent the mean and the error bars represent the standard error of the mean. * = $p < .05$

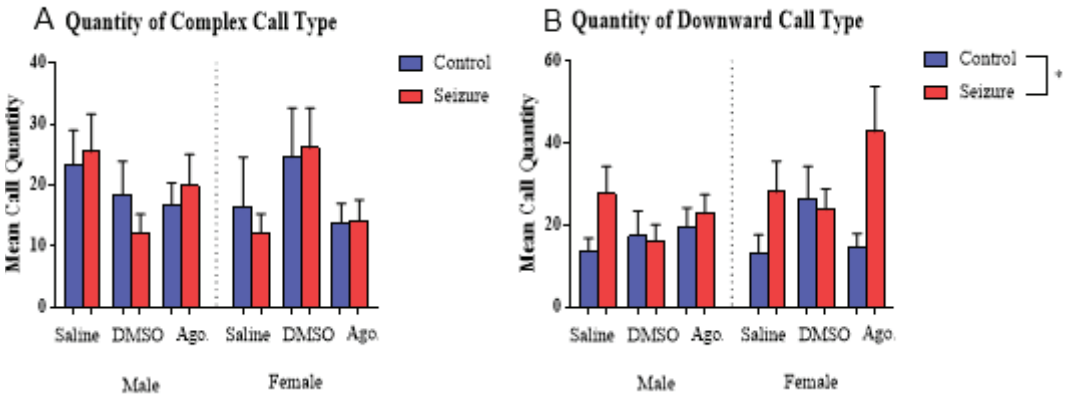
Quantity of USVs per Call Type: 1-hour Post SE Treatment

PD 11: 1-hour Timepoint

Multivariate analysis of Variance MANOVA's were run on PD 11 and PD 12 with seizure, sex, and treatment as the independent variables and the total number of calls for complex, upward, downward, chevron, composite, and frequency steps call types as the dependent variables. Analysis revealed significant main effects of seizure for downward ($F(1,121) = 6.81$, $p = .01$), and frequency steps call types ($F(1,121) = 4.92$, $p = .03$), and a trend for composite call types ($F(1,121) = 3.57$, $p = .06$). Seizures were shown to increase the quantity of downward, frequency steps, and composite call types relative to controls (Figure 7 B,C,F). No main effects of seizure were found for complex ($F(1,121) = .027$, $p = .87$), upward ($F(1,121) = 1.57$, $p = .21$), or chevron ($F(1,121) = .38$, $p = .38$) call types (Figure 7 A,D,E). When assessing sex, there were main effects for upward ($F(1,121) = 4.52$, $p = .04$), and composite ($F(1,121) = 7.20$, $p < .008$), call types but not for complex ($F(1,121) = .22$, $p = .64$), downward ($F(1,121) = 2.12$, $p = .15$), chevron ($F(1,121) = 1.39$, $p = .24$), or frequency steps ($F(1,121) = .83$, $p = .37$), call types. Females were shown to emit more upward and composite call types than males (Figure 7 D,F). There were no main effects of treatment for complex ($F(2,121) = .52$, $p = .52$), upward ($F(2,121) = .17$, $p = .84$), downward ($F(2,121) = .63$, $p = .54$), chevron ($F(2,121) = .43$, $p > .65$), composite ($F(1,121) = .14$, $p = .87$), or frequency steps ($F(2,121) = 3.45$, $p = .99$) call types. There was an interaction between sex and treatment for complex ($F(2,121) = 3.65$, $p < .05$) and chevron ($F(2,121) = 4.94$, $p = .03$) call types but no interaction for upward ($F(2,121) = .79$, $p = .45$), downward ($F(1,121) = .55$, $p = .58$), composite ($F(1,121) = .26$, $p = .26$), or frequency steps ($F(1,121) = 2.03$, $p = .14$)

call types. No interactions were present between sex and seizure for complex ($F(2,121) = .008$, $p = .93$), upward ($F(2,121) = .064$, $p = .80$), downward ($F(2,121) = 1.29$, $p = .26$) chevron ($F(2,121) = .033$, $p = .86$) composite ($F(2,121) = 1.26$, $p = .26$), or frequency steps call types ($F(2,121) = 00$, $p = .00$). There were also no interactions found between seizure and treatment for complex ($F(2,121) = .16$, $p = .86$), upward ($F(2,121) = .70$, $p = .80$) downward ($F(2,121) = 2.46$, $p = .26$) chevron ($F(2,121) = .48$, $p = .50$) composite ($F(2,121) = .77$, $p = .47$) or frequency steps call types ($F(2,121) = .27$, $p = .76$) nor between sex, seizure, and treatment for complex ($F(2,121) = .46$, $p = .63$), upward ($F(2,121) = .25$, $p = .78$), downward ($F(2,121) = 1.37$, $p = .26$), chevron ($F(2,121) = .74$, $p = .48$), composite ($F(2,121) = .27$, $p = .77$), and frequency steps call types ($F(2,121) = 2.43$, $p = .09$).

Due to the interaction between sex and treatment for complex and chevron call types, we created a grouping variable and ran a Tukey's HSD post hoc analysis. Upon assessment no differences were detected between the groups for complex and chevron call type $p > .05$.



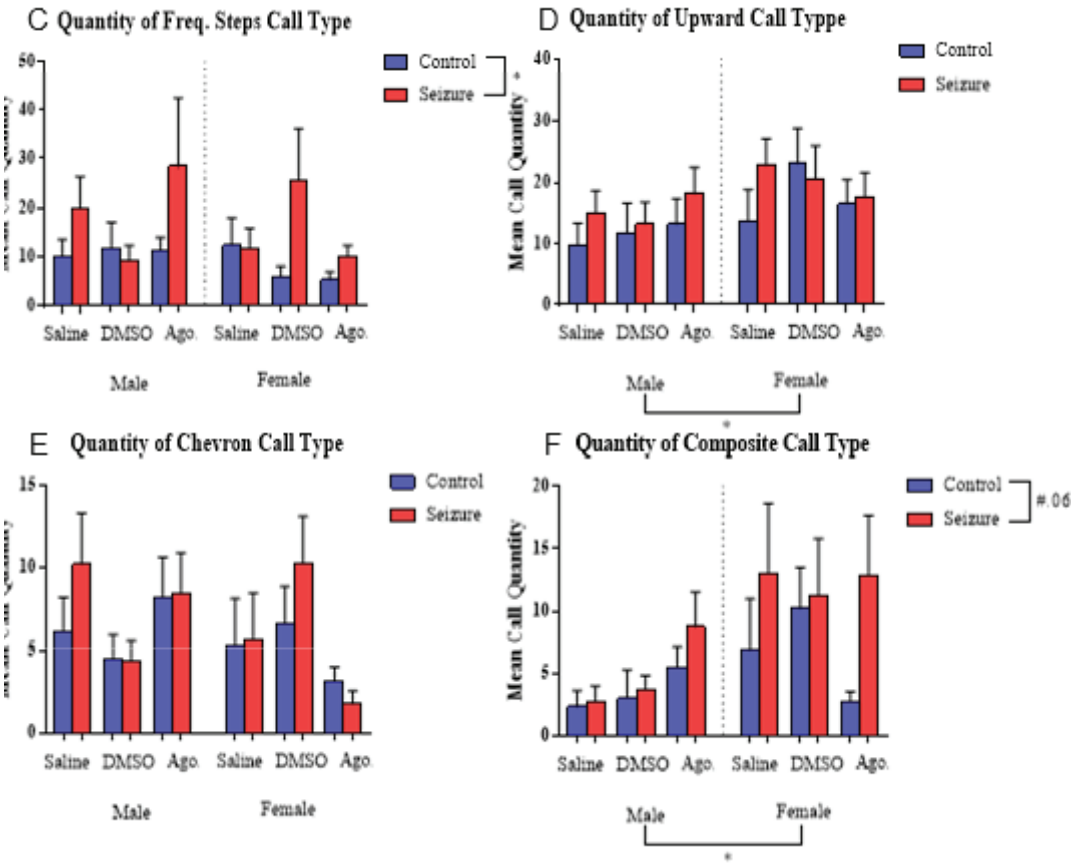


Figure 7. The total quantity of USVs per the complex, downward, frequency steps, upward, chevron, and composite call types per the PD 11 1-hour timepoint. A. There were no significant differences per seizure group, sex or treatment for complex USVs. B. Seizure treated animals were found to emit more downward call types than control mice, with no differences observed per sex or treatment group. C. Seizure treated animals were also found to emit more frequency steps call types than control mice, with no differences observed per sex or treatment groups. D. Females emitted more upward call types than males but no differences per seizure or treatment group were found. E. No significant differences per seizure group, sex or treatment for chevron USVs were found. F. Seizure animals trended towards emitting more composite calls than controls and females were found to emit more USVs than males, with no difference present per treatment. Bars represent the mean and the error bars represent the standard error of the mean. * = $p < .05$

PD 12: 1-hour Timepoint

On PD 12, MANOVA's revealed no main effect of seizure for complex ($F(1,121) = 1.33$, $p = .25$) upward ($F(1,121) = .09$, $p = .77$) downward ($F(1,121) = .057$, $p = .81$) chevron ($F(1,121) = .00$, $p = .99$), or frequency steps ($F(1,121) = .067$, $p = .80$) call types. However, there was a trend present for composite call types ($F(1,121) = 2.95$, $p = .08$), wherein seizure animals emitted more composite calls than controls (Figure 8 F). There

was a main effect of sex for downward ($F(1,121) = 6.70$, $p = .01$), but not for complex ($F(1,121) = 3.06$, $p = .08$) upward ($F(1,121) = 1.92$, $p = .17$), chevron ($F(1,121) = 1.83$, $p = .18$), composite ($F(1,121) = 2.11$, $p = .15$), or frequency steps call types ($F(1,121) = .43$, $p = .51$). Specifically, males emitted more downward call types than females (Figure 8 B). There was no main effect for treatment for complex ($F(2,121) = .09$, $p = .91$), upward ($F(2,121) = .014$, $p = .77$), downward ($F(2,121) = .37$, $p = .69$), chevron ($F(2,121) = .07$, $p > .05$), composite ($F(2,121) = 1.58$, $p = .21$), or frequency steps call types ($F(2,121) = .37$, $p = .05$). There was no interaction between seizure and sex for complex ($F(2,121) = 1.62$, $p = .21$), upward ($F(2,121) = .003$, $p = .95$), downward ($F(2,121) = 1.34$, $p = .25$), chevron ($F(2,121) = 2.41$, $p = .12$), composite ($F(2,121) = .093$, $p = .76$), or frequency steps call types ($F(2,121) = 2.14$, $p = .15$). Similarly, no interactions were found between sex and treatment for complex ($F(2,121) = .33$, $p = .72$), upward ($F(2,121) = .75$, $p = .18$), downward ($F(2,121) = .067$, $p = .94$), chevron ($F(2,121) = .85$, $p = .43$), composite ($F(2,121) = 1.92$, $p = .15$), or frequency steps call types ($F(2,121) = 2.53$, $p = .08$). No interaction was present between seizure and treatment for complex ($F(2,121) = 1.11$, $p = .33$), upward ($F(2,121) = 1.55$, $p = .22$), downward ($F(2,121) = 1.75$, $p = .18$), chevron ($F(2,121) = .54$, $p = .58$), composite ($F(2,121) = .89$, $p = .41$), or frequency steps call types ($F(2,121) = .29$, $p = .75$). There was also no interaction between seizure, sex, and treatment for complex ($F(2,121) = .64$, $p = .53$), upward ($F(2,121) = .96$, $p = .39$), downward ($F(2,121) = .46$, $p = .64$), chevron ($F(2,121) = .79$, $p = .45$), composite ($F(2,121) = 2.01$, $p = .14$) or frequency steps call types ($F(2,121) = .028$, $p = .97$).

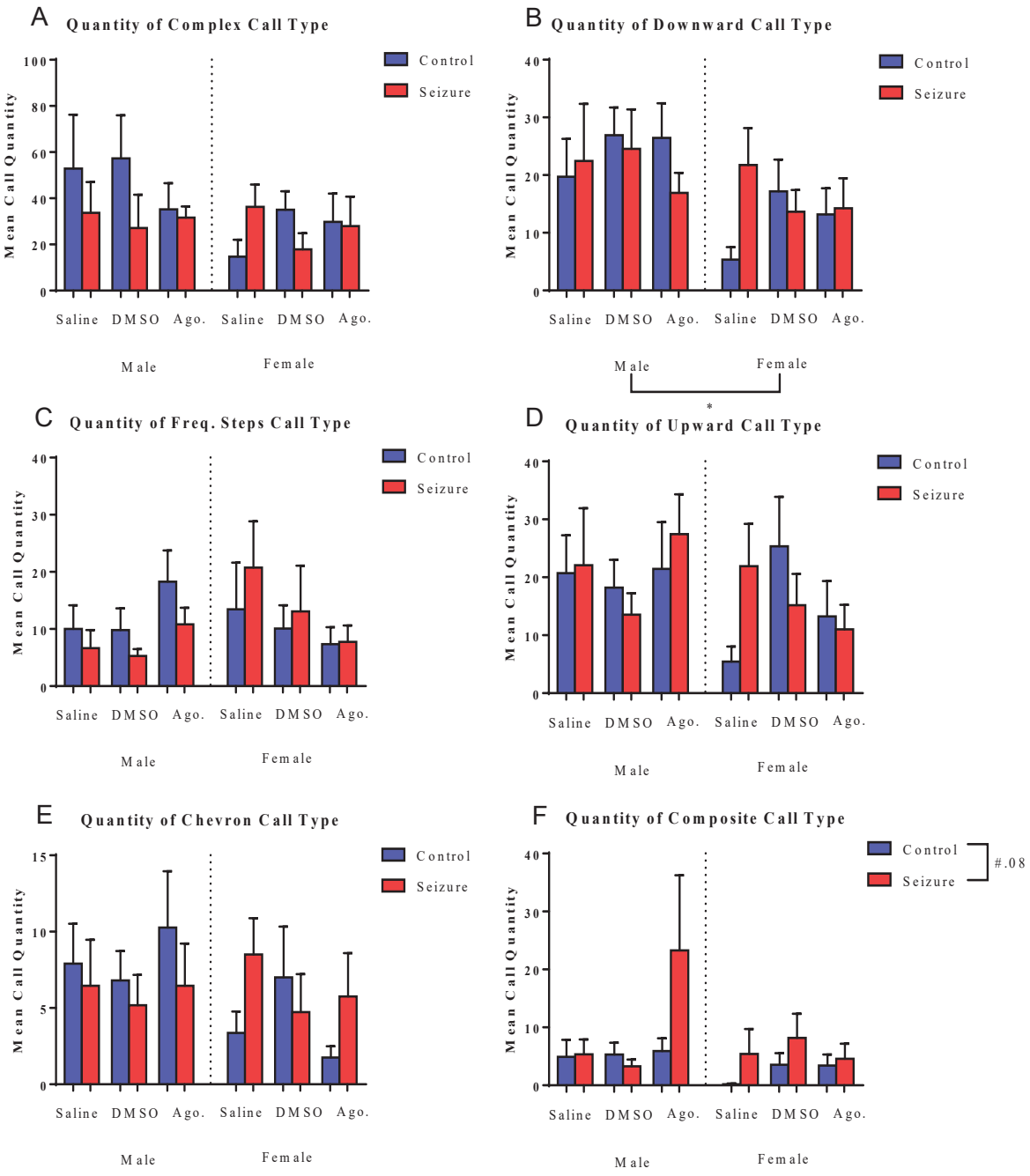


Figure 8. The total quantity of USVs per the complex, downward, frequency steps, upward, chevron, and composite call types per the PD 12 1-hour timepoint. A. There were no significant differences per seizure group, sex or treatment for complex USVs. B. Males were found to emit more downward call types than female mice, with no differences observed per seizure or treatment group. C No differences in frequency steps call types were observed per seizure, sex, or treatment groups. D. No differences were found in upward call types per seizure, sex, or treatment groups. E. No significant differences per seizure group, sex or treatment for chevron USVs were found. F. Seizure animals trended towards emitting more composite calls than controls with no difference present per sex or treatment. Bars represent the mean and the error bars represent the standard error of the mean. * = $p < .05$

Quantity of Calls per Call Type: 24 Hours Post SE Treatment

PD 11: 24-hour Timepoint

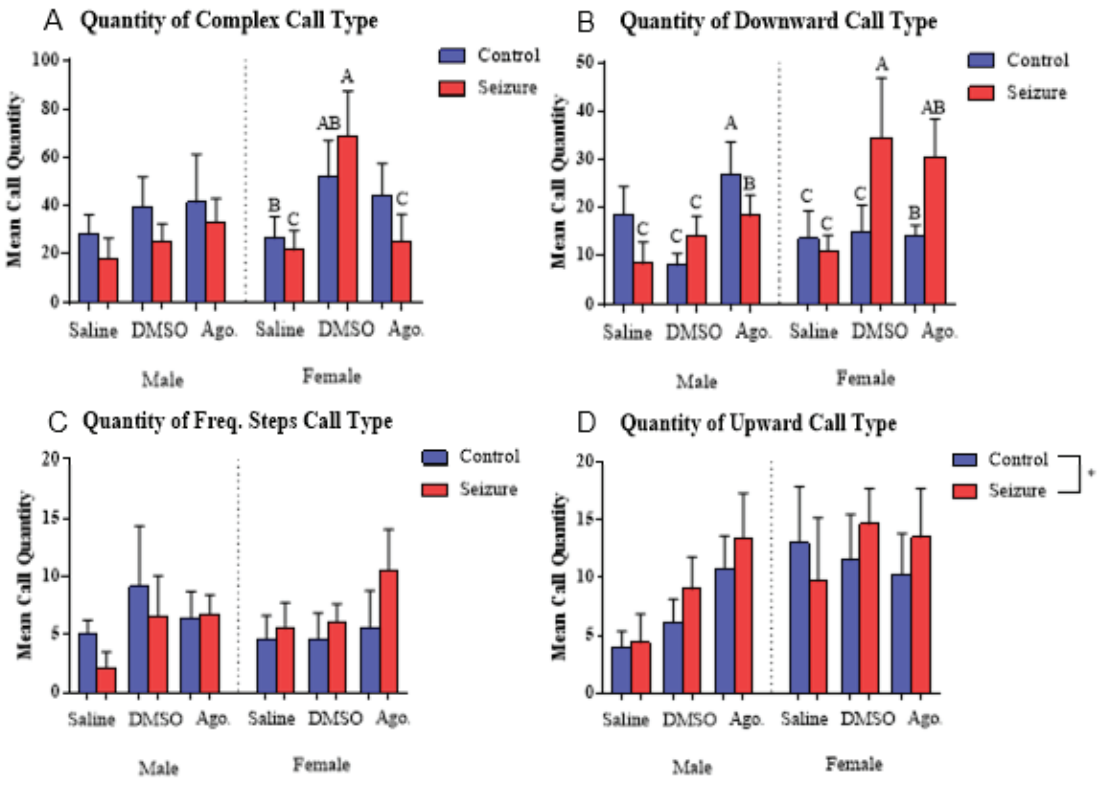
Multivariate analysis of Variance MANOVA's were again run on PD 11 and PD 12 for the 24-hour time-point with seizure, sex and treatment as the independent variables and the total number of calls for complex, upward, downward, chevron, composite, and frequency steps call types as the dependent variables. Analyses found a main effect of seizure for upward ($F(1,121) = 4.60$, $p = .03$), downward ($F(1,121) = 4.73$, $p = .03$), and composite ($F(1,121) = 23.75$, $p = .00$) call types and a trending effect for chevron call types ($F(1,121) = 3.49$, p = .06). Mice administered KA emitted more upward, downward, composite, and chevron call types than control animals (Figure 9 D,E). No main effects of seizure were found for complex ($F(1,121) = 1.35$, $p = .25$), or frequency steps call types ($F(1,121) = .75$, $p = .39$). There were also main effects of sex for upward ($F(1,121) = 13.37$, $p = .00$), and downward ($F(1,121) = 4.49$, $p = .04$), call types, but no main effect for complex ($F(1,121) = 2.89$, $p = .09$), chevron ($F(1,121) = 3.66$, $p = .06$), composite ($F(1,121) = .59$, $p = .44$), or frequency steps call types ($F(1,121) = .013$, $p = .91$). Females emitted more upward and downward call types than males. For treatment, main effects were found for complex ($F(2,121) = 10.75$, $p = .00$), upward ($F(2,121) = 7.10$, $p = .001$), downward ($F(2,121) = 12.44$, $p = .00$), composite ($F(2,121) = 11.78$, $p = .00$), but not for frequency steps ($F(2,121) = 2.16$, $p = .12$), or chevron call types ($F(2,121) = 2.39$, $p = .09$). Mice administered DMSO emitted more complex call types than saline or agomelatine treated mice, whereas agomelatine treated mice emitted more upward call and frequency steps call types than the other treatments (Figure 9 A,C,D). There was a sex by seizure interaction for downward ($F(2,121) = 10.36$, $p = .001$), call

types but not for complex ($F(2,121) = .13$, $p = .72$), upward ($F(2,121) = 2.06$, $p = .15$), chevron ($F(2,121) = 2.45$, $p = .12$), composite ($F(2,121) = 2.50$, $p = .11$), or frequency step ($F(2,121) = 3.77$, $p = .053$) call types. There were sex by treatment interactions for complex ($F(2,121) = 5.76$, $p = .003$), upward ($F(2,121) = 6.86$, $p = .001$), downward ($F(2,121) = 7.25$, $p = .001$), chevron ($F(2,121) = 15.87$, $p = .00$) and composite ($F(2,121) = 4.14$, $p = .02$), but not for frequency steps ($F(2,121) = 2.17$, $p = .12$) call types. There were also seizure by treatment interactions for downward ($F(2,121) = 10.61$, $p = .00$), and composite ($F(2,121) = 3.06$, $p = .05$) call types but not for complex ($F(2,121) = .93$, $p = .40$), upward ($F(2,121) = 2.56$, $p = .08$), chevron ($F(2,121) = .094$, $p = .91$), or frequency steps ($F(2,121) = 2.13$, $p = .12$), call types. A sex by seizure by treatment interaction was found for complex ($F(2,121) = 3.29$, $p = .04$) call types but not for upward ($F(2,121) = .20$, $p = .82$), downward ($F(2,121) = .61$, $p = .55$), chevron ($F(2,121) = 1.05$, $p = .35$), composite ($F(2,121) = .72$, $p = .49$) or frequency steps ($F(2,121) = .25$, $p = .78$) call types.

Due to the interactions between sex and treatment, seizure and treatment, and sex by seizure by treatment, we created a grouping variable and ran a Tukey's HSD post hoc analysis. In order to further clarify the interactions the data was split by sex to isolate variables of interest. When examining males, for the downward call type, saline and agomelatine treated mice emitted more USVs than saline/DMSO, KA/DMSO, and KA/saline treated mice $p > .05$ (Figure 9 B). Additionally, KA/agomelatine mice emitted more USVs than saline/DMSO, KA/DMSO, or KA/saline treated mice $p > .05$ (Figure 9 B). For the composite call type, males administered KA and agomelatine emitted a

greater quantity of calls than saline/DMSO, KA/DMSO, saline/saline, and KA/saline treated mice $p > .05$ (Figure 9 F).

For females, mice administered KA/DMSO emitted more complex USVs than the KA/agomelatine, saline/saline, and KA/saline groups $p > .05$, additionally, saline/DMSO mice emitted more complex USVS than KA/agomelatine and KA/saline treated mice $p > .05$ (Figure 9 A). For the downward call type, mice given KA and DMSO emitted more USVs than saline/agomelatine, saline/DMSO, KA/saline, and saline/saline treated mice, $p > .05$ (Figure 9 B). The KA/agomelatine group also emitted more USVs than the saline/DMSO, saline/saline, and KA/saline groups $p > .05$ (Figure 9 B). Lastly, for the composite call type, female mice administered KA and agomelatine emitted more USVs than saline/agomelatine, and saline/saline mice $p > .05$ (Figure 9 F). Saline/saline mice also emitted fewer USVs than saline/DMSO, and KA/DMSO mice $p > .05$ (Figure 9 F).



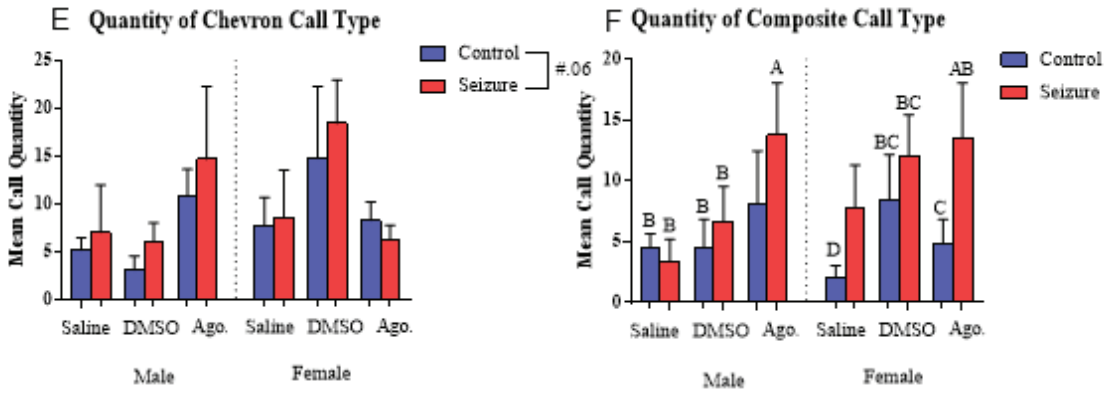


Figure 9. The total quantity of USVs per the complex, downward, frequency steps, upward, chevron, and composite call types per the PD 11 24-hour timepoint. A. Female KA/DMSO mice emitted more complex USVs than KA/ago, KA/saline, and saline/saline mice. Saline/DMSO mice emitted more USVs than KA/ago and KA/saline treated mice. No other effects were found. B. Male mice treated with saline and ago emitted more downward USVs than KA/ago, KA/DMSO, KA/saline, and KA/saline mice. KA/ago mice emitted more USVs than KA/DMSO, saline/DMSO, and KA/saline mice. Female KA/DMSO mice emitted more downward USVs than saline/ago, saline/DMSO, KA/saline, and saline/saline mice. KA/ago mice also emitted more downward USVs than saline/DMSO, KA/saline, and saline/saline mice. C No differences in frequency steps call types were observed per seizure, sex, or treatment groups. D. Seizure animals emitted more upward call types than controls, with no other effects observed. E. Seizure animals trended towards emitting more chevron call types than controls, with no other differences being observed. F. Male KA/ago mice emitted more composite USVs than KA/DMSO, saline/DMSO, KA/saline, and saline/saline mice. Female KA/ago mice emitted more composite USVs than saline/ago, and saline/saline mice. KA/DMSO and saline/DMSO mice also emitted more USVs than saline/saline mice. Bars represent the mean and the error bars represent the standard error of the mean. * = $p < .05$

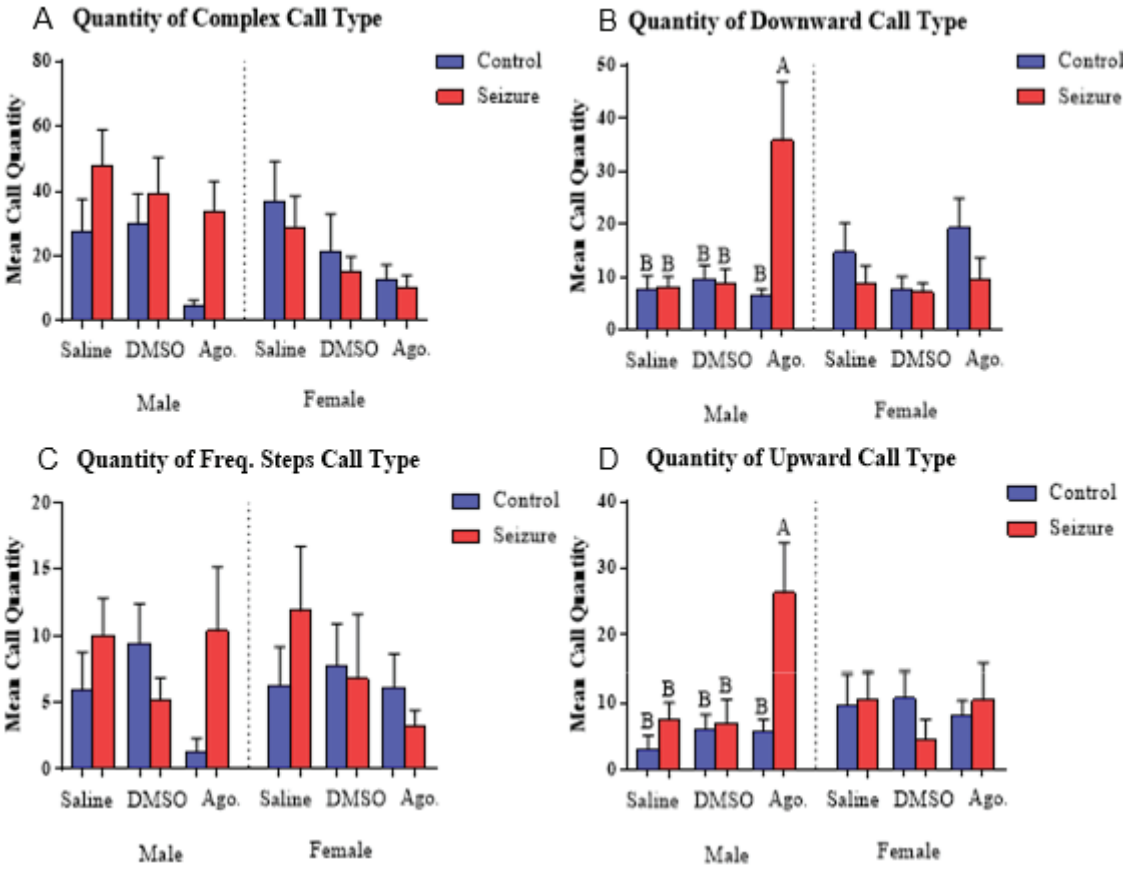
PD 12: 24-hour Timepoint

On PD 12 for the 24-hour timepoint, MANOVA's revealed no main effect of seizure for complex ($F(1,121) = 1.66$, $p = .20$), downward ($F(1,121) = .75$, $p = .08$), chevron ($F(1,121) = 2.20$, $p = .14$), composite ($F(1,121) = 2.23$, $p = .14$), or frequency steps ($F(1,121) = .94$, $p = .33$) call types. However, there was a trend for upward ($F(1,121) = 3.06$, $p = .08$) call types, with KA mice emitting more upward calls than controls (Figure 10 A-F). There was a main effect of sex for composite ($F(1,121) = 4.83$, $p = .03$) call types but not for complex ($F(1,121) = 2.93$, $p = .09$), upward ($F(1,121) = .011$, $p = .92$), downward ($F(1,121) = .37$, $p = .54$), chevron ($F(1,121) = 1.00$, $p = .32$), or frequency steps ($F(1,121) = .00$, $p = .98$) call types. Males were found to emit more

composite call types than females (Figure 10 F). There was also a main effect of treatment for complex ($F(2,121) = 4.10$, $p = .02$), and downward ($F(2,121) = 5.46$, $p = .006$) call types but not for upward ($F(2,121) = 2.44$, $p = .09$), chevron ($F(2,121) = .36$, $p = .70$), composite ($F(2,121) = .14$, $p = .87$), or frequency step ($F(2,121) = 1.03$, $p = .36$) call types. Specifically, saline treated animals emitted more complex calls than agomelatine or DMSO treated mice, whereas the agomelatine groups emitted more downward call types than the other treatments (Figure 10 A,B). There was a sex by seizure interaction for complex ($F(2,121) = 5.10$, $p = .03$), upward ($F(2,121) = 4.80$, $p = .03$), downward ($F(2,121) = 9.13$, $p = .003$), and chevron ($F(2,121) = 8.67$, $p = .004$) call types but not for composite ($F(2,121) = 2.47$, $p = .12$), or frequency step ($F(2,121) = .43$, $p = .51$) call types. There were no sex by treatment interactions for complex ($F(2,121) = .39$, $p = .68$), upward ($F(2,121) = 2.30$, $p = .10$), downward ($F(2,121) = 1.45$, $p = .24$), chevron ($F(2,121) = .92$, $p = .40$), composite ($F(2,121) = .02$, $p = .98$), or frequency steps ($F(2,121) = .12$, $p = .88$) call types. A seizure by treatment interaction was found for upward ($F(2,121) = 3.40$, $p = .04$) call types but not for complex ($F(2,121) = .39$, $p = .69$), downward ($F(2,121) = 1.45$, $p = .11$), chevron ($F(2,121) = .92$, $p = .23$), composite ($F(2,121) = 2.30$, $p = .11$), or frequency steps ($F(2,121) = .12$, $p = .20$) call types. Similarly, there was an interaction between sex, seizure, and treatment for downward ($F(2,121) = 5.87$, $p = .004$) call types but not for complex ($F(2,121) = .21$, $p = .81$), upward ($F(2,121) = .96$, $p = .39$), chevron ($F(2,121) = .92$, $p = .40$), composite ($F(2,121) = .14$, $p = .14$), or frequency step ($F(2,121) = 1.66$, $p = .20$) call types.

Due to the interactions between seizure and treatment, and sex by seizure by treatment, we created a grouping variable and ran a Tukey's HSD post hoc analysis,

again splitting the files by sex to isolate variables of interest. For males, mice administered KA and agomelatine emitted increased USVs for the upward call type relative to saline/agomelatine, KA/DMSO, saline/DMSO, KA/saline, and saline/saline treated mice (Figure 10 D). Similarly, for the downward call type, the KA/agomelatine group emitted increased USVs as compared to the saline/agomelatine, KA/DMSO, saline/DMSO, KA/saline, saline/saline groups (Figure 10 B). For females, there were no significant differences between groups for complex, upward, downward, or chevron call types.



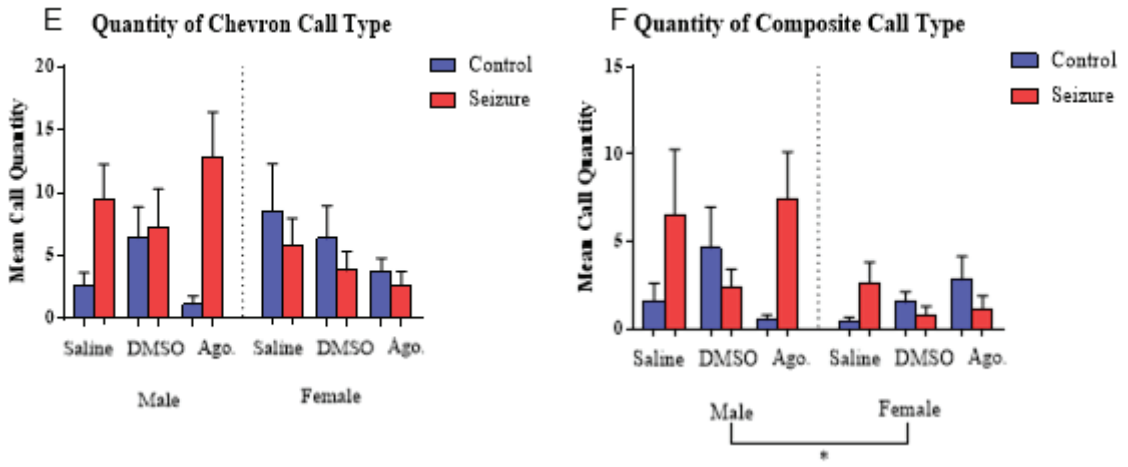


Figure 10. The total quantity of USVs per the complex, downward, frequency steps, upward, chevron, and composite call types per the PD 12 24-hour timepoint. A. There were no significant differences per seizure group, sex or treatment for complex USVs. B. Male mice treated with KA/ago emitted more downward USVs than the saline/ago, KA/DMSO, saline/DMSO, KA/saline, and saline/saline mice, no other differences were observed. C. No differences in frequency steps call types were observed per seizure, sex, or treatment groups. D. Male KA/ago mice emitted more upward call types than saline/saline, KA/DMSO, saline/DMSO, KA/saline, and saline/saline mice, with no other differences being observed. E. No significant differences per seizure group, sex or treatment for chevron USVs were observed. F. Male mice emitted more composite call types than female mice with no additional effects being observed. Bars represent the mean and the error bars represent the standard error of the mean. * = $p < .05$.

Spectral Characteristics Per Call Type: 1-hour Time Point

PD 11: 1-hour Timepoint

Multivariate analysis of Variance MANOVA's were run on PD 11 and PD 12 with seizure and treatment as the independent variables and the mean duration, peak frequency, fundamental frequency, and peak amplitude for complex, upward, downward, chevron, composite, and frequency steps call types as the dependent variables. To clarify any interactions the files were split according to sex. Significant main effects and interactions are discussed below, all other analyses were non-significant and can be found in Tables 1, 3,5, and 7. The mean ± SEM for each call type and group is found in Tables 2,4,6, and 8. The statistics for the PD 11 1-hour timepoint for males and females is contained within Table 1. At the 1-hour time point for male mice, an interaction was

found between seizure and treatment for the peak amplitude of composite call types ($F(1,25) = 3.68$, $p = .04$). For PD 11 females receiving treatment 1-hour post SE, a main effect of seizure was found for the duration of upward call types ($F(1,31) = 6.09$, $p = .02$) and the peak amplitude of composite call types ($F(1,31) = 4.59$, $p = .04$). KA treated animals displayed upward calls of a longer duration than control mice, while also exhibiting calls of a higher peak amplitude than control mice (Table 2). When a Tukey's post hoc test to clarify the interaction between seizure and treatment for males, no difference between groups was observed, $p > .05$.

Table 1. Statistics for the Spectral Characteristics PD 11 1 hr. Timepoint

Characteristics	Call Type	Seizure	Treatment	Interaction
Male Duration				
	Complex	$F(1,25) = .55$ $p = .47$	$F(1,25) = .28$ $p = .76$	$F(1,25) = .24$ $p = .79$
	Upward	$F(1,25) = .21$ $p = .65$	$F(1,25) = .44$ $p = .65$	$F(1,25) = .91$ $p = .42$
	Downward	$F(1,25) = 1.75$ $p = .20$	$F(1,25) = .78$ $p = .47$	$F(1,25) = .62$ $p = .55$
	Chevron	$F(1,25) = 2.07$ $p = .16$	$F(1,25) = .06$ $p = .95$	$F(1,25) = 1.59$ $p = .22$
	Composite	$F(1,25) = .29$ $p = .60$	$F(1,25) = .22$ $p = .80$	$F(1,25) = 1.74$ $p = .20$
	Frequency steps	$F(1,25) = .13$ $p = .73$	$F(1,25) = .12$ $p = .89$	$F(1,25) = .12$ $p = .89$
Male Peak Frequency				
	Complex	$F(1,25) = .027$ $p = .87$	$F(1,25) = 1.87$ $p = .18$	$F(1,25) = 1.34$ $p = .28$
	Upward	$F(1,25) = .47$ $p = .50$	$F(1,25) = 1.97$ $p = .16$	$F(1,25) = .37$ $p = .70$
	Downward	$F(1,25) = .14$ $p = .72$	$F(1,25) = 2.59$ $p = .10$	$F(1,25) = .13$ $p = .88$
	Chevron	$F(1,25) = 3.16$ $p = .09$	$F(1,25) = .46$ $p = .64$	$F(1,25) = 2.42$ $p = .11$
	Composite	$F(1,25) = .41$ $p = .53$	$F(1,25) = .51$ $p = .88$	$F(1,25) = .44$ $p = .65$
	Frequency steps	$F(1,25) = .08$ $p = .78$	$F(1,25) = .36$ $p = .70$	$F(1,25) = .33$ $p = .72$
Male Fundamental Frequency				
	Complex	$F(1,25) = .013$ $p = .91$	$F(1,25) = 2.62$ $p = .09$	$F(1,25) = 1.06$ $p = .36$

(Continued)

Characteristics	Call Type	Seizure	Treatment	Interaction
	Upward	$F(1,25) = 1.88$ $p = .18$	$F(1,25) = 1.35$ $p = .27$	$F(1,25) = .62$ $p = .55$
	Downward	$F(1,25) = .002$ $p = .97$	$F(1,25) = 2.90$ $p = .07$	$F(1,25) = 1.59$ $p = .22$
	Chevron	$F(1,25) = .003$ $p = .96$	$F(1,25) = .42$ $p = .66$	$F(1,25) = 1.85$ $p = .17$
	Composite	$F(1,25) = .46$ $p = .50$	$F(1,25) = 1.73$ $p = .20$	$F(1,25) = .36$ $p = .70$
	Frequency steps	$F(1,25) = .15$ $p = .82$	$F(1,25) = 1.89$ $p = .17$	$F(1,25) = 1.89$ $p = .17$
Male Amplitude				
	Complex	$F(1,25) = .018$ $p = .89$	$F(1,25) = .13$ $p = .88$	$F(1,25) = 2.23$ $p = .13$
	Upward	$F(1,25) = .31$ $p = .58$	$F(1,25) = .29$ $p = .75$	$F(1,25) = .74$ $p = .49$
	Downward	$F(1,25) = .95$ $p = .78$	$F(1,25) = .17$ $p = .85$	$F(1,25) = 2.68$ $p = .09$
	Chevron	$F(1,25) = .51$ $p = .48$	$F(1,25) = 1.15$ $p = .33$	$F(1,25) = 2.85$ $p = .08$
	Composite	$F(1,25) = .17$ $p = .69$	$F(1,25) = .27$ $p = .77$	$F(1,25) = 3.68$ $p = .04$
	Frequency steps	$F(1,25) = .01$ $p = .94$	$F(1,25) = .15$ $p = .86$	$F(1,25) = 2.40$ $p = .11$
Female Duration				
	Complex	$F(1,31) = .049$ $p = .83$	$F(1,31) = .95$ $p = .40$	$F(1,31) = 1.18$ $p = .32$
	Upward	$F(1,31) = 6.09$ $p = .02$	$F(1,31) = .79$ $p = .46$	$F(1,31) = .50$ $p = .61$
	Downward	$F(1,31) = .12$ $p = .73$	$F(1,31) = .22$ $p = .80$	$F(1,31) = .26$ $p = .78$
	Chevron	$F(1,31) = .25$ $p = .62$	$F(1,31) = 2.70$ $p = .08$	$F(1,31) = 2.26$ $p = .12$
	Composite	$F(1,31) = 1.02$ $p = .32$	$F(1,31) = 1.37$ $p = .27$	$F(1,31) = 2.62$ $p = .09$
	Frequency steps	$F(1,31) = .00$ $p = .99$	$F(1,31) = .35$ $p = .71$	$F(1,31) = 1.12$ $p = .34$
Female Peak Frequency				
	Complex	$F(1,31) = 2.52$ $p = .12$	$F(1,31) = .69$ $p = .51$	$F(1,31) = .34$ $p = .72$
	Upward	$F(1,31) = 1.21$ $p = .28$	$F(1,31) = .52$ $p = .60$	$F(1,31) = .03$ $p = .97$
	Downward	$F(1,31) = .24$ $p = .63$	$F(1,31) = 2.96$ $p = .07$	$F(1,31) = .14$ $p = .87$
	Chevron	$F(1,31) = .62$ $p = .44$	$F(1,31) = .49$ $p = .62$	$F(1,31) = .27$ $p = .76$
	Composite	$F(1,31) = 3.47$ $p = .07$	$F(1,31) = .48$ $p = .62$	$F(1,31) = .41$ $p = .67$
	Frequency steps	$F(1,31) = .28$ $p = .60$	$F(1,31) = 1.51$ $p = .24$	$F(1,31) = .72$ $p = .49$
Female Fundamental Frequency				

(Continued)

Characteristics	Call Type	Seizure	Treatment	Interaction
Female Amplitude	Complex	$F(1,31) = .49$ $p = .49$	$F(1,31) = .17$ $p = .84$	$F(1,31) = .86$ $p = .43$
	Upward	$F(1,31) = 3.33$ $p = .08$	$F(1,31) = 1.62$ $p = .21$	$F(1,31) = .13$ $p = .88$
	Downward	$F(1,31) = 1.45$ $p = .24$	$F(1,31) = .28$ $p = .76$	$F(1,31) = .56$ $p = .58$
	Chevron	$F(1,31) = 1.77$ $p = .19$	$F(1,31) = .67$ $p = .52$	$F(1,31) = 1.06$ $p = .36$
	Composite	$F(1,31) = 2.43$ $p = .13$	$F(1,31) = .25$ $p = .78$	$F(1,31) = .42$ $p = .66$
	Frequency steps	$F(1,31) = .27$ $p = .61$	$F(1,31) = .27$ $p = .76$	$F(1,31) = 1.34$ $p = .28$
	Complex	$F(1,31) = 3.01$ $p = .09$	$F(1,31) = 2.62$ $p = .09$	$F(1,31) = 1.06$ $p = .36$
	Upward	$F(1,31) = 3.79$ $p = .06$	$F(1,31) = 1.35$ $p = .27$	$F(1,31) = .62$ $p = .55$
	Downward	$F(1,31) = 1.82$ $p = .19$	$F(1,31) = 2.90$ $p = .07$	$F(1,31) = 1.59$ $p = .22$
	Chevron	$F(1,31) = .05$ $p = .83$	$F(1,31) = .42$ $p = .66$	$F(1,31) = 1.85$ $p = .17$
	Composite	$F(1,31) = 4.59$ $p = .04$	$F(1,31) = 1.73$ $p = .20$	$F(1,31) = .36$ $p = .70$
	Frequency steps	$F(1,31) = 2.51$ $p = .12$	$F(1,31) = 1.89$ $p = .17$	$F(1,31) = 1.03$ $p = .37$

Table 2. Mean and SEM for the Spectral Characteristics of USVs at PD 11 1 hr. Timepoint

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
Male Duration							
	Complex	.022± .0049	.021± .0026	.027± .0045	.02± .002	.022± .0038	.018± .0023
	Upward	.010± .002	.0081± .0008	.0089±. 001	.0088± .0009	.01± .0016	.0079± .0017
	Downward	.017± .0037	.012± 0009	.1032±. 0018	.0097± .0013	.016± .0034	.0084± .002
	Chevron	.014± .003	.0134 ± .0015	.016± .0014	.013± .0021	.013± .0019	.013± .0022
	Composite	.026± .008	.017± .005	.012± .005	.025± .0096	.0199± .0032	.037± .013
	Frequency steps	.027± .0067	.021± .003	.027± .0047	.022± .0035	.024± .005	.018± .0047
Male Peak Frequency							

(Continued)

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
Male Fundamental Frequency	Complex	80082 ±2408	84005 ±1766	84917 ±2284	85156 ±1390	81472± 1968	83982± 2067
	Upward	84715 ±2078	85658 ±1348	86049 ±1661	85974 ±2248	85247± 1736	85276± 3267
	Downward	82594 ±1368	81212 ±1543	83592 ±1398	86215 ±2845	81360± 1777	82637± 2131
	Chevron	86034 ±2542	83863 ±1703	83965 ±1863	84663 ±2166	80028± 1584	85392± 2445
	Composite	73852 ±2945	79129 ±4815	76311 ±2120	74861 ±4663	80937± 1332	77350± 7007
	Frequency steps	77414 ±3362	77311 ±2593	79292 ±2095	80995 ±2927	78934± 2067	79703± 2837
Male Amplitude	Complex	62250± 1589	58067± 2541	61487± 3458	64272± 4267	62622± 3044	3243± 10255
	Upward	62661± 2825	3123±9 369	64726± 2445	58026± 7173	60876± 1600	4249± 12748
	Downward	60629± 2210	55172± 2180	60281± 2163	56761± 3088	54487± 3392	3729± 11188
	Chevron	6821±3 671	63061± 3894	67576± 3911	65963± 4717	66402± 2674	2779± 8338
	Composite	49225± 5878	46073± 3378	36341± 9078	40314± 6150	46550± 4377	4171± 9326
	Frequency steps	51383± 2765	50879± 5059	51359± 4000	58299± 2446	56657± 4394	4666± 13198
Female Duration	Complex	-58.98± 1.83	-63.40± 1.16	-60.99± 1.34	-62.19± 1.35	-62.68± 1.49	-63.02± 1.18
	Upward	-60.00± .98	-62.70± 1.78	-61.99± .83	-64.21± 1.54	-62.06± 1.12	-64.3± 1.57
	Downward	-60.78± 1.07	-63.60± 1.06	-62.34± 1.03	-63.47± 1.25	-63.90± 1.23	-64.07± 1.38
	Chevron	-58.12± 1.22	-63.08± 1.35	-61.04± 1.21	-63.51± 1.38	-59.66± .87	-63.44± .92
	Composite	-55.70± 2.03	-61.89± 1.91	-62.31± 2.25	-56.47± 4.64	-59.66± .87	-56.80± 3.66
	Frequency steps	-57.73± 2.17	-63.43± 1.43	-60.73± 1.44	-60.78± 1.85	-62.48± 1.27	-62.71± 1.68
	Complex	.025± .0038	.018± .0015	.02± .005	.02± .0046	.026± .0041	.025± .0067
	Upward	.012± .0017	.009± .0013	.012± .0016	.0088± .0012	.012± .0013	.0069± .0009
	Downward	.015± .0023	.012± .0024	.013± .0023	.012± .0024	.014± .003	.013± .0034

(Continued)

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
	Chevron	.0094± .0017	.012± .0017	.013± .0022	0.11± .0016	.016± .0026	.012± .0014
	Composite	.037± .0072	.016± .0039	.036± .0062	.043± .011	.026± .0056	.024± .0075
	Frequency steps	.028± .0022	.022± .0039	.017± .0035	.028± .0050	.024± .0064	.019± .0043
Female Peak Frequency	Complex	83444± 2700	86382± 1872	85169± 2444	83590± 2105	80225± 1732	84315± 1455
	Upward	85948± 2281	87818± 1628	86451± 1880	89840± 2658	83956± 1923	88223± 1827
	Downward	85535± 1955	88367± 1416	84286± 1931	87325± 2215	82919± 697	84000± 1307
	Chevron	85722± 3561	85488± 2218	84175± 2129	80410± 4124	83695± 2084	81170± 2800
	Composite	73566± 2951	8415±3 044	73799± 2298	76130± 2024	74882± 1590	75167± 3928
	Frequency steps	78516± 3207	82647± 2331	83296± 4297	75350± 4214	76951± 1839	79400± 3762
Female Fundamental Frequency	Complex	56730± 2395	58699± 2148	54385± 5815	51197± 6998	60987± 3278	60036± 4160
	Upward	59760± 2995	52477± 2894	57727± 2947	66012± 5985	63386± 1987	57816± 3140
	Downward	61056± 2396	58675± 3722	60920± 2337	52812± 5493	57954± 2675	60317± 2905
	Chevron	73095± 4731	57213± 5650	63258± 2623	66024± 3953	60334± 4298	61892± 5238
	Composite	54155± 2226	48147± 4824	49859± 4348	42881± 4675	49389± 3593	46214± 7448
	Frequency steps	51590± 3226	59270± 6324	54554± 6455	46583± 2604	56309± 3341	47223± 8086
Female Amplitude	Complex	-62.63± 1.18	-63.9± .75	-61.04± 1.44	-63.54± 2.03	-58.99± 1.24	-63.67± 1.70
	Upward	-62.74± .72	-63.31± .55	-61.79± 1.14	-61.61± 1.66	-59.49± 1.02	-63.09± 1.07
	Downward	-64.13± .57	-63.27± .54	-61.72± .95	-63.88± 1.08	-60.29± .85	-62.61± 1.47
	Chevron	-63.65± 1.19	-60.90± .20	-60.06± 1.15	-61.72± 2.02	-59.62± 1.61	-61.42± 2.38
	Composite	-60.03 ±1.44	-61.57± .78	-58.03± 1.49	-61.17± 1.68	-56.23± 1.88	-58.9± 1.60
	Frequency steps	-61.18± .94	-62.15± .90	-60.74± 1.25	-60.82± 2.01	-57.62± 2.17	-61.49± 1.96

PD 12: 1-hour Timepoint

The statistics for the PD 12 1-hour timepoint for males and females is contained within Table 3. On PD 12 male mice receiving treatment 1 hour after SE, a main effect of seizure was found for the duration of frequency steps call types ($F(1,31) = 4.96$, $p = .03$) and downward call types for fundamental frequency ($F(1,31) = 4.46$, $p = .04$). There was also a main effect of treatment for the duration of frequency steps call types ($F(1,31) = 4.04$, $p = .03$), the peak frequency of frequency steps call types. *(*$F(1,31) = 3.45$, $p = .04$), and the peak amplitude of downward call type ($F(1,31) = 3.45$, $p = .04$). Male KA treated mice emitted downward calls of a higher fundamental frequency than control mice. Additionally, animals in the DMSO group emitted frequency steps calls of a shorter duration than agomelatine or saline treated animals (Table 4). However, saline treated mice emitted frequency steps of a lower duration than DMSO or agomelatine treated mice. Saline treated mice also emitted calls of an increased peak amplitude relative to the other treatments (Table 4). A significant interaction between seizure and treatment was found for the duration of frequency steps call types ($F(1,31) = 5.58$, $p = .009$). A Tukey's post hoc analysis was run to clarify the interaction between seizure and treatment, but no significant differences were found for the duration of frequency steps calls between groups, $p > .05$.

For PD 12 females receiving treatment 1 hour after SE, a main effect of seizure was observed for the fundamental frequency of frequency step call types ($F(1,14) = 5.59$, $p = .03$). There was also a main effect of treatment for the peak frequency of frequency steps call types ($F(1,14) = 5.68$, $p = .02$) and for the fundamental frequency of frequency steps call types ($F(1,14) = .85$, $p = .004$). Saline treated mice emitted calls of a higher

peak frequency than DMSO or agomelatine treated mice, $p > .05$ (Table 4). Additionally, interactions between seizure and treatment were found for the peak frequency of composite call types ($F(1,14) = 4.19$, $p = .04$), and the fundamental frequency of frequency steps call types ($F(1,14) = 5.61$, $p = .02$). A post hoc test was again run, however no differences were detected between groups for the peak frequency of composite call types, or the fundamental frequency of frequency steps call types (Table 4).

Table 3. Statistics for the Spectral Characteristics PD 12 1 hr. Timepoint

Characteristics	Call Type	Seizure	Treatment	Interaction
Male Duration				
	Complex	$F(1,31) = 2.48$ $p = .13$	$F(1,31) = .76$ $p = .47$	$F(1,31) = 1.33$ $p = .28$
	Upward	$F(1,31) = 1.74$ $p = .20$	$F(1,31) = .70$ $p = .51$	$F(1,31) = .13$ $p = .88$
	Downward	$F(1,31) = .02$ $p = .90$	$F(1,31) = 1.67$ $p = .21$	$F(1,31) = .47$ $p = .63$
	Chevron	$F(1,31) = 2.81$ $p = .10$	$F(1,31) = .54$ $p = .59$	$F(1,31) = 1.20$ $p = .32$
	Composite	$F(1,31) = 3.18$ $p = .09$	$F(1,31) = .88$ $p = .43$	$F(1,31) = .22$ $p = .81$
	Frequency steps	$F(1,31) = 4.96$ $p = .03$	$F(1,31) = 4.04$ $p = .03$	$F(1,31) = 5.58$ $p = .009$
Male Peak Frequency				
	Complex	$F(1,31) = .94$ $p = .34$	$F(1,31) = 1.03$ $p = .37$	$F(1,31) = 1.08$ $p = .35$
	Upward	$F(1,31) = 1.11$ $p = .30$	$F(1,31) = .45$ $p = .64$	$F(1,31) = .10$ $p = .91$
	Downward	$F(1,31) = .16$ $p = .69$	$F(1,31) = .83$ $p = .45$	$F(1,31) = .58$ $p = .57$
	Chevron	$F(1,31) = .06$ $p = .80$	$F(1,31) = 1.92$ $p = .16$	$F(1,31) = .99$ $p = .38$
	Composite	$F(1,31) = .41$ $p = .53$	$F(1,31) = 2.89$ $p = .07$	$F(1,31) = .18$ $p = .84$
	Frequency steps	$F(1,31) = .08$ $p = .78$	$F(1,31) = 3.45$ $p = .04$	$F(1,31) = .08$ $p = .93$
Male Fundamental Frequency				
	Complex	$F(1,31) = .01$ $p = .94$	$F(1,31) = .41$ $p = .67$	$F(1,31) = .75$ $p = .48$
	Upward	$F(1,31) = .00$ $p = .99$	$F(1,31) = .08$ $p = .93$	$F(1,31) = .32$ $p = .73$

(Continued)

Characteristics	Call Type	Seizure	Treatment	Interaction
	Downward	$F(1,31) = 4.46$ $p = .04$	$F(1,31) = .80$ $p = .46$	$F(1,31) = .18$ $p = .84$
	Chevron	$F(1,31) = .27$ $p = .61$	$F(1,31) = .39$ $p = .68$	$F(1,31) = 2.06$ $p = .14$
	Composite	$F(1,31) = .03$ $p = .87$	$F(1,31) = .04$ $p = .96$	$F(1,31) = .25$ $p = .78$
	Frequency steps	$F(1,31) = .00$ $p = .99$	$F(1,31) = 1.11$ $p = .34$	$F(1,31) = .02$ $p = .98$
Male Amplitude				
	Complex	$F(1,31) = .60$ $p = .44$	$F(1,31) = .33$ $p = .73$	$F(1,31) = .35$ $p = .70$
	Upward	$F(1,31) = 2.81$ $p = .10$	$F(1,31) = .51$ $p = .61$	$F(1,31) = 1.86$ $p = .17$
	Downward	$F(1,31) = 1.13$ $p = .30$	$F(1,31) = 3.45$ $p = .04$	$F(1,31) = .26$ $p = .78$
	Chevron	$F(1,31) = 3.33$ $p = .08$	$F(1,31) = .65$ $p = .53$	$F(1,31) = .87$ $p = .43$
	Composite	$F(1,31) = .37$ $p = .55$	$F(1,31) = 1.06$ $p = .36$	$F(1,31) = .10$ $p = .90$
	Frequency steps	$F(1,31) = .03$ $p = .88$	$F(1,31) = 1.65$ $p = .21$	$F(1,31) = 1.65$ $p = .21$
Female Duration				
	Complex	$F(1,14) = .60$ $p = .45$	$F(1,14) = 2.15$ $p = .15$	$F(1,14) = .69$ $p = .52$
	Upward	$F(1,14) = 3.84$ $p = .07$	$F(1,14) = .75$ $p = .49$	$F(1,14) = 2.21$ $p = .15$
	Downward	$F(1,14) = .19$ $p = .67$	$F(1,14) = .17$ $p = .85$	$F(1,14) = .17$ $p = .85$
	Chevron	$F(1,14) = 3.07$ $p = .10$	$F(1,14) = 1.87$ $p = .19$	$F(1,14) = 1.43$ $p = .27$
	Composite	$F(1,14) = 1.79$ $p = .20$	$F(1,14) = .87$ $p = .44$	$F(1,14) = .38$ $p = .69$
	Frequency steps	$F(1,14) = 1.10$ $p = .31$	$F(1,14) = 1.03$ $p = .38$	$F(1,14) = 1.12$ $p = .36$
Female Peak Frequency				
	Complex	$F(1,14) = .28$ $p = .61$	$F(1,14) = 1.66$ $p = .23$	$F(1,14) = 2.01$ $p = .17$
	Upward	$F(1,14) = 2.72$ $p = .12$	$F(1,14) = 1.56$ $p = .25$	$F(1,14) = 1.84$ $p = .20$
	Downward	$F(1,14) = .33$ $p = .58$	$F(1,31) = .03$ $p = .97$	$F(1,14) = .86$ $p = .45$
	Chevron	$F(1,14) = .09$ $p = .77$	$F(1,31) = .47$ $p = .63$	$F(1,14) = .10$ $p = .91$
	Composite	$F(1,14) = 2.23$ $p = .16$	$F(1,14) = 1.11$ $p = .36$	$F(1,14) = 4.19$ $p = .04$
	Frequency steps	$F(1,14) = 1.18$ $p = .30$	$F(1,14) = 5.68$ $p = .02$	$F(1,14) = 1.18$ $p = .34$
Female Fundamental Frequency				

(Continued)

Characteristics	Call Type	Seizure	Treatment	Interaction
Female Amplitude	Complex	$F(1,14) = .13$ $p = .72$	$F(1,14) = 1.27$ $p = .31$	$F(1,14) = .032$ $p = .97$
	Upward	$F(1,14) = .23$ $p = .64$	$F(1,14) = .35$ $p = .17$	$F(1,14) = .20$ $p = .82$
	Downward	$F(1,14) = .27$ $p = .61$	$F(1,14) = 2.15$ $p = .15$	$F(1,14) = .26$ $p = .78$
	Chevron	$F(1,14) = 1.15$ $p = .30$	$F(1,31) = 2.61$ $p = .11$	$F(1,14) = .60$ $p = .57$
	Composite	$F(1,14) = .51$ $p = .49$	$F(1,14) = 2.76$ $p = .10$	$F(1,14) = .69$ $p = .52$
	Frequency steps	$F(1,14) = 5.59$ $p = .03$	$F(1,14) = .85$ $p = .004$	$F(1,14) = 5.61$ $p = .02$
	Complex	$F(1,14) = .12$ $p = .74$	$F(1,14) = .20$ $p = .83$	$F(1,14) = .28$ $p = .76$
	Upward	$F(1,14) = .36$ $p = .56$	$F(1,14) = .75$ $p = .49$	$F(1,14) = .00$ $p = .99$
	Downward	$F(1,14) = .74$ $p = .40$	$F(1,14) = .29$ $p = .76$	$F(1,14) = .24$ $p = .79$
	Chevron	$F(1,14) = .46$ $p = .51$	$F(1,31) = .24$ $p = .79$	$F(1,14) = .13$ $p = .88$
	Composite	$F(1,14) = .69$ $p = .42$	$F(1,14) = .06$ $p = .95$	$F(1,14) = .28$ $p = .76$
	Frequency steps	$F(1,14) = .004$ $p = .95$	$F(1,14) = .79$ $p = .48$	$F(1,14) = .014$ $p = .99$

Table 4. Mean and SEM for the Spectral Characteristics of USVs at PD 12 1 hr. Timepoint

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
Male Duration							
	Complex	.026± .0037	.023± .004	.034± .0046	.023± .004	.023± .003	.018± .0031
	Upward	.009± .0010	.008± .0009	.0097± .0014	.007± .0014	.0081± .0017	.0081± .0008
	Downward	.019± .002	.014± .0031	.019± .0031	.014± .0022	.011± .0017	.012± .0022
	Chevron	.017± .0035	.011± .001	.015± .0014	.014± .002	.011± .0020	.011± .001
	Composite	.037± .0066	.024± .007	.033± .0075	.024± .009	.028± .0073	.019± .006
	Frequency steps	.021± .0032	.023± .003	.041± .0073	.015± .004	.016± .0024	.015± .003
Male Peak Frequency							
	Complex	84124± 1381	83620± 1032	84406± 1952	87219± 1635	83143± 1339	87544± 1057

(Continued)

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
	Upward	88940± 1511	86349± 3049	86993± 1834	91830± 2635	88009± 2140	93759± 1336
	Downward	82713± 1116	82758± 1430	84939± 1277	86406± 2385	85483± 2638	85611± 1316
	Chevron	83750± 1566	81646± 2067	87409± 1960	85727± 978	83708± 2848	89426± 1584
	Composite	67907± 5691	69971± 3135	74609± 3837	79257± 4421	72232± 3692	75497± 5120
	Frequency steps	78372± 2306	76807± 2761	76984± 3959	82120± 3492	79313± 2641	86276± 2570
Male Fundamental Frequency							
	Complex	56271± 3180	56292± 2023	57682± 3288	65804± 2313	52583± 4030	61310± 4153
	Upward	53076± 3679	58244± 5770	54560± 2872	61343± 3454	58536± 4209	58724± 3614
	Downward	56797± 1809	53807± 2692	59315± 4551	61587± 4693	55469± 3287	51820± 2133
	Chevron	68950± 3681	59598± 4861	59423± 8357	69328± 4200	68020± 4931	58745± 6695
	Composite	39776± 2790	36392± 6540	41285± 7433	34731± 9678	36084± 5036	39165± 4416
	Frequency steps	39259± 4637	44366± 2502	45723± 5205	44995± 6075	38804± 5322	38069± 5120
Male Amplitude							
	Complex	-64.0± 1.27	-63.68± 1.14	-63.97± 1.27	-64.45± 1.49	-65.72± 1.54	-64.87± .67
	Upward	-62.62± 1.51	-64.01± .76	-62.62± 1.51	-65.11± 1.41	-64.55± 1.47	-64.54± 1.09
	Downward	-62.45± 2.34	-63.78± 1.02	-62.45± 2.34	-65.48± 1.44	-64.56± 1.47	-64.77± .91
	Chevron	-63.51± 1.50	-63.15± 1.12	-63.51± 1.50	-66.00± 1.80	-63.35± 1.98	-64.23± .94
	Composite	-62.23± 1.83	-60.53± 2.40	-62.23± 1.83	-62.54± 2.22	-59.70± 2.21	-62.83± 2.4
	Frequency steps	-62.50± 1.77	-61.62± 1.60	-62.46± 1.77	-62.56± 1.20	-63.49± 2.44	-63.10± 1.57
Female Duration							
	Complex	.022± .004	.027± .0068	.032± .0030	.033± .0052	.033± .0039	.031± .0049
	Upward	.007± .0016	.009± .001	.0097± .0001	.010± .0017	.011± .0015	.009± .0007
	Downward	.012± .0027	.015± .0027	.018± .0032	.015± .0040	.022± .0053	.016± .0036
	Chevron	.011± .0015	.015± .0045	.016± .002	.017± .0050	.018± .0023	.014± .001

(Continued)

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
	Composite	.033± .012	.022± .0032	.045± .012	.044± .0400	.036± .011	.036± .011
	Frequency steps	.02± .0032	.023± .0032	.038± .0054	.024± .0038	.030± .0035	.027± .008
Female Peak Frequency	Complex	84297± 3891	85041± 1943	84969± 1611	85925± 1197	81709± 2541	84044± 1685
	Upward	85474± 1997	88516± 2250	86285± 2143	86895± 3528	85986± 3346	87007± 1840
	Downward	85189± 2831	83779± 2197	82965± 1724	83985± 3528	81660± 2623	82486± 1630
	Chevron	87409± 1960	83373± 3084	86513± 994	86239± 1707	79863± 4324	87099± 1817
	Composite	74609± 3837	74946± 4803	69862± 7729	59600± 2500	79862± 1894	67608± 6782
	Frequency steps	76984± 3959	76862± 4341	75937± 2189	79631± 2922	80054± 3021	77246± 3247
Female Fundamental Frequency							
	Complex	61618± 3166	58737± 3862	57012± 4475	64341± 3959	57701± 4564	57552± 4623
	Upward	57669± 5729	52465± 5358	57963± 4475	62665± 3289	52212± 3885	52287± 7010
	Downward	62749± 4412	50654± 5341	57963± 3586	64035± 4546	52101± 4925	50347± 5324
	Chevron	67984± 5534	62505± 6404	62144± 5302	74970± 1945	58856± 4538	62124± 5864
	Composite	37566± 5327	28762± 6391	48786± 1881	55950± 1250	30505± 9825	27705± 7560
	Frequency steps	47641± 5843	38383± 3153	42173± 5212	45490± 11578	45244± 6256	42725± 5736
Female Amplitude							
	Complex	-63.09± 1.55	-64.36± 1.46	-64.64± 1.45	-63.06± 1.85	-61.19± 4.09	-65.72± 1.54
	Upward	-65.65± .94	-63.75± 2.35	-63.49± 1.30	-62.91± 1.96	-62.18± 2.22	-64.55± 1.18
	Downward	-65.63± 1.03	-63.86± 2.77	-63.59± 1.38	-62.60± 3.39	-60.20± 2.32	-64.56± 1.47
	Chevron	-64.61± 1.30	-65.68± 2.18	-63.00± 1.30	-59.36± 3.83	-59.30± 3.94	-63.65± 1.98
	Composite	-60.22± 2.38	-65.37± 2.35	-62.79± 1.95	-52.90± 10.88	-62.50± 2.48	- 65.60± 1.32
	Frequency steps	-63.67± 1.09	-64.14± 2.60	-60.96± 1.54	-61.49± 3.46	-60.80± 3.37	-63.49± 2.44

PD 11: 24-hour Timepoint

Multivariate analysis of Variance MANOVA's were run on PD 11 and PD 12 for the 24-hour time-points. All of the statistics for the PD 11 24-hour timepoint for males and females is contained within Table 5. On PD 11 for males, a main effect of seizure was observed for the duration of frequency steps call types ($F(1,158) = 9.26$, $p = .003$), the peak frequency of complex call types ($F(1,158) = 4.40$, $p = .04$), the peak frequency of composite call types ($F(1,158) = 5.69$, $p = .02$), the peak frequency of frequency steps ($F(1,158) = 10.88$, $p = .001$), the peak frequency of upward call types ($F(1,158) = 9.13$, $p = .003$, and the peak amplitude for frequency steps call types ($F(1,158) = 9.41$, $p = .003$). KA treated mice emitted frequency step call types of a longer duration, a lower peak frequency of complex, composite and frequency steps call types, as well as a higher amplitude of frequency steps call types relative to controls (Table 6). Main effects of treatments were observed for the duration of complex calls ($F(1,158) = 15.61$, $p = .00$), and composite calls ($F(1,158) = 14.69$, $p = .00$). There was also a main effect of treatment for the peak frequency of complex calls ($F(1,158) = 4.83$, $p = .009$), composite calls ($F(1,25) = 15.89$, $p = .00$), frequency steps call types ($F(1,158) = 4.44$, $p = .013$), and for the fundamental frequency of upward call types ($F(1,158) = 6.94$, $p = .001$), downward call types ($F(1,25) = 3.53$, $p = .03$), and composite call types ($F(1,158) = 6.37$, $p = .002$). Saline treated mice emitted complex, composite, and frequency steps call types of a lower peak frequency than agomelatine or DMSO treated animals (Table 6). Saline injected mice also emitted upward call types of a lower fundamental frequency than the other groups, whereas agomelatine treated mice emitted downward call types of a higher fundamental frequency (Table 6). Lastly, for peak amplitude, there was a main

effect of treatment for complex ($F(1,158) = 12.04$, $p = .00$), downward ($F(1,158) = 6.79$, $p = .001$), and chevron ($F(1,25) = 5.88$, $p = .003$) call types. Additionally, interactions between seizure and treatment were found for the duration of chevron call types ($F(1,158) = 4.36$, $p = .01$) and the peak frequency of upward ($F(1,158) = 4.84$ $p = .009$ downward ($F(1,158) = 4.31$, $p = .02$), and chevron call types ($F(1,158) = 7.31$, $p = .001$), as well as for the fundamental frequency of composite call types and the peak amplitude of downward ($F(1,158) = 5.17$, $p = .007$), and chevron ($F(1,158) = 7.08$, $p = .001$) call types.

Upon running a Tukey's HSD post hoc analysis to assess the interactions, we found that for the upward call type males administered KA and saline emitted upward calls of a longer duration than males administered saline and DMSO, additionally, KA/DMSO mice also had upward calls of a longer duration than saline/saline mice, $p > .05$ (Table 6). There was no significant difference between the duration of downward call types between groups when assessed with the Tukey's post hoc, $p < .05$. When assessing the duration of the chevron call type, male mice administered KA/saline exhibited calls of a longer duration than saline/saline mice (Table 6). Differences in the fundamental frequency of composite call types were found between groups, with saline/DMSO mice emitting calls of a higher fundamental frequency than saline agomelatine mice $p > .05$. Saline agomelatine mice also emitted a higher fundamental frequency than saline/saline mice $p > .05$ (Table 6). When assessing the amplitude of downward call types, mice administered KA/agomelatine emitted calls of a higher peak amplitude than saline/saline and saline/DMSO mice, $p > .05$ (Table 6).

For females on PD 11 receiving treatment 24 hours post SE a main effect of

seizure was observed for the duration of upward ($F(1,157) = 5.62$, $p = .02$), downward ($F(1,157) = 4.22$, $p = .04$), chevron ($F(1,157) = 4.72$, $p = .03$), and composite ($F(1,157) = 7.81$, $p = .006$) call types. Control mice emitted upward and chevron calls of a longer duration than KA mice, but downward and composite calls of a shorter duration than KA mice (Table 6). A main effect for seizure was also found for the peak frequency of complex ($F(1,157) = 11.37$, $p = .001$) and frequency steps call types ($F(1,157) = 11.85$, $p = .001$), as well as for the peak amplitude of composite ($F(1,157) = 8.77$, $p = .004$), and frequency steps ($F(1,157) = 10.44$, $p = .002$) call types. KA mice emitted frequency steps calls of a lower peak frequency steps than control mice (Table 6). There was a main effect of treatment for the duration of upward ($F(1,157) = 7.62$, $p = .001$) call types and for the peak frequency of downward ($F(1,157) = 5.99$, $p = .003$), and chevron ($F(1,157) = 3.59$, $p = .03$) call types. Saline treated mice emitted upward calls of a shorter duration than agomelatine or DMSO treated mice (Table 6). A main effect of treatment was also found for the fundamental frequency of complex ($F(1,157) = 12.47$, $p = .00$), upward ($F(1,157) = 4.95$, $p = .00$), downward ($F(1,157) = 16.38$, $p = .00$), chevron ($F(1,157) = 5.01$, $p = .008$), composite ($F(1,157) = 4.83$, $p = .009$), and frequency steps ($F(1,157) = 3.28$, $p = .04$) call types. Saline treated females emitted complex calls of a higher fundamental frequency and frequency steps calls of a lower fundamental frequency than the other treatments, whereas DMSO treated mice emitted upward and chevron calls of a lower fundamental frequency when compared to saline and agomelatine treated animals (Table 6). Similarly, a main effect of treatment was found for the peak amplitude of upward ($F(1,157) = 7.35$, $p = .001$), downward $F(1,157) = 9.56$, $p = .00$), and frequency steps ($F(1,157) = 10.44$, $p = .002$) call types. Seizure by treatment interactions were

observed for the peak frequency of complex ($F(1,157) = 5.62$, $p = .004$), downward ($F(1,157) = 9.56$, $p = .00$), chevron ($F(1,157) = 3.59$, $p = .03$), and frequency steps ($F(1,157) = 5.09$, $p = .007$) call types. There was also an interaction for the fundamental frequency of downward ($F(1,157) = 16.38$, $p = .00$) and composite ($F(1,157) = 4.83$, $p = .009$) call types as well as for the amplitude of complex ($F(1,157) = 3.80$, $p = .03$), upward ($F(1,157) = 13.88$, $p = .00$), downward ($F(1,157) = 3.59$, $p = .03$), composite ($F(1,157) = 5.26$, $p = .006$), and frequency steps ($F(1,157) = 3.25$, $p = .04$) call types.

When post hoc analyses were run in response to the interactions, it was found that for the peak frequency of complex call types, the KA/saline group emitted calls of a lower frequency than the saline/agomelatine, saline/DMSO, and saline/saline groups. Additionally, KA/saline animals emitted a lower frequency of complex calls than Saline/DMSO mice, $p < .05$ (Table 6). There were also differences in peak frequency for downward call types, with KA/agomelatine treated mice emitting calls of a higher peak frequency than saline/DMSO, KA/DMSO, and KA/saline treated mice. Saline/saline mice also emitted downward calls of a higher peak frequency than KA/saline and saline/DMSO mice $p < .05$ (Table 6). No difference in the peak frequency of chevron call types was found upon post hoc analysis $p > .05$. However, for the peak frequency of frequency steps call types there were differences between groups, with KA/saline animals emitting a lower peak frequency than saline/agomelatine, KA/agomelatine, and Saline/DMSO animals $p < .05$ (Table 6). Next, we examined the fundamental frequency of downward call types, finding that saline/saline mice had calls of a lower fundamental frequency than saline/agomelatine, saline/DMSO, and KA/agomelatine mice $p < .05$. Additionally, KA/agomelatine and saline/DMSO mice emitted downward calls of a

higher fundamental frequency than KA/saline mice, $p < .05$. No differences in fundamental frequency were found for composite call types upon post hoc analysis, $p > .05$ (Table 6). Lastly the amplitude of the call types was assessed, for complex calls. KA/saline mice emitted calls of higher peak amplitude than KA/DMSO and saline/agomelatine mice. When the amplitude of upward calls were assessed, it was found that KA/saline mice emitted louder calls than saline/agomelatine, KA/agomelatine, saline/DMSO, KA/DMSO, and saline/saline mice, $p < .05$ (Table 6). KA/agomelatine treated mice also emitted calls of a lower amplitude than saline DMSO mice, $p < .05$. For the amplitude of downward call types, the KA/saline group emitted calls of a higher peak amplitude than the saline/agomelatine, saline/DMSO, KA/DMSO, and KA/DMSO groups, $p < .05$. When the amplitude for composite call types was assessed, KA/saline treated mice were found to emit calls of a higher peak amplitude than saline/agomelatine, saline/DMSO, KA/DMSO, and saline/saline treated mice $p < .05$ (Table 6). Lastly, the amplitude of frequency steps call types for the KA/saline groups was found to be lower than saline/agomelatine, KA/agomelatine, saline/DMSO, KA/DMSO, and saline/saline groups $p < .05$ (Table 6).

Table 5. Statistics for the Spectral Characteristics PD 11 24 hr. Timepoint

Characteristics	Call Type	Seizure	Treatment	Interaction
Male Duration				
	Complex	$F(1,27) = .69$ $p = .41$	$F(1,27) = 15.61$ $p = .00$	$F(1,27) = 2.55$ $p = .08$
	Upward	$F(1,27) = .00$ $p = .99$	$F(1,27) = .26$ $p = .77$	$F(1,27) = 3.62$ $p = .03$
	Downward	$F(1,27) = .68$ $p = .41$	$F(1,27) = .97$ $p = .38$	$F(1,27) = 2.07$ $p = .13$
	Chevron	$F(1,27) = .00$ $p = .98$	$F(1,27) = 1.04$ $p = .36$	$F(1,27) = 4.36$ $p = .01$
	Composite	$F(1,27) = 3.31$ $p = .07$	$F(1,27) = 14.69$ $p = .00$	$F(1,27) = 9.89$ $p = .00$
	Frequency steps	$F(1,27) = 9.26$ $p = .003$	$F(1,27) = 2.38$ $p = .10$	$F(1,27) = 1.84$ $p = .16$

(Continued)

Characteristics	Call Type	Seizure	Treatment	Interaction
Male Peak Frequency				
	Complex	$F(1,27) = 4.40$ $p = .04$	$F(1,27) = 4.83$ $p = .009$	$F(1,27) = .19$ $p = .83$
	Upward	$F(1,27) = 9.13$ $p = .003$	$F(1,27) = .04$ $p = .96$	$F(1,27) = 4.84$ $p = .009$
	Downward	$F(1,27) = .00$ $p = .99$	$F(1,27)=2.08$ $p = .13$	$F(1,27) =4.31$ $p = .02$
	Chevron	$F(1,27) = 3.46$ $p = .07$	$F(1,25)=.02$ $p = .98$	$F(1,27) = 7.31$ $p = .001$
	Composite	$F(1,27) = 5.69$ $p = .02$	$F(1,25) = 15.89$ $p = .00$	$F(1,27) = 1.19$ $p = .31$
	Frequency steps	$F(1,27)=10.88$ $p = .001$	$F(1,27) =4.44$ $p = .013$	$F(1,27) =2.19$ $p = .12$
Male Fundamental Frequency				
	Complex	$F(1,27) = 1.28$ $p = .26$	$F(1,27)=1.46$ $p = .24$	$F(1,27) =.07$ $p = .93$
	Upward	$F(1,27) = 1.09$ $p = .30$	$F(1,27) = 6.94$ $p = .001$	$F(1,27) =.61$ $p = .54$
	Downward	$F(1,27) = 1.35$ $p = .25$	$F(1,25) = 3.53$ $p = .03$	$F(1,27) = .11$ $p = .90$
	Chevron	$F(1,27) = .99$ $p = .32$	$F(1,25) = 1.58$ $p = .21$	$F(1,27) = .36$ $p = .70$
	Composite	$F(1,27) = 1.96$ $p = .16$	$F(1,27) = 6.37$ $p = .002$	$F(1,27) = 5.52$ $p = .005$
	Frequency steps	$F(1,27) = 3.61$ $p = .06$	$F(1,25) = .08$ $p = .93$	$F(1,27) = .24$ $p = .78$
Male Amplitude				
	Complex	$F(1,27) = 2.68$ $p = .10$	$F(1,27) = 12.04$ $p = .00$	$F(1,27) = 1.68$ $p = .19$
	Upward	$F(1,27) = .48$ $p = .49$	$F(1,25) = 2.29$ $p = .11$	$F(1,27) = 2.11$ $p = .13$
	Downward	$F(1,27) = .40$ $p = .53$	$F(1,27) = 6.79$ $p = .001$	$F(1,27) = 5.17$ $p = .007$
	Chevron	$F(1,27) = 1.88$ $p = .17$	$F(1,25) = 5.88$ $p = .003$	$F(1,27) = 7.08$ $p = .001$
	Composite	$F(1,27) = 2.71$ $p = .10$	$F(1,25) = 2.83$ $p = .06$	$F(1,27) = 1.75$ $p = .18$
	Frequency steps	$F(1,27) = 9.41$ $p = .003$	$F(1,25) =.15$ $p = .86$	$F(1,27) =.28$ $p = .76$
Female Duration				
	Complex	$F(1,30) = 1.43$ $p = .23$	$F(1,30) = .22$ $p = .81$	$F(1,30) = 1.15$ $p = .32$
	Upward	$F(1,30) = 5.62$ $p = .02$	$F(1,30) = 7.62$ $p = .001$	$F(1,30) = .79$ $p = .46$
	Downward	$F(1,30) = 4.22$ $p = .04$	$F(1,30) =11.25$ $p = .00$	$F(1,30) = .30$ $p = .74$
	Chevron	$F(1,30) = 4.72$ $p = .03$	$F(1,30) = 2.92$ $p = .06$	$F(1,30) = 2.96$ $p = .06$

(Continued)

Characteristics	Call Type	Seizure	Treatment	Interaction
	Composite	$F(1,30) = 7.81$ $p = .006$	$F(1,30) = 1.23$ $p = .30$	$F(1,30) = .99$ $p = .37$
	Frequency steps	$F(1,30) = .23$ $p = .63$	$F(1,30) = 1.53$ $p = .22$	$F(1,30) = .026$ $p = .97$
Female Peak Frequency				
	Complex	$F(1,30) = 11.37$ $p = .001$	$F(1,30) = .46$ $p = .63$	$F(1,30) = 5.62$ $p = .004$
	Upward	$F(1,30) = .36$ $p = .55$	$F(1,30) = .90$ $p = .41$	$F(1,30) = .53$ $p = .59$
	Downward	$F(1,30) = .17$ $p = .68$	$F(1,30) = 5.99$ $p = .003$	$F(1,30) = 9.56$ $p = .00$
	Chevron	$F(1,30) = 1.36$ $p = .25$	$F(1,30) = 3.59$ $p = .03$	$F(1,30) = 3.59$ $p = .03$
	Composite	$F(1,30) = .62$ $p = .43$	$F(1,30) = 2.14$ $p = .12$	$F(1,30) = 1.42$ $p = .24$
	Frequency steps	$F(1,30) = 11.85$ $p = .001$	$F(1,30) = .46$ $p = .63$	$F(1,30) = 5.09$ $p = .007$
Female Fundamental Frequency				
	Complex	$F(1,30) = .043$ $p = .84$	$F(1,30) = 12.47$ $p = .00$	$F(1,30) = 1.35$ $p = .26$
	Upward	$F(1,30) = .52$ $p = .47$	$F(1,30) = 4.95$ $p = .00$	$F(1,30) = .20$ $p = .82$
	Downward	$F(1,30) = .022$ $p = .88$	$F(1,30) = 16.38$ $p = .00$	$F(1,30) = 4.68$ $p = .01$
	Chevron	$F(1,30) = .028$ $p = .87$	$F(1,30) = 5.01$ $p = .008$	$F(1,30) = .20$ $p = .82$
	Composite	$F(1,30) = .25$ $p = .62$	$F(1,30) = 4.83$ $p = .009$	$F(1,30) = 4.83$ $p = .009$
	Frequency steps	$F(1,30) = 1.69$ $p = .20$	$F(1,30) = 3.28$ $p = .04$	$F(1,30) = 1.84$ $p = .16$
Female Amplitude				
	Complex	$F(1,30) = .90$ $p = .34$	$F(1,30) = 2.09$ $p = .13$	$F(1,30) = 3.80$ $p = .03$
	Upward	$F(1,30) = .07$ $p = .80$	$F(1,30) = 7.35$ $p = .001$	$F(1,30) = 13.88$ $p = .00$
	Downward	$F(1,30) = 2.58$ $p = .11$	$F(1,30) = 9.56$ $p = .00$	$F(1,30) = 3.59$ $p = .03$
	Chevron	$F(1,30) = 1.21$ $p = .27$	$F(1,30) = .76$ $p = .47$	$F(1,30) = .15$ $p = .87$
	Composite	$F(1,30) = 8.77$ $p = .004$	$F(1,30) = 2.25$ $p = .11$	$F(1,30) = 5.26$ $p = .006$
	Frequency steps	$F(1,30) = 10.44$ $p = .002$	$F(1,30) = 4.02$ $p = .02$	$F(1,30) = 3.25$ $p = .04$

Table 6. Mean and SEM for the Spectral Characteristics of USVs at PD 11 24 hr. Timepoint

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
Male Duration							
	Complex	.031± .0016	.032± .002	.019± .0016	.023± .0018	.024± .0020	.018± .0014
	Upward	.011± .0007	.008± .0005	.0088± .0007	.010± .0008	.0098± .0008	.009± .0006
	Downward	.018± .001	.014± .0012	.017± .0014	.017± .0012	.015± .0010	.015± .0014
	Chevron	.014± .0006	.013± .0008	.013± .00078	.014± .0014	.014± .0007	.011± .0006
	Composite	.045± .002	.03± .004	.035± .0036	.018± .0019	.026± .0020	.034± .0032
	Frequency steps	.035± .002	.022± .0026	.0308± .0035	.026± .0020	.032± .0029	.028± .003
Male Peak Frequency							
	Complex	80159± 765	81151± 924	82235± 948	82089 ± 639	80087± 938	82266 ± 896
	Upward	84002± 967	86855± 759	85605± 1152	83833± 1191	84542± 993	84416 ± 921
	Downward	80238± 718	84584± 627	81186± 1012	81848± 847	81085± 725	80441 ± 808
	Chevron	80936± 1052	84392± 800	82609± 1099	82423± 790	83848± 715	81483 ± 845
	Composite	67341± 1265	74381± 2480	81497± 1317	80682± 1914	76322± 1798	76339 ± 1970
	Frequency steps	72329± 1077	75683± 1734	74923± 1698	79087± 1576	72626± 1131	78926 ± 1566
Male Fundamental Frequency							
	Complex	55209± 1992	59605± 1485	49411± 2999	55311± 1463	57519± 1494	59102 ± 1133
	Upward	59822± 1574	60095± 2478	52696± 2708	58177± 2555	61449± 2289	58437 ± 2077
	Downward	55514± 1100	53722± 1830	56512± 1831	52477± 1620	54335± 1530	52556 ± 1884
	Chevron	65144± 1507	67652± 1993	65006± 1937	63402± 2454	66088± 1589	65270 ± 2069
	Composite	38014± 2591	32178± 1455	44032± 3037	42182± 3450	68140± 1193	47319 ± 2338
	Frequency steps	37246± 2927	46251± 3895	42180± 3110	37471± 2108	39458± 1580	45065 ± 1581
Male Amplitude							
	Complex	-65.00± .87	-66.29± .70	-63.55± 1.01	-60.96± 1.54	-62.18± .68	-62.64 ± .49

(Continued)

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
	Upward	-63.91± .75	-64.12± .61	-62.95± .75	-62.46± .87	-59.50± .81	- 62.59± .42
	Downward	-65.66± .67	-64.01± .62	-59.59± .1.22	-62.30± .62	-62.52± .70	-64.88± .46
	Chevron	-63.32± .70	-62.43± 1.06	-60.77± .87	-61.50± .52	-62.28± .43	- 63.52± .63
	Composite	-59.93± 1.21	-62.91± .77	-59.24± 1.29	-60.58± .79	-63.76± .60	-58.43± 1.04
	Frequency steps	-63.19± .86	-64.99± .84	-60.87± 1.27	-63.52± .83	-60.57± .92	-61.20± .97
Female Duration							
	Complex	.023± .0012	.023± .0011	.023± .0016	.024± .0018	.026± .0017	.025± .0018
	Upward	.0097± .00046	.008± .003	.010± .00088	.0093± .00068	.0098± .0005	.0094± .00039
	Downward	.013± .0008	.012± .00076	.015± .00098	.014± .0011	.019± .0012	.018± .0016
	Chevron	.013± .00062	.013± .00069	.012± .00089	.014± .0089	.015± .00079	.014± .00072
	Composite	.028± .0025	.025± .0030	.0065± .00093	.025± .0024	.033± .003	.027± .0030
	Frequency steps	.029± .0021	.026± .0024	.031± .0024	.034± .0039	.027± .0021	.025± .0026
Female Peak Frequency							
	Complex	85033± 1031	82103± 713	81282± 1088	83197± 445	83190± 525	84177± 581
	Upward	87644± 1111	83811± 863	85773± 978	88918± 735	86559± 845	87422± 893
	Downward	86534± 770	84256± 635	81203± 941	85087± 560	82599± 547	82512± 815
	Chevron	80812± 1675	81887± 894	83235± 1111	83472± 919	85030± 701	84468± 700
	Composite	73972± 951	74839± 1421	76435± 2179	77950± 1416	71684± 1246	74811± 1241
	Frequency steps	77214± 1295	78250± 1020	71060± 1875	80198± 1889	76813± 1339	78800± 1457
Female Fundamental Frequency							
	Complex	59582± 1836	61030± 852	45030± 3333	55310± 2195	58363± 2024	59102± 1133
	Upward	56881± 2743	58822± 1935	57410± 3020	46291± 2060	57764± 2316	58437± 2077
	Downward	52273± 770	57487± 1465	50545± 1972	50026± 1661	53778± 1649	52556± 1884

(Continued)

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
Female Amplitude	Chevron	62261± 1675	67066± 1135	57829± 3458	59092± 1637	65081± 1889	65270± 2069
	Composite	39105± 2260	32626± 3134	42710± 3147	41455± 3656	38652± 3200	47319± 2338
	Frequency steps	41487± 2574	41250± 3196	43983± 2471	41811± 2261	38731± 3257	45065± 1581
	Complex	-65.49± .55	-65.36± .42	-63.37± .65	-61.79± .70	-64.68± .83	-62.57± .48
	Upward	-67.40± .46	-64.10± .44	-59.58± .85	-62.50± .64	-64.65± .59	-62.28± .34
	Downward	-67.27± .44	-65.93± .30	-62.15± .50	-62.67± .63	-63.33± .80	-64.16± .45
	Chevron	-65.24± .54	-64.97± .54	-62.36± .32	-60.97± .72	-63.33± .80	-61.78± .53
	Composite	-62.40± .95	-62.82± .64	-57.12± 1.14	-61.51± .75	-61.81± 1.04	-60.80± .62
	Frequency steps	-65.40± .91	-64.80± .83	-59.28± 1.09	-60.77± .87	-63.49± .87	-63.68± .69

PD 12: 24-hour Timepoint

All of the statistics for the PD 12 24-hour timepoint for males and females is contained within Table 7. On PD 12, males receiving treatment 24 hours after SE displayed a main effect of seizure for the duration of upward call types ($F(1,15) = 8.99$, $p = .009$). Specifically, KA treated mice emitted upward calls of a longer duration than control mice (Table 8). Meanwhile, PD 12 females exhibited a main effect of treatment for the peak frequency of complex ($F(1,13) = 3.98$, $p = .05$) call types. Saline treated females emitted complex calls of a lower peak frequency than agomelatine or DMSO treated females (Table 8). A seizure by treatment interaction was also observed for the peak amplitude of upward ($F(1,13) = 3.83$, $p = .05$) and composite ($F(1,13) = 4.73$, $p =$

.03) call types. However, when a Tukey's post hoc test was conducted no differences in peak amplitude were observed for upward or composite call types, $p > .05$.

Table 7. Statistics for the Spectral Characteristics PD 12 24 hr. Timepoint

Characteristics	Call Type	Seizure	Treatment	Interaction
Male Duration				
	Complex	$F(1,15) = 2.47$ $p = .14$	$F(1,15) = 1.75$ $p = .21$	$F(1,15) = .42$ $p = .53$
	Upward	$F(1,15) = 8.99$ $p = .009$	$F(1,15) = .65$ $p = .54$	$F(1,15) = .11$ $p = .74$
	Downward	$F(1,15) = 3.28$ $p = .09$	$F(1,15) = 1.32$ $p = .30$	$F(1,15) = .02$ $p = .89$
	Chevron	$F(1,15) = 1.73$ $p = .21$	$F(1,15) = .06$ $p = .95$	$F(1,15) = .24$ $p = .63$
	Composite	$F(1,15) = .79$ $p = .39$	$F(1,15) = 1.61$ $p = .23$	$F(1,15) = 1.17$ $p = .30$
	Frequency steps	$F(1,15) = 3.54$ $p = .08$	$F(1,15) = 1.21$ $p = .33$	$F(1,15) = .53$ $p = .48$
Male Peak Frequency				
	Complex	$F(1,15) = .022$ $p = .88$	$F(1,15) = .68$ $p = .52$	$F(1,15) = .00$ $p = .99$
	Upward	$F(1,15) = 6.68$ $p = .02$	$F(1,15) = 1.22$ $p = .32$	$F(1,15) = .008$ $p = .93$
	Downward	$F(1,15) = .032$ $p = .86$	$F(1,15) = .89$ $p = .43$	$F(1,15) = 1.02$ $p = .33$
	Chevron	$F(1,15) = .27$ $p = .61$	$F(1,15) = .003$ $p = .99$	$F(1,15) = 1.12$ $p = .31$
	Composite	$F(1,15) = .013$ $p = .91$	$F(1,15) = .55$ $p = .59$	$F(1,15) = .55$ $p = .59$
	Frequency steps	$F(1,15) = 1.80$ $p = .20$	$F(1,15) = .66$ $p = .53$	$F(1,15) = .25$ $p = .62$
Male Fundamental Frequency				
	Complex	$F(1,15) = .86$ $p = .37$	$F(1,15) = .16$ $p = .86$	$F(1,15) = 2.07$ $p = .17$
	Upward	$F(1,15) = .43$ $p = .52$	$F(1,15)=1.24$ $p = .32$	$F(1,15) = 2.05$ $p = .17$
	Downward	$F(1,15) = 2.32$ $p = .15$	$F(1,15) = .92$ $p = .42$	$F(1,15) = .75$ $p = .40$
	Chevron	$F(1,15) = 1.29$ $p = .28$	$F(1,15) = .95$ $p = .41$	$F(1,15) = .04$ $p = .85$
	Composite	$F(1,15) = .13$ $p = .72$	$F(1,15) = 1.94$ $p = .18$	$F(1,15) = .21$ $p = .65$
	Frequency steps	$F(1,15) = .00$ $p = .99$	$F(1,15) = .11$ $p = .90$	$F(1,15) = .37$ $p = .55$
Male Amplitude				
	Complex	$F(1,15) = .00$ $p = .99$	$F(1,15) = 1.48$ $p = .26$	$F(1,15) = .40$ $p = .54$

(Continued)

Characteristics	Call Type	Seizure	Treatment	Interaction
	Upward	$F(1,15) = .07$ $p = .80$	$F(1,15) = 1.02$ $p = .38$	$F(1,15) = .08$ $p = .78$
	Downward	$F(1,15) = .031$ $p = .86$	$F(1,15) = .86$ $p = .44$	$F(1,15) = .008$ $p = .93$
	Chevron	$F(1,15) = .040$ $p = .84$	$F(1,15) = 1.09$ $p = .36$	$F(1,15) = .07$ $p = .4780$
	Composite	$F(1,15) = .001$ $p = .98$	$F(1,15) = .98$ $p = .40$	$F(1,15) = .13$ $p = .72$
	Frequency steps	$F(1,15) = .56$ $p = .47$	$F(1,15) = .82$ $p = .46$	$F(1,15) = .27$ $p = .61$
Female Duration				
	Complex	$F(1,13) = .24$ $p = .63$	$F(1,13) = 2.97$ $p = .09$	$F(1,13) = .276$ $p = .21$
	Upward	$F(1,13) = .01$ $p = .94$	$F(1,13) = 1.46$ $p = .27$	$F(1,13) = 2.77$ $p = .10$
	Downward	$F(1,13) = .07$ $p = .80$	$F(1,13) = .52$ $p = .61$	$F(1,13) = 1.03$ $p = .38$
	Chevron	$F(1,13) = .26$ $p = .62$	$F(1,13) = .89$ $p = .44$	$F(1,13) = 2.12$ $p = .16$
	Composite	$F(1,13) = .42$ $p = .53$	$F(1,13) = .28$ $p = .76$	$F(1,13) = .21$ $p = .81$
	Frequency steps	$F(1,13) = .17$ $p = .69$	$F(1,13) = .02$ $p = .98$	$F(1,13) = 1.73$ $p = .22$
Female Peak Frequency				
	Complex	$F(1,13) = 3.67$ $p = .08$	$F(1,13) = 3.98$ $p = .05$	$F(1,13) = .245$ $p = .79$
	Upward	$F(1,13) = .022$ $p = .89$	$F(1,13) = .97$ $p = .41$	$F(1,13) = .43$ $p = .66$
	Downward	$F(1,13) = 3.89$ $p = .07$	$F(1,13) = 1.26$ $p = .32$	$F(1,13) = .61$ $p = .56$
	Chevron	$F(1,13) = .27$ $p = .61$	$F(1,13) = 2.88$ $p = .09$	$F(1,13) = .40$ $p = .68$
	Composite	$F(1,13) = 1.01$ $p = .33$	$F(1,13) = 2.23$ $p = .15$	$F(1,13) = 1.23$ $p = .32$
	Frequency steps	$F(1,13) = .003$ $p = .96$	$F(1,13) = 2.69$ $p = .11$	$F(1,13) = .20$ $p = .82$
Female Fundamental Frequency				
	Complex	$F(1,13) = .002$ $p = .97$	$F(1,13) = 1.79$ $p = .21$	$F(1,13) = .10$ $p = .90$
	Upward	$F(1,13) = .29$ $p = .60$	$F(1,13) = .52$ $p = .61$	$F(1,13) = .81$ $p = .47$
	Downward	$F(1,13) = .18$ $p = .68$	$F(1,13) = 1.65$ $p = .23$	$F(1,13) = 1.07$ $p = .37$
	Chevron	$F(1,13) = 3.01$ $p = .11$	$F(1,13) = .14$ $p = .87$	$F(1,13) = .40$ $p = .68$
	Composite	$F(1,13) = 1.99$ $p = .18$	$F(1,13) = 2.09$ $p = .16$	$F(1,13) = .244$ $p = .65$
	Frequency steps	$F(1,13) = 2.23$ $p = .16$	$F(1,13) = 3.61$ $p = .06$	$F(1,13) = 1.05$ $p = .38$

(Continued)

Characteristics	Call Type	Seizure	Treatment	Interaction
Female Amplitude				
	Complex	$F(1,13) = .082$ $p = .78$	$F(1,13) = .54$ $p = .60$	$F(1,13) = 1.11$ $p = .36$
	Upward	$F(1,13) = .039$ $p = .85$	$F(1,13) = .47$ $p = .64$	$F(1,13) = 3.83$ $p = .05$
	Downward	$F(1,13) = .00$ $p = .99$	$F(1,13) = 1.79$ $p = .21$	$F(1,13) = 2.56$ $p = .12$
	Chevron	$F(1,13) = .10$ $p = .75$	$F(1,13) = .17$ $p = .85$	$F(1,13) = .71$ $p = .51$
	Composite	$F(1,13) = 2.84$ $p = .10$	$F(1,13) = 2.84$ $p = .10$	$F(1,13) = 4.73$ $p = .03$
	Frequency steps	$F(1,13) = .015$ $p = .90$	$F(1,13) = .80$ $p = .47$	$F(1,13) = .80$ $p = .47$

Table 8. Mean and SEM for the Spectral Characteristics of USVs at PD 12 24 hr.Timepoint

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
Male Duration							
	Complex	.032± .0020	.030± .0045	.017± .0037	.015± .0025	.018± .0027	.014± .0022
	Upward	.0099± .0013	.0066± .001	.0096± .0016	.011± .0035	.011± .0012	.0089± .0019
	Downward	.019± .0022	.010± .0022	.015± .0033	.013± .0035	.017± .0030	.016± .0044
	Chevron	.015± .0016	.015± .0029	.014± .0014	.015± .0010	.014± .0020	.011± .0014
	Composite	.0040± .0007	.0077± .0042	.032± .010	.0094± .0028	.013± .0034	.011± .0038
	Frequency steps	.033± .0025	.021± .0075	.024± .0048	.018± .0038	.029± .0052	.021± .0038
Male Peak Frequency							
	Complex	81610± 1976	84290± 2051	85836± 1340	84307± 1375	83197± 1512	84391± 1919
	Upward	85204± 2437	89812± 1908	87884± 3270	83008± 2716	89186± 2275	86170± 1998
	Downward	81979± 2055	87797± 2267	86283± 1718	83416± 1688	83833± 1541	84815± 1553
	Chevron	84084± 2852	90422± 6837	86913± 1276	84896± 2073	83089± 1799	82320± 2407
	Composite	74515± 5543	93711± 7007	78602± 4113	94800± 3047	88918± 4263	80885± 2076
	Frequency steps	75534± 2589	70720± 8526	80765± 3062	82643± 2014	79586± 2501	85548± 4453
Male							

(Continued)

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
Fundamental Frequency							
	Complex	51934± 3240	59137± 5506	58226± 4182	58318± 5562	55110± 4449	58868± 3989
	Upward	54178± 3253	49963± 9157	58212± 7105	69889± 7391	56238± 5135	55121± 6500
	Downward	50790± 2471	45267± 6695	50968± 3423	48533± 5253	51007± 3769	50116± 4463
	Chevron	64107± 3479	79394± 11973	67798± 5235	62931± 6197	58987± 2598	56999± 5506
	Composite	39016± 6931	36155± 11349	30779± 7543	33217± 8991	23780± 6438	39125± 8982
	Frequency steps	30625± 7431	26353± 11893	40092± 6207	36765± 6488	38667± 4719	44089± 7367
Male Amplitude							
	Complex	-67.23± .91	-69.03± 2.91	-64.63± 1.11	-65.37± 1.57	-64.36± .91	-63.39± 1.17
	Upward	-64.70± 1.00	-65.64± .99	-62.28± 1.53	-64.57± 3.98	-61.82± .82	-62.12± 1.61
	Downward	-67.03± .70	-65.43± 1.69	-64.22± 1.29	-62.83± 1.29	-62.69± .75	-63.34± .71
	Chevron	-64.88± 1.01	-61.81± 6.52	-63.83± 1.14	-61.01± 1.00	-63.12± .80	-61.62± 1.31
	Composite	-62.51± 1.56	-63.42± 3.10	-61.83± 1.14	-63.77± 2.05	-64.66± 2.17	-60.72± 1.60
	Frequency steps	-62.91± 1.56	-67.19± 4.56	-63.91± 1.56	-65.46± 1.43	-63.11± 1.12	-61.18± 1.49
Female Duration							
	Complex	.017± .0037	.022± .0028	.026± .0055	.024± .0032	.023± .0042	.028± .0054
	Upward	.0071± .0073	.0079± .0011	.010± .0021	.012± .0022	.010± .0026	.0099± .0014
	Downward	.013± .0025	.013± .0031	.013± .0029	.016± .0028	.019± .0026	.019± .0059
	Chevron	.012± .0023	.014± .002	.015± .0028	.016± .0024	.014± .0027	.012± .0016
	Composite	.024± .0051	.022± .0093	.027± .0091	.011± .0043	.017± .0079	.018± .007
	Frequency steps	.028± .0039	.024± .0066	.023± .0043	.024± .0032	.024± .0043	.026± .0051
Female Peak Frequency							
	Complex	84121± 2087	82946± 935	86937± 869	86359± 742	84241± 1599	86360± 1710
	Upward	89912± 2846	84424± 2328	89606± 1703	87844± 1874	87267± 1775	87771± 2415

(Continued)

Call Aspects	Call Type	KA/Sal	Sal/Sal	KA/Ago	Sal/Ago	KA/DM	Sal/DM
Female Fundamental Frequency	Downward	84018± 2945	82924± 1499	85106± 1650	87326± 1375	83394± 1288	87139± 1823
	Chevron	88904± 2269	81493± 2515	89904± 1693	85088± 1078	83573± 2639	84065± 2109
	Composite	71190± 4704	75770± 6457	78867± 3508	80575± 7941	77955± 7166	79370± 1734
	Frequency steps	76013± 3189	75998± 2191	84634± 1547	81862± 1514	79903± 2936	80745± 4085
	Complex	60589± 4158	53787± 2276	53812± 5300	53473± 5654	67028± 5138	51330± 6423
	Upward	44564± 7691	52652± 3879	61701± 7510	42989± 7059	60730± 10348	57681± 5461
	Downward	45447± 2874	53901± 5002	46439± 5495	46489± 3832	61301± 4206	58428± 4590
	Chevron	67637± 3122	62131± 3835	72737± 6858	53860± 4641	71641± 5026	57701± 7138
	Composite	47021± 6787	70272± 1644	24527± 5908	30625± 11334	40405± 14018	30416± 8414
	Frequency steps	48708± 6393	41643± 6784	41215± 5474	38210± 4811	41098± 7182	48893± 8803
Female Amplitude							
	Complex	-67.05± .77	-66.62± 1.58	-63.62± .74	-64.46± 1.42	-64.52± 1.43	-63.84± 1.40
	Upward	-67.76± 1.21	-64.83± .59	-62.57± .89	-64.62± 2.12	-65.72± 1.64	-62.5± 1.08
	Downward	-67.42± .79	-65.12± .59	-62.23± 1.12	-63.01± .97	-64.10± 1.75	-63.8± 1.35
	Chevron	-64.91± .47	-63.48± 1.70	-62.18± .91	-63.05± 1.59	-64.82± 1.28	-62.62± 1.74
	Composite	-62.23± 2.42	-62.37± .95	-61.20± 1.63	-65.30± 2.51	-64.68± 3.69	-61.11± 1.57
	Frequency steps	-66.79± 1.16	-64.26± 1.44	-64.98± 1.20	-64.80± 2.08	-65.53± 1.69	-63.95± 1.87

Western Blot Analysis

1-hour Timepoint

To assess protein expression, we split the files by sex and ran a MANOVA with seizure and treatment as the fixed factors and the expression of Iba1, GFAP, GluR1, and

mGluR1/5 as the dependent variables. For males, no main effect of seizure was found for Iba1 ($F(1,30) = 1.06$, $p = .31$), GFAP ($F(1,30) = .56$, $p = .46$), GluR1 ($F(1,30) = .19$, $p = .66$), or mGluR1/5 ($F(1,30) = .05$, $p = .83$) (Figure 11 A-D). No main effects of treatment were observed for Iba1 ($F(1,30) = 1.19$, $p = .32$), GFAP ($F(1,30) = .45$, $p = .64$), GluR1 ($F(1,30) = .23$, $p = .80$), or mGluR1/5 ($F(1,30) = .43$, $p = .66$) (Figure 11 A-D). There were also no seizure by treatment interactions for Iba1 ($F(1,30) = .08$, $p = .93$), GFAP ($F(1,30) = .51$, $p = .61$), GluR1 ($F(1,30) = .10$, $p = .91$), or mGluR1/5 ($F(1,30) = .09$, $p = .91$) (Figure 11 A-D).

For females receiving treatment 1 hour post SE there were no main effects of seizure for Iba1 ($F(1,30) = .03$, $p = .86$), GluR1 ($F(1,30) = 1.99$, $p = .17$), or mGluR1/5 ($F(1,30) = .47$, $p = .50$) (Figure 11 E-H). However, there was a trend present for GFAP ($F(1,30) = 3.24$, $p = .08$) (Figure 11 H). No main effects of treatment were found for Iba1 ($F(1,30) = 1.67$, $p = .21$), GFAP ($F(1,30) = .18$, $p = .83$), GluR1 ($F(1,30) = .08$, $p = .93$), or mGluR1/5 ($F(1,30) = .17$, $p = .84$) (Figure 11 E-H). There were also no seizure by treatment interactions for Iba1 ($F(1,30) = .91$, $p = .41$), GFAP ($F(1,30) = .20$, $p = .83$), GluR1 ($F(1,30) = .34$, $p = .72$), or mGluR1/5 ($F(1,30) = .33$, $p = .72$) (Figure 11 E-H).

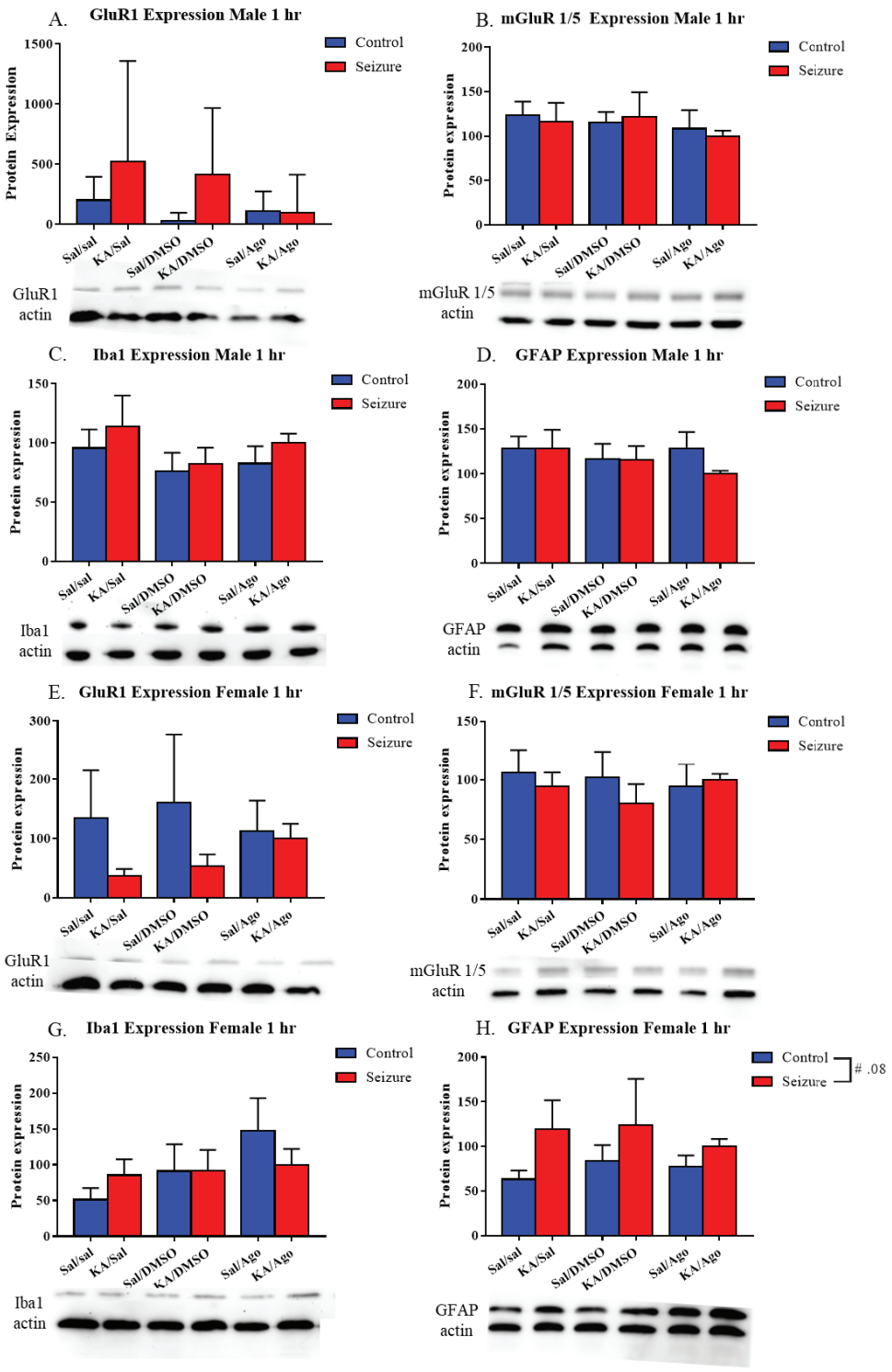


Figure 11. Expression of GluR1, mGluR1/5, Iba1, and GFAP in male and female mice at the 1-hour time point. A-D. There were no differences inGluR1, mGluR1/5, Iba1, or GFAP expression per seizure or treatment for males. E-G. For females there were no differences in protein expression per seizure or treatment for GluR1, mGluR1/5, or Iba1. H. KA treated females did express more GFAP than control animals, with no difference present between treatments. Bars represent the mean and the error bars represent the standard error of the mean. * = $p < .05$

24-hour Timepoint

For the 24-hour timepoint, the files were again split by sex and a MANOVA was run. Males displayed a main effect of seizure for GluR1 *(F*(1,30) = 4.60, *p* = .04) and a trend for GFAP (*F*(1,30) = 3.80, *p* = .06), with no main effects present for Iba1 *(F*(1,30) = .00, *p* = .99) or mGlur1/5 (*F*(1,30) = 2.52, *p* = .12) (Figure 12 A-D). Specifically, mice in the seizure group displayed increased levels of GluR1 relative to mice in the control group (Figure 12 A). No main effects for treatment were observed for Iba1 (*F*(1,30) = .016, *p* = .98), GFAP (*F*(1,30) = .35, *p* = .71), GluR1 (*F*(1,30) = .04, *p* = .96), or mGluR1/5 (*F*(1,30) = .04, *p* = .97) (Figure 12 A-D). There were also no seizure by treatment interactions for Iba1 5 (*F*(1,30) = 2.14, *p* = .14), GFAP (*F*(1,30) = .79, *p* = .46), GluR1 (*F*(1,30) = .87, *p* = .43), or mGluR1/5 (*F*(1,30) = .42, *p* = .66) (Figure 12 A-D).

For females receiving treatment 24 hours after SE there was a tending main effect of seizure for Iba1 (*F*(1,30) = 3.91, *p* = .06), but no main effects for GFAP *F*(1,30) = .23, *p* = .64), GluR1 (*F*(1,30) = 2.08, *p* = .16), or mGluR1/5 (*F*(1,30) = 1.47, *p* = .24) (Figure 12 E-H). The trend indicated an increase in Iba1 expression levels for KA treated mice relative to controls (Figure 12 G). No main effects for treatment were observed for Iba1 (*F*(1,30) = 30, *p* = .74), GFAP (*F*(1,30) = .11, *p* = .90), GluR1 (*F*(1,30) = .11, *p* = .90, or mGluR1/5 (*F*(1,30) = .32, *p* = .73) (Figure 12 E-H). Similarly, there were no seizure by treatment interactions for Iba1 (*F*(1,30) = 1.77, *p* = .19), GFAP (*F*(1,30) = .80, *p* = .46), GluR1 (*F*(1,30) = .23, *p* = .80), or mGluR1/5 (*F*(1,30) = .78, *p* = .47) (Figure 12 E-H).

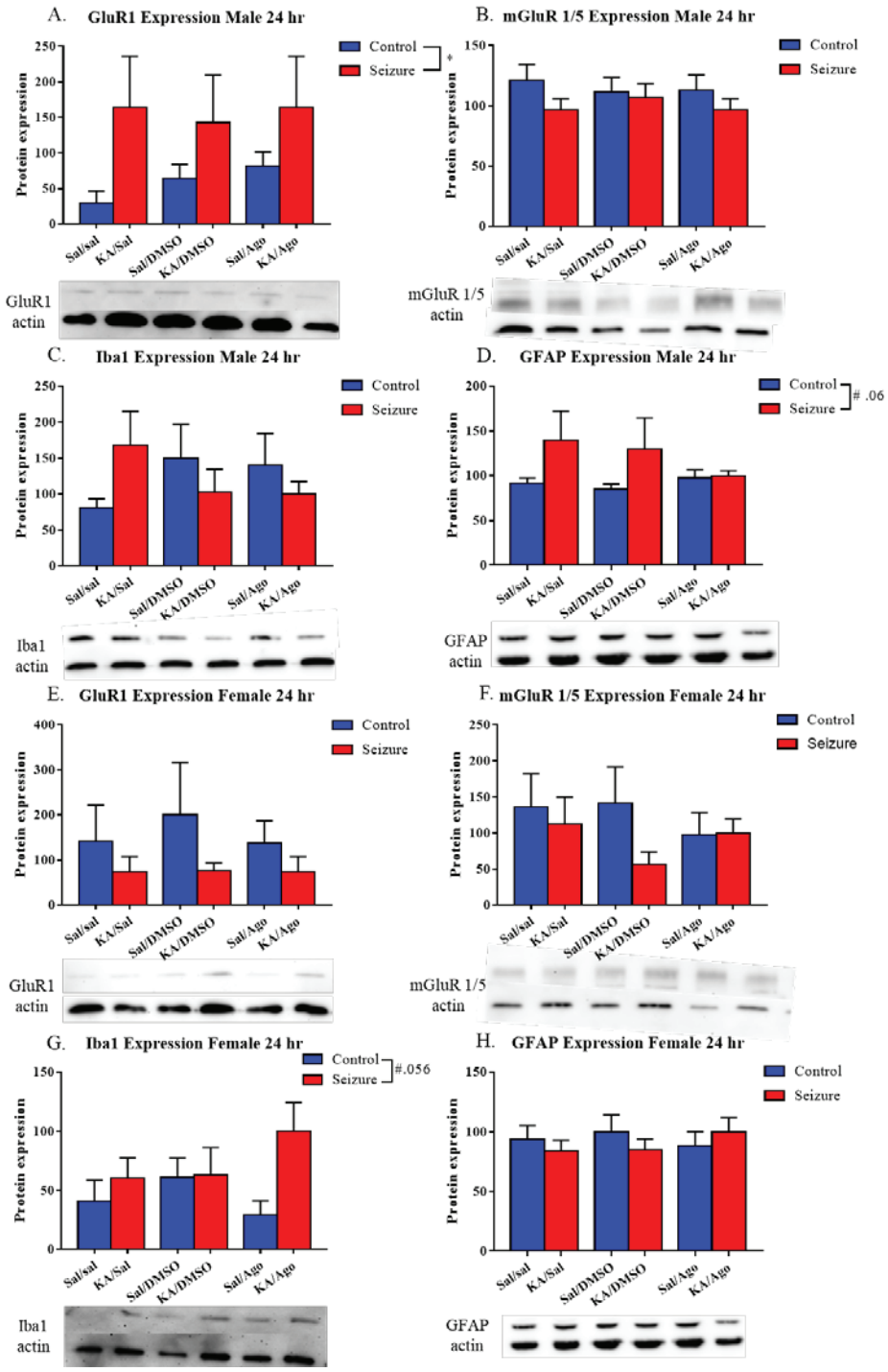


Figure 12. Expression of GluR1, mGluR1/5, Iba1, and GFAP in male and female mice at the 24-hour time point. A. KA treated males displayed an increase in the expression of GluR1 relative to controls. B-C. No differences in the expression of mGluR1/5 or Iba1 was found per seizure or treatment for males. D. KA males displayed an increase in GFAP relative to controls but no difference per treatment. E,F H. For females, no differences in the protein expression of GluR1, mGluR1/5 or GFAP was found per seizure or treatment. G. KA treated females displayed an increased expression of Iba1 relative to controls with no differences per treatment. Bars represent the mean and the error bars represent the standard error of the mean. * = $p < .05$.

CHAPTER FIVE

Discussion

This study was the first to assess the efficacy of agomelatine to treat communication deficits, increased excitation, and inflammation in neonates administered the chemoconvulsant kainic acid. Agomelatine administered both 1-hour, as well as 24 hours, following SE was unable to attenuate the seizure-induced increases in the total quantity of vocalizations emitted overall and per the downward, frequency steps, upward, chevron, and composite call types. Furthermore, acute agomelatine administration did not mitigate the seizure-induced alterations in the duration, peak frequency, fundamental frequency, and amplitude observed per call type. In addition to not attenuating the behavioral deficits that accompany ELS, agomelatine also did not normalize the increased expression of Iba1, GFAP, and GluR1 observed in the seizure groups, displaying little affinity to serve as a therapeutic treatment option for the comorbidities of neonates suffering from seizures.

Due to its high theoretical potential, we expected agomelatine to effectively attenuate the increased quantity of vocalizations, aberrant spectral characteristics, and increased inflammation and excitotoxicity present in seizure treated mice. One possible reason these effects were not observed in our study may be due to the acute administration paradigm used. Previous studies have shown that agomelatine exerts neuroprotective, antioxidant, anti-inflammatory, and anticonvulsant effects in various models of acquired epilepsy; however, each used a chronic administration paradigm, with

agomelatine injections ranging from 10 to 20 weeks (Demir Ozkay et al., 2015; Ethemoglu et al., 2019; Tchekalarova et al., 2018; Tchekalarova et al., 2017). Only one prior study has assessed an acute administration of agomelatine, with Gressens et al. (2008) finding that a single injection of agomelatine was sufficient to reduce white matter excitotoxicity in the hippocampus of a mouse model of cerebral palsy (Gressens et al., 2008). Importantly, cerebral palsy is characterized by increased neuroinflammation which selectivity damages the white matter tracts in the developing brain (Bax, Tydeman, & Flodmark, 2006; Pingel et al., 2019). Conversely, kainic acid administration targets primarily gray matter in the brain, specifically damaging mossy fiber synapses located on pyramidal neurons in the CA3 and CA1 regions of the hippocampus (Nadler, Perry, Gentry, & Cotman, 1980; Turner & Wheal, 1991). This indicates that agomelatine has differential effects dependent upon its administration paradigm. Specifically, an acute administration of agomelatine may have a greater affinity towards protecting the brain against white matter insults but a lesser affinity towards gray matter insults, such as those observed in models of ELS. However, when agomelatine is administered chronically, its neuroprotective effects seemingly become much more robust, extending to gray matter damage (Tchekalarova et al., 2018; Tchekalarova et al., 2017). The limitations of an acute injection of agomelatine also highlight a potential complication to both its therapeutic assessment in neonates and its overall utility. Since USVs begin on PD 5 and conclude on PD 14, a chronic administration paradigm in ELS models examining USV deficits is not tenable, therefore an effective treatment targeting behavioral deficits would need to confer beneficial effects after an acute administration, which agomelatine appears not to do (Bowers et al., 2013; Branchi et al., 2001; Elwood & Keeling, 1982).

Altogether, the available evidence suggests that while agomelatine may display therapeutic effects when administered chronically, its therapeutic efficacy following an acute administration is constrained.

Another factor that may have contributed to agomelatine's relative inefficiency in the present study may be the time point chosen to assess its efficacy. The majority of studies that have established agomelatine's therapeutic effects were conducted in adult models of acquired seizures. Due to the significant differences in brain structure and neurological milieu between neonates and adults, it is possible that agomelatine is most effective when administered in an older animal. Perhaps the most prudent neurological difference between neonates and adults that may account for this discrepancy is the relative disparity in melatonin levels. In adults, there is a small baseline quantity of melatonin that helps to reduce oxidative stress in mitochondria, with a large surge of melatonin occurring in tandem with the circadian rhythm (Jou et al., 2007; Kennaway & Wright, 2002). Upon insult, particularly seizures, melatonin levels dramatically increase, as it is produced to help reduce inflammation and oxidative stress (Esposito & Cuzzocrea, 2010; Reiter et al., 2016). In neonates, melatonin plays a vital role in neurodevelopment in addition to the roles it plays in the circadian rhythm and the immune response. Specifically, melatonin has been shown to regulate intracellular processes and the activity of second messengers in order to modulate neural gene responses and neuronal differentiation (de Faria Poloni, Feltes, & Bonatto, 2011). Due to its diversified function in neonates, endogenous baseline levels of melatonin are consistently elevated throughout early development (Munoz-Hoyos et al., 2007). Therefore, it is possible that adding a melatonin agonist in an environment with already high endogenous levels of melatonin

may result in diminishing returns, as competition for binding sites and the rate of melatonin metabolism would increase. This problem would be lessened in adults due to the lower relative amount of endogenous melatonin in the brain. Thus, while administration of agomelatine may be effective in adults, it may be less effective in neonates due to the fundamental differences in neural architecture and relative hormone levels, in part explaining agomelatine's inability to attenuate the neonatal behavioral and molecular deficits induced by kainic acid.

An additional entity that may have contributed to agomelatine's low efficacy in the current study is the complex role melatonin plays in the brain, both before and after seizures. Studies have shown that patients with intractable epilepsy have low baseline levels of melatonin relative to controls but exhibit a threefold increase in melatonin levels during and after seizures (Bazil et al., 2000). Melatonin agonists have also been shown to inhibit audiogenic and electrical seizures, in addition to reducing the convulsant activity induced via pentetrazole, pilocarpine, L-cysteine, and kainite administration, strongly implicating the hormone as a promising therapeutic target (Banach et al., 2011; Costa-Lotufo et al., 2002; Savina et al., 2006; Yahyavi-Firouz-Abadi et al., 2007). However, a smaller subset of studies have found melatonin agonists to display minimal therapeutic value and to even promote proconvulsant effects and induce epileptiform activity in the hippocampus (Musshoff & Speckmann, 2003; Sandyk, Tsagas, & Anninos, 1992). Altogether, the discrepancy in findings across studies indicates that melatonin does play a significant role in seizures, both early in life and in adulthood, however, it also indicates that this relationship is intricate and may be susceptible to influence by the type of seizure that is present, the administration paradigm, the age of the animal, the timing of

the circadian rhythm relative to injection, endogenous melatonin levels, or other as of yet undefined confounding variables. In sum, the relationship between melatonin and seizures cannot be reduced to a strict binary correlation; further research is needed in order to better understand its respective nuances.

In addition to failing to mitigate seizure-induced deficits in USVs, agomelatine administration was also shown to selectively exacerbate some of the deficits present. For instance, when assessing the total quantity of USVs emitted per the downward and composite call types on PD 11, and the downward call type on PD 12, at the 24-hour timepoint, agomelatine and kainic acid treated mice emitted significantly greater quantities of USVs than the other groups, exceeding the KA/saline and KA/DMSO mice. Similarly, when assessing the spectral and temporal characteristics of the calls a similar pattern was observed, with KA/agomelatine treated mice on PD 11 at the 1-hour timepoint emitting composite calls of a greater amplitude than comparison groups. Furthermore, at the PD 12 1-hour timepoint, KA/agomelatine mice emitted frequency steps call types of an increased duration when compared across groups. Since no differences were observed in the saline/agomelatine groups, this suggests that agomelatine can selectively worsen existing deficits. As previously discussed, this may be due the complex relationship between seizures and melatonin levels. Altogether, although the exacerbation of seizure-induced deficits by agomelatine was not present across all parameters assessed; it does provide additional support indicating that agomelatine displays little affinity to treat deficits in communication following ELS.

In regard to the observed seizure effects, our study aligns well with previous studies assessing ultrasonic vocalizations in neonates presenting with early life seizures.

Similar to our findings, *Tsc1* heterogenous mice, a model of genetic seizures, display an increase in the quantity of vocalizations emitted relative to controls (Tsai et al., 2012). Additionally, Lopez-Meraz (2014) assessed vocalizations following pilocarpine-induced status epilepticus in pups and also reported an increase in the quantity of USVs produced (López-Meraz et al., 2014). Conversely, Reynolds (2017) administered the chemoconvulsant kainic acid and assessed USVs on PD 12 and found a reduction in the quantity of vocalizations emitted in the seizure group (Reynolds et al., 2017). Taken together, the literature indicates that the particular quantity of vocalizations elicited is not always constant across early life seizure models, however, the underlying discrepancies in the quantity of USVs produced in seizure mice relative to controls is strikingly stable. Thus, our findings of an increased production of USVs both overall and per the downward, upward, composite, and frequency steps call types is in line with the established literature.

When assessing the spectral and temporal parameters of the vocalizations following KA administration, a similar trend of seizures altering communicative behaviors is observed in our study and the literature. Specifically, Reynolds (2017) found changes in the amplitude of upward, downward, and complex call types as well as alterations in the duration of short and two-syllable call types (Reynolds et al., 2017). The present study found seizure specific increases in the amplitude of composite and frequency step call types in addition to increases in the duration of upward, downward, chevron, composite, and frequency steps call types. No other study has assessed the spectral and temporal changes of vocalizations in ELS models, however, the available data suggests that early life seizures result in marked changes to the various parameters of

the calls relative to controls, albeit in a seemingly indiscriminate manner. Therefore, there is a similar pattern to that observed in the production of vocalizations in ELS animals, wherein differences in call characteristics are dynamic but are more broadly, and more importantly, indictive of early life seizures fundamentally altering communication in neonates. Altogether, our findings corroborate this result and further illustrate the effect of seizures on USVs.

In accordance with our behavioral data, western blot analysis revealed changes in protein expression commiserate with the seizure literature. Specifically, we observed an increase in GFAP in females at the 1-hour timepoint and increases in GluR1 and GFAP in males at the 24-hour timepoint, as well as an increase in Iba1 in females. Increases in in microglia, astrogliosis, and excitation following seizures have been well established in the epilepsy literature for both genetic and acquired seizures, providing solidarity to our findings (Hiragi et al., 2018; Vargas-Sánchez et al., 2018). However, while we did observe the requisite increases in protein expression associated with ELS, we did expect more consistent increases in markers of inflammation and excitation across groups and sex. One reason for instances of subtler findings may be due to the variability in protein expression following KA administration. For example, Jarvela (2010) and Hong (2010) reported an increase in Iba1 expression after KA injection but no significant increase in GFAP levels (Hong et al., 2010; Jarvela et al., 2011). However, Sabilallah (2016) did not find KA administration to increase Iba1 levels, whereas Jorgensen (1993) found KA to significantly increase GFAP in CA1 of the hippocampus but decrease expression in the CA3 region (Jorgensen et al., 1993; Sabilallah et al., 2016). This discrepancy across findings also extends to markers of excitotoxicity as well, since GluR1 has been shown to

be repeatedly increased following KA administration in some studies (Rakhade et al., 2012; Sommer et al., 2001) yet unaltered in others (Friedman et al., 1994; Grooms, Opitz, Bennett, & Zukin, 2000), with mGluR1/5 presenting with a similarly variable assessment (Aronica et al., 1997; Blumcke et al., 2000). Taken together, these findings suggest that the relationship between KA administration and increases in inflammation and excitation is complex and subject to change based on numerous outside variables. Of these, perhaps the most pertinent influence is the time of assessment, as protein analysis timeframes in the literature ranged from hours up to 21 days post insult (Grooms et al., 2000; Jorgensen et al., 1993). For our study, brains were collected on PD 15 for western blotting in order to align with the peak of microglia levels in neonates (Kim et al., 2015). However, it is possible that a greater excitatory and inflammatory response would have been observed at an earlier time point that more closely followed brain insult. The 5-day interim between KA injection and tissue collection may be sufficient for compensatory mechanisms to reduce inflammation and excitation in the hippocampus, leading to the apparent normalization of protein expression in some groups. Due to this ambiguity, and the variability of tissue collection timepoints in the literature, future studies investigating the ideal time frame to assess proteins related to inflammation and excitation following ELS is recommended. Nonetheless, while more modest than expected, our results do display an inflammatory and excitatory profile that is in agreement with the seizure literature.

When assessing saline and DMSO, the vehicle of agomelatine, no ability to attenuate KA-induced deficits was observed. Specifically, the treatments did not reduce the seizure induced increases in the total quantity of vocalizations emitted overall, the total quantity of vocalizations emitted per the downward, upward, composite, and

frequency steps call types, nor the spectral characteristics of the calls. Furthermore, the increases in GluR1, Iba1, and GFAP observed in western blotting were also unaffected. This indicates that saline and DMSO functioned as intended and formed valid controls, establishing a baseline from which agomelatine's effects could be reliably compared.

In order to get a comprehensive measure of the effects of ELS on ultrasonic vocalizations, as well as to best assess agomelatine's potential effectiveness, sex specific effects were assessed. Females were found to emit fewer total USVs at the PD 12 1-hour timepoint than males. Additionally, when examining the USVs emitted per call type, females produced fewer downward and composite call types at the PD 12 1 and 24-hour timepoints but more upward and composite call types on PD 11 at the 1-hour timepoint. Although our study was the first to assess vocalizations in both neonatal males and females injected with kainic acid, our results are consistent with the broader vocalization literature that has shown differences in both the quantity of USVs produced, as well as differences in the spectral and temporal characteristics of the vocalizations, between the sexes (Binder & Lugo, 2017). Future studies investigating USVs in both male and female pups following ELS, as well as studies examining the underlying mechanisms that account for the observed differences, would help to provide context for, and inform, our study in addition to related investigations. Importantly, when assessing treatment effects per males and females, no changes were observed. This suggests that our control injections were valid across sex and further indicates that agomelatine does not comprise an effective therapy for males nor females.

Overall, while our study did find seizure specific effects on USVs, inflammation, and excitation that were congruent with prior findings, these effects were not always

present at each time point assessed (Jarvela et al., 2011; Reynolds et al., 2017). Specifically, when examining the overall quantity of vocalizations emitted, a main effect of seizure was found in the PD 11 group receiving treatment 1-hour post SE but not at the other timepoints and, as previously discussed, western blotting did not display the same pattern of pathological upregulation across all assessments. These slight discrepancies across groups and time points may be attributable to the effects of acute stress. The literature has reliably demonstrated that glucocorticoids have prominent anti-inflammatory effects, in addition to one study reporting anti-excitatory effects; therefore, the acute stressors of the intraperitoneal injections may be counteracting the prominent underlying mechanisms associated with adverse pathology in seizure models (Barnes, 1998; Di, Maxson, Franco, & Tasker, 2009). This in turn could lead to subtler comorbidities following ELS than those observed in studies wherein mice received fewer acute stressors, explaining some of the observed results in the present study (Gressens et al., 2008; Reynolds et al., 2017). However, despite instances of relatively modest effects of early life seizures, kainic acid treated mice did present with a phenotype congruent with the established literature and were sufficient to assess agomelatine's therapeutic potential. Furthermore, the present study may highlight the role that acute stressors play in attenuating comorbidities of the seizure phenotype, though additional research is needed.

Conclusion

Our study is the first to assess the therapeutic potential of agomelatine to attenuate deficits in communication, excitation, and inflammation commensurate with an early life injection of kainic acid. Minimal therapeutic affinity was found for any aspect of the

deficits in behavior and changes in protein expression. Factors such as the acute administration paradigm, agomelatine's injection in neonates, and the complex relationship between melatonin and seizures may all comprise principal contributing factors to the observed results. Altogether, our study helped elucidate a potential treatment option for the comorbidities of ELS, providing evidence that agomelatine's therapeutic efficacy to treat aberrant vocalizations, excitation, and neuroinflammation in neonates is limited.

REFERENCES

Aaberg, K. M., Bakken, I. J., Lossius, M. I., Lund Søraas, C., Håberg, S. E., Stoltenberg, C., . . . Chin, R. (2016). Comorbidity and Childhood Epilepsy: A Nationwide Registry Study. *Pediatrics*.

Acuna-Castroviejo, D., Escames, G., Venegas, C., Diaz-Casado, M. E., Lima-Cabello, E., Lopez, L. C., . . . Reiter, R. J. (2014). Extrapineal melatonin: sources, regulation, and potential functions. *Cell Mol Life Sci, 71*(16), 2997-3025. doi:10.1007/s00018-014-1579-2

Alliot, F., Godin, I., & Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res Dev Brain Res, 117*(2), 145-152. doi:10.1016/s0165-3806(99)00113-3

Aronica, E. M., Gorter, J. A., Paupard, M.-C., Grooms, S. Y., Bennett, M. V. L., & Zukin, R. S. (1997). Status Epilepticus-Induced Alterations in Metabotropic Glutamate Receptor Expression in Young and Adult Rats. *The Journal of Neuroscience, 17*(21), 8588. doi:10.1523/JNEUROSCI.17-21-08588.1997

Auvin, S., Mazarati, A., Shin, D., & Sankar, R. (2010). Inflammation enhances epileptogenesis in the developing rat brain. *Neurobiol Dis, 40*(1), 303-310.

Auvin, S., Porta, N., Nehlig, A., Lecointe, C., Vallée, L., & Bordet, R. (2009). Inflammation in rat pups subjected to short hyperthermic seizures enhances brain long-term excitability. *Epilepsy Res, 86*(2-3), 124-130.

Aydogan, S., Yerer, M. B., & Goktas, A. (2006). Melatonin and nitric oxide. *Journal of Endocrinological Investigation, 29*(3), 281-287. doi:10.1007/BF03345555

Azedi, F., Mehrpour, M., Talebi, S., Zendedel, A., Kazemnejad, S., Mousavizadeh, K., . . . Joghataei, M. T. (2019). Melatonin regulates neuroinflammation ischemic stroke damage through interactions with microglia in reperfusion phase. *Brain Research, 1723*, 146401. doi:https://doi.org/10.1016/j.brainres.2019.146401

Babaee, A., Eftekhar-Vaghefi, S. H., Asadi-shekaari, M., Shahrokhi, N., Soltani, S. D., Malekpour-Afshar, R., & Basiri, M. (2015). Melatonin treatment reduces astrogliosis and apoptosis in rats with traumatic brain injury. *Iranian Journal of Basic Medical Sciences, 18*(9), 867-872.

Baio, J. (2014). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2010. *MMWR Surveillance Summaries, 63*(2), 1-21.

Banach, M., Gurdziel, E., Jedrych, M., & Borowicz, K. K. (2011). Melatonin in experimental seizures and epilepsy. *Pharmacol Rep, 63*(1), 1-11. doi:10.1016/s1734-1140(11)70393-0

Banks, W. A., & Erickson, M. A. (2010). The blood–brain barrier and immune function and dysfunction. *Neurobiol Dis, 37*(1), 26-32. doi:https://doi.org/10.1016/j.nbd.2009.07.031

Bardgett, M. E., Jackson, J. L., Taylor, B. M., & Csernansky, J. G. (1998). The effects of kainic acid lesions on locomotor responses to haloperidol and clozapine. *Psychopharmacology, 135*(3), 270-278. doi:10.1007/s002130050509

Barnes, P. J. (1998). Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond), 94*(6), 557-572. doi:10.1042/cs0940557

Barr, R. G., Konner, M., Bakeman, R., & Adamson, L. (1991). Crying in! Kung San infants: A test of the cultural specificity hypothesis. *Developmental Medicine & Child Neurology, 33*(7), 601-610.

Bast, T., Pezze, M., & McGarrity, S. (2017). Cognitive deficits caused by prefrontal cortical and hippocampal neural disinhibition. *British Journal of Pharmacology, 174*(19), 3211-3225.

Bax, M., Tydeman, C., & Flodmark, O. (2006). Clinical and MRI correlates of cerebral palsy: the European Cerebral Palsy Study. *Jama, 296*(13), 1602-1608. doi:10.1001/jama.296.13.1602

Bazil, C. W., Short, D., Crispin, D., & Zheng, W. (2000). Patients with intractable epilepsy have low melatonin, which increases following seizures. *Neurology, 55*(11), 1746-1748.

Beghi, E., & Giussani, G. (2018). Aging and the Epidemiology of Epilepsy. *Neuroepidemiology, 51*(3-4), 216-223. doi:10.1159/000493484

Ben-Ari, Y., & Dudek, F. E. (2010). Primary and secondary mechanisms of epileptogenesis in the temporal lobe: there is a before and an after. *Epilepsy Currents, 10*(5), 118-125. doi:10.1111/j.1535-7511.2010.01376.x

Berg, A. T., Jallon, P., & Preux, P. M. (2013). The epidemiology of seizure disorders in infancy and childhood: definitions and classifications. *Handb Clin Neurol, 111*, 391-398. doi:10.1016/b978-0-444-52891-9.00043-9

Binder, M. S., & Lugo, J. N. (2017). NS-Pten knockout mice show sex-and age-specific differences in ultrasonic vocalizations. *Brain and Behavior*.

Blevins, J. E., Stanley, B. G., & Reidelberger, R. D. (2002). DMSO as a vehicle for central injections: tests with feeding elicited by norepinephrine injected into the paraventricular nucleus. *Pharmacol Biochem Behav, 71*(1-2), 277-282. doi:10.1016/s0091-3057(01)00659-1

Blumcke, I., Becker, A. J., Klein, C., Scheiwe, C., Lie, A. A., Beck, H., . . . Wiestler, O. D. (2000). Temporal lobe epilepsy associated up-regulation of metabotropic glutamate receptors: correlated changes in mGluR1 mRNA and protein expression in experimental animals and human patients. *J Neuropathol Exp Neurol, 59*(1), 1-10. doi:10.1093/jnen/59.1.1

Bowers, J. M., Perez-Pouchoulen, M., Edwards, N. S., & McCarthy, M. M. (2013). Foxp2 mediates sex differences in ultrasonic vocalization by rat pups and directs order of maternal retrieval. *J Neurosci, 33*(8), 3276-3283. doi:10.1523/jneurosci.0425-12.2013

Bradley, W. G. (2004). *Neurology in clinical practice: principles of diagnosis and management* (Vol. 1): Taylor & Francis.

Branchi, I., Santucci, D., & Alleva, E. (2001). Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behavioural Brain Research, 125*(1–2), 49-56. doi:http://dx.doi.org/10.1016/S0166-4328(01)00277-7

Branchi, I., Santucci, D., Vitale, A., & Alleva, E. (1998). Ultrasonic vocalizations by infant laboratory mice: A preliminary spectrographic characterization under different conditions. *Developmental Psychobiology, 33*(3), 249-256. doi:10.1002/(SICI)1098-2302(199811)33:3<249::AID-DEV5>3.0.CO;2-R

Bubenik, G., Brown, G., & Grota, L. (1977). Immunohistological localization of melatonin in the rat digestive system. *Experientia, 33*(5), 662-663.

Burda, J. E., & Sofroniew, M. V. (2014). Reactive gliosis and the multicellular response to CNS damage and disease. *Neuron, 81*(2), 229-248. doi:10.1016/j.neuron.2013.12.034

Burgess, H. J., & Fogg, L. F. (2008). Individual differences in the amount and timing of salivary melatonin secretion. *PLoS ONE, 3*(8), e3055-e3055. doi:10.1371/journal.pone.0003055

Camfield, P., & Camfield, C. (2015). Incidence, prevalence and aetiology of seizures and epilepsy in children. *Epileptic Disord, 17*(2), 117-123. doi:10.1684/epd.2015.0736

Carbajo-Pescador, S., Garcia-Palomo, A., Martin-Renedo, J., Piva, M., Gonzalez-Gallego, J., & Mauriz, J. L. (2011). Melatonin modulation of intracellular signaling pathways in hepatocarcinoma HepG2 cell line: role of the MT1 receptor. *J Pineal Res, 51*(4), 463-471. doi:10.1111/j.1600-079X.2011.00910.x

Chen, C.-Q., Fichna, J., Bashashati, M., Li, Y.-Y., & Storr, M. (2011). Distribution, function and physiological role of melatonin in the lower gut. *World journal of gastroenterology: WJG, 17*(34), 3888.

Cherian, A., & Thomas, S. V. (2009). Status epilepticus. *Annals of Indian Academy of Neurology, 12*(3), 140-153. doi:10.4103/0972-2327.56312

Chung, S.-Y., & Han, S.-H. (2003). Melatonin attenuates kainic acid-induced hippocampal neurodegeneration and oxidative stress through microglial inhibition. *J Pineal Res, 34*(2), 95-102. doi:10.1034/j.1600-079X.2003.00010.x

Clarke, D. F., Roberts, W., Daraksan, M., Dupuis, A., McCabe, J., Wood, H., . . . Weiss, S. K. (2005). The prevalence of autistic spectrum disorder in children surveyed in a tertiary care epilepsy clinic. *Epilepsia, 46*(12), 1970-1977. doi:10.1111/j.1528-1167.2005.00343.x

Clemett, D. A., Punhani, T., Duxon, M. S., Blackburn, T. P., & Fone, K. C. (2000). Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. *Neuropharmacology, 39*(1), 123-132. doi:10.1016/s0028-3908(99)00086-6

Coppola, A., & Moshé, S. L. (2012). Chapter 4 - Animal models. In H. Stefan & W. H. Theodore (Eds.), *Handb Clin Neurol* (Vol. 107, pp. 63-98): Elsevier.

Costa-Lotufo, L. V., Fonteles, M. M., Lima, I. S., de Oliveira, A. A., Nascimento, V. S., de Bruin, V. M., & Viana, G. S. (2002). Attenuating effects of melatonin on pilocarpine-induced seizures in rats. *Comp Biochem Physiol C Toxicol Pharmacol, 131*(4), 521-529. doi:10.1016/s1532-0456(02)00037-6

Coulter, D. A., & Steinhäuser, C. (2015). Role of astrocytes in epilepsy. *Cold Spring Harbor perspectives in medicine, 5*(3), a022434-a022434. doi:10.1101/cshperspect.a022434

Curia, G., Longo, D., Biagini, G., Jones, R. S. G., & Avoli, M. (2008). The pilocarpine model of temporal lobe epilepsy. *Journal of Neuroscience Methods, 172*(2), 143-157. doi:10.1016/j.jneumeth.2008.04.019

Cuzzocrea, S., Costantino, G., Mazzon, E., & Caputi, A. P. (1999). Regulation of prostaglandin production in carrageenan-induced pleurisy by melatonin. *J Pineal Res, 27*(1), 9-14.

Dagyte, G., Trentani, A., Postema, F., Luiten, P., Boer, J., Gabriel, C., . . . Van der Zee, E. (2010). The Novel Antidepressant Agomelatine Normalizes Hippocampal Neuronal Activity and Promotes Neurogenesis in Chronically Stressed Rats. *CNS neuroscience & therapeutics, 16*, 195-207. doi:10.1111/j.1755-5949.2009.00125.x

Davis, B. M., Salinas-Navarro, M., Cordeiro, M. F., Moons, L., & De Groef, L. (2017). Characterizing microglia activation: a spatial statistics approach to maximize information extraction. *Scientific reports, 7*(1), 1576. doi:10.1038/s41598-017-01747-8

de Bodinat, C., Guardiola-Lemaitre, B., Mocaër, E., Renard, P., Muñoz, C., & Millan, M. J. (2010). Agomelatine, the first melatonergic antidepressant: discovery, characterization and development. *Nature Reviews Drug Discovery, 9*, 628. doi:10.1038/nrd3140

de Faria Poloni, J., Feltes, B. C., & Bonatto, D. (2011). Melatonin as a central molecule connecting neural development and calcium signaling. *Functional & Integrative Genomics, 11*(3), 383-388. doi:10.1007/s10142-011-0221-8

de Lanerolle, N. C., Lee, T. S., & Spencer, D. D. (2010). Astrocytes and epilepsy. *Neurotherapeutics, 7*(4), 424-438. doi:10.1016/j.nurt.2010.08.002

DeLorenzo, Pellock, Towne, & Boggs. (1995). Epidemiology of status epilepticus. *J Clin Neurophysiol, 12*(4), 316-325.

Delorenzo, Sun, D., & Deshpande. (2005). Cellular mechanisms underlying acquired epilepsy: the calcium hypothesis of the induction and maintainance of epilepsy. *Pharmacol Ther, 105*(3), 229-266. doi:10.1016/j.pharmthera.2004.10.004

Demir Ozkay, U., Soztutar, E., Can, O. D., Ucel, U. I., Ozturk, Y., & Ulupinar, E. (2015). Effects of long-term agomelatine treatment on the cognitive performance and hippocampal plasticity of adult rats. *Behav Pharmacol, 26*(5), 469-480. doi:10.1097/fbp.0000000000000153

DeMyer, M. K., Barton, S., DeMyer, W. E., Norton, J. A., Allen, J., & Steele, R. (1973). Prognosis in autism: A follow-up study. *Journal of autism and childhood schizophrenia, 3*(3), 199-246. doi:10.1007/BF01538281

Deng, W.-G., Tang, S.-T., Tseng, H.-P., & Wu, K. K. (2006). Melatonin suppresses macrophage cyclooxygenase-2 and inducible nitric oxide synthase expression by inhibiting p52 acetylation and binding. *Blood, 108*(2), 518.

Devinsky, O., Schein, A., & Najjar, S. (2013). Epilepsy associated with systemic autoimmune disorders. *Epilepsy Currents, 13*(2), 62-68. doi:10.5698/1535-7597-13.2.62

Di, S., Maxson, M. M., Franco, A., & Tasker, J. G. (2009). Glucocorticoids regulate glutamate and GABA synapse-specific retrograde transmission via divergent nongenomic signaling pathways. *J Neurosci, 29*(2), 393-401. doi:10.1523/JNEUROSCI.4546-08.2009

Ding, K., Wang, H., Xu, J., Lu, X., Zhang, L., & Zhu, L. (2014). Melatonin reduced microglial activation and alleviated neuroinflammation induced neuron degeneration in experimental traumatic brain injury: Possible involvement of mTOR pathway. *Neurochemistry International, 76*, 23-31. doi:https://doi.org/10.1016/j.neuint.2014.06.015

Domin, H., Zieba, B., Golembiowska, K., Kowalska, M., Dziubina, A., & Smialowska, M. (2010). Neuroprotective potential of mGluR5 antagonist MTEP: effects on kainate-induced excitotoxicity in the rat hippocampus. *Pharmacol Rep, 62*(6), 1051-1061. doi:10.1016/s1734-1140(10)70367-4

DSM-5. Diagnostic and statistical manual of mental disorders (5th ed.). (Arlington, VA: American Psychiatric Publishing. ... (American Psychoatric Association, 2013).

DSM-5. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). (Arlington, VA: American Psychiatric Publishing. ... (American Psychiatric Association, 2013).

Dubé, C. M., Brewster, A. L., Richichi, C., Zha, Q., & Baram, T. Z. (2007). Fever, febrile seizures and epilepsy. *Trends Neurosci, 30*(10), 490-496. doi:https://doi.org/10.1016/j.tins.2007.07.006

Ebadi, M., & Govitrapong, P. (1986). Neural pathways and neurotransmitters affecting melatonin synthesis. *Journal of neural transmission. Supplementum, 21*, 125-155.

Elwood, R. W., & Keeling, F. (1982). Temporal organization of ultrasonic vocalizations in infant mice. *Developmental Psychobiology, 15*(3), 221-227.

Emet, M., Ozcan, H., Ozel, L., Yayla, M., Halici, Z., & Hacimuftuoglu, A. (2016). A Review of Melatonin, Its Receptors and Drugs. *The Eurasian journal of medicine, 48*(2), 135-141. doi:10.5152/eurasianjmed.2015.0267

Eng, L. F., & Ghirnikar, R. S. (1994). GFAP and Astrogliosis. *Brain Pathology, 4*(3), 229-237. doi:10.1111/j.1750-3639.1994.tb00838.x

England, M. J., Liverman, C. T., Schultz, A. M., & Strawbridge, L. M. (2012). *Epilepsy Across the Spectrum: Promoting Health and Understanding*. Washington, D.C.: The National Academies Press.

Esposito, & Cuzzocrea. (2010). Antiinflammatory Activity of Melatonin in Central Nervous System. *Current Neuropharmacology, 8*(3), 228-242. doi:10.2174/157015910792246155

Esposito, Hiroi, & Scattoni. (2017). Cry, Baby, Cry: Expression of Distress As a Biomarker and Modulator in Autism Spectrum Disorder. *International Journal of Neuropsychopharmacology, 20*(6), 498-503. doi:10.1093/ijnp/pyx014

Esposito, Nakazawa, Venuti, & Bornstein. (2012). Perceptions of distress in young children with autism compared to typically developing children: a cultural comparison between Japan and Italy. *Research in Developmental Disabilities, 33*(4), 1059-1067. doi:10.1016/j.ridd.2012.01.014

Esposito, Valenzi, Islam, & Bornstein. (2015). Three physiological responses in fathers and non-fathers' to vocalizations of typically developing infants and infants with Autism Spectrum Disorder. *Research in Developmental Disabilities, 43-44*, 43-50. doi:10.1016/j.ridd.2015.06.007

Esposito, & Venuti. (2008). How is crying perceived in children with Autistic Spectrum Disorder. *Research in Autism Spectrum Disorders, 2*(2), 371-384. doi:https://doi.org/10.1016/j.rasd.2007.09.003

Esposito, & Venuti. (2009). Comparative Analysis of Crying in Children with Autism, Developmental Delays, and Typical Development. *Focus on Autism and Other Developmental Disabilities, 24*(4), 240-247. doi:10.1177/1088357609336449

Esteban-Zubero, E., Alatorre-Jiménez, M. A., López-Pingarrón, L., Reyes-Gonzales, M. C., Almeida-Souza, P., Cantín-Golet, A., . . . Reiter, R. J. (2016). Melatonin's role in preventing toxin-related and sepsis-mediated hepatic damage: a review. *Pharmacological Research, 105*, 108-120.

Ethemoglu, M. S., Kutlu, S., Seker, F. B., Erdogan, C. S., Bingol, C. A., & Yilmaz, B. (2019). Effects of agomelatine on electrocorticogram activity on penicillin-induced seizure model of rats. *Neurosci Lett, 690*, 120-125. doi:10.1016/j.neulet.2018.09.014

Falcicchia, C., Paolone, G., Emerich, D. F., Lovisari, F., Bell, W. J., Fradet, T., . . . Simonato, M. (2018). Seizure-Suppressant and Neuroprotective Effects of Encapsulated BDNF-Producing Cells in a Rat Model of Temporal Lobe Epilepsy. *Molecular Therapy. Methods & Clinical Development, 9*, 211-224. doi:10.1016/j.omtm.2018.03.001

Felger, J. C. (2019). Role of Inflammation in Depression and Treatment Implications. *Handb Exp Pharmacol, 250*, 255-286. doi:10.1007/164_2018_166

Forrester, J. V., McMenamin, P. G., & Dando, S. J. (2018). CNS infection and immune privilege. *Nature Reviews Neuroscience, 19*(11), 655-671. doi:10.1038/s41583-018-0070-8

Friedman, L. K., Pellegrini-Giampietro, D. E., Sperber, E. F., Bennett, M. V., Moshe, S. L., & Zukin, R. S. (1994). Kainate-induced status epilepticus alters glutamate and GABAA receptor gene expression in adult rat hippocampus: an in situ hybridization study. *J Neurosci, 14*(5 Pt 1), 2697-2707.

Frodi, A. (1985). When empathy fails *Infant crying* (pp. 263-277): Springer.

Gagnon, K., & Godbout, R. (2018). Melatonin and Comorbidities in Children with Autism Spectrum Disorder. *Current developmental disorders reports, 5*(3), 197-206. doi:10.1007/s40474-018-0147-0

Galano, A., Tan, D. X., & Reiter, R. J. (2014). Cyclic 3-hydroxymelatonin, a key metabolite enhancing the peroxyl radical scavenging activity of melatonin. *RSC Advances, 4*(10), 5220-5227.

Galic, M. A., Riazi, K., Heida, J. G., Mouihate, A., Fournier, N. M., Spencer, S. J., . . . Pittman, Q. J. (2008). Postnatal inflammation increases seizure susceptibility in adult rats. *Journal of Neuroscience, 28*(27), 6904-6913.

Galley, H. F., Lowes, D. A., Allen, L., Cameron, G., Aucott, L. S., & Webster, N. R. (2014). Melatonin as a potential therapy for sepsis: a phase I dose escalation study and an ex vivo whole blood model under conditions of sepsis. *J Pineal Res, 56*(4), 427-438.

Garthwaite, J., & Wilkin, G. P. (1982). Kainic acid receptors and neurotoxicity in adult and immature rat cerebellar slices. *Neuroscience, 7*(10), 2499-2514. doi:https://doi.org/10.1016/0306-4522(82)90210-X

Gilad, E., Wong, H. R., Zingarelli, B., Virag, L., O'Connor, M., Salzman, A. L., & Szabo, C. (1998). Melatonin inhibits expression of the inducible isoform of nitric oxide synthase in murine macrophages: role of inhibition of NFkappaB activation. *Faseb j, 12*(9), 685-693. doi:10.1096/fasebj.12.9.685

Gimeno, M. F., Landa, A., Sterin-Speziale, N., Cardinali, D. P., & Gimeno, A. L. (1980). Melatonin blocks in vitro generation of prostaglandin by the uterus and hypothalamus. *Eur J Pharmacol, 62*(4), 309-317.

Gladysz, D., Krzywdzinska, A., & Hozyasz, K. K. (2018). Immune Abnormalities in Autism Spectrum Disorder-Could They Hold Promise for Causative Treatment? *Mol Neurobiol, 55*(8), 6387-6435. doi:10.1007/s12035-017-0822-x

Goldberg-Stern, H., Oren, H., Peled, N., & Garty, B. Z. (2012). Effect of melatonin on seizure frequency in intractable epilepsy: a pilot study. *J Child Neurol, 27*(12), 1524-1528. doi:10.1177/0883073811435916

Goodwin, S. W., Lambrinos, A. I., Ferro, M. A., Sabaz, M., & Speechley, K. N. (2015). Development and assessment of a shortened Quality of Life in Childhood Epilepsy Questionnaire (QOLCE-55). *Epilepsia, 56*(6), 864-872. doi:10.1111/epi.13000

Green, J. A., Whitney, P. G., & Potegal, M. (2011). Screaming, yelling, whining, and crying: categorical and intensity differences in vocal expressions of anger and sadness in children's tantrums. *Emotion (Washington, D.C.), 11*(5), 1124-1133. doi:10.1037/a0024173

Gressens, P., Schwendimann, L., Husson, I., Sarkozy, G., Mocaer, E., Vamecq, J., & Spedding, M. (2008). Agomelatine, a melatonin receptor agonist with 5-HT2C receptor antagonist properties, protects the developing murine white matter against excitotoxicity. *European Journal of Pharmacology, 588*(1), 58-63. doi:https://doi.org/10.1016/j.ejphar.2008.04.016

Grivas, T. B., & Savvidou, O. D. (2007). Melatonin the "light of night" in human biology and adolescent idiopathic scoliosis. *Scoliosis, 2*(1), 6. doi:10.1186/1748-7161-2-6

Grooms, S. Y., Opitz, T., Bennett, M. V., & Zukin, R. S. (2000). Status epilepticus decreases glutamate receptor 2 mRNA and protein expression in hippocampal pyramidal cells before neuronal death. *Proceedings of the National Academy of Sciences, 97*(7), 3631-3636.

Guaiana, G., Gupta, S., Chiodo, D., Davies, S. J., Haederle, K., & Koesters, M. (2013). Agomelatine versus other antidepressive agents for major depression. *Cochrane Database Syst Rev*(12), Cd008851. doi:10.1002/14651858.CD008851.pub2

Guardiola-Lemaitre, B., De Bodinat, C., Delagrange, P., Millan, M. J., Munoz, C., & Mocaër, E. (2014). Agomelatine: mechanism of action and pharmacological profile in relation to antidepressant properties. *British Journal of Pharmacology, 171*(15), 3604-3619. doi:10.1111/bph.12720

Hamani, C., & Mello, L. E. (1997). Status epilepticus induced by pilocarpine and picrotoxin. *Epilepsy Res, 28*(1), 73-82. doi:10.1016/s0920-1211(97)00030-2

Hardeland, R. (2010). Melatonin metabolism in the central nervous system. *Current Neuropharmacology, 8*(3), 168-181. doi:10.2174/157015910792246244

Heida, J. G., & Pittman, Q. J. (2005). Causal links between brain cytokines and experimental febrile convulsions in the rat. *Epilepsia, 46*(12), 1906-1913.

Herrick-Davis, K., Grinde, E., Lindsley, T., Teitler, M., Mancia, F., Cowan, A., & Mazurkiewicz, J. E. (2015). Native serotonin 5-HT2C receptors are expressed as homodimers on the apical surface of choroid plexus epithelial cells. *Molecular pharmacology, 87*(4), 660-673. doi:10.1124/mol.114.096636

Hesdorffer, D. C., Logroscino, G., Benn, E. K., Katri, N., Cascino, G., & Hauser, W. A. (2011). Estimating risk for developing epilepsy: a population-based study in Rochester, Minnesota. *Neurology, 76*(1), 23-27. doi:10.1212/WNL.0b013e318204a36a

Hill, R. A., Murray, S. S., Halley, P. G., Binder, M. D., Martin, S. J., & van den Buuse, M. (2011). Brain-derived neurotrophic factor expression is increased in the hippocampus of 5-HT(2C) receptor knockout mice. *Hippocampus, 21*(4), 434-445. doi:10.1002/hipo.20759

Hiragi, T., Ikegaya, Y., & Koyama, R. (2018). Microglia after Seizures and in Epilepsy. *Cells, 7*(4), 26. doi:10.3390/cells7040026

Holmes, G. L., & Ben-Ari, Y. (2001). The neurobiology and consequences of epilepsy in the developing brain. *Pediatr Res, 49*(3), 320-325. doi:10.1203/00006450-200103000-00004

Hong, J., Cho, I. H., Kwak, K. I., Suh, E. C., Seo, J., Min, H. J., . . . Lee, S. J. (2010). Microglial Toll-like receptor 2 contributes to kainic acid-induced glial activation and hippocampal neuronal cell death. *J Biol Chem, 285*(50), 39447-39457. doi:10.1074/jbc.M110.132522

Hu, Wang, Pan, Zhang, Fang, Jiang, . . . Lin. (2017). Melatonin protects against blood-brain barrier damage by inhibiting the TLR4/ NF-kappaB signaling pathway after LPS treatment in neonatal rats. *Oncotarget, 8*(19), 31638-31654. doi:10.18632/oncotarget.15780

Hu, Zhang, Wen, Liu, Cai, Du, . . . Xia. (2019). Melatonin decreases M1 polarization via attenuating mitochondrial oxidative damage depending on UCP2 pathway in prorenin-treated microglia. *PLoS ONE, 14*(2), e0212138-e0212138. doi:10.1371/journal.pone.0212138

Huang, H., Lee, S.-C., & Yang, X.-L. (2005). Modulation by melatonin of glutamatergic synaptic transmission in the carp retina. *The Journal of Physiology, 569*(Pt 3), 857-871. doi:10.1113/jphysiol.2005.098798

Iwasaki, A., & Medzhitov, R. (2010). Regulation of Adaptive Immunity by the Innate Immune System. *Science, 327*(5963), 291. doi:10.1126/science.1183021

Iwasaki, A., & Medzhitov, R. (2015). Control of adaptive immunity by the innate immune system. *Nature Immunology, 16*(4), 343-353. doi:10.1038/ni.3123

Jafarpour, S., Stredny, C. M., Piantino, J., & Chapman, K. E. (2018). Baseline and outcome assessment in pediatric status epilepticus. *Seizure*. doi:https://doi.org/10.1016/j.seizure.2018.04.019

Jarvela, J. T., Ruohonen, S., Kukko-Lukjanov, T. K., Plysjuk, A., Lopez-Picon, F. R., & Holopainen, I. E. (2011). Kainic acid-induced neurodegeneration and activation of inflammatory processes in organotypic hippocampal slice cultures: treatment with cyclooxygenase-2 inhibitor does not prevent neuronal death. *Neuropharmacology, 60*(7-8), 1116-1125. doi:10.1016/j.neuropharm.2010.09.024

Jenwitheesuk, A., Nopparat, C., Mukda, S., Wongchitrat, P., & Govitrapong, P. (2014). Melatonin regulates aging and neurodegeneration through energy metabolism, epigenetics, autophagy and circadian rhythm pathways. *International Journal of Molecular Sciences, 15*(9), 16848-16884. doi:10.3390/ijms150916848

Jin, X. T., & Smith, Y. (2011). Localization and functions of kainate receptors in the basal ganglia. *Adv Exp Med Biol, 717*, 27-37. doi:10.1007/978-1-4419-9557-5_3

Jockers, R., Maurice, P., Boutin, J. A., & Delagrange, P. (2008). Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? *British Journal of Pharmacology, 154*(6), 1182-1195. doi:10.1038/bjp.2008.184

Jorgensen, M. B., Finsen, B. R., Jensen, M. B., Castellano, B., Diemer, N. H., & Zimmer, J. (1993). Microglial and astroglial reactions to ischemic and kainic acid-induced lesions of the adult rat hippocampus. *Exp Neurol, 120*(1), 70-88. doi:10.1006/exnr.1993.1041

Jou, M.-J., Peng, T.-I., Yu, P.-Z., Jou, S.-B., Reiter, R. J., Chen, J.-Y., . . . Hsu, L.-F. (2007). Melatonin protects against common deletion of mitochondrial DNA-augmented mitochondrial oxidative stress and apoptosis. *J Pineal Res, 43*(4), 389-403. doi:10.1111/j.1600-079X.2007.00490.x

Jyonouchi, H., Sun, S., & Le, H. (2001). Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol, 120*(1-2), 170-179. doi:10.1016/s0165-5728(01)00421-0

Kanner, A. M. (2006). Depression and Epilepsy: A New Perspective on Two Closely Related Disorders. *Epilepsy Currents, 6*(5), 141-146. doi:10.1111/j.1535-7511.2006.00125.x

Kasari, C., & Sigman, M. (1997). Linking Parental Perceptions to Interactions in Young Children with Autism. *Journal of Autism and Developmental Disorders, 27*(1), 39-57. doi:10.1023/A:1025869105208

Kennaway, D. J., & Wright, H. (2002). Melatonin and circadian rhythms. *Current topics in medicinal chemistry, 2*(2), 199-209. doi:10.2174/1568026023394380

Khaliq, S., Haider, S., Saleem, S., Memon, Z., & Haleem, D. J. (2012). Influence of serotonergic 5-HT2C receptor antagonist mesulergine in the reversal of memory deficits induced by mCPP. *J Coll Physicians Surg Pak, 22*(2), 75-79. doi:02.2012/jcpsp.7579

Kim, I., Mlsna, L. M., Yoon, S., Le, B., Yu, S., Xu, D., & Koh, S. (2015). A postnatal peak in microglial development in the mouse hippocampus is correlated with heightened sensitivity to seizure triggers. *Brain and Behavior, 5*(12), e00403-e00403. doi:10.1002/brb3.403

Köhler, O., Benros, M. E., Nordentoft, M., Farkouh, M. E., Iyengar, R. L., Mors, O., & Krogh, J. (2014). Effect of Anti-inflammatory Treatment on Depression, Depressive Symptoms, and Adverse Effects: A Systematic Review and Meta-analysis of Randomized Clinical Trials. *JAMA Psychiatry, 71*(12), 1381-1391. doi:10.1001/jamapsychiatry.2014.1611

Komaram, R. B., Nukala, S., Palla, J., Nambaru, L. R., & Kasturi, S. M. (2015). A Comparative Study of Efficacy and Safety of Agomelatine and Escitalopram in Major Depressive Disorder. *Journal of clinical and diagnostic research : JCDR, 9*(6), VC05-VC08. doi:10.7860/JCDR/2015/12371.6092

Krishnakumar, A., Nandhu, M. S., & Paulose, C. S. (2009). Upregulation of 5-HT2C receptors in hippocampus of pilocarpine-induced epileptic rats: Antagonism by Bacopa monnieri. *Epilepsy & Behavior, 16*(2), 225-230. doi:https://doi.org/10.1016/j.yebeh.2009.07.031

Lee, H.-S., Wang, S.-Y., Salter, D. M., Wang, C.-C., Chen, S.-J., & Fan, H.-C. (2013). The impact of the use of antiepileptic drugs on the growth of children. *BMC Pediatrics, 13*(1), 211. doi:10.1186/1471-2431-13-211

Lewis, M. L., Kesler, M., Candy, S. A., Rho, J. M., & Pittman, Q. J. (2018). Comorbid epilepsy in autism spectrum disorder: Implications of postnatal inflammation for brain excitability. *Epilepsia, 59*(7), 1316-1326. doi:10.1111/epi.14440

Ling, E. A., & Wong, W. C. (1993). The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia, 7*(1), 9-18. doi:10.1002/glia.440070105

Liu, Clough, Hutchinson, Adamah-Biassi, Popovska-Gorevski, & Dubocovich. (2016). MT1 and MT2 Melatonin Receptors: A Therapeutic Perspective. *Annual Review of Pharmacology and Toxicology, 56*(1), 361-383. doi:10.1146/annurev-pharmtox-010814-124742

Liu, Yu, & Lü. (2016). The causes of new-onset epilepsy and seizures in the elderly. *Neuropsychiatric disease and treatment, 12*, 1425-1434. doi:10.2147/NDT.S107905

Liu, Zhuang, Zhu, Shen, Tan, & Zhou. (2007). Cultured rat cortical astrocytes synthesize melatonin: absence of a diurnal rhythm. *J Pineal Res, 43*(3), 232-238. doi:10.1111/j.1600-079X.2007.00466.x

López-Meraz, M.-L., Medel-Matus, J.-S., Morgado-Valle, C., Beltrán-Parrazal, L., Pérez-Estudillo, C., & Manzo, J. (2014). Effect of lithium-pilocarpine-induced status epilepticus on ultrasonic vocalizations in the infant rat pup. *Epilepsy & Behavior, 31*, 263-266. doi:https://doi.org/10.1016/j.yebeh.2013.10.006

Lugo, Barnwell, L. F., Ren, Y., Lee, W. L., Johnston, L. D., Kim, R., . . . Anderson, A. E. (2008). Altered phosphorylation and localization of the A-type channel, Kv4.2 in status epilepticus. *J Neurochem, 106*(4), 1929-1940. doi:10.1111/j.1471-4159.2008.05508.x

Malkova, N. V., Yu, C. Z., Hsiao, E. Y., Moore, M. J., & Patterson, P. H. (2012). Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain Behav Immun, 26*(4), 607-616. doi:10.1016/j.bbi.2012.01.011

Malow, B., Adkins, K. W., McGrew, S. G., Wang, L., Goldman, S. E., Fawkes, D., & Burnette, C. (2012). Melatonin for sleep in children with autism: a controlled trial examining dose, tolerability, and outcomes. *Journal of Autism and Developmental Disorders, 42*(8), 1729-1737.

Mandell, D. S., Novak, M. M., & Zubritsky, C. D. (2005). Factors associated with age of diagnosis among children with autism spectrum disorders. *Pediatrics, 116*(6), 1480-1486. doi:10.1542/peds.2005-0185

Mazarati, A. M., Lewis, M. L., & Pittman, Q. J. (2017). Neurobehavioral comorbidities of epilepsy: Role of inflammation. *Epilepsia, 58*(S3), 48-56. doi:10.1111/epi.13786

McDuffie, A., Yoder, P., & Stone, W. (2005). Prelinguistic predictors of vocabulary in young children with autism spectrum disorders. *Journal of Speech, Language, and Hearing Research, 48*, 1080+.

McFarlane, H. G., Kusek, G., Yang, M., Phoenix, J., Bolivar, V., & Crawley, J. (2008). Autism-like behavioral phenotypes in BTBR T+ tf/J mice. *Genes, Brain and Behavior, 7*(2), 152-163.

McTighe, S. M., Neal, S. J., Lin, Q., Hughes, Z. A., & Smith, D. G. (2013). The BTBR Mouse Model of Autism Spectrum Disorders Has Learning and Attentional Impairments and Alterations in Acetylcholine and Kynurenic Acid in Prefrontal Cortex. *PLoS ONE, 8*(4), e62189. doi:10.1371/journal.pone.0062189

Medzhitov, R., & Janeway, C. A. (1997). Innate immunity: impact on the adaptive immune response. *Current Opinion in Immunology, 9*(1), 4-9. doi:https://doi.org/10.1016/S0952-7915(97)80152-5

Milanese, M., Tardito, D., Musazzi, L., Treccani, G., Mallei, A., Bonifacino, T., . . . Bonanno, G. (2013). Chronic treatment with agomelatine or venlafaxine reduces depolarization-evoked glutamate release from hippocampal synaptosomes. *BMC Neuroscience, 14*(1), 75. doi:10.1186/1471-2202-14-75

Millan, M. J. (2005). Serotonin 5-HT2C receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. *Therapie, 60*(5), 441-460. doi:10.2515/therapie:2005065

Millichap, J. (2013). Cowden syndrome with cortical malformation and epilepsy. *Pediatric Neurology Briefs, 27*(1).

Mishra, A., & Goel, R. K. (2012). Age dependent learning and memory deficit in Pentylenetetrazol kindled mice. *Eur J Pharmacol, 674*(2-3), 315-320. doi:10.1016/j.ejphar.2011.11.010

Mogenson, G. J., Wu, M., & Jones, D. L. (1980). Locomotor activity elicited by injections of picrotoxin into the ventral tegmental area is attenuated by injections of GABA into the globus pallidus. *Brain Res, 191*(2), 569-571. doi:10.1016/0006-8993(80)91308-6

Morley-Fletcher, S., Mairesse, J., Soumier, A., Banasr, M., Fagioli, F., Gabriel, C., . . . Maccari, S. (2011). Chronic agomelatine treatment corrects behavioral, cellular, and biochemical abnormalities induced by prenatal stress in rats. *Psychopharmacology, 217*(3), 301. doi:10.1007/s00213-011-2280-x

Moulton, E., Barton, M., Robins, D. L., Abrams, D. N., & Fein, D. (2016). Early Characteristics of Children with ASD Who Demonstrate Optimal Progress Between Age Two and Four. *Journal of Autism and Developmental Disorders, 46*(6), 2160-2173. doi:10.1007/s10803-016-2745-1

Moy, S. S., Nadler, J. J., Young, N. B., Perez, A., Holloway, L. P., Barbaro, R. P., . . . Lauder, J. M. (2007). Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. *Behavioural Brain Research, 176*(1), 4-20.

Müller, C. J., Gröticke, I., Bankstahl, M., & Löscher, W. (2009). Behavioral and cognitive alterations, spontaneous seizures, and neuropathology developing after a pilocarpine-induced status epilepticus in C57BL/6 mice. *Exp Neurol, 219*(1), 284-297. doi:https://doi.org/10.1016/j.expneurol.2009.05.035

Munoz-Hoyos, A., Bonillo-Perales, A., Avila-Villegas, R., Gonzalez-Ripoll, M., Uberos, J., Florido-Navio, J., & Molina-Carballo, A. (2007). Melatonin levels during the first week of life and their relation with the antioxidant response in the perinatal period. *Neonatology, 92*(3), 209-216. doi:10.1159/000102957

Musshoff, U., & Speckmann, E. J. (2003). Diurnal actions of melatonin on epileptic activity in hippocampal slices of rats. *Life Sci, 73*(20), 2603-2610. doi:10.1016/s0024-3205(03)00614-3

Nabavi, S. M., Nabavi, S. F., Sureda, A., Xiao, J., Dehpour, A. R., Shirooie, S., . . . Daglia, M. (2019). Anti-inflammatory effects of Melatonin: A mechanistic review. *Critical Reviews in Food Science and Nutrition, 59*(sup1), S4-S16. doi:10.1080/10408398.2018.1487927

Nabbout, R., Belousova, E., Benedik, M. P., Carter, T., Cottin, V., Curatolo, P., . . . Investigators, T. (2018). Epilepsy in tuberous sclerosis complex: Findings from the TOSCA Study. *Epilepsia open, 4*(1), 73-84. doi:10.1002/epi4.12286

Nadeem, A., Ahmad, S. F., El-Sherbeeny, A. M., Al-Harbi, N. O., Bakheet, S. A., & Attia, S. M. (2018). Systemic inflammation in asocial BTBR T+ tf/J mice predisposes them to increased psoriatic inflammation. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 83*, 8-17. doi:https://doi.org/10.1016/j.pnpbp.2017.12.015

Nadler, J. V., Perry, B. W., Gentry, C., & Cotman, C. W. (1980). Degeneration of hippocampal CA3 pyramidal cells induced by intraventricular kainic acid. *Journal of Comparative Neurology, 192*(2), 333-359. doi:10.1002/cne.901920209

Nayak, D., Roth, T. L., & McGavern, D. B. (2014). Microglia development and function. *Annual review of immunology, 32*, 367-402. doi:10.1146/annurev-immunol-032713-120240

Ngugi, A. K., Bottomley, C., Kleinschmidt, I., Sander, J. W., & Newton, C. R. (2010). Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. *Epilepsia, 51*(5), 883-890. doi:10.1111/j.1528-1167.2009.02481.x

Niles. (1991). Melatonin interaction with the benzodiazepine-GABA receptor complex in the CNS. *Adv Exp Med Biol, 294*, 267-277. doi:10.1007/978-1-4684-5952-4_24

Niles, Pickering, & Arciszewski. (1987). Effects of chronic melatonin administration on GABA and diazepam binding in rat brain. *J Neural Transm, 70*(1-2), 117-124. doi:10.1007/bf01252513

Okatani, Y., Wakatsuki, A., & Kaneda, C. (2000). Melatonin increases activities of glutathione peroxidase and superoxide dismutase in fetal rat brain. *J Pineal Res, 28*(2), 89-96.

Olsen, R. W. (2006). Picrotoxin-like channel blockers of GABA_A receptors. *Proceedings of the National Academy of Sciences, 103*(16), 6081. doi:10.1073/pnas.0601121103

Opal, M. D., Klenotich, S. C., Morais, M., Bessa, J., Winkle, J., Doukas, D., . . . Dulawa, S. M. (2013). Serotonin 2C receptor antagonists induce fast-onset antidepressant effects. *Molecular Psychiatry, 19*, 1106. doi:10.1038/mp.2013.144 https://www.nature.com/articles/mp2013144#supplementary-information

Ortiz, F., García, J. A., Acuña-Castroviejo, D., Doerrier, C., López, A., Venegas, C., . . . Escames, G. (2014). The beneficial effects of melatonin against heart mitochondrial impairment during sepsis: inhibition of i NOS and preservation of n NOS. *J Pineal Res, 56*(1), 71-81.

Panayiotopoulos, C. (2005). Neonatal seizures and neonatal syndromes *The Epilepsies: Seizures, Syndromes and Management*: Bladon Medical Publishing.

Park, D., Joo, S. S., Kim, T. K., Lee, S. H., Kang, H., Lee, H. J., . . . Kim, Y.-B. (2012). Human neural stem cells overexpressing choline acetyltransferase restore cognitive function of kainic acid-induced learning and memory deficit animals: SAGE Publications Sage CA: Los Angeles, CA.

Peled, N., Shorer, Z., Peled, E., & Pillar, G. (2001). Melatonin Effect on Seizures in Children with Severe Neurologic Deficit Disorders. *Epilepsia, 42*(9), 1208-1210. doi:10.1046/j.1528-1157.2001.28100.x

Pingel, J., Barber, L., Andersen, I. T., Walden, F. V., Wong, C., Døssing, S., & Nielsen, J. B. (2019). Systemic inflammatory markers in individuals with cerebral palsy. *European Journal of Inflammation, 17*, 2058739218823474. doi:10.1177/2058739218823474

Racagni, G., Riva, M. A., Molteni, R., Musazzi, L., Calabrese, F., Popoli, M., & Tardito, D. (2011). Mode of action of agomelatine: synergy between melatonergic and 5-HT2C receptors. *World J Biol Psychiatry, 12*(8), 574-587. doi:10.3109/15622975.2011.595823

Racine, R. J. (1972). Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalography and Clinical Neurophysiology, 32*(3), 281-294. doi:https://doi.org/10.1016/0013-4694(72)90177-0

Rakhade, S. N., Fitzgerald, E. F., Klein, P. M., Zhou, C., Sun, H., Huganir, R. L., & Jensen, F. E. (2012). Glutamate receptor 1 phosphorylation at serine 831 and 845 modulates seizure susceptibility and hippocampal hyperexcitability after early life seizures. *J Neurosci, 32*(49), 17800-17812. doi:10.1523/jneurosci.6121-11.2012

Ramani, T., Auletta, C. S., Weinstock, D., Mounho-Zamora, B., Ryan, P. C., Salcedo, T. W., & Bannish, G. (2015). Cytokines: The Good, the Bad, and the Deadly. *Int J Toxicol, 34*(4), 355-365. doi:10.1177/1091581815584918

Rana, A., & Musto, A. E. (2018). The role of inflammation in the development of epilepsy. *Journal of Neuroinflammation, 15*(1), 144-144. doi:10.1186/s12974-018-1192-7

Ransohoff, R. M., Kivisäkk, P., & Kidd, G. (2003). Three or more routes for leukocyte migration into the central nervous system. *Nature Reviews Immunology, 3*(7), 569-581. doi:10.1038/nri1130

Reisinger, S., Khan, D., Kong, E., Berger, A., Pollak, A., & Pollak, D. D. (2015). The Poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. *Pharmacol Ther, 149*, 213-226. doi:https://doi.org/10.1016/j.pharmthera.2015.01.001

Reiter, R. J., Calvo, J. R., Karbownik, M., Qi, W., & Tan, D. X. (2000). Melatonin and its relation to the immune system and inflammation. *Ann N Y Acad Sci, 917*, 376-386.

Reiter, R. J., Mayo, J. C., Tan, D. X., Sainz, R. M., Alatorre-Jimenez, M., & Qin, L. (2016). Melatonin as an antioxidant: under promises but over delivers. *J Pineal Res, 61*(3), 253-278. doi:10.1111/jpi.12360

Reiter, R. J., Tan, D. X., Manchester, L. C., Lopez-Burillo, S., Sainz, R. M., & Mayo, J. C. (2003). Melatonin: detoxification of oxygen and nitrogen-based toxic reactants. *Adv Exp Med Biol, 527*, 539-548.

Ressmeyer, A.-R., Mayo, J. C., Zelosko, V., Sáinz, R. M., Tan, D.-X., Poeggeler, B., . . . Hardeland, R. (2003). Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox report, 8*(4), 205-213.

Reynolds, Nolan, Huebschman, Hodges, & Lugo. (2017). Early-life status epilepticus acutely impacts select quantitative and qualitative features of neonatal vocalization behavior: Spectrographic and temporal characterizations in C57BL/6 mice. *Epilepsy Behav, 72*, 58-62. doi:10.1016/j.yebeh.2017.04.025

Reynolds, Smith, Jefferson, & Lugo. (2016). The effect of early life status epilepticus on ultrasonic vocalizations in mice. *Epilepsia, 57*(9), 1377-1385. doi:10.1111/epi.13450

Robinson, J. H., & Deadwyler, S. A. (1981). Kainic acid produces depolarization of CA3 pyramidal cells in the in vitro hippocampal slice. *Brain Research, 221*(1), 117-127. doi:https://doi.org/10.1016/0006-8993(81)91067-2

Rodriguez, J. I., & Kern, J. K. (2011). Evidence of microglial activation in autism and its possible role in brain underconnectivity. *Neuron glia biology, 7*(2-4), 205-213. doi:10.1017/S1740925X12000142

Rose Priel, M., dos Santos, N. F., & Cavalheiro, E. A. (1996). Developmental aspects of the pilocarpine model of epilepsy. *Epilepsy Res, 26*(1), 115-121. doi:https://doi.org/10.1016/S0920-1211(96)00047-2

Rothgänger, H. (2003). Analysis of the sounds of the child in the first year of age and a comparison to the language. *Early Human Development, 75*(1-2), 55-69.

Roy, H., Lippé, S., Lussier, F., Sauerwein, H. C., Lortie, A., Lacroix, J., & Lassonde, M. (2011). Developmental outcome after a single episode of status epilepticus. *Epilepsy & Behavior, 21*(4), 430-436. doi:10.1016/j.yebeh.2011.05.009

Sabilallah, M., Fontanaud, P., Linck, N., Boussadia, B., Peyroutou, R., Lasgouzes, T., . . . Hirbec, H. E. (2016). Evidence for Status Epilepticus and Pro-Inflammatory Changes after Intranasal Kainic Acid Administration in Mice. *PLoS ONE, 11*(3), e0150793. doi:10.1371/journal.pone.0150793

Sainz, R. M., Mayo, J. C., Rodriguez, C., Tan, D. X., Lopez-Burillo, S., & Reiter, R. J. (2003). Melatonin and cell death: differential actions on apoptosis in normal and cancer cells. *Cellular and Molecular Life Sciences CMLS, 60*(7), 1407-1426. doi:10.1007/s00018-003-2319-1

Samokhina, E., & Samokhin, A. (2018). Neuropathological profile of the pentylenetetrazol (PTZ) kindling model. *Int J Neurosci, 128*(11), 1086-1096. doi:10.1080/00207454.2018.1481064

Sandyk, R., Tsagas, N., & Anninos, P. A. (1992). Melatonin as a proconvulsive hormone in humans. *Int J Neurosci, 63*(1-2), 125-135. doi:10.3109/00207459208986662

Sarkisian, M. R., Tandon, P., Liu, Z., Yang, Y., Hori, A., Holmes, G. L., & Stafstrom, C. E. (1997). Multiple Kainic Acid Seizures in the Immature and Adult Brain: Ictal Manifestations and Long–Term Effects on Learning and Memory. *Epilepsia, 38*(11), 1157-1166. doi:10.1111/j.1528-1157.1997.tb01211.x

Savina, T. A., Balashova, O. A., & Shchipakina, T. G. (2006). Effect of chronic consumption of sodium valproate and melatonin on seizure activity in Krushinskii-Molodkina rats. *Bull Exp Biol Med, 142*(5), 601-604. doi:10.1007/s10517-006-0429-0

Sayyah, M., Javad-Pour, M., & Ghazi-Khansari, M. (2003). The bacterial endotoxin lipopolysaccharide enhances seizure susceptibility in mice: involvement of proinflammatory factors: nitric oxide and prostaglandins. *Neuroscience, 122*(4), 1073-1080.

Scattoni, M. L., Gandhy, S. U., Ricceri, L., & Crawley, J. N. (2008). Unusual Repertoire of Vocalizations in the BTBR T+tf/J Mouse Model of Autism. *PLoS ONE, 3*(8), e3067. doi:10.1371/journal.pone.0003067

Scheller, J., Chalaris, A., Schmidt-Arras, D., & Rose-John, S. (2011). The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1813*(5), 878-888. doi:https://doi.org/10.1016/j.bbamcr.2011.01.034

Schultz, R. T. (2005). Developmental deficits in social perception in autism: the role of the amygdala and fusiform face area. *International Journal of Developmental Neuroscience, 23*(2), 125-141. doi:https://doi.org/10.1016/j.ijdevneu.2004.12.012

Seidenberg, M., Pulsipher, D. T., & Hermann, B. (2009). Association of epilepsy and comorbid conditions. *Future neurology, 4*(5), 663-668. doi:10.2217/fnl.09.32

Serafini, A., Lukas, R. V., VanHaerents, S., Warnke, P., Tao, J. X., Rose, S., & Wu, S. (2016). Paraneoplastic epilepsy. *Epilepsy Behav, 61*, 51-58. doi:10.1016/j.yebeh.2016.04.046

Shair, H. N. (2007). Acquisition and expression of a socially mediated separation response. *Behavioural Brain Research, 182*(2), 180-192. doi:http://dx.doi.org/10.1016/j.bbr.2007.02.016

Sheinkopf, S. J., Iverson, J. M., Rinaldi, M. L., & Lester, B. M. (2012). Atypical Cry Acoustics in 6-Month-Old Infants at Risk for Autism Spectrum Disorder. *Autism Research, 5*(5), 331-339.

Sheldon, P. W., & Aghajanian, G. K. (1991). Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex: evidence for mediation by 5-HT1C receptors in pyramidal cells and 5-HT2 receptors in interneurons. *Synapse, 9*(3), 208-218. doi:10.1002/syn.890090307

Simões, L. R., Sangiogo, G., Tashiro, M. H., Generoso, J. S., Faller, C. J., Dominguini, D., . . . Barichello, T. (2018). Maternal immune activation induced by lipopolysaccharide triggers immune response in pregnant mother and fetus, and induces behavioral impairment in adult rats. *Journal of Psychiatric Research, 100*, 71-83. doi:https://doi.org/10.1016/j.jpsychires.2018.02.007

Siniscalco, Schultz, Brigida, & Antonucci. (2018). Inflammation and Neuro-Immune Dysregulations in Autism Spectrum Disorders. *Pharmaceuticals (Basel), 11*(2), 56. doi:10.3390/ph11020056

Slanar, O., Pelisek, V., & Vanecek, J. (2000). Melatonin inhibits pituitary adenylyl cyclase-activating polypeptide-induced increase of cyclic AMP accumulation and [Ca2+]i in cultured cells of neonatal rat pituitary. *Neurochem Int, 36*(3), 213-219. doi:10.1016/s0197-0186(99)00120-5

Sofroniew, M. V. (2014). Astrogliosis. *Cold Spring Harbor perspectives in biology, 7*(2), a020420-a020420. doi:10.1101/cshperspect.a020420

Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathologica, 119*(1), 7-35. doi:10.1007/s00401-009-0619-8

Soltis, J. (2005). The signal functions of early infant crying. *Behavioral and Brain Sciences, 27*(4), 443-458. doi:10.1017/S0140525X0400010X

Sommer, C., Roth, S. U., & Kiessling, M. (2001). Kainate-induced epilepsy alters protein expression of AMPA receptor subunits GluR1, GluR2 and AMPA receptor binding protein in the rat hippocampus. *Acta Neuropathol, 101*(5), 460-468. doi:10.1007/s004010000310

Sperk, G., Lassmann, H., Baran, H., Kish, S. J., Seitelberger, F., & Hornykiewicz, O. (1983). Kainic acid induced seizures: Neurochemical and histopathological changes. *Neuroscience, 10*(4), 1301-1315. doi:https://doi.org/10.1016/0306-4522(83)90113-6

Srinivas, H. V., & Shah, U. (2017). Comorbidities of epilepsy. *Neurol India, 65*(Supplement), S18-s24. doi:10.4103/neuroindia.NI_922_16

Srinivasan, V., Mohamed, M., & Kato, H. (2012). Melatonin in bacterial and viral infections with focus on sepsis: a review. *Recent Pat Endocr Metab Immune Drug Discov, 6*(1), 30-39.

Stafstrom, C. E., & Carmant, L. (2015). Seizures and epilepsy: an overview for neuroscientists. *Cold Spring Harbor perspectives in medicine, 5*(6), a022426. doi:10.1101/cshperspect.a022426

Stein, D. J., Ahokas, A., Jarema, M., Avedisova, A. S., Vavrusova, L., Chaban, O., . . . de Bodinat, C. (2017). Efficacy and safety of agomelatine (10 or 25 mg/day) in non-depressed out-patients with generalized anxiety disorder: A 12-week, double-blind, placebo-controlled study. *European Neuropsychopharmacology, 27*(5), 526-537. doi:https://doi.org/10.1016/j.euroneuro.2017.02.007

Steinlein, O. K. (2008). Genetics and epilepsy. *Dialogues in clinical neuroscience, 10*(1), 29-38.

Tan, Hardeland, Manchester, Galano, & Reiter. (2014). Cyclic-3-hydroxymelatonin (C3HOM), a potent antioxidant, scavenges free radicals and suppresses oxidative reactions. *Current medicinal chemistry, 21*(13), 1557-1565.

Tan, Manchester, Qin, & Reiter. (2016). Melatonin: A Mitochondrial Targeting Molecule Involving Mitochondrial Protection and Dynamics. *International Journal of Molecular Sciences, 17*(12), 2124. doi:10.3390/ijms17122124

Tan, Manchester, Reiter, Plummer, Limson, Weintraub, & Qi. (2000). Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. *Free Radical Biology and Medicine, 29*(11), 1177-1185.

Tan, Reiter, Manchester, Yan, El-Sawi, Sainz, . . . Hardeland. (2002). Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. *Current topics in medicinal chemistry, 2*(2), 181-197.

Tang, Y., & Le, W. (2016). Differential Roles of M1 and M2 Microglia in Neurodegenerative Diseases. *Mol Neurobiol, 53*(2), 1181-1194. doi:10.1007/s12035-014-9070-5

Tardito, D., Milanese, M., Bonifacino, T., Musazzi, L., Grilli, M., Mallei, A., . . . Bonanno, G. (2010). Blockade of stress-induced increase of glutamate release in the rat prefrontal/frontal cortex by agomelatine involves synergy between melatonergic and 5-HT2C receptor-dependent pathways. *BMC Neuroscience, 11*, 68-68. doi:10.1186/1471-2202-11-68

Tavares, L. R. R., Baptista-de-Souza, D., & Canto-de-Souza, A. (2018). Activation of 5-HT2C (but not 5-HT1A) receptors in the amygdala enhances fear-induced antinociception: Blockade with local 5-HT2C antagonist or systemic fluoxetine. *Neuropharmacology, 135*, 376-385. doi:10.1016/j.neuropharm.2018.03.008

Tchekalarova, Atanasova, Kortenska, Atanasova, & Lazarov. (2018). Chronic agomelatine treatment prevents comorbid depression in the post-status epilepticus model of acquired epilepsy through suppression of inflammatory signaling. *Neurobiol Dis, 115*, 127-144. doi:10.1016/j.nbd.2018.04.005

Tchekalarova, Atanasova, Nenchovska, Atanasova, Kortenska, Gesheva, & Lazarov. (2017). Agomelatine protects against neuronal damage without preventing epileptogenesis in the kainate model of temporal lobe epilepsy. *Neurobiol Dis, 104*, 1-14. doi:https://doi.org/10.1016/j.nbd.2017.04.017

Tian, Y., Yabuki, Y., Moriguchi, S., Fukunaga, K., Mao, P.-J., Hong, L.-J., . . . Han, F. (2014). Melatonin reverses the decreases in hippocampal protein serine/threonine kinases observed in an animal model of autism. *J Pineal Res, 56*(1), 1-11. doi:10.1111/jpi.12081

Tordjman, S., Najjar, I., Bellissant, E., Anderson, G. M., Barburoth, M., Cohen, D., . . . Lagdas, E. (2013). Advances in the research of melatonin in autism spectrum disorders: literature review and new perspectives. *International Journal of Molecular Sciences, 14*(10), 20508-20542.

Treiman, D. M. (2001). GABAergic mechanisms in epilepsy. *Epilepsia, 42 Suppl 3*, 8-12. doi:10.1046/j.1528-1157.2001.042suppl.3008.x

Tsai, P. T., Hull, C., Chu, Y., Greene-Colozzi, E., Sadowski, A. R., Leech, J. M., . . . Sahin, M. (2012). Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature, 488*(7413), 647-651.

Turner, D. A., & Wheal, H. V. (1991). Excitatory synaptic potentials in kainic acid-denervated rat CA1 pyramidal neurons. *The Journal of Neuroscience, 11*(9), 2786. doi:10.1523/JNEUROSCI.11-09-02786.1991

Turvey, S. E., & Broide, D. H. (2010). Innate immunity. *The Journal of allergy and clinical immunology, 125*(2 Suppl 2), S24-S32. doi:10.1016/j.jaci.2009.07.016

Varga, E. A., Pastore, M., Prior, T., Herman, G. E., & McBride, K. L. (2009). The prevalence of PTEN mutations in a clinical pediatric cohort with autism spectrum disorders, developmental delay, and macrocephaly. *Genetics In Medicine, 11*, 111. doi:10.1097/GIM.0b013e31818fd762

Vargas-Sánchez, K., Mogilevskaya, M., Rodríguez-Pérez, J., Rubiano, M. G., Javela, J. J., & González-Reyes, R. E. (2018). Astroglial role in the pathophysiology of status epilepticus: an overview. *Oncotarget, 9*(42), 26954-26976. doi:10.18632/oncotarget.25485

Varin, A., & Gordon, S. (2009). Alternative activation of macrophages: immune function and cellular biology. *Immunobiology, 214*(7), 630-641. doi:10.1016/j.imbio.2008.11.009

Vezzani, A. (2015). Anti-inflammatory drugs in epilepsy: does it impact epileptogenesis? *Expert Opin Drug Saf, 14*(4), 583-592. doi:10.1517/14740338.2015.1010508

Vezzani, A., French, J., Bartfai, T., & Baram, T. Z. (2011). The role of inflammation in epilepsy. *Nat Rev Neurol, 7*(1), 31-40. doi:10.1038/nrneurol.2010.178

Vignoli, A., La Briola, F., Peron, A., Turner, K., Vannicola, C., Saccani, M., . . . Canevini, M. P. (2015). Autism spectrum disorder in tuberous sclerosis complex: searching for risk markers. *Orphanet journal of rare diseases, 10*, 154-154. doi:10.1186/s13023-015-0371-1

Vijitruth, R., Liu, M., Choi, D.-Y., Nguyen, X. V., Hunter, R. L., & Bing, G. (2006). Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease. *Journal of Neuroinflammation, 3*(1), 6. doi:10.1186/1742-2094-3-6

Wang. (2009). The anti-apoptotic activity of melatonin in neurodegenerative diseases. *CNS neuroscience & therapeutics, 15*(4), 345-357. doi:10.1111/j.1755-5949.2009.00105.x

Wang, Yu, Simonyi, Sun, & Sun. (2005). Kainic acid-mediated excitotoxicity as a model for neurodegeneration. *Mol Neurobiol, 31*(1), 3-16. doi:10.1385/MN:31:1-3:003

WHO. (2017, February 2017). Epilepsy Fact Sheet. Retrieved from http://www.who.int/mediacentre/factsheets/fs999/en/

Wolff, P. H. (1969). The natural history of crying and other vocalizations in early infancy. *Determinants of infant behavior*, 81-111.

Wright, B., Sims, D., Smart, S., Alwazeer, A., Alderson-Day, B., Allgar, V., . . . Jardine, J. (2011). Melatonin versus placebo in children with autism spectrum conditions and severe sleep problems not amenable to behaviour management strategies: a randomised controlled crossover trial. *Journal of Autism and Developmental Disorders, 41*(2), 175-184.

Wu, U. I., Mai, F. D., Sheu, J. N., Chen, L. Y., Liu, Y. T., Huang, H. C., & Chang, H. M. (2011). Melatonin inhibits microglial activation, reduces pro-inflammatory cytokine levels, and rescues hippocampal neurons of adult rats with acute Klebsiella pneumoniae meningitis. *J Pineal Res, 50*(2), 159-170. doi:10.1111/j.1600-079X.2010.00825.x

Xie, Z., Chen, F., Li, W. A., Geng, X., Li, C., Meng, X., . . . Yu, F. (2017). A review of sleep disorders and melatonin. *Neurological Research, 39*(6), 559-565. doi:10.1080/01616412.2017.1315864

Yahyavi-Firouz-Abadi, N., Tahsili-Fahadan, P., Riazi, K., Ghahremani, M. H., & Dehpour, A. R. (2007). Melatonin enhances the anticonvulsant and proconvulsant effects of morphine in mice: role for nitric oxide signaling pathway. *Epilepsy Res, 75*(2-3), 138-144. doi:10.1016/j.eplepsyres.2007.05.002

Yohn, C. N., Gergues, M. M., & Samuels, B. A. (2017). The role of 5-HT receptors in depression. *Molecular brain, 10*(1), 28-28. doi:10.1186/s13041-017-0306-y

Young, D., & Dragunow, M. (1994). Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. *Neuroscience, 58*(2), 245-261. doi:10.1016/0306-4522(94)90032-9

Yu, H., Dickson, E. J., Jung, S.-R., Koh, D.-S., & Hille, B. (2016). High membrane permeability for melatonin. *The Journal of general physiology, 147*(1), 63-76. doi:10.1085/jgp.201511526

Zanassi, P., Paolillo, M., Feliciello, A., Avvedimento, E. V., Gallo, V., & Schinelli, S. (2001). cAMP-dependent protein kinase induces cAMP-response element-binding protein phosphorylation via an intracellular calcium release/ERK-dependent pathway in striatal neurons. *Journal of Biological Chemistry, 276*(15), 11487-11495.

Zeskind, P. S., & Marshall, T. R. (1988). The relation between variations in pitch and maternal perceptions of infant crying. *Child Development*, 193-196.

Zhang, Hu, Qian, O'Callaghan, & Hong. (2010). Astrogliosis in CNS pathologies: is there a role for microglia? *Mol Neurobiol, 41*(2-3), 232-241. doi:10.1007/s12035-010-8098-4

Zhang, & Zhu. (2011). Kainic Acid-induced neurotoxicity: targeting glial responses and glia-derived cytokines. *Current Neuropharmacology, 9*(2), 388-398. doi:10.2174/157015911795596540

Zhang, J.-M., & An, J. (2007). Cytokines, inflammation, and pain. *International anesthesiology clinics, 45*(2), 27-37. doi:10.1097/AIA.0b013e318034194e

Zheng, X.-Y., Zhang, H.-L., Luo, Q., & Zhu, J. (2011). Kainic Acid-Induced Neurodegenerative Model: Potentials and Limitations. *Journal of Biomedicine and Biotechnology, 2011*. doi:10.1155/2011/457079

Zisapel, N. (2018). New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. *British Journal of Pharmacology, 175*(16), 3190-3199. doi:10.1111/bph.14116