DOES CONSUMPTION OF A WESTERN DIET DURING EARLY DEVELOPMENT EXACERBATE HIPPOCAMPAL MICROGLIOSIS AFTER PILOCARPINE-INDUCED STATUS EPILEPTICUS?

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ABSTRACT

Rationale: Western diet (WD) consumption has been identified as a contributing source of proinflammatory responses in the brain, as demonstrated by elevated cytokines and microgliosis. Evidence from rodent studies suggests that these proinflammatory effects are seen globally in the brain, including in the hippocampus, suggesting a role for WD contributing to pathophysiological disruptions to brain function. Moreover, hippocampal microgliosis is a hallmark not only in experimental models of seizure disorders but also in acquired epilepsy in humans. Inflammatory responses of the brain to insults, as in the case of epilepsy, are modulated by microglia—the resident immune cells of the brain. Activation and proliferation of microglia contributes to the development and progression of spontaneous recurring seizures, and ultimately epilepsy. Because WD consumption also engages microglia in inflammatory responses in rodent models, WD consumption that occurs prior to a seizure-related insult might potentially prime the hippocampus for exacerbated responses.

The potential interaction between WD consumption, epilepsy and hippocampal pathology has been relatively unexplored. Furthermore, the effects of early developmental exposure to WD on seizures or acquired epilepsies have not been studied. These interactions are important to examine in light of increasing rates of WD consumption in the U.S. According to the latest USDA data, 70% of the population exceed the recommended dietary intake of added sugars and saturated fats (Flegal et al., 2016). Along with rising rates of WD consumption, rates of epilepsy in children in the U.S. have increased by 5% in the past decade, and there are currently close to 500,000 cases (Zack & Kobau, 2015). The goal of the present experiments is to use a rat model of acquired temporal lobe epilepsy—pilocarpine-induced SE—to test the hypothesis that WD exacerbates the inflammatory changes following SE neuronal injury in hippocampus of rats. Our *central hypothesis* is that consumption of WD during early development will produce an inflammatory state which will prime the hippocampus for greater neuroinflammation following SE.

Methods: The goal of this study is to use a rat model of acquired temporal lobe epilepsy, pilocarpine-induced status epilepticus (SE), to test the hypothesis that WD exacerbates the inflammatory consequences of SE in hippocampus of male rats. Testing this hypothesis in animals consuming WD from early in life may provide insight into how a modifiable environmental factor could influence the development of seizure disorders.

Results: We found no significant differences in body weight at induction, or latency to SE after pilocarpine injection in animals on a WD compared to a standard control diet. There were no differences in time to sedation after diazepam injection or in time to recovery from diazepam in animals that reached SE across the diets. Analysis of Iba1+ cells number revealed no differences in microgliosis between diet or seizure groups or across hippocampal regions of interest. Morphological assessment per hippocampal area did not show significant differences between diet or seizure groups across hippocampal areas. Thus, under the current conditions, microgliosis was not produced by consumption of a WD during early development; it was not observed 4 hours after seizure induction in animals consuming a control diet; and there was no evidence that WD exacerbated any effects of pilocarpine.

INTRODUCTION

Status Epilepticus

Status epilepticus (SE) is a sustained convulsive state that lasts longer than 5 minutes in humans and longer than 30 minutes in animals. SE is a medical emergency that can be fatal if not promptly treated. Different types of injuries can result in an episode of SE, including traumatic brain injury, high fever, stroke, infection or encephalitis, drug withdrawal, among others. An episode of SE contributes to the onset of spontaneous recurrent seizures that can eventually develop into epilepsy (Chapman et al., 2001; Lowenstein & Alldredge, 1998). There are different animal models used to study SE and the development of epilepsy, of which the pilocarpine model is one of the most widely used.

The Pilocarpine Model of SE and Temporal Lobe Epilepsy

Temporal lobe epilepsy (TLE) is the most common seizure disorder seen in adults (Allen Hauser & Annegers, 1996; Wieser, 2004), and is characterized by: (i) the location of seizure foci particularly in the hippocampus, entorhinal cortex, and amygdala (Bartolomei et al., 2005); (ii) onset typically produced by initial "precipitating injury"; (iii) a latent period without seizures after the initial injury; and (iv) pronounced hippocampal sclerosis (neuronal death) in DG and CA regions (Mathern et al., 2002).

There are several experimental rodent models of epilepsy, but the pilocarpine model is one of the most widely used (Kandratavicius et al., 2014). The pilocarpine animal model of SE was first described by Turski and colleagues in 1983 (W. A. Turski et al., 1983). The model has been used for decades as a tool to study multiple aspects of TLE and its progression because it recapitulates the pathophysiological and histopathological hallmarks of human TLE including hippocampal sclerosis and gliosis (Curia et al., 2008; Eyo et al., 2014, 2017; Lévesque et al., 2016; L. Turski et al., 1989).

Pilocarpine acts as a receptor agonist at the muscarinic acetylcholine receptor 1 (M1). Cholinergic hyperactivation induces uncontrolled smooth muscle contraction that results in convulsive tonic and clonic seizures in an episode of SE. Sustained dysregulation of cholinergic systems, and excess levels of acetylcholine in the brain following SE are known contributors to

the development of spontaneous recurrent seizures (Freitas et al., 2006; W. A. Turski et al., 1983). Pilocarpine also target M1 receptors in the brain vasculature before and after SE onset, which promotes leakage of unwanted compounds into the brain and propagates an inflammatory response (Marchi et al., 2007). An additional mechanism that contributes to the induction of SE after pilocarpine involves signaling of the nucleoside adenosine triphosphate (ATP) in the brain. Neuronal hyperactivity that follows seizures increase extracellular ATP levels in hippocampal neurons. This activity-dependent change leads to the release of ATP, which increases excitability via purinergic P2 receptors (Beamer et al., 2019). Notably, microglia respond to all of the contributing factors described above and become reactive to alleviate the effects of SE injury (Wyatt-Johnson & Brewster, 2020) after systemic pilocarpine delivery.

Pilocarpine can be administered via intraperitoneal (i.p.) or intra-hippocampal injection, both producing effective SE induction. When administered via i.p. injection, pilocarpine induces SE rapidly—approximately 30 mins after (Hamilton et al., 1997; W. A. Turski et al., 1983). Researchers have employed different pilocarpine doses depending on the protocol or pretreatment procedures, and rodent strain. However, dosages of 300-400 mg/kg pilocarpine i.p. in rats are able to reproduce the spectrum of pilocarpine-induced symptoms that model TLE (Curia et al., 2008; W. A. Turski et al., 1983). There are dose-dependent benefits and disadvantages of pilocarpine. Although higher doses of pilocarpine shorten the time to SE during induction and increase the likelihood of developing robust TLE symptoms, they also significantly increase the mortality rate in rats. Jope and colleagues observed 83% SE induction rate in adult male Sprague-Dawley rats given 400 mg/kg, but also a null survival rate (Jope et al., 1986). Doses between 300-320 mg/kg have been shown to produce lower mortality rates (15-30%) in Sprague-Dawley rats, which also vary with SE duration (Curia et al., 2008).

The Racine Scale

The Racine scale is an established set of behavioral scoring parameters widely employed as the standard scoring guide in experimental models of seizure disorders(Racine, 1972). This tool is used to track the behavioral symptoms and intensity of pharmacologically- or electrically-induced seizures that produce SE. It has been previously shown that higher seizure intensity and therefore Racine scores during SE induction correlate with microglial alterations that correspond to the pathophysiology of TLE and have been extensively validated in the field and by our

collaborators (Brewster et al., 2013; Schartz et al., 2016b; Wyatt et al., 2017; Wyatt-Johnson et al., 2017)

Microglia and Neuroinflammation

Microglia are the resident immune cells of the central nervous system. Microglia originate as a type of monocyte, migrate to the brain early in embryonic development and become permanently enclosed in the brain (Alliot et al., 1991; Arcuri et al., 2017; Ashwell, 1991). Microglia survey their microenvironment throughout the brain and transition from a quiescent to a reactive and proliferating state known as microgliosis. During microgliosis, microglia change their cytoskeletal structure from highly ramified to hypertrophic, amoeboid, bushy, and rod (Dubbelaar et al., 2018; Hanisch, 2002; Ramlackhansingh et al., 2011; Wyatt-Johnson et al., 2017; Wyatt-Johnson & Brewster, 2020) shaped as they proliferate, make contacts with, phagocytose, and clear unwanted cellular elements (Sierra et al., 2013). *In vivo* two-photon microscopy of adult mouse microglia at rest revealed that microglial processes extend and retract on a time scale of minutes to constantly examine in their microenvironment. Time-lapse imaging in the presence of acute laser lesion to brain capillaries triggered microglia to immediately begin extending their processes to the injured point at a mean rate of 2 mm/min. Microglia were observed to shield the lesioned point within 10 min, while accumulating and engulfing elements appeared approximately 30-60 mins post injury (Nimmerjahn et al., 2005). Microgliosis and inflammatory responses are seen following injury from neurological conditions like epilepsy (Wyatt-Johnson & Brewster, 2020).

Microglial activity is modulated by a variety of neuroimmune signaling mediators, including chemokines; cytokines; ATP; and complement proteins; to signal a disruption to the homeostatic state that begins an inflammatory response, or signals a pathological condition. Microglia can communicate with adjacent glial cells and neurons via these inflammatory markers and receive a variety of signals that ultimately instruct microglia to find and eat, or to not eat, cell debris, and stressed or dying/apoptotic neurons (Li & Barres, 2018).

Microglia can be immunolabeled and visualized with ionized calcium-binding adapter molecule 1 (Iba1), which is a microglia/macrophage specific marker that facilitates membrane and actin remodeling, and phagocytosis. Iba1 expression is upregulated upon microglial activation due to insult, and Iba1+ cell number increases according to the degree of injury. This

response is seen throughout the brain and is characteristic of neuronal injury and disease. In adult male Sprague-Dawley rats, evidence from transient focal cerebral ischemia in rats shows Iba1 upregulation at the ischemic core (site of injury) and at peri-ischemic cortical areas starting at 3.5 h post injury, and peaking after 7 days post injury (Ito et al., 2001).

Microglia and Status Epilepticus

Microglia respond to neuronal hyperactivity during seizures before there is neuronal death. In the pilocarpine model of SE, the chemoconvulsant facilitates the preceding insult that triggers hyperexcitability of the seizure focus. The causal relationship between microglia and seizure onset remains an open question for study, yet there are overarching mechanisms that are known to promote microglial reactivity. Elevated levels of ATP act as proliferation signal to microglia (Beamer et al., 2019). In addition, changes in microglial morphology have been observed within 40 min after either i.p. or intracerebroventricular kainic acid administration (Eyo et al., 2014). During kainate-induced seizure activity, ATP release stimulates PY2Y12 receptors on microglia, which leads to extension and outgrowth of microglia processes shortly after stimulation (5-10 min) (Dissing-Olesen et al., 2014; Eyo et al., 2014). In addition, proinflammatory cytokine signaling, and disruption of vasculature homeostasis in the brain following SE lead to microglial reactivity.

Using the pilocarpine model of SE, Schartz et al., (2016) mapped the spatiotemporal profile of hippocampal Iba1 immunostaining after SE induction (Schartz et al., 2016b). Their work using adult male Sprague-Dawley rats examined microglia reactivity at 4 h, as well as 1, 3, 14, and 35 days after SE in hippocampal CA1, CA1 and DG. They found a significantly higher density of Iba1+ microglia at 4hr and 14d after SE in all three regions compared to controls. In a separate experiment, they counted and characterized the morphological differences in Iba1+ microglia in each of these hippocampal areas in male Sprague-Dawley rats after a single episode of SE using pilocarpine. At 4 h specifically, they found significantly greater numbers of bushy-shaped microglia, characterized by a diameter of 20-25 mm with short, thick processes, in all 3 areas compared to predominantly ramified morphology in control animals (Wyatt-Johnson et al., 2017).

Morphological changes in microglia have also been reported in other studies that used the pilocarpine model of SE, or different models of acquired epilepsy. These changes happen in

response to proinflammatory cytokine signaling, and result in rapid cytoskeletal remodeling (Vezzani et al., 2011). Adult male Sprague Dawley rats treated with pilocarpine displayed significant increases in hypertrophic microglia in CA1, CA3 and hilus starting 1 day after SE (Shapiro et al., 2008). Moreover, male and female mice treated with pilocarpine had significantly greater levels of CD68+/Iba1+ cells in CA1, CA3 and DG compared to Sham controls 3 days after SE, which indicates these were activated/reactive microglia based on lysosomal marker colocalization (Zhao et al., 2020). Kainic acid (KA) induction of SE in transgenic mice with GFP-labeled microglia found significantly higher levels of activated microglia within 24 h of SE measured by percent of fluorescent area and soma counts in the DG (Abraham et al., 2012).

Furthermore, similar microgliosis and changes in morphology have also been reported as a result of proinflammatory signaling elicited by consumption of diets high in saturated fats and refined sugars (Guillemot-Legris & Muccioli, 2017; Thaler et al., 2011). This suggests that proinflammatory effects of diets can contribute to an exacerbated microglial response to seizure insult.

Types of Diets

Several different types of fat-rich diets—western, high-fat, and ketogenic—that have distinct macronutrient profiles have been used experimentally for different purposes (see Table 1). WD and HFD are both diets typically used to study the pathophysiology of obesity, cardiovascular disease, metabolic syndrome, and type 2 diabetes. The composition of these two diets are intended to mimic the properties of typical, modern-day diets consumed by Westernized (e.g. American) societies. Both WD and HFD have been demonstrated to produce inflammation in the brain (Guillemot-Legris et al., 2016; Guillemot-Legris & Muccioli, 2017). Conversely, ketogenic diets (KD) drive the body to use lipids rather than glucose as fuel. KD have been extensively investigated and are sometimes used as therapeutic tools in the treatment of neurological disorders including seizures and have been purported to be tools to aid in weight loss (Murugan & Boison, 2020; Zarnowska, 2020).

Table 1. Diet Compositions

Diet	Macronutrient % kcal		
	Fat	Carbs	Protein
Ketogenic Diet (KD)	75-80	2-5	20
High-Fat Diet (HFD)	60	20	20
Western Diet (WD)	40-45	40-45	10-20

	Macronutrient % kcal		
	Fat	Carbs	Protein
WD (TD.88137)	42	42.7	15.2
Kcal/g	4.5		
Formula	g/KG		
Casein	195.0		
DL-Methionine	3.0		
Sucrose	341.5		
Corn Starch	150.0		
Andrydrous Milk	210.0		
Cholesterol	1.5		
Cellulose	50.0		
Mineral Mix	35.0		
Calcium Carbonate	4.0		
Vitamin Mix	10		
Ethoxyquin	0.04		
Fatty Acid Profile	% of Diet		
Total	20.7		
Saturated Fat	12.8		
Monounsaturated Fat	5.6		
Polyunsaturated Fat	1.0		
Unknown	1.3		

For proposed studies, we chose the WD because the composition and macronutrient ratios are more translatable, in particular the high ratio of simple sugars, to what many Americans consume compared to HFDs (Bortolin et al., 2018). Further, rodent studies during early developmental stages have indicated that WD elicits neuroinflammation in early life and in adulthood, along with cognitive impairments in adulthood. The translatable quality of a WD also makes it a useful, suitable tool to study early developmental dietary effects given the current rates of childhood obesity, and the incidence of epilepsy in children (Flegal et al., 2016; Zack & Kobau, 2015).

Our WD is comprised predominantly of saturated fatty acids and simple carbohydrates like sucrose (see Table 1). Consuming high amounts of saturated fatty acids and simple sugars contributes to the initiation and propagation of proinflammatory signals in peripheral organs like the liver, adipose tissue, and pancreas via toll-like receptor (TLR) activation. TLRs are one of the principal mechanisms of inflammatory cytokine activation in our cells. Therefore, activation of TLR signaling due to WD consumption leads to increases in proinflammatory cytokines like tumor necrosis factor-alpha (TNFα) and interleukin 1-beta (IL-1β) to name a few. These inflammatory effects are seen in the periphery and in the brain, where they activate microglia and potentiate inflammation (Guillemot-Legris & Muccioli, 2017; Könner & Brüning, 2011; Myles, 2014).

Dietary Effects in the Hippocampus

Investigation into the effects of fat-rich diets on neuroinflammation has been predominantly focused on the hypothalamus. More recent evidence indicates that similar effects of WD and HFD are seen in many areas of the brain, including the hippocampus (Guillemot-Legris & Muccioli, 2017) an area of particular interest because it is a predominant area affected in TLE. While microglia are found ubiquitously in the brain, microgliosis in hippocampal regions has been found to specifically influence epileptogenesis, circuit remodeling, and the development of TLE (Eyo et al., 2017; Wyatt-Johnson & Brewster, 2020). Furthermore, hippocampal microglia develop innate immune memory based on their responses to previous insults through which they undergo molecular reprogramming that influences their response to subsequent events—a concept termed “priming” (Neher & Cunningham, 2019). Hippocampal functions that are affected

by WD consumption, may become predisposed to more pronounced damage in the context of TLE.

Rodent studies that have investigated the effects of WDs on hippocampal function have often focused on outcomes related to hippocampal-dependent cognitive abilities (Kanoski & Davidson, 2011). Nevertheless, these studies have also reported gliosis and inflammatory increases in the hippocampus. Adult male C57/BL/6J mice fed a 45% or a 60% fat diet chronically displayed whole hippocampal alterations including: a reduction of synaptic integrity markers SNAP-25 and PSD-95, and reductions in vesicular glutamate transporter and GABA transporters (vGlut1, vGAT; Lizarbe et al., 2019). Adult male Wistar rats exposed chronically (12 weeks) to HFD showed significantly higher GFAP in the DG (but not in CA1 and CA3) compared to chow controls, as well as greater number of Iba1+ cells in all 3 areas compared to controls (Rivera et al., 2013). Importantly, they also reported increased hippocampal apoptosis as seen by higher total and cleaved caspase-3 levels. WD consumption leads to additional detrimental neurophysiological changes in adult humans and rodents including impaired glucoregulation, reduced levels of neurotrophins, alterations in blood-brain barrier (BBB) integrity, and neuroinflammation (Gross et al., 2004; Kanoski & Davidson, 2010). HFD gives rise to impaired insulin response to excess simple carbohydrates and this has been linked to impaired memory performance in adult male Sprague Dawley rats that received HFD for 1 month (Pathan et al., 2008). Moreover, WD consumption in adult male Sprague-Dawley rats for 12 weeks reduced levels of brain derived neurotrophic factor (BDNF) in the ventral hippocampus (CA1) and altered learning compared to chow controls (Kanoski et al., 2007). Lastly, adult male Sprague-Dawley rats that received WD for 12 weeks showed BBB disruption evidenced by reduced expression of structural and tight-junction proteins that preserve BBB integrity(Kanoski & Davidson, 2010) and disruption of BBB integrity is linked to hippocampal pathology.

These findings indicate that WDs and HFDs produce significant and detrimental neurobiological alterations in the rodent hippocampus (Noble & Kanoski, 2016) but effects of WDs have been less explored in conjunction with neurological diseases like TLE. For example, WD was fed for 6 weeks to adult male Wistar rats prior to pentylenetetrazol (PTZ)-induced seizures. Their results showed a significant decrease in seizure threshold in the WD-fed group, accompanied by increased oxidative stress markers. This suggests that prolonged WD

consumption increases susceptibility to seizures in this model (Alzoubi, Hasan, Khabour, Mayyas, Al Yacoub, et al., 2019).

Early Developmental Effects of WD and HFD

Evidence from studies that examined the effects of prenatal exposure to high-fat diets shows that at weaning, offspring of dams that consumed high-fat diets display increased proinflammatory cytokine levels in the hippocampus, including IL-1β (Bilbo & Tsang, 2010). They also reported microglial activation throughout the hippocampus (CA1, CA3, DG) of P20 pups from high-fat diet dams, as reflected by denser Iba1 immunostaining. Further, Boitard and colleagues compared the effects of postnatal WD consumption in rats starting the juvenile period (P20) or adulthood (P80) and found that juvenile, but not adult, WD exposure produced hippocampal-dependent cognitive and memory impairments (Boitard et al., 2014). Importantly, despite no significant basal differences in cytokine levels between juveniles and adults exposed to WD, they report that only juvenile WD exposure induces a potentiated proinflammatory response to peripheral insult (LPS) as seen by elevated IL-1β and TNFα specifically in the hippocampus. They propose that microglia undergo priming following early postnatal WD consumption. Developmental programming of metabolic and neurological outcomes by immune response (infection or trauma) during early-postnatal stages has been linked to increased cytokine expression in the brain (Bilbo & Schwarz, 2009; Schwarz & Bilbo, 2011).

Perinatal exposure to WD and HFD can alter programing of brain pathways when influenced by inflammatory stimuli such as HFDs, as these diets have been shown to initiate and propagate inflammatory signaling cascades (Bolton & Bilbo, 2014). Nutritional components of the maternal diet, including saturated fats, influence the composition of the dam's milk (Burnol et al., 1987; Swithers et al., 2001; Wolff & Sébédio, 2003). Maternal dietary fats are incorporated into the offspring's myelin sheaths, synaptic terminals and brain vasculature (Grandgirard et al., 1994). Pups exclusively consume the dam's milk in the early post-natal days until they begin to sample chow at approximately postnatal day 15 (P15) (Doerflinger & Swithers, 2004; Swithers, 2000). Consequently, the pup's post-natal neuronal and glial development is affected by diet both through effects on the dam's milk composition and through its own intake before and after weaning.

Maternal HFD consumption in Long Evans dams beginning 4 weeks prior to breeding, continued through gestation, lactation, and until weaning produced evidence of neuroinflammation in male offspring that were also fed a HFD in adulthood; females were not included in the study (White et al., 2009). HFD offspring had elevated Iba1 expression in the frontal cortex (microgliosis), and elevated IL-6 levels in whole brain homogenates. Another study that examined the effects of prenatal exposure to HFD showed that at weaning, offspring of HFD dams displayed increased IL-1β levels in the hippocampus (Bilbo & Tsang, 2010). They also report microglial activation throughout the hippocampus (CA1, CA3, DG) of P20 pups from HFD dams, as reflected by denser Iba1 immunostaining. Further, Boitard and colleagues compared the effects of postnatal WD consumption in rats starting in the juvenile period (P20) or at adulthood (P80)(Boitard et al., 2014). They found that juvenile, but not adult, WD exposure produced hippocampal-dependent cognitive and memory impairments. While no significant basal differences in inflammatory cytokine levels between juveniles and adults exposed to WD were reported, only juvenile WD exposure induced a potentiated proinflammatory response to peripheral immune challenge (LPS) as seen by elevated IL-1β and TNFα specifically in the hippocampus. These results are consistent with the idea that early postnatal WD consumption elicits microglial priming, which produces an exacerbated response to insult (LPS) later in life.

These findings support the idea that inflammatory mediators found in the mother's milk Rnd early diet consumed by the pup can influence or alter the offspring's early post-natal brain development. Further, these data suggest early perinatal exposure to WD may prime the brain for exacerbated central immune response and produce greater activation of brain immune cells and mediators in the hippocampus at later stages.

Taken together, these results indicate that early exposure to WD can produce an increase in levels of proinflammatory cytokines in the brains of young and adult male rats, and notably in the hippocampus (Bilbo & Tsang, 2010; Boitard et al., 2014; White et al., 2009). In addition, supporting evidence indicates that WD-induced cytokine increases can prime microglia for a more robust inflammatory response when presented with injury later in life. However, few studies have examined the effects of WD consumption on injury caused by neurological conditions, and the neuroinflammatory consequences of the two remain unexplored. Fewer studies have examined the relation of these overlapping inflammatory responses in the context of acquired epilepsy or SE (Alzoubi, Hasan, Khabour, Mayyas, Al Yacoub, et al., 2019; Kang et al., 2015a). This is an

important area of study given that in the United States alone, 41 out of 100,000 individuals have reported an SE episode (Lawson & Yeager, 2016), and given the prevalence of WD consumption in the U.S. population (Myles, 2014). Therefore, the proposed work can produce valuable knowledge to increase our understanding of the interactions of diet and seizure disorders.

Hippocampal Regional Differences

The literature suggests that microgliosis is a consequence of inflammatory effects produced by diet and seizure insult can be seen across CA1, CA3 and the DG. Nevertheless, these results can be region specific as described above as a result of WD or SE alone. In addition, we propose to examine both microglia number and morphology, which are microglial properties that do not necessarily change together or uniformly across hippocampal areas as mentioned above. Furthermore, evaluating hippocampal regional differences in our experiments could help us better understand potential functional changes if specific areas of interest display exacerbated outcomes following WD consumption and SE.

HYPOTHESIS

The potential interaction between WD consumption, epilepsy and hippocampal pathology has been relatively unexplored. Furthermore, the effects of early developmental exposure to WD on seizures or acquired epilepsies have not been studied. These interactions are important to examine in light of increasing rates of WD consumption in the U.S. According to the latest USDA data, 70% of the population exceed the recommended dietary intake of added sugars and saturated fats (Flegal et al., 2016). Along with rising rates of WD consumption, rates of epilepsy in children in the U.S. have increased by 5% in the past decade, and there are currently close to 500,000 cases (Zack & Kobau, 2015). The goal of the present experiments was to use a rat model of acquired temporal lobe epilepsy, pilocarpine-induced SE, to test the hypothesis that WD exacerbates inflammatory changes following SE neuronal injury in hippocampus of rats. Our central hypothesis was that consumption of WD during early development would produce an inflammatory state which would prime the hippocampus for greater neuroinflammation following SE.

Specific Aim #1: Determine the role of WD consumption on microglial proliferation in the hippocampal CA1, CA3 and DG following SE induction. Our working hypothesis was that the deleterious effects of SE would be exacerbated in WD-consuming animals. To test this, Sprague-Dawley rats were bred and after birth, dams were exposed to a control or a WD. Offspring remained on the diet for 4 weeks post-weaning, at which time SE was induced with pilocarpine. Four hours following induction, brain tissue was collected for IHC analysis of microglia. We predicted that WDSE animals would display elevated microgliosis Sham controls 4 h following pilocarpine injection. Hippocampal Iba1+ cells were quantified across CA1, CA3 and DG.

Specific Aim #2: Determine the role of WD consumption on microglial morphological changes after SE across hippocampal regions. Our working hypothesis was that WD consumption would lead to an increase in the proportion of reactive microglial phenotypes (hypertrophic, bushy, amoeboid) and that these outcomes would be exacerbated by SE induction compared to CD controls. To test this, we morphologically categorized Iba1+ cells in CA1, CA3, and DG.

MATERIALS AND METHODS

Ethics Statement

All animal studies were approved by the Purdue Animal Care and Use Committee and followed the approved IRB guidelines.

Feeding and Housing

Sprague-Dawley rats were bred in the lab, maintained in temperature-controlled rooms (21°C) with 12 h light and 12 h dark (0700-1900 hours) cycles, and had unlimited access to standard chow and water. Pregnant dams were monitored daily during the last week of gestation, and litters found by 1700 hours were considered to be born on that day (P0). Litters were culled to 10 pups each (5 males, 5 females where possible) on the day after birth and each dam was then assigned to either standard Chow Diet (CD; TD.2018 Envigo; 3.1 kcal/g, 18% kcal fat, 58% kcal carbohydrate, 24% kcal protein) or Western Diet (WD; TD.88137 Envigo; 4.5 kcal/g, 42% kcal fat, 43% kcal carbohydrate, and 15.2% kcal protein). Water was available *ad libitum*. The dam received the assigned diet until pups were weaned on postnatal day 21 (P21). Pups were grouped housed in standard polycarbonate cages with 1 or 2 littermates per cage. We only utilized male rats for this experiment. After weaning, animals continued to receive *ad libitum* access to WD or CD (same as the dam) in the home cage until P50-55 when inductions began.

Pup Group Assignment

At P21, animals were weaned and maintained on the same diet as the dam. One male from each litter was assigned to either a baseline (BL), Sham, or SE group. The BL group was used to examine effects of the dietary manipulation alone on basal levels of microgliosis. BL animals were sacrificed between P50-55—the time point immediately prior to pilocarpine induction. Sham animals were handled the same as our SE animals on induction day, and given the same i.p. injections as SE animals, except that sterile saline was administered in place of pilocarpine. There were n=8-10 males per experimental group for a total of $n = 55$ animals. A sample size of at least n=6 animals per group was determined by power analysis as sufficient to detect differences in hippocampal areas in our analyses based a power level of 0.80, and $\alpha = 0.05$.

Pilocarpine Induction of SE

On the day of induction, animals in SE and Sham groups were weighed and moved to the testing room 30 mins prior to testing and kept in plastic tubs with access to water. Figure 1 illustrates the experimental design. Experimenters identified and retrieved the rats from the animal based on litter number before bringing them to the testing room. The litter numbering system did not correspond to dietary assignment; however experimenters were unblinded to the diet condition in the animal room because the CD and WD food pellets were visibly different. However, during the induction protocol, experimenters were blinded to the diet condition, and could only see the animal's ID which was randomized. The experimenters remained blinded for the rest of the procedures: tissue collection, sectioning, immunohistochemistry, imaging, counts, and morphological assessment. The experimenters were unblinded during the statistical analysis.

Inductions were performed on separate consecutive days, and litters were counterbalanced across test days. At P50-55, animals in the SE and Sham groups received an intraperitoneal (i.p.) scopolamine methylbromide injection (1 mg/kg, i.p.; 1 ml/kg). Thirty minutes later, Sham animals an i.p. injection of isotonic saline (3ml/kg) while SE animals received an injection of the chemoconvulsant pilocarpine hydrochloride (300 mg/kg; i.p.; 3 ml/kg). This dose was chosen because it has been extensively used and shown to produce a high rate in the desired intensity of behavioral seizures (5 or 6 on the Racine Scale), a low rate of non-responders (~25%), and a low mortality rate according to previous published experiments (Schartz et al., 2016a, 2018; Wyatt-Johnson et al., 2017). All SE animals were video recorded using a Flip Video Ultra HD camcorder (Pure Digital Technologies) for the first 60 min after pilocarpine administration. The experimenter began scoring seizure activity of SE animals beginning 3 min after pilocarpine injection. Seizure stage was recorded every minute for 30 min, followed by every 2 min for 10 min, and then every 5 minutes for the remainder of the 60 min induction period. Behavioral assessment and seizure scoring using the Racine scale, monitoring for rearing, falling, or tonic clonic seizures. The scale of scores used in the present experiments are: [1] rigid posture or immobility, mouth moving, [2] tail clonus, [3] partial body clonus with fore- or hind-limb clonus, head bobbing, [4] rearing, [4.5] severe whole body clonic seizures while rearing retaining posture, [5] rearing and falling, [6] tonic-clonic with loss of posture or jumping (Racine, 1972). A score of 3 indicated seizure activity, and a 5 and above were considered reaching SE within the timed protocol.

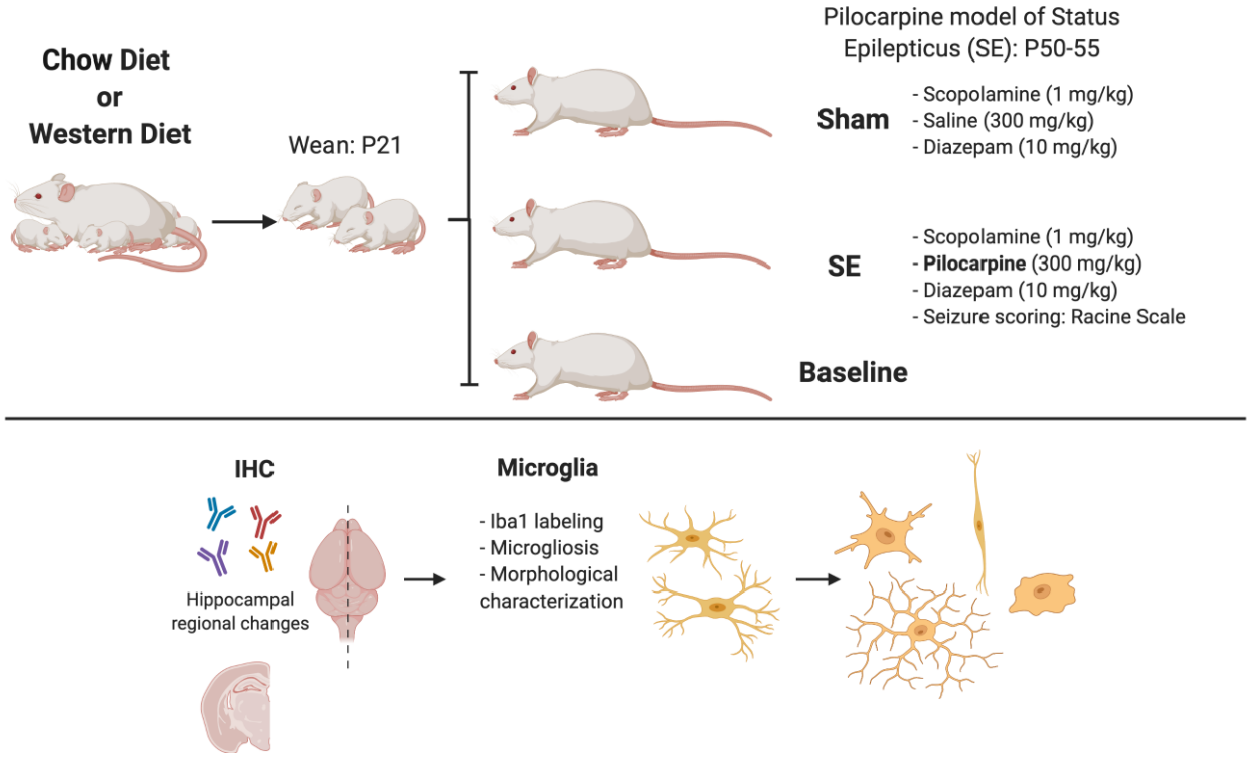

Figure 1. Diagram of experimental groups and design.

Both Sham and SE animals received diazepam 60 minutes after pilocarpine or saline administration (10 mg/kg; i.p.; 2 ml/kg). Behavioral scores were recorded for SE animals that received pilocarpine every 5 min for 90 min, followed by every 10 min during the final 90 min. Following diazepam administration we monitored animals until we collected tissue to ensure that animals maintained their respiratory rate and to help restore body temperature. We administered an additional half dose of diazepam (5 mg/kg i.p.; 1 ml/kg) to animals that displayed neck jerking and body shaking (behavior D) 1 h after the first dose. During this period the animals displayed continuous movement patterns before they became sedated, and seizures stopped. Therefore, we designed the following behavioral ethogram, scored the range of behaviors, and recorded the time at which animals became sedated, and then recovered from the drug to determine possible behavioral group differences. Although these measures have not been previously externally validated or correlated to quantitative measures of seizure intensity, they comprised the behavioral response to SE induction.

The behaviors we observed include mouth movement, fore- or hind-limb twitching, head bobbing, neck jerking, and sometimes whole body shaking. These descriptions were part of our scale but were observed in the context of sedation (or reaching sedation). Therefore, these observations were scored using the same descriptors, but appeared lower in intensity, and the rats looked different than during the induction period due to the effects of diazepam. These behaviors are further explained below.

A. *Mouth moving.* Determined by light mouth movement that does not engage the jaw or entire face, with no other signs of shaking of the head, body, or extremities. The rat remains the prone position or lying on its back, aside from the mouth movement it appears to be slightly sedated.

B. *Tail clonus and/or leg twitching*. The rat remains lying on its side or in the prone position, and only light twitching of 1 or more of the fore- or hind-limbs (typically hindlimbs). The body is not shaking. Mouth movement may or may not be observed with this behavior. The rat remains in the same spot and does not move around.

C. *Head bobbing.* The rat is lying down on its side or in the prone position and light repeated head bobbing continues. This may be seen with or without mouth movement, and/or leg twitching. The rest of the body is not shaking, and the rat remains in the same spot without moving around.

D. *Neck jerking*. The rat is prone or lying on its side and the neck repeatedly jerks back and movement looks stronger than just head bobbing. The rest of the body does not shake. The forearms will sometimes be forward and the hindlimbs sometimes will be abducted. There may or may not be repeated mouth movement.

E. *Neck jerking and body shaking*. The rat is prone, the forelimbs point forward, the hindlimbs may or may not be abducted, there is repeated neck jerking backwards like in stage D, shaking of the body may also be observed. There may or may not be mouth moving, and sometimes the tail is clonus, but not always. The repeated shaking may also make the animal move around the tub, and in some cases give a few "steps" which look more like stumbles. Rat does not have full postural control.

F. *Sedated*. The rat does not show signs of shaking or twitching on the head, body, or extremities, while lying on its side or in the prone position. The rat appears to be asleep or does not startle with noise or light tapping of the tub. Note that some rats do not immediately become sedated, and will stumble around in the tub, or try to reach up on the sides of the tub before remaining in one spot.

G. *Awake/Recovered*. The animal regains postural control and may briefly give a couple steps around the tub or move away from the heated area of the tub, and then remain still. Startle reflex (ear flinching for example) with noise or light tapping of the tub is observed. Walking around the tub may increase with time post-diazepam, along with grooming.

Tissue Collection and Processing

Animals were deeply sedated with Beuthanasia (0.77 mg/kg), transcardially perfused with ice cold (1-4°C) 1X PBS to remove circulating macrophages and blood from meninges, and the brain was rapidly removed. One brain hemisphere was fixed with 4% paraformaldehyde (PFA) for 24 h. This was followed by 30% sucrose for at least 5 days. Brains were then frozen using dry ice and stored at -50°C until ready to section and were kept at -20°C at least 1 h prior to sectioning.

Tissue Preparation for Immunohistochemistry

Brains were covered with OCT mounting medium and sectioned at 50 μm in the coronal plane using a Leica CM1860 cryostat. Sections were stored at 4°C in 1X PBS with 0.1% sodium azide.

Iba1 Immunohistochemistry

Six brain sections containing dorsal hippocampus were selected per animal (Bregma -2.80 to -4.30 mm). IHC was done at RT in which free-floating sections were washed in 1X PBS for 5 min, incubated for 30 min in 3% H_2O_2, and for 20 min in 1X PBS+3% Triton (1XPBS-3%T). Then, sections were placed in 5% goat immuno buffer for 1 h. Next, sections were incubated overnight in tubes with primary Iba1 antibody (rabbit, 1:1000, Cat# 190-19741, Wako) at 4°C and placed on a rotating platform. This was followed by three 10-min washes with 1XPBS-0.1%T at RT, and incubation in goat anti-rabbit biotinylated secondary antibody (1:1000; Cat # BA-1000, Vector Laboratories) for 1 h at RT. Next, sections were washed for 10 min 3 times with 1XPBS-0.1%T, and then incubated for 30 min in ABC Avidin/Biotin complex. After 3 additional 10-min washes with 1XPBS-0.1%T, sections were developed for 1-2 min using the DAB Peroxidase (HRP) Substrate Kit, 3,3'-diaminobenzidine (Vector Laboratories). DAB reaction was stopped with deionized water, and sections were then placed in 1X PBS to be mounted onto gelatin-coated slides. Nissl stain was performed, followed by series dehydration in alcohol (50, 70, 95, 100%) followed by Xylene, and cover slipped using Permount mounting media (ThermoFisher Scientific). Slides were allowed to dry for at least 24 h before imaging.

Iba1 Imaging

Iba1 immunostaining was visualized using a Leica DFC365FX microscope and images were captured at 40X magnification using the LASX 4.4 software. Three or four brain slices were imaged per animal. Our hippocampal regions of interest were stratum radiatum of CA1, stratum lucidum of CA3, and the granule cell layer and hilus of the dentate gyrus (DG). Three non-overlapping frames of each hippocampal area were imaged per brain slice, for a total of 27-36 frames imaged per animal (Fig. 2). Microglial counts and morphological assessment were performed manually as outlined in Wyatt-Johnson et al., 2017. We used Image J to add counters and circle outlines of 12.5, 25, and 50 μm diameters for reference. Counts were reported as the average per hippocampal area per frame area. The percentage of each morphology type was calculated as percentage of total Iba1+ cells per hippocampal area.

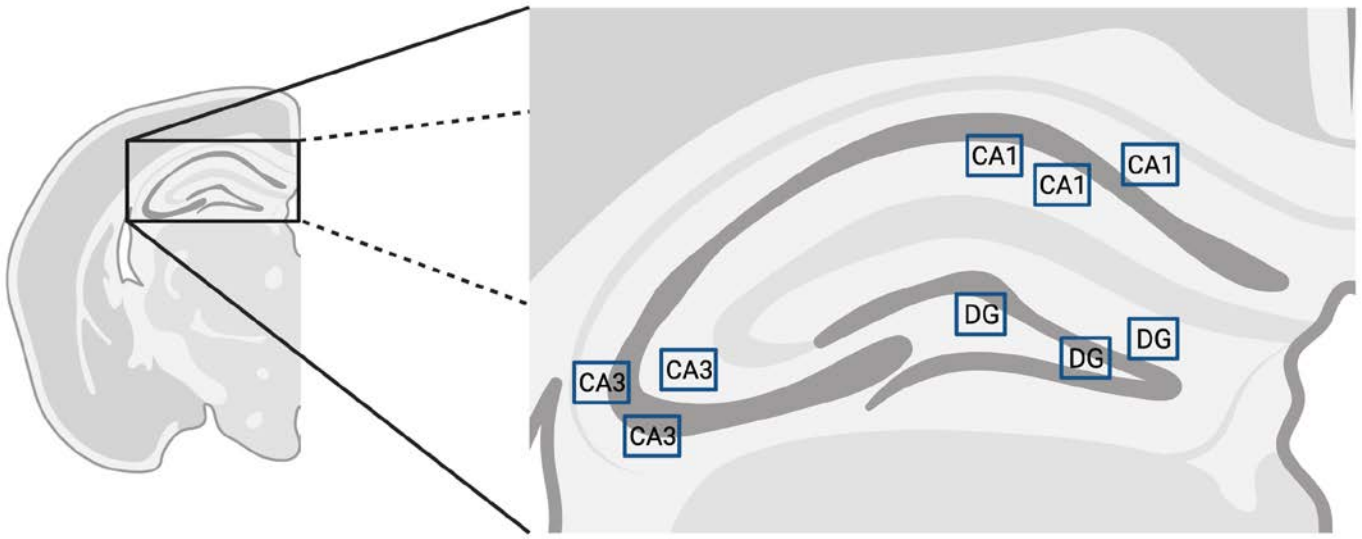

Figure 2. Diagram of hippocampal imaging areas for Iba1 morphological analysis per brain slice.

Total Area of Iba1+DAB Signal

Total area of Iba1+DAB signal was measured using ImageJ in the CA1 images that were captured as described above. There were 3-4 brain slices per animal, and 3 non-overlapping frames of CA1 per brain slice, for a total of 9-12 frames per animal (see Figure 2). For each frame we adjusted color threshold. In the Threshold Color menu, we selected the Thresholding Method option as *Default*, selected *Red* for Threshold color, and *RGB* for Color Space. These parameters selected the DAB-stained areas and excluded the blue Nissl-stained areas. We then clicked on the *Select* button, and in the main menu used the *Analyze→Measure* tool to quantify the selected area. Finally, we calculated the average of all area measurements per animal.

Statistical Analyses

Microglia counts and percent morphologies were analyzed using t-tests, two- and three-way ANOVA to compare all groups using SPSS, and GraphPad Prism 9 software. Statistical significance was set at $\alpha < .05$. Data was reported as median or mean (M) $\pm$ standard error of the mean (SEM).

RESULTS

Body Weights at Induction

Intake of CD or WD for 8 weeks after birth (P50-55) did not produce significant differences in body weights across the CD and WD animals that reached SE ($F(3, 34) = 0.482$, $p = 0.69$) (Fig. 3A). This finding was the same in we compared all animals that received pilocarpine and their Sham counterparts ($F(3, 20) = 0.264$, $p = 0.85$) (Fig. 3B). We found no differences between CD and WD Baseline groups ($t(13.11) = 1.098$, $p = 0.291$) (Fig. 3C).

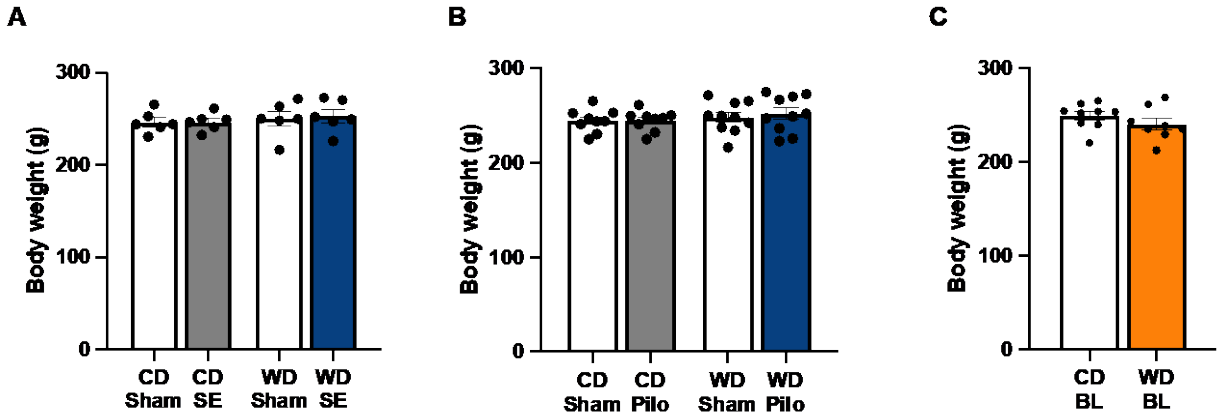

Figure 3. WD did not produce changes in body weights at P50-55 across groups. (A) SE and Sham controls (CD $n = 6$, WD $n = 6$). (B) WD did not change body weights across all induced animals and Sham controls (CD $n = 9$, WD $n = 10$). (C) There were no differences in body weight among Baseline groups (CD BL $n = 9$, WD BL $n = 8$), data shown as Mean ± SEM.

Latency to SE

There were no significant differences in time to SE ($t(8.227) = 1.834$, $p = 1.029$) between CD and WD animals (Fig. 4A). There were no significant differences in frequency of stage 5 or stage 6 seizures between CD SE and WD SE groups ($t(8.41) = 0.295$, $p = 0.775$) (Fig. 4B).

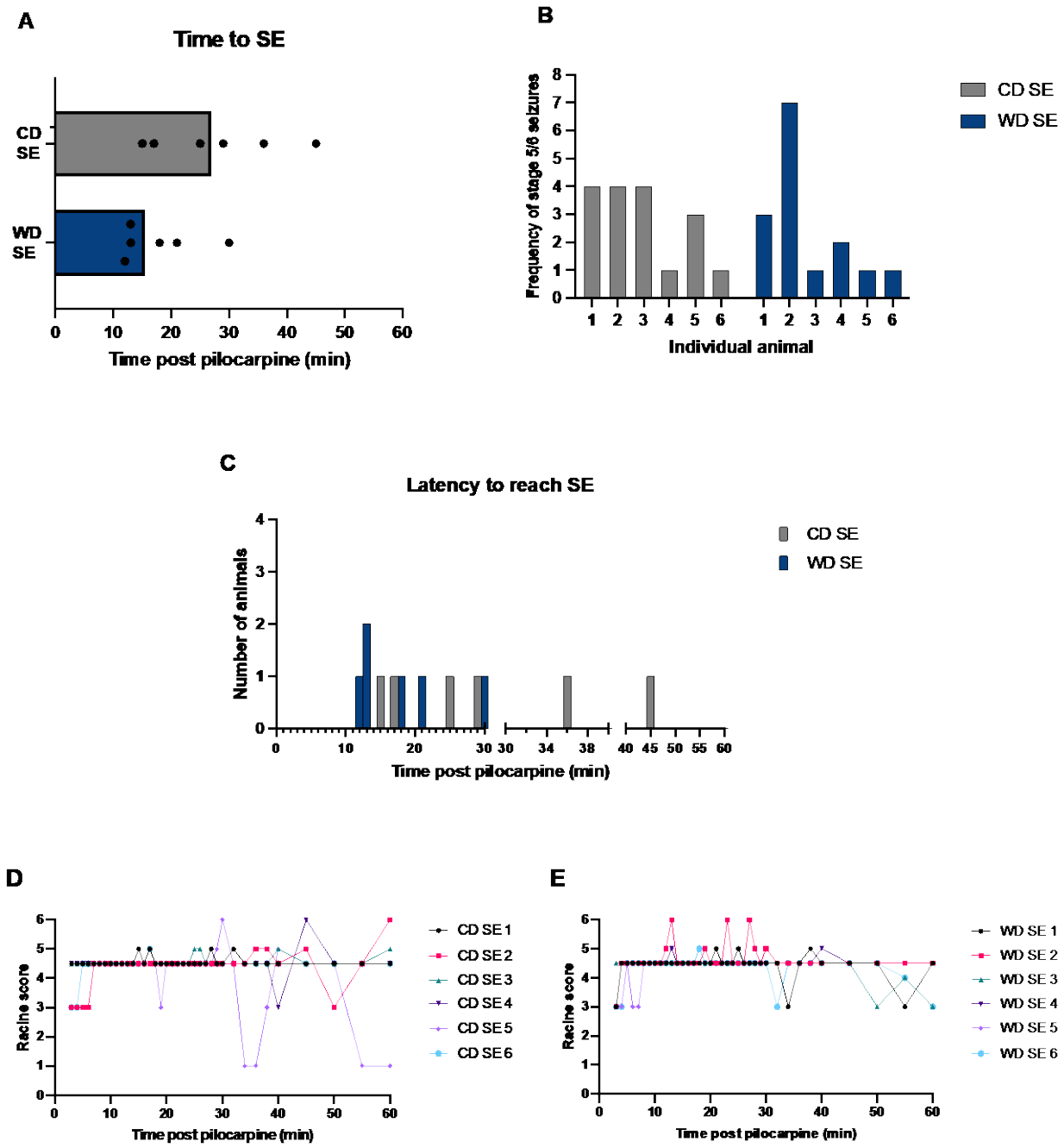

Figure 4. Latency to SE was not different between SE groups. (A) Time to reach a stage 5 seizure was not significantly different between CD SE (n = 6) and WD SE (n = 6) animals, data shown as Median. (B) There was no difference in frequency to reach stage 5 seizures. (C) Distribution of latency to reach SE across groups. (D) Racine scores plotted for the 60 min protocol of SE induction for CD SE, and (E) WD SE animals, data shown as Median.

Reachers and Non-Reachers

A total of 9 CD, and 10 WD animals were given pilocarpine (CD Pilo and WD Pilo), of which 6 in each group reached stage 5 seizures, which was our criteria for SE (CD SE and WD SE). Therefore, there was a 66% and 60% response rate respectively (Table 2). All CD and WD Pilo animals reached at least 4.5 on the Racine scale.

Table 2. Distribution of Animals in Pilocarpine-Induced SE Protocol

Group	Induced	Reached 5	Reached Sedation	Woke from Diazepam
CD SE	9	6	4	3
CD Pilo		3	3	3
WD SE	10	6	3	3
WD Pilo		4	4	3

Note. Induced animals received Pilocarpine injection. Reached 5 (stage 5 seizure). Reached sedation after Diazepam injection. Woke from Diazepam regained motor control and did not display behavioral seizures until the end of the protocol.

Time to Sedation, and to Recovery After Diazepam

There were no significant differences in time to sedation after diazepam between CD SE and WD SE groups ($t(5) = 1.132$, $p = 0.243$) (Fig. 5A). There were no significant differences in time to sedation between CD Pilo and WD Pilo animals ($t(8.63) = 1.299$), $p = 0.226$) (Fig. 5B). Six animals in the CD SE group, and 6 animals in the WD SE group reached stage 5 seizures. Of the 6 animals per group only 4 and 3 became fully sedated in the CD and WD groups respectively. The remaining 2 CD SE and 3 WD SE animals did not reach full sedation and still displayed mild observable seizure activity that was scored until 180 min post diazepam. The frequency of the types of behaviors (A-G) post diazepam were compared, and there was a main effect of Behavior ($F(6, 70) = 6.063$, $p = 0.0001$) post diazepam, but no significant differences between CD SE and

WD SE groups (Fig. 5C). The same comparison was done for CD and WD Pilo animals and we also found a main effect of behavior ($F(6, 119) = 17.87$, $p < 0.0001$), but no differences between diet groups (Fig. 5D).

Then, time to recover from diazepam was measured in CD SE and WD SE groups. We found no significant differences in time to recover from diazepam among SE animals ($t(2.3) = 2.94$, $p = 0.083$) (Fig. 6 A,B). However, when we compared CD Pilo and WD Pilo animals that regained consciousness after diazepam we found that WD animals took significantly longer to recover ($t(9.828) = 3.737$, $p = 0.004$) (Fig. 6C). This is also illustrated in the frequency plot (Fig. 6D).

Microgliosis

Total Average Iba1+ Cells in Hippocampal Regions of Interest

There were no differences in average Iba1+ cell number between Sham and SE groups irrespective of diet, but there was a main effect of hippocampal area ($F(1.5, 32) = 25.46$, $p < 0.001$ (Fig. 7A). Among all the animals that received pilocarpine we only observed a main effect of hippocampal Area ($F(2, 68) = 45.13$, $p < 0.0001$, but did not observe evidence of increased average Iba1+ cell number in the WD groups compared to CD groups (Fig. 7B). Furthermore, we found no significant differences in average microglial counts in CA1, CA3, or DG. There were no significant differences in average microglial number between the CD and WD BL groups ($F(1, 15) = 1.172$, $p = 0.296$), or in our hippocampal regions of interest ($F(1.855, 27.83) = 0.769$, $p = 0.464$) (Fig 8).

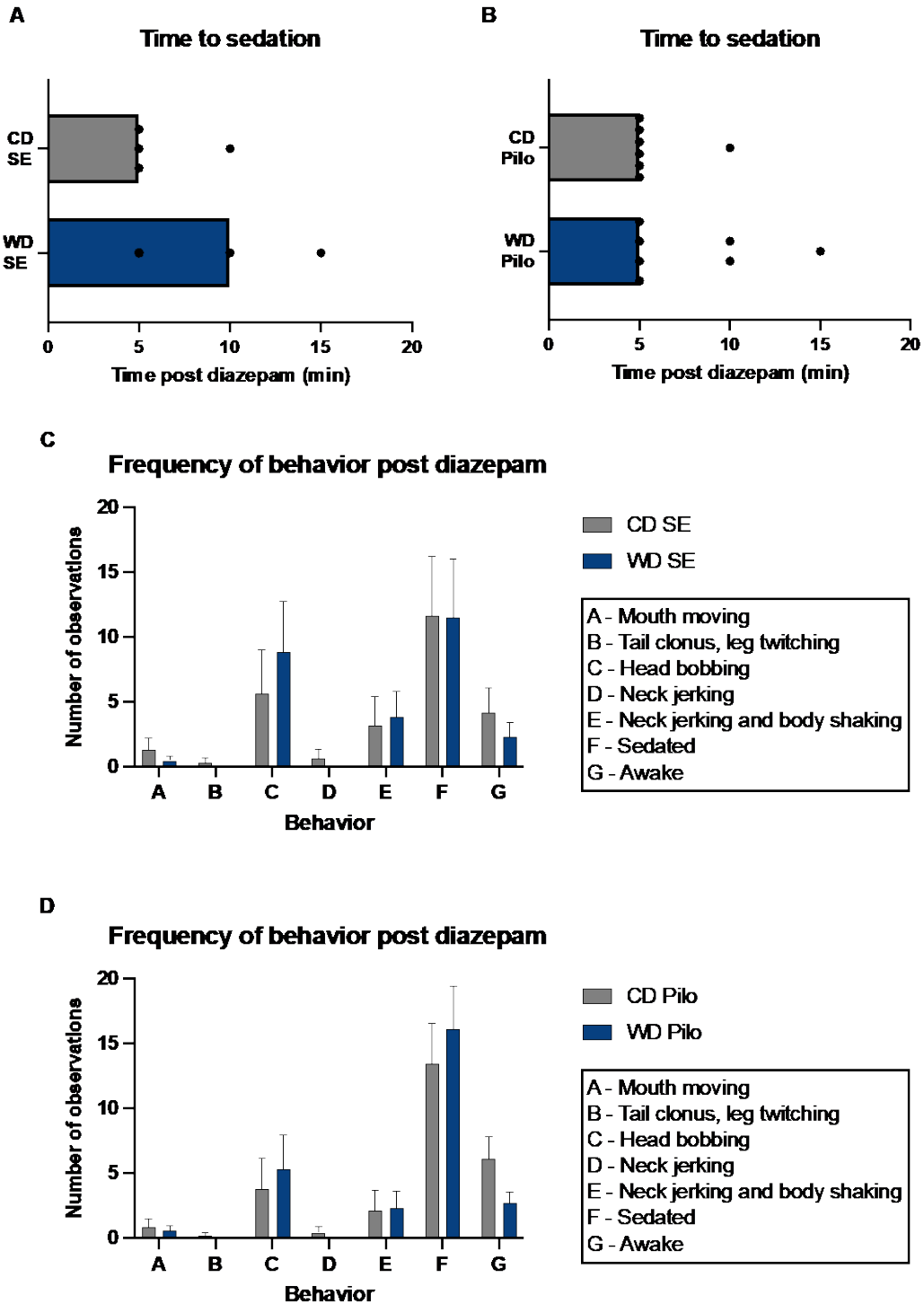

Figure 5. No significant differences in time to sedation after Diazepam injection. (A) No differences in time to reach behavioral sedation in CD SE (n = 4) and WD SE (n = 3), data shown as Median. Only a fraction of the animals that reached SE also became sedated after Diazepam, while the remainder displayed mild seizure activity post Diazepam (D). (B) No significant differences in time to sedation between CD Pilo (n = 7) and WD Pilo (n = 7) animals, data shown as Median. (C) No significant differences in frequency of behaviors observed after diazepam in SE and (D) Pilo animals, data shown as Mean ± SEM.

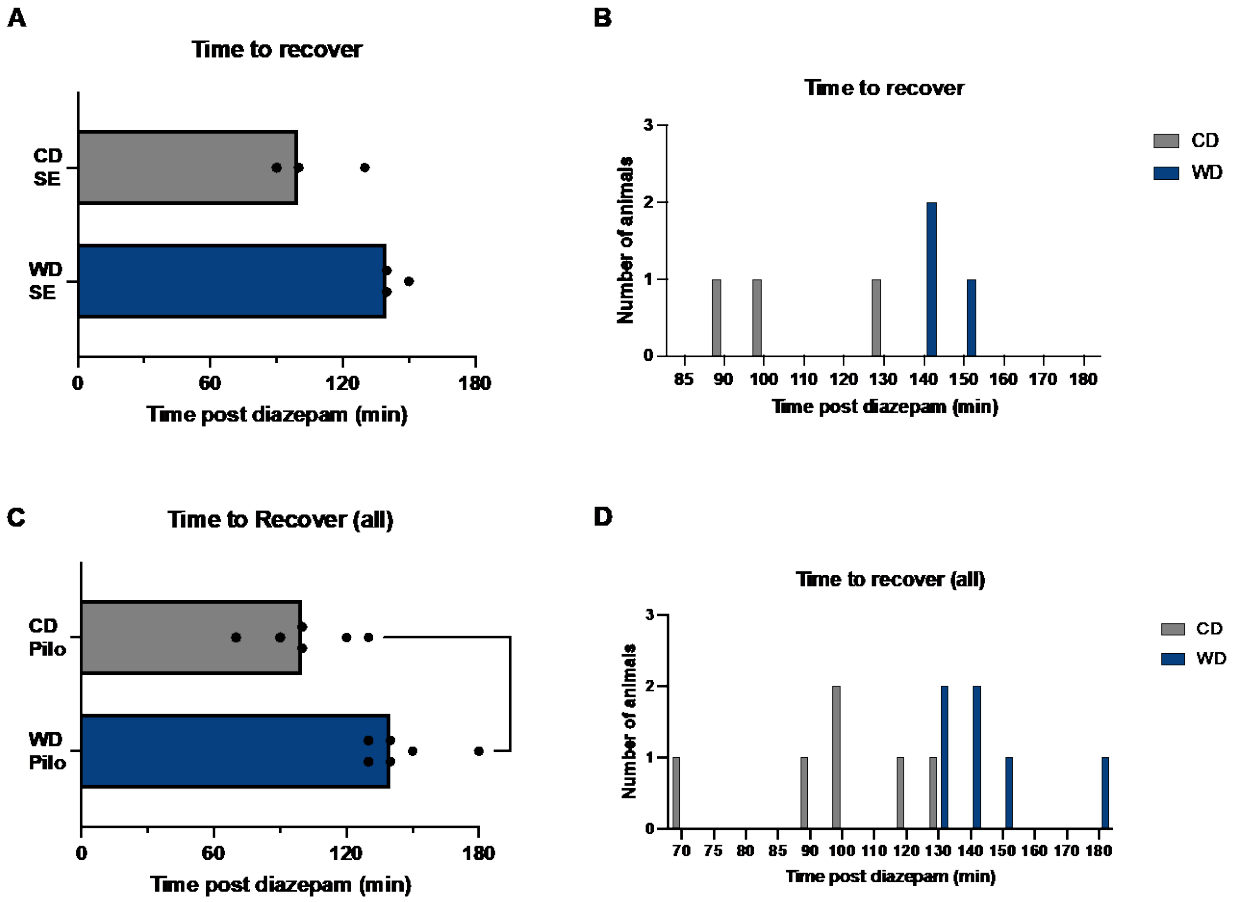

Figure 6. Time to recovery from Diazepam does not differ in animals that reach SE, but significantly differs in Pilo animals. (A, B) CD SE (n = 3) and WD SE (n = 3) animals do not show significant differences in time to wake from Diazepam, data shown as Median. (C, D) WD Pilo (n = 6) animals show significantly longer times to recover from diazepam compared to CD Pilo animals (n = 6).

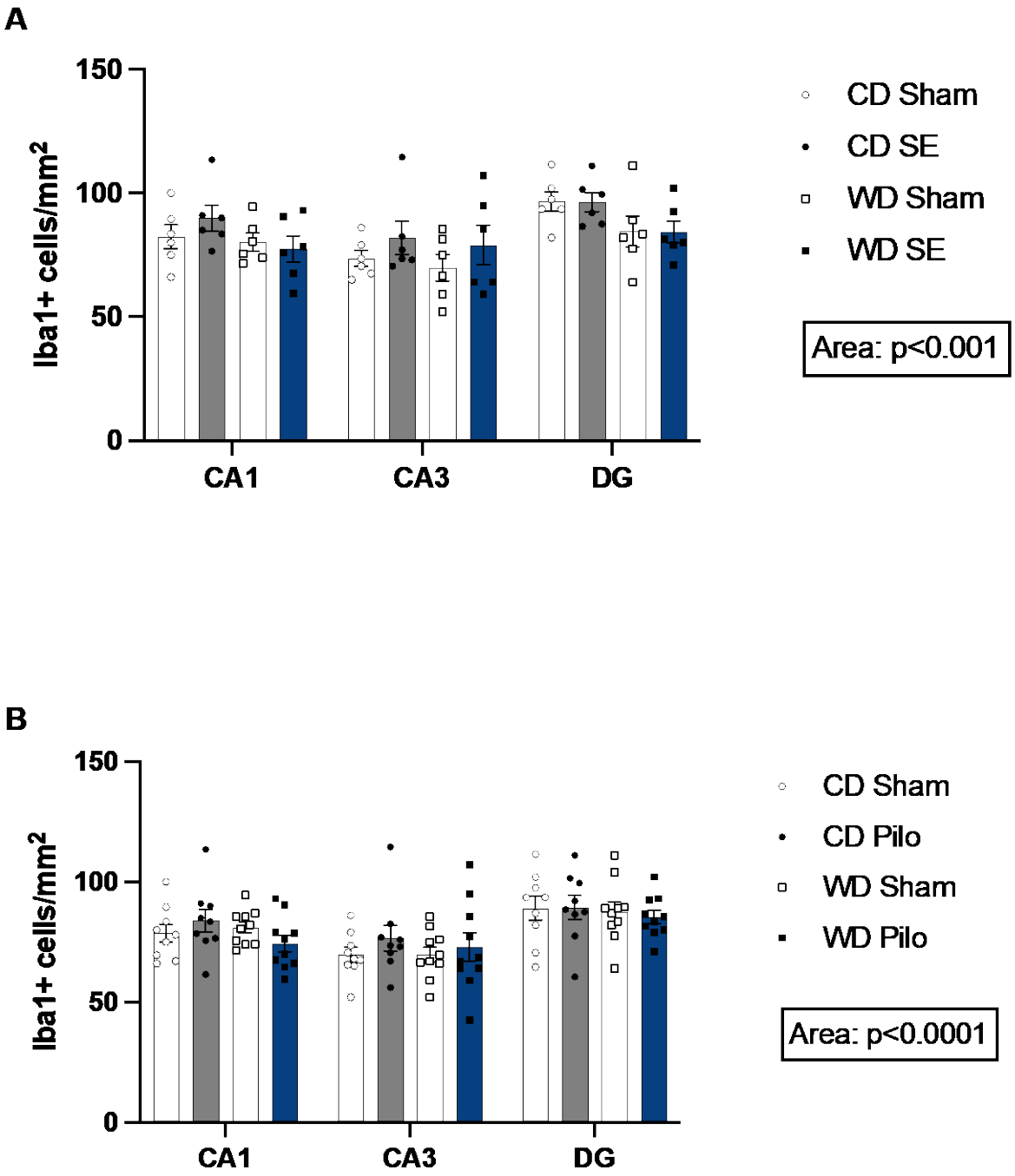

Figure 7. No significant difference in average Iba1+ cells/mm^2 in the hippocampus across groups. (A) Average Iba1+ cells in Sham and SE groups (n = 6 per group) in CA1, CA3, and DG not significantly different, data shown as Mean ± SEM. (B) Grouped means of Iba1+cells/mm^2 by hippocampal region of Sham and Pilo animals (CD n = 9, WD n = 10) with no significant differences across groups, data shown as Mean ± SEM.

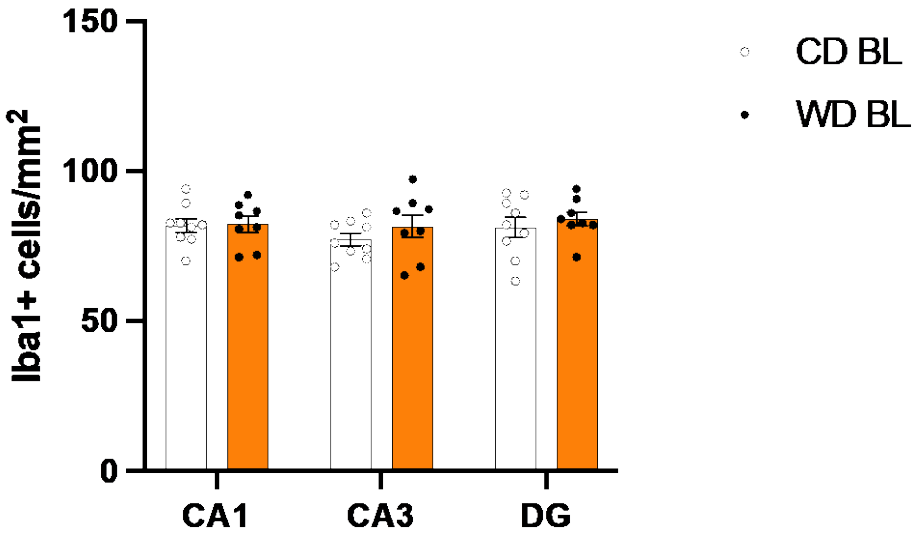

Figure 8. Average Iba1+ cells/mm^2 did not differ between BL groups. Individual means (CD *n* = 9, WD *n* = 8). Data shown as Mean ± SEM.

Microglial Morphology

Percent Microglia Morphologies in Sham and SE Groups

There was a main effect of morphology (F(1.613, 54.84) = 164.1, $p < 0.0001$) in CA1 (Fig. 9). Ramified cells were the predominant phenotype across groups. Our results did not show a significant increase in the activated hypertrophic or bushy morphologies between Sham and SE groups. In CA3 we also found a main effect of morphology ($F(2.218, 75.41) = 148.9$, $p < =0.0001$), and the predominant morphology was ramified (Fig. 10). In the DG we found a main effect of morphology ($F(2.221, 75.51) = 116.7$, $p < 0.0001$), and an interaction of morphology x diet ($F(4, 136) = 3.727$, $p = 0.065$) and ramified cells were also the predominant phenotype (Fig. 11).

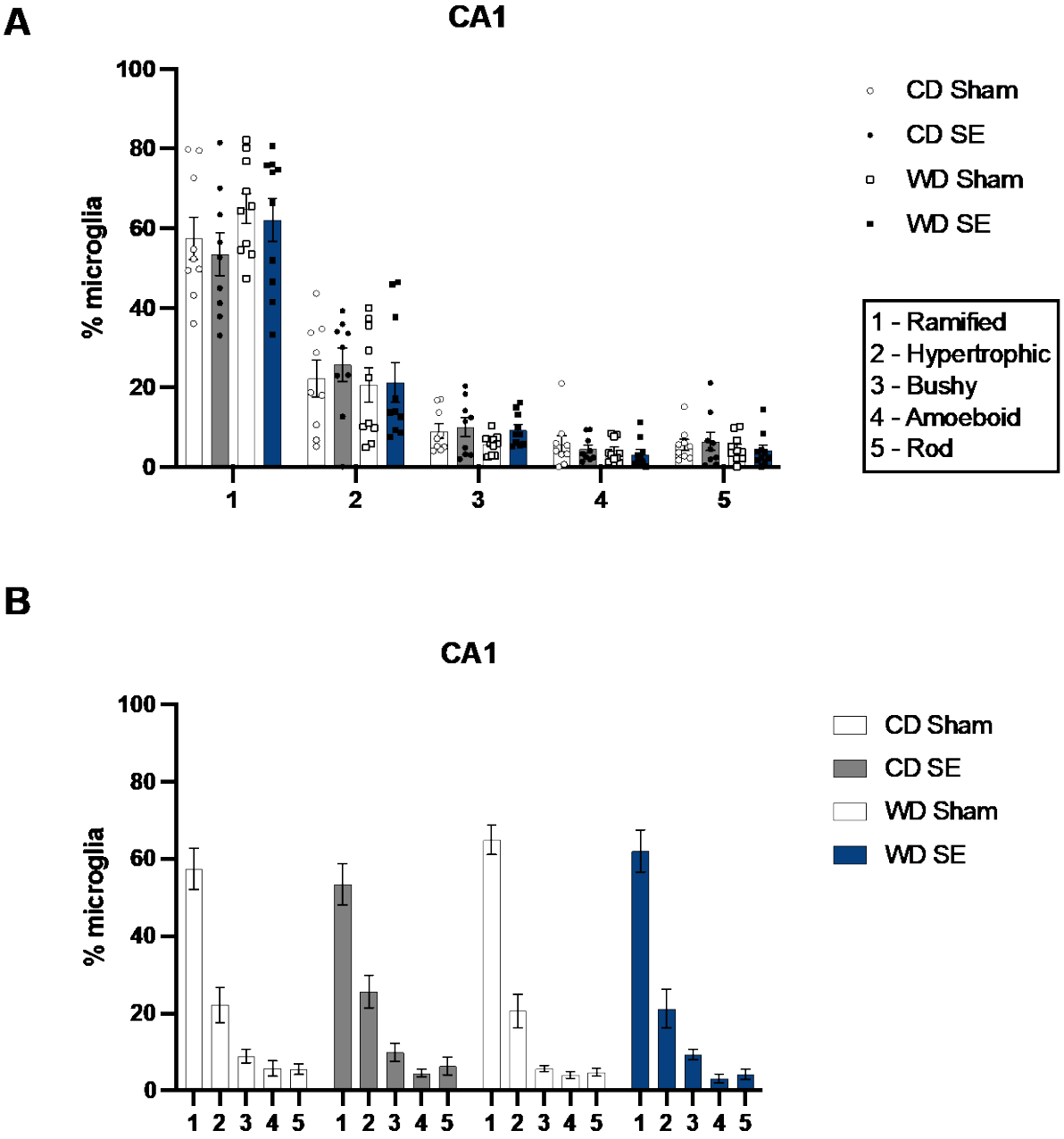

Figure 9. Percent microglial morphological phenotypes were not significantly different in CD compared to WD groups in CA1. (A) Individual distribution of percent microglia per morphology (CD *n* = 9, WD *n* = 10). (B) Mean percent morphology by group. Data shown as Mean ± SEM.

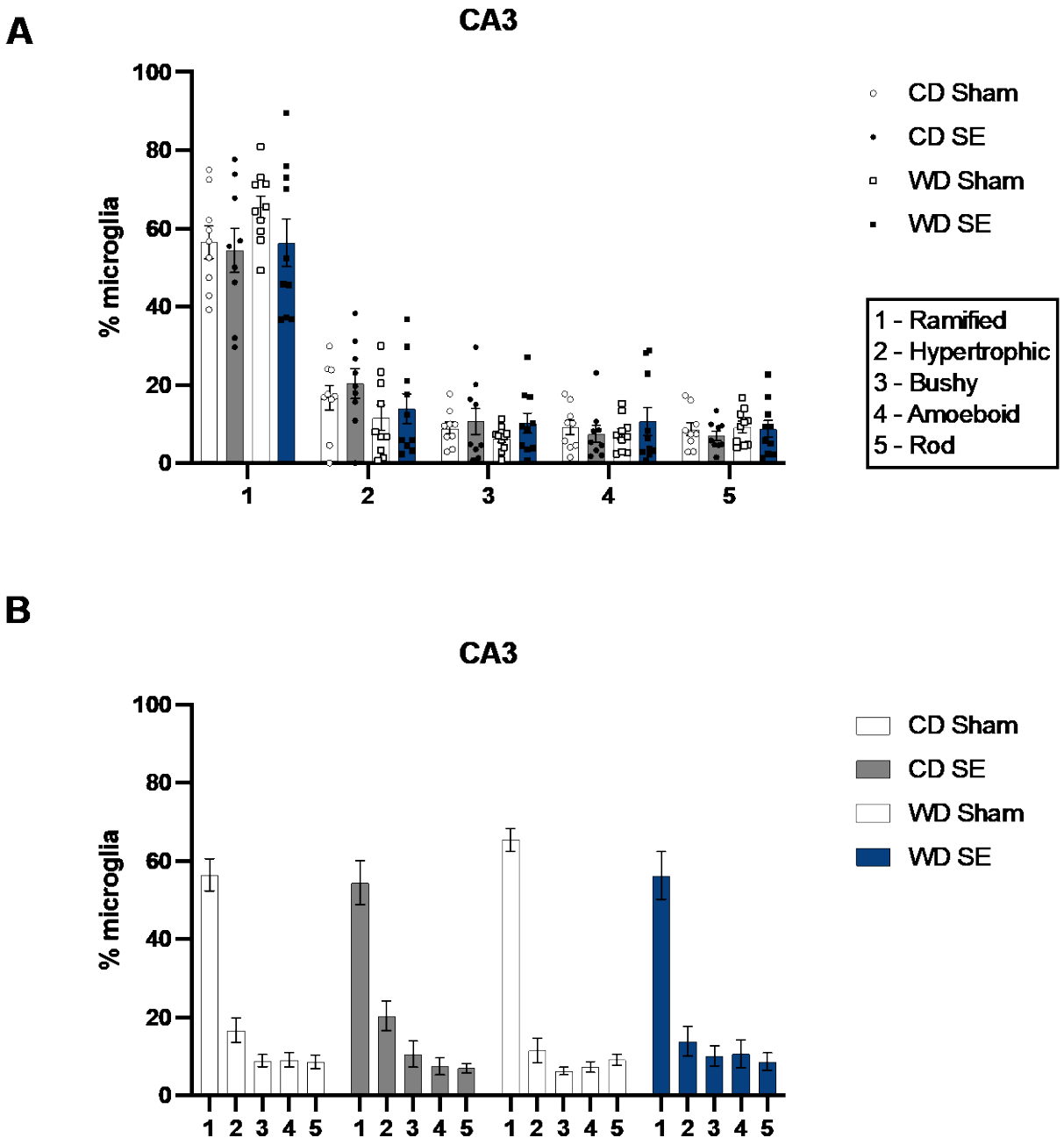

Figure 10. Percent microglial morphological phenotypes were not significantly different in CD compared to WD groups in CA3. (A) Individual distribution of percent microglia per morphology (CD *n* = 9, WD *n* = 10). (B) Mean percent morphology by group. Data shown as Mean ± SEM.

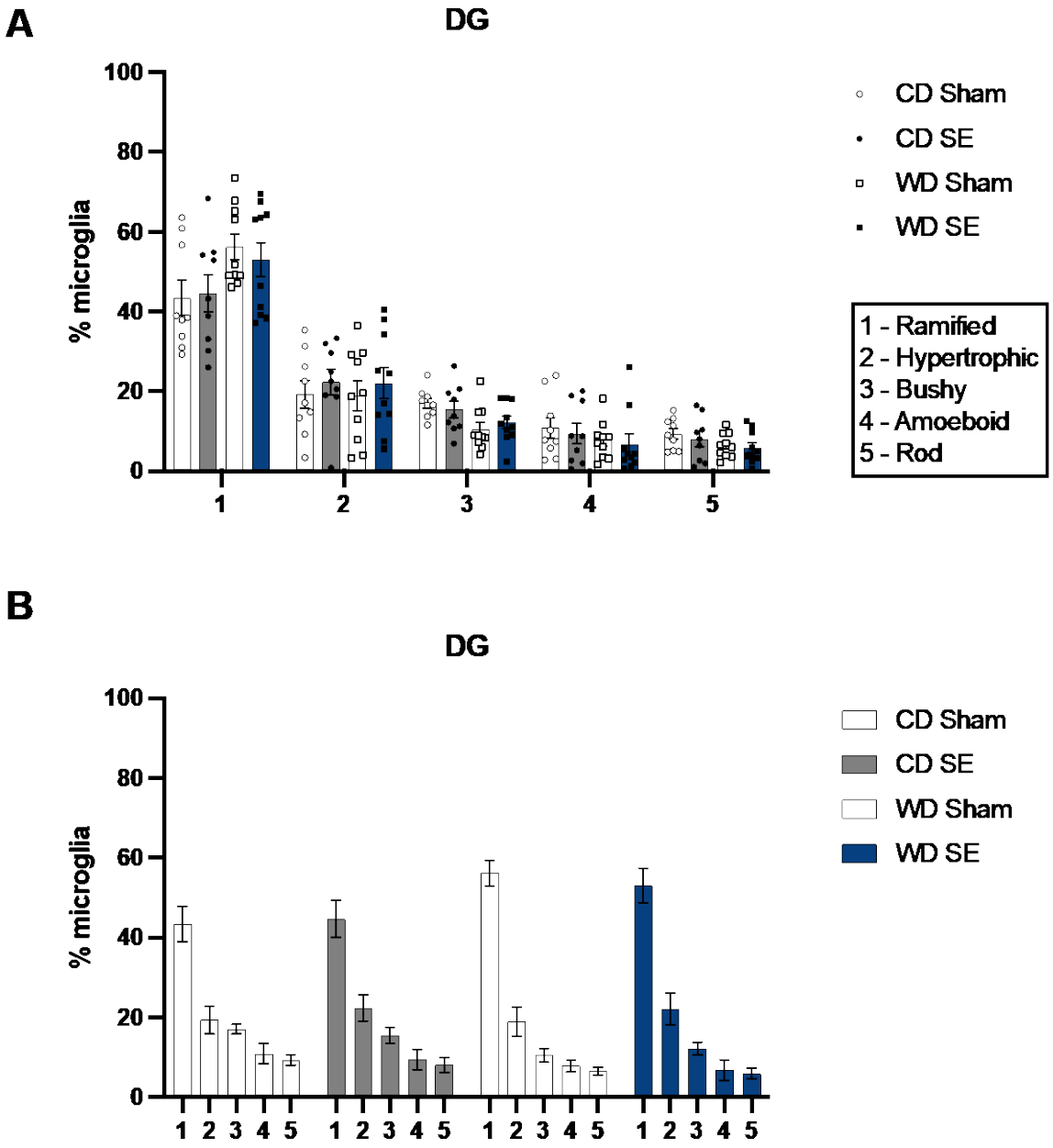

Figure 11. Percent microglial morphological phenotypes were not significantly different in CD compared to WD groups in DG. (A) Individual distribution of percent microglia per morphology (CD *n* = 9, WD *n* = 10). (B) Mean percent morphology by group. Data shown as Mean ± SEM.

Percent Microglial Morphologies in BL Groups

In order to examine differences in morphology caused by diet alone, we compared CD and WD BL groups. We found a main effects of morphology in CA1 ($F(1.88, 17.82) = 237.2$, $p < 0.0001$), CA3 ($F(1.285, 19.29) = 357.5$, $p < 0.0001$), and DG ($F(1.332, 19.98) = 222.7$, $p < 0.0001$). However, we did not find significant differences between diet groups for the different morphologies. The predominant phenotype across hippocampal areas was ramified, and we saw low percentages of bushy, amoeboid, and rod microglia in all areas (Fig. 12).

Analysis of Total Iba1+DAB Area (Densitometry)

We found a main effect of seizure in the treated groups ($F(1, 34) = 5.29$, $p = 0.027$), but no significant differences between CD Sham and SE, or WD Sham and WD SE groups (Fig. 13). Among the BL groups there were no significant differences in total Iba1 area between CD and WD groups ($t(8.297) = 1.324$, $p = 0.2209$) (Fig. 13B).

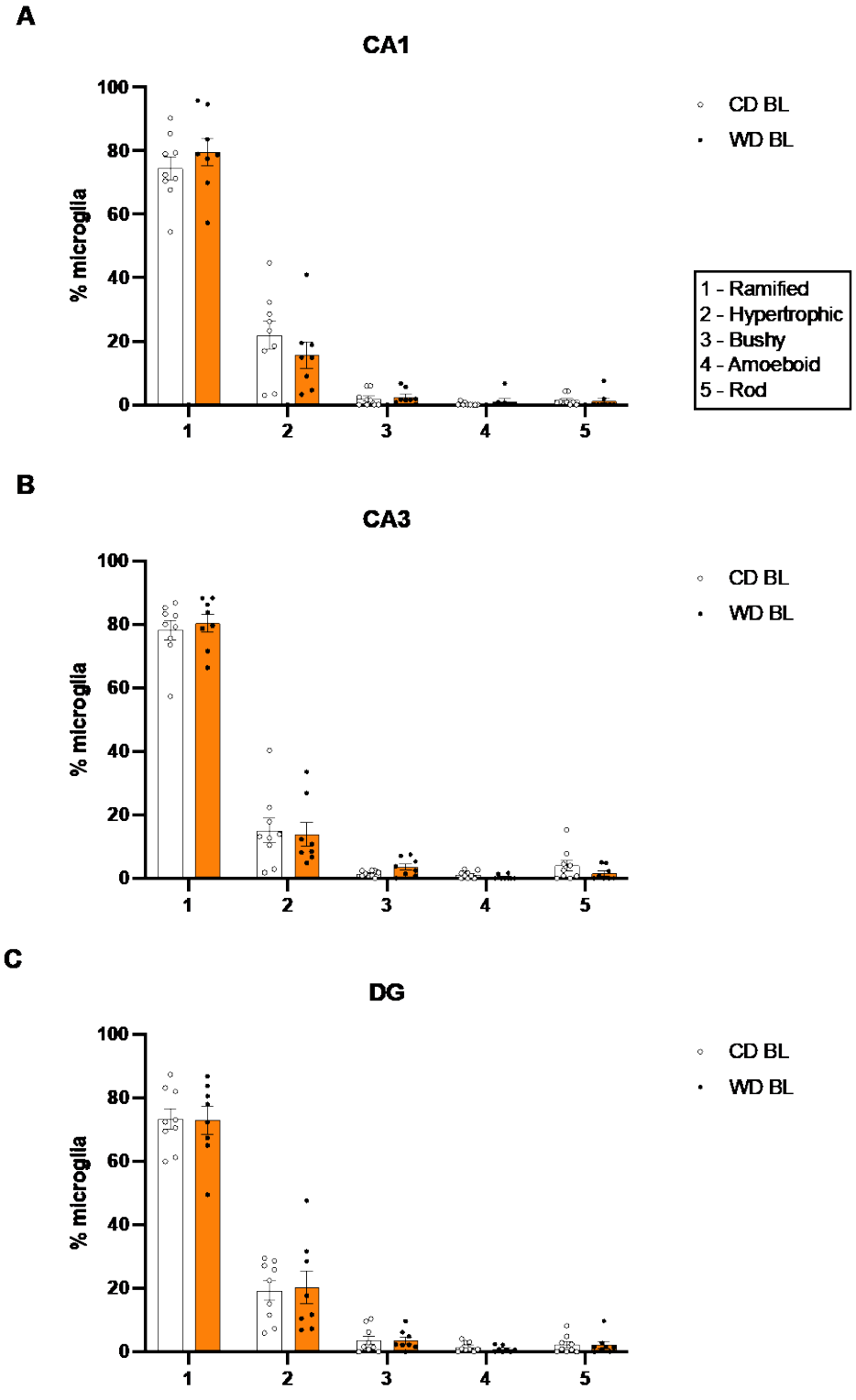

Figure 12. Percent microglial morphological phenotypes were not significantly different in CD compared to WD BLs across hippocampal areas. (A) Individual distribution and mean of percent microglia per morphology in CA1, (B) CA3, and (C) DG (CD n = 9, WD n = 8). Data shown as Mean ± SEM

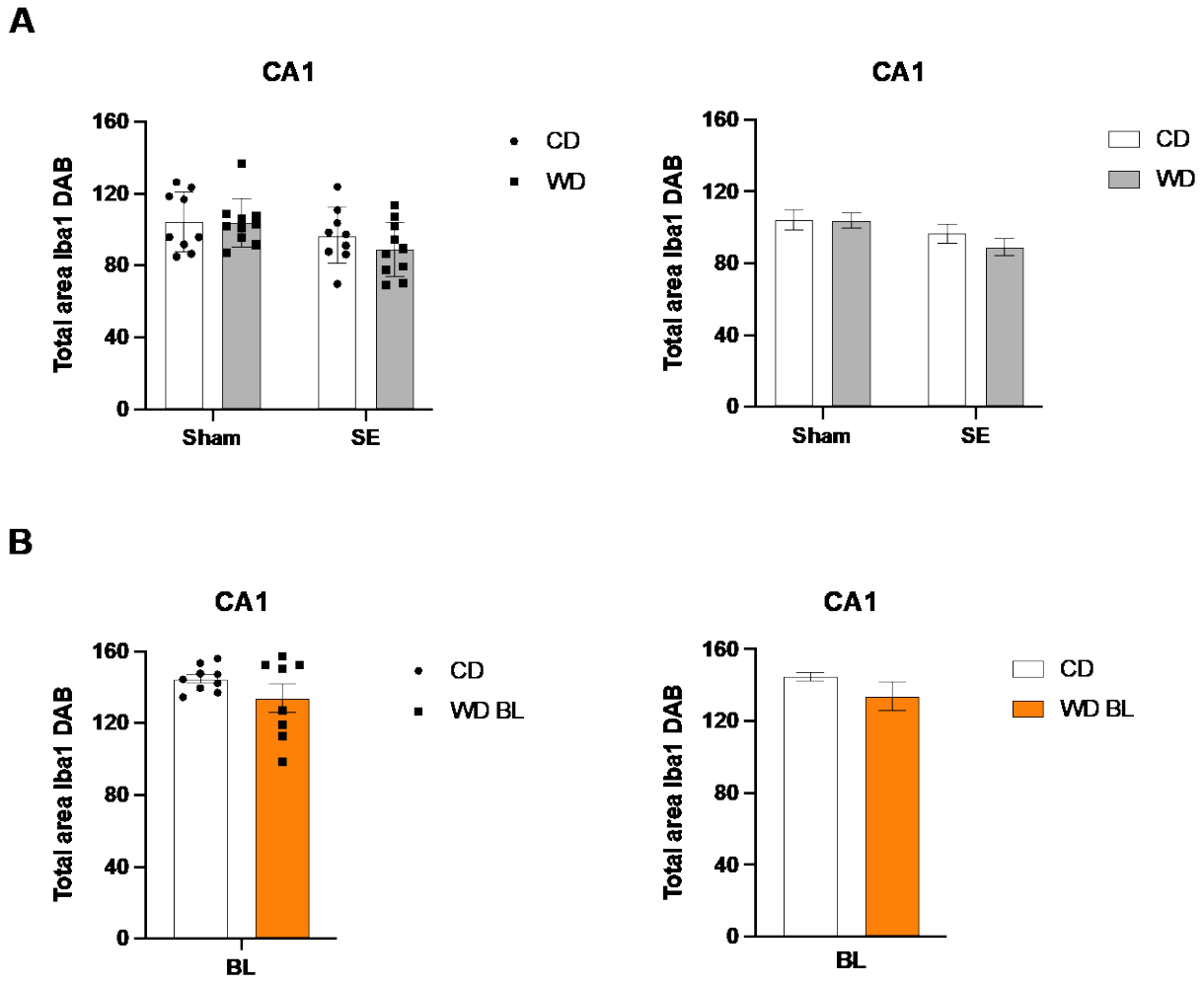

Figure 13. Total DAB area of Iba1+ cells in CA1. (A) No significant differences in CD vs. WD Sham and SE groups. (CD *n* = 9, WD *n* = 10). (B) No significant differences in CD and WD BL groups (CD *n* = 9, WD *n* = 8). Data shown as Mean ± SEM

DISCUSSION

This study examined the role of early developmental WD consumption on behavioral and neuroimmune outcomes after pilocarpine-induced seizures in male rats. WD consumption has been identified as a contributing source of proinflammatory responses in the hippocampus including microgliosis. The potential priming effects of WD to exacerbate outcomes after SE remain underexplored, along with the interaction between early developmental WD consumption, and SE. We hypothesized that early developmental consumption of a WD would produce hippocampal neuroinflammation and exacerbate outcomes after seizure injury. However, our results showed no differences in body weights, latency to SE, or frequency of SE after pilocarpine injection. We did not observe differences in time to sedation after diazepam, but found differences in time to recover from diazepam in WD Pilo animals. WD did not produce an increase in average microglial number in the hippocampus, and did not increase the proportion of reactive microglial phenotypes.

Body Weights

We found no differences in body weight at induction between Sham and SE, or between BL groups. This finding was not expected since the literature suggests that WD consumption produces differences in body weight, depending on when the diet was introduced, and for how low it was given (Boitard et al., 2014; Daly et al., 2020). Studies that find robust changes in body weight or body weight change typically test the effects of 60% fat diets (HFD) and use mice in their experiments (Reichelt et al., 2021). However, some rodent studies that examined the effects on WD on hippocampal neuroinflammation have reported no differences in body weight between WD and CD animals. A study in male mice consumed WD from P21 to P60 found no significant differences in body weight at P60, despite finding evidence of metabolic dysfunction and microglial reactivity (Vinuesa et al., 2019a). The same group also found that 1 month-old male mice that consumed WD from 4 months did not show differences in final body weight compared to CD controls, despite also reporting differences in metabolic markers (Vinuesa et al., 2016). Furthermore, the few studies that have examined the role of WD and HFD on SE did not always measure or report body weight data (Alzoubi et al., 2018; Alzoubi, Hasan, Khabour, Mayyas, al

Yacoub, et al., 2019; Nogueira et al., 2019). The study that reported body weight data used a 60% HFD in mice and found differences at 7 and 8 weeks of consumption (Kang et al., 2015b). Notably, these last studies did find differences in SE measures between diet groups. Together, this evidence suggests that not all early exposures to WD produce differences in body weight, which suggests that weight is not the only factor driving these effects, and perhaps other metabolic processes play more significant roles under specific experimental conditions. Lastly, these contrasting findings indicate that neuroinflammatory changes elicited by WD consumption could be age-dependent or have differing impact across developmental stages and age of diet intervention.

Latency to SE

We hypothesized that animals in the WD SE group would display a reduced time to SE compared to CD SE. However, in our experiments, early developmental exposure to WD did not significantly reduce the latency to reach SE compared to CD SE animals (Fig. 4A-C). In the present study, WD does not significantly alter the frequency to reach SE during the 60 min scoring period, suggesting that there were no differences in observable intensity of behavioral seizures. Originally, we chose the stage 5 seizure as our definition of SE based on the outcomes associated with this stage, and on previous studies from our collaborators that found differences in microgliosis and morphology. Stage 5 and 6 seizures correspond to severe brain damage and/or injury and the development of SE (Racine, 1972). In addition, electrographic analysis of pilocarpine induced SE in male mice showed that stage 5 seizures were associated with the highest ictal EEG activity (Phelan et al., 2015). It has been previously shown that only SE animals that reach scores of 5 and 6 show microglial alterations that correspond to the pathophysiology of TLE and have been extensively validated by our collaborators (Brewster et al., 2013; Schartz et al., 2016b; Wyatt et al., 2017; Wyatt-Johnson et al., 2017). In addition, according to the pilocarpine model, rats that reach SE are more likely to develop spontaneous recurring seizures after the epileptogenic period, and these stages more accurately mimic the neurological changes seen in humans with acquired TLE.

Only a few studies have tested whether WD or HFD changes seizure outcomes and inflammatory responses in the brain. One study examined the effect of 60% HFD on KA-induced seizures in a mouse model of SE. The HFD group showed “greater susceptibility” to seizures and seizure severity. Both behavioral and electrographic seizures were reported as more severe based

on more pronounced observable seizure behavior, higher spike number, more spontaneous seizure activity, and higher and longer spike trains in HFD mice starting 5 min after the KA injection (Kang et al., 2015b). A different study that measured EEG response after pilocarpine in male rats that consumed a WD from P60 to P120 found higher electrical activity and electrical wave patterns compared to controls (Nogueira et al., 2019). In a different model of SE that uses multiple doses of the chemoconvulsant PTZ, male rats in the WD+PTZ group needed fewer PTZ doses to reach SE compared to CD controls (Alzoubi et al., 2018; Alzoubi, Hasan, Khabour, Mayyas, al Yacoub, et al., 2019). This is supporting evidence for altered latency and severity of seizures promoted by WD consumption.

However, our experiment does not reveal significant differences in latency to reach SE. In the other SE studies, the diet manipulation started at P35 (KA) and P60 (Pilo) respectively, and for different durations (8 weeks, 60 days, and 6 weeks). This suggests that latency to SE and seizure severity in animals that receive high-fat or WD are influenced by age of first exposure and duration of diet consumption. In our study we introduced the diet from birth and predicted a priming proinflammatory effect that would worsen seizure outcomes. However, this may have resulted in adaptation in the neurodevelopmental environment of WD animals, or possible compensatory mechanisms in our animals. In addition, one limitation of our study is the lack of electrographic measures of seizure parameters, which would be a more sensitive tool to measure temporal differences and seizure profiles between groups.

Reachers and Non-Reachers

Since we did not find significant differences between animals that reached SE and their litter matched Sham controls as hypothesized, we decided to include CD and WD Pilo animals in our analyses to determine if these effects persisted regardless of behavioral seizure status. All animals that received a pilocarpine injection reached stage 4.5 seizures, and some remained in this state for most of the induction protocol. Grouping SE and Pilo animals along with litter-matched Sham controls also increased our group numbers for analyses of time to sedation, and to recovery post-diazepam. It is important to note that not all SE animals reached sedation after diazepam, and that not all the animals that became completely sedated recovered within the 180 min of scoring post-diazepam. This was also true for NRs that were scored post-diazepam. Details are shown in Table 2.

Time to Sedation, and to Recovery After Diazepam

Diazepam administration is a well-established treatment to stop seizures, both in episodes of seizures in humans and in rodent models of SE. Diazepam is a benzodiazepine that allosterically modulates (γ-aminobutyric acid) $GABA_A$ receptors and promotes the inhibitory effects of GABA neurotransmission. In our experiments we administered an i.p, injection of (10 mg/kg) diazepam to all rats 60 minutes after pilocarpine (or saline) to terminate the seizures and induce sedation. The behavioral effects of diazepam after pilocarpine-induced SE have not been as widely reported as the behavioral effects of pilocarpine. Diazepam effects such as absence of observable seizure activity may vary depending on the dose, pharmacological SE model, and rodent species (Buckmaster & Haney, 2012; Khan et al., 1999; Lüttjohann et al., 2009). Depending on the intensity, frequency, and time to develop SE the behavioral response to diazepam varies among animals that receive pilocarpine. Rats that reached a 5 or a 6, multiple times, or later in the induction period have been reported to show attenuated responses to diazepam and can exhibit persistent seizure activity before reaching a sedated state, and while experiencing gradual sedative effects (Goodkin et al., 2003; Jones et al., 2002). In addition, one group examined the electrographic seizure activity in male Wistar rats using the lithium-pilocarpine model reported that after rats reached stage 5 behavioral and electrographic seizures and diazepam (5 mg/kg) was given, no further seizure behaviors were observed, although it is not specified how many minutes after i.p. diazepam (Khan et al., 1999). However, other WD and HFD SE studies did not report behavioral outcomes after diazepam, so to our knowledge this study is the first one to report these observations. In our study, one WD SE animal received a supplementary half-dose of diazepam 1 h after the first dose. This animal displayed milder movement patterns after the second diazepam dose but did not become sedated by the end of the protocol. This could be a confounding factor for the behavioral measures and microglial analyses since this animal had prolonged seizure behavior and additional exposure to the diazepam effects compared to the rest. Potential differences in neuronal excitation and inhibition could alter microglial activity.

In this study, time to sedation after diazepam was not different between CD and WD animals irrespective of seizure status (Fig 5.A-C). Moreover, time to recover or wake from sedation was measured. CD and WD SE animals did not differ in time to recovery (Fig. 6A, B). However, WD Pilo animals took significantly longer to recover from diazepam than their CD

counterparts (Fig. 6C, D). This means that WD animals that received diazepam and reached sedation took longer to recover than CD Pilo animals. This finding suggests that WD influences the dynamics of diazepam after seizure injury. This behavioral difference could result from changes in the peripheral pharmacokinetics or pharmacodynamics of diazepam that arise from WD consumption. The lipophilic property of diazepam, which facilitates its passage through cell membranes, would in turn promote rapid absorption and targeted effects (Arendt et al., 1987). The lipophilicity of a diazepam is positively related to its pharmacokinetic volume of distribution, which has been shown to increase in obese individuals (Bruno et al., 2021). This is an important factor to consider since lipophilic drugs that are administered i.p. (as in the present study) can diffuse more easily in adipose tissue, which is typically higher in individuals with obesity. In our case, WD animals could have higher adiposity, and therefore higher rates of diazepam diffusion or metabolism compared to CD animals. Higher peripheral lipophilicity could also promote easier diffusion of diazepam into the brain and affect the binding and subsequent sedative effects (Arendt et al., 1987). However, in our study we cannot determine differences in the magnitude of sedative effects of diazepam after seizure injury, or the temporal response in the brain since that would require EEG recordings.

Other potential mechanisms that could explain differences in response to diazepam and other drugs include blood-brain barrier (BBB) alterations in WD animals. WD and HFD consumption has been shown to produce BBB disruption that result in leakage into the brain (Hargrave et al., 2015, 2016; Hsu & Kanoski, 2014; Kanoski et al., 2010; Sharma, 2021). This disruption can affect the amount of drug that enters the brain and influences sedation and recovery times. The effects of WD could interact with the effects of seizures on BBB integrity as well. Evidence suggests that pilocarpine-treated (320 mg/kg) stage 5 seizure/SE animals had higher concentrations of Evans blue dye in the brain, indicative of BBB leakage 1 day after SE (Yu et al., 2019). The blood protein albumin is not supposed to be found inside the brain, so detection of this protein in brain tissue is indicative of barrier leakage, and that has been shown to increase after SE (Swissa et al., 2019). Further analysis of albumin levels in hippocampal tissue using Western blotting would help us identify BBB leakage in our animals. Lastly, one limitation of our study is that we did not score Sham animals after their diazepam injection. This would have been helpful to directly compare both diet groups and better understand the behavioral effects of diet on diazepam response after SE induction.

Microglial Proliferation

Based on previous evidence that SE and WD produce microgliosis in the hippocampus independently, we hypothesized that WD would produce microgliosis in Sham animals and a greater response in SE rats. However, our experiment does not show differences in average microglial counts between Sham and SE animals irrespective of diet 4 h after pilocarpine injection. We do not observe differences in microgliosis when we grouped all animals per group either (Fig. 7). This is true across hippocampal regions of interest. This finding is consistent to previous work that used manual quantification of Iba1+ cells to examine the spatiotemporal changes in microglial proliferation up to 2 weeks post-Pilocarpine and found no significant differences at 4 h between CD Sham and CD SE animals in CA1, CA3, or DG(Wyatt-Johnson et al., 2017). In their analysis, Wyatt-Johnson *et al.* (2017) found that peak microglial proliferation was not observed acutely after SE, but rather observed starting days after, and significantly greater 2 weeks after SE.

Analysis of Total Iba1+DAB Area (Densitometry)

Previous studies have examined changes in hippocampal microgliosis and microglia morphology using densitometric analyses have found significant differences due the induction of seizures, and WD respectively(Schartz et al., 2016a; Vinuesa et al., 2016, 2019b). Specifically in the first study that characterized the progression of microgliosis up to 35 days after pilocarpine-induced SE, Schartz *et al.* (2016) found significantly higher mean pixel intensity of Iba1+ cells in CA1, CA3, and DG of SE animals 4 h after pilocarpine, which was most prominent in CA1. Therefore, it is possible that some of the differences in Iba1+ density reported across studies may be due to the method of analysis for densitometry. Some studies performed densitometry of broadly defined hippocampal regions at lower magnification, while others captured images at higher magnification with the same frame area across images. Schartz *et al.* (2016) performed semi-quantitative densitometry of Iba1 immunoreactive cells labeled with DAB at 4X magnification in bilateral hippocampus.

To test whether our lack of differences in average microglial number were due to differences in methods of analysis/quantification we performed color thresholding analysis of Iba1+ DAB-labeled images in CA1 in all groups. Our findings show no differences between Sham

and Pilo groups in CA1 (Fig. 13A). Moreover, we did not find significant differences in BL groups, which is contrary to what the diet literature would suggest. Nevertheless, these two methods are not identical and only direct comparison could explain if there are indeed differing results. Our findings indicate that early developmental consumption of WD does not increase average microglial number across hippocampal areas, and it does not increase Iba1+ DAB area in CA1 after pilocarpine (Fig. 13A) or at P50-55 (Fig. 13B). However, the age of first exposure and length of diet exposure may account for variability in inflammatory responses to WD that begin or occur in early life (Bilbo & Tsang, 2010; Bolton & Bilbo, 2014; Noble & Kanoski, 2016; Tsan et al., 2021).

Microglial Morphology

Microglia respond rapidly to changes in their microenvironment and to changes caused by neuronal insult, and diet respectively by modifying their cytoskeletal structure (Nimmerjahn et al., 2005; Sierra et al., 2013; Wyatt-Johnson & Brewster, 2020). Microglia shift from ramified (1) to hypertrophic (2), bushy (3), and amoeboid (4) morphologies after insult reflecting a reactive state. We predicted we would replicate previous findings using only CD animals showed significant increases in bushy microglial morphology in CA1 and CA3 4 h after Pilocarpine (Wyatt-Johnson et al., 2017). Our results do not show a significant increase in the activated hypertrophic, bushy, or amoeboid morphologies between Sham and SE groups or between CD and WD (Fig. 9-11). In addition, we do not observe morphological differences between CD and WD BL groups (Fig. 12). This pattern is consistent across CA1, CA3, and DG. Our predicted outcomes in CD Sham and SE animals are not supported by our results. Together, this indicates that our dietary manipulation does not increase the proportion of reactive microglia in the hippocampus at baseline or after pilocarpine-induced SE.

There are other morphological features that we did not analyze in our study such as number of processes, and branch length, which can change with diet and SE. One study tested the effects of long term WD consumption on hippocampal microglial function and morphology in 3-month-old rats after and found increased branch length and branch number in WD animals compared to controls, but no significant differences in Iba1+ cell number (Daly et al., 2020). Early exposure (from P21 to P60) to WD in male mice resulted in no differences in Iba1+ density in the

DG or CA1 compared to CD controls but did produce an increase in microglial soma area in both regions (Vinuesa et al., 2016, 2019b).

CONCLUSION

Western diet consumption has been identified as a contributing source of proinflammatory responses in the brain including microgliosis. Evidence from rodent studies indicates that these proinflammatory effects are seen in the hippocampus, suggesting a role for WD contributing to pathophysiological disruptions to brain function. Moreover, hippocampal microgliosis is a hallmark not only in experimental models of seizure disorders but also in acquired epilepsy in humans. Activation and proliferation of microglia contributes to the development and progression of spontaneous recurring seizures, and ultimately epilepsy. The interaction between WD consumption, epilepsy and hippocampal pathology has been relatively unexplored, along with the potential priming effects of WD to exacerbate outcomes after status epilepticus.

This study examined the role of early developmental WD consumption on behavioral and inflammatory outcomes after pilocarpine-induced seizures in male rats. Our experiments show no differences in body weights at the time of induction (P50-55), latency to seizures, or frequency of SE after pilocarpine injection. These data suggest that WD does not exacerbate behavioral outcomes after pilocarpine. To our knowledge, we are the first to report observations after diazepam in studies that examine the effects of high-fat diets on chemically-induced seizures. Further, we do not observe behavioral differences in time to sedation after diazepam injection. We report no differences in time to recover from diazepam in SE animals. However, WD animals that received pilocarpine and became sedated after diazepam did experience longer recovery times than their CD counterparts. This finding suggests that WD plays a role in the behavioral response to diazepam that follows systemic pilocarpine injection. Moreover, our experiments do not reveal proinflammatory increases measured by microgliosis analyses 4 h after pilocarpine, and WD consumption from birth to P50-55 does not exacerbate these outcomes in our experiments. In all our groups, our results indicate that the predominant microglial phenotype is ramified or in a surveillance state.

These findings help us better understand the ways in which inflammatory diets that are consumed early in development influence neuroimmune responses by microglia. They also illustrate that not all WD exposures result in different responses by hippocampal microglia, and that not all neuroinflammatory changes that are observed in the hippocampus are produced in a similar fashion. Rather, there are variable outcomes when we examine the role of developmental

WD on seizure and inflammatory outcomes, which support the rationale that multiple or alternative factors play a role in the development of these pathologies.

REFERENCES

Abraham, J., Fox, P. D., Condello, C., Bartolini, A., & Koh, S. (2012). Minocycline attenuates microglia activation and blocks the long-term epileptogenic effects of early-life seizures. https://doi.org/10.1016/j.nbd.2012.02.006

Allen Hauser, W., & Annegers, J. F. (1996). Descriptive epidemiology of epilepsy: Contributions of population-based studies from Rochester, Minnesota. *Mayo Clinic Proceedings*, *71*(6), 576–586. https://doi.org/10.4065/71.6.576

Alliot, F., Lecain, E., Grima, B., & Pessac, B. (1991). Microglial progenitors with a high proliferative potential in the embryonic and adult mouse brain. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(4), 1541-1545. https://doi.org/10.1073/pnas.88.4.1541

Alzoubi, K. H., Hasan, Z. A., Khabour, O. F., Mayyas, F. A., al Yacoub, O. N., Banihani, S. A., Alomari, M. A., & Alrabadi, N. N. (2019). Vitamin E modifies high-fat diet-induced reduction of seizure threshold in rats: Role of oxidative stress. *Physiology and Behavior*, *206*, 200-205. https://doi.org/10.1016/j.physbeh.2019.04.011

Alzoubi, K. H., Hasan, Z. A., Khabour, O. F., Mayyas, F. A., al Yacoub, O. N., Banihani, S. A., Azab, M. A., & Alrabadi, N. (2018). The effect of high-fat diet on seizure threshold in rats: Role of oxidative stress. *Physiology and Behavior*, *196*, 1-7. https://doi.org/10.1016/J.PHYSBEH.2018.08.011

Arcuri, C., Mecca, C., Bianchi, R., Giambanco, I., & Donato, R. (2017). The pathophysiological role of microglia in dynamic surveillance, phagocytosis and structural remodeling of the developing CNS. *Frontiers in Molecular Neuroscience*, *10*, 191). https://doi.org/10.3389/fnmol.2017.00191

Arendt, R. M., Greenblatt, D. J., Liebisch, D. C., Luu, M. D., & Paul, S. M. (1987). Determinants of benzodiazepine brain uptake: lipophilicity versus binding affinity. *Psychopharmacology*, *93*(1), 72-76.

Ashwell, K. (1991). The distribution of microglia and cell death in the fetal rat forebrain. *Developmental Brain Research*, *58*(1), 1-12. https://doi.org/10.1016/0165-3806(91)90231-7

Bartolomei, F., Khalil, M., Wendling, F., Sontheimer, A., Régis, J., Ranjeva, J. P., Guye, M., & Chauvel, P. (2005). Entorhinal cortex involvement in human mesial temporal lobe epilepsy: An electrophysiologic and volumetric study. *Epilepsia*, *46*(5), 677-687. https://doi.org/10.1111/j.1528-1167.2005.43804.x

Beamer, E., Conte, G., & Engel, T. (2019). ATP release during seizures-A critical evaluation of the evidence. *Brain Research Bulletin*, *151*, 65-73. https://doi.org/10.1016/j.brainresbull.2018.12.021

Bilbo, S. D., & Schwarz, J. M. (2009). Early-life programming of later-life brain and behavior: A critical role for the immune system. *Frontiers in Behavioral Neuroscience*, *3*(AUG), 1-14. https://doi.org/10.3389/neuro.08.014.2009

Bilbo, S. D., & Tsang, V. (2010). Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. *The FASEB Journal*, *24*(6), 2104-2115. https://doi.org/10.1096/fj.09-144014

Boitard, C., Cavaroc, A., Sauvant, J., Aubert, A., Castanon, N., Layé, S., & Ferreira, G. (2014). Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats. *Brain Behavior and Immunity*, *40*, 9-17. https://doi.org/10.1016/j.bbi.2014.03.005

Bolton, J. L., & Bilbo, S. D. (2014). Developmental programming of brain and behavior by perinatal diet: Focus on inflammatory mechanisms. *Dialogues in Clinical Neuroscience*, *16*(3), 307-320. www.dialogues-cns.org

Bortolin, R. C., Vargas, A. R., Gasparotto, J., Chaves, P. R., Schnorr, C. E., Da Boit Martinello, K., Silveira, A. K., Rabelo, T. K., Gelain, D. P., & Moreira, J. (2018). A new animal diet based on human Western diet is a robust diet-induced obesity model: comparison to high-fat and cafeteria diets in term of metabolic and gut microbiota disruption. *International Journal of Obesity*, *42*, 525-534. https://doi.org/10.1038/ijo.2017.225

Brewster, A. L., Lugo, J. N., Patil, V. V., Lee, W. L., Qian, Y., Vanegas, F., & Anderson, A. E. (2013). Rapamycin reverses status epilepticus-induced memory deficits and dendritic damage. *PLoS ONE*, *8*(3). https://doi.org/10.1371/journal.pone.0057808

Bruno, C. D., Harmatz, J. S., Duan, S. X., Zhang, Q., Chow, C. R., Greenblatt, D. J., & David Greenblatt, C. J. (2021). Effect of lipophilicity on drug distribution and elimination: Influence of obesity. *British Journal of Clinical Pharmacology, 87*(6) https://doi.org/10.1111/bcp.14735

Buckmaster, P. S., & Haney, M. M. (2012). Factors affecting outcomes of pilocarpine treatment in a mouse model of temporal lobe epilepsy. *Epilepsy Research, 102*(3), 153-159. https://doi.org/10.1016/j.eplepsyres.2012.05.012

Burnol, A. F., Leturque, A., De Saintaurin, M. A., Penicaud, L., & Girard, J. (1987). Glucose turnover rate in the lactating rat: Effect of feeding a high fat diet. *Journal of Nutrition, 117*(7), 1275-1279. https://doi.org/10.1093/jn/117.7.1275

Chapman, M. G., Smith, M., & Hirsch, N. P. (2001). Status epilepticus. *Anaesthesia, 56*, 648-659.

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. https://doi.org/10.1016/j.jneumeth.2008.04.019

Daly, C. M., Saxena, J., Singh, J., Bullard, M. R., Bondy, E. O., Saxena, A., Buffalino, R. E., Melville, M. F., & Freeman, L. R. (2020). Sex differences in response to a high fat, high sucrose diet in both the gut microbiome and hypothalamic astrocytes and microglia. *Nutritional Neuroscience, 25*(2), 321-335. https://doi.org/10.1080/1028415X.2020.1752996

Dissing-Olesen, L., LeDue, J. M., Rungta, R. L., Hefendehl, J. K., Choi, H. B., & MacVicar, B. A. (2014). Activation of neuronal NMDA receptors triggers transient ATP-mediated microglial process outgrowth. *Journal of Neuroscience, 34*(32), 10511-10527. https://doi.org/10.1523/JNEUROSCI.0405-14.2014

Doerflinger, A., & Swithers, S. E. (2004). Effects of diet and handling on initiation of independent ingestion in rats. *Developmental Psychobiology, 45*(2), 72-82. https://doi.org/10.1002/dev.20015

Dubbelaar, M. L., Kracht, L., Eggen, B. J. L., & Boddeke, E. W. G. M. (2018). The kaleidoscope of microglial phenotypes. *Frontiers in Immunology, 9*, 1753). https://doi.org/10.3389/fimmu.2018.01753

Eyo, U. B., Murugan, M., & Wu, L. J. (2017). Microglia-neuron communication in epilepsy. *Glia, 65*(1), 5-18. https://doi.org/10.1002/glia.23006

Eyo, U. B., Peng, J., Swiatkowski, P., Mukherjee, A., Bispo, A., & Wu, L. J. (2014). Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. *Journal of Neuroscience, 34*(32), 10528-10540. https://doi.org/10.1523/JNEUROSCI.0416-14.2014

Flegal, K. M., Kruszon-Moran, D., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2016). Trends in obesity Among adults in the United States, 2005 to 2014. *Jama, 315*(21), 2284. https://doi.org/10.1001/jama.2016.6458

Freitas, R. M., Sousa, F. C. F., Viana, G. S. B., & Fonteles, M. M. F. (2006). Acetylcholinesterase activities in hippocampus, frontal cortex and striatum of Wistar rats after pilocarpine-induced status epilepticus. *Neuroscience Letters*, *399*(1-2), 76-78. https://doi.org/10.1016/j.neulet.2006.01.028

Goodkin, H. P., Liu, X., & Holmes, G. L. (2003). Diazepam terminates brief but not prolonged seizures in young, naïve rats. *Epilepsia*, *44*(8), 1109-1112.

Grandgirard, A., Bourre, J. M., Julliard, F., Homayoun, P., Dumont, O., Piciotti, M., & Sebedio, J. L. (1994). Incorporation of trans long-chain n-3 polyunsaturated fatty acids in rat brain structures and retina. *Lipids*, *29*(4), 251-258. https://doi.org/10.1007/BF02536329

Gross, L. S., Li, L., Ford, E. S., & Liu, S. (2004). Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: An ecologic assessment. *American Journal of Clinical Nutrition*, *79*(5), 774-779. https://doi.org/10.1093/ajcn/79.5.774

Guillemot-Legris, O., Masquelier, J., Everard, A., Cani, P. D., Alhouayek, M., & Muccioli, G. G. (2016). High-fat diet feeding differentially affects the development of inflammation in the central nervous system. *Journal of Neuroinflammation*, *13*(1). https://doi.org/10.1186/s12974-016-0666-8

Guillemot-Legris, O., & Muccioli, G. G. (2017). Obesity-induced neuroinflammation: Beyond the hypothalamus. *Trends in Neurosciences*, *40*(4), 237-253. https://doi.org/10.1016/j.tins.2017.02.005

Hamilton, S. E., Loose, M. D., Qi, M., Levey, A. I., Hille, B., Mcknight, G. S., Idzerda, R. L., & Nathanson, N. M. (1997). Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(24), 13311-13316. https://doi.org/10.1073/pnas.94.24.13311

Hanisch, U. K. (2002). Microglia as a source and target of cytokines. *Glia*, *40*(2), 140-155. https://doi.org/10.1002/GLIA.10161

Hargrave, S. L., Davidson, T. L., Lee, T. J., & Kinzig, K. P. (2015). Brain and behavioral perturbations in rats following Western diet access. *Appetite*, *93*, 35-43. https://doi.org/10.1016/J.APPET.2015.03.037

Hargrave, S. L., Davidson, T. L., Zheng, W., Kinzig, K. P., & Hargrave, S. (2016). Western diets induce blood-brain barrier leakage and alter spatial strategies in rats HHS public access. *Behavioral Neuroscience*, *130*(1), 123-135. https://doi.org/10.1037/bne0000110

Hsu, T. M., & Kanoski, S. E. (2014). Blood-brain barrier disruption: Mechanistic links between western diet consumption and dementia. *Frontiers in Aging Neuroscience*, 6(MAY). https://doi.org/10.3389/fnagi.2014.00088

Ito, D., Tanaka, K., Suzuki, S., Dembo, T., & Fukuuchi, Y. (2001). Enhanced expression of iba1, ionized calcium-binding adapter molecule 1, after transient focal cerebral ischemia in rat brain. *Stroke*, *32*(5), 1208-1215. https://doi.org/10.1161/01.STR.32.5.1208

Jones, D. M., Esmaeil, N., Maren, S., & Macdonald, R. L. (2002). Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. *Epilepsy Research*, *50*(3), 301-312. https://doi.org/10.1016/S0920-1211(02)00085-2

Jope, R. S., Morrisett, R. A., & Snead, O. C. (1986). Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. *Experimental Neurology*, *91*(3), 471-480. https://doi.org/10.1016/0014-4886(86)90045-2

Kandratavicius, L., Balista, P., Lopes-Aguiar, C., Ruggiero, R., Umeoka, E., Garcia-Cairasco, N., Bueno-Junior, L., & Leite, J. (2014). Animal models of epilepsy: use and limitations. *Neuropsychiatric Disease and Treatment*, *10*, 1693. https://doi.org/10.2147/NDT.S50371

Kang, D. H., Heo, R. W., Yi, C. ok, Kim, H., Choi, C. H., & Roh, G. S. (2015a). High-fat diet-induced obesity exacerbates kainic acid-induced hippocampal cell death. *BMC Neuroscience*, *16*(1), 72. https://doi.org/10.1186/s12868-015-0202-2

Kanoski, S. E., & Davidson, T. L. (2010). Different patterns of memory impairments accompany short- and longer-term maintenance on a high-energy diet. *Journal of Experimental Psychology: Animal Behavior Processes*, *36*(2), 313-319. https://doi.org/10.1037/a0017228

Kanoski, S. E., & Davidson, T. L. (2011). Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity. *Physiology and Behavior*, *103*(1), 59-68. https://doi.org/10.1016/j.physbeh.2010.12.003

Kanoski, S. E., Meisel, R. L., Mullins, A. J., & Davidson, T. L. (2007). The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat. *Behavioural Brain Research*, *182*(1), 57-66. https://doi.org/10.1016/j.bbr.2007.05.004

Kanoski, S. E., Zhang, Y., Zheng, W., & Davidson, T. L. (2010). The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat. *Journal of Alzheimer's Disease* , *21*(1), 207-219. https://doi.org/10.3233/JAD-2010-091414

Khan, G. M., Smolders, I., Lindekens, H., Manil, J., Ebinger, G., & Michotte, Y. (1999). Effects of diazepam on extracellular brain neurotransmitters in pilocarpine-induced seizures in rats. *European Journal of Pharmacology*, *373*(2-3), 153-161. https://doi.org/10.1016/S0014-2999(99)00209-5

Könner, A. C., & Brüning, J. C. (2011). Toll-like receptors: Linking inflammation to metabolism. *Trends in Endocrinology and Metabolism*, *22*(1), 16-23. https://doi.org/10.1016/J.TEM.2010.08.007

Lawson, T., & Yeager, S. (2016). Status epilepticus in adults: A review of diagnosis and treatment. *Critical Care Nurse*, *36*(2), 62-73. https://doi.org/10.4037/ccn2016892

Lévesque, M., Avoli, M., & Bernard, C. (2016). Animal models of temporal lobe epilepsy following systemic chemoconvulsant administration. *Journal of Neuroscience Methods*, *260*, 45-52. Elsevier. https://doi.org/10.1016/j.jneumeth.2015.03.009

Li, Q., & Barres, B. A. (2018). Microglia and macrophages in brain homeostasis and disease. *Nature Reviews Immunology*, *18*(4), 225-242. https://doi.org/10.1038/nri.2017.125

Lizarbe, B., Soares, A. F., Larsson, S., & Duarte, J. M. N. (2019). Neurochemical modifications in the hippocampus, cortex and hypothalamus of mice exposed to long-term high-fat diet. *Frontiers in Neuroscience*, *12*(JAN), 985. https://doi.org/10.3389/fnins.2018.00985

Lowenstein, D. H., & Alldredge, B. K. (1998). Status epilepticus. *The New England Journal of Medicine*, 970-976. https://doi.org/10.1142/9789814273596_0013

Lüttjohann, A., Fabene, P. F., & van Luijtelaar, G. (2009). A revised Racine's scale for PTZ-induced seizures in rats. *Physiology and Behavior*, *98*(5), 579-586. https://doi.org/10.1016/J.PHYSBEH.2009.09.005

Marchi, N., Oby, E., Batra, A., Uva, L., de Curtis, M., Hernandez, N., van Boxel-Dezaire, A., Najm, I., & Janigro, D. (2007). In vivo and in vitro effects of pilocarpine: Relevance to ictogenesis. *Epilepsia*, *48*(10), 1934-1946. https://doi.org/10.1111/j.1528-1167.2007.01185.x

Mathern, G. W., Adelson, P. D., Cahan, L. D., & Leite, J. P. (2002). Hippocampal neuron damage in human epilepsy: Meyer's hypothesis revisited. *Progress in Brain Research*, *135*, 237-251. https://doi.org/10.1016/S0079-6123(02)35023-4

Murugan, M., & Boison, D. (2020). Ketogenic diet, neuroprotection, and antiepileptogenesis. *Epilepsy Research*, *167*, 106444. https://doi.org/10.1016/j.eplepsyres.2020.106444

Myles, I. A. (2014). Fast food fever: Reviewing the impacts of the Western diet on immunity. *Nutrition Journal*, *13*(1), 61. https://doi.org/10.1186/1475-2891-13-61

Neher, J. J., & Cunningham, C. (2019). Priming microglia for innate immune memory in the brain. *Trends in Immunology*, *40*(4), 358-374. https://doi.org/10.1016/j.it.2019.02.001

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Sciences*, *308*(May), 1314–1319. https://doi.org/10.1126/science.1110647Title & authors

Noble, E. E., & Kanoski, S. E. (2016). Early life exposure to obesogenic diets and learning and memory dysfunction. *Current Opinion in Behavioral Sciences*, *9*, 7-14. https://doi.org/10.1016/j.cobeha.2015.11.014

Nogueira, R. de A., Pessoa, D. T., da Silva, E. L. A., & Costa, E. V. L. (2019). Can a hypercholesterolemic diet change the basal brain electrical activity and during status epilepticus in rats? *Metabolic Brain Disease*, *34*(1), 71-77. https://doi.org/10.1007/s11011-018-0321-4

Pathan, A. R., Gaikwad, A. B., Viswanad, B., & Ramarao, P. (2008). Rosiglitazone attenuates the cognitive deficits induced by high fat diet feeding in rats. *European Journal of Pharmacology*, *589*(1-3), 176-179. https://doi.org/10.1016/j.ejphar.2008.06.016

Phelan, K. D., Shwe, U. T., Williams, D. K., Greenfield, L. J., & Zheng, F. (2015). Pilocarpine-induced status epilepticus in mice: A comparison of spectral analysis of electroencephalogram and behavioral grading using the Racine scale. *Epilepsy Research*, *117*, 90-96. https://doi.org/10.1016/j.eplepsyres.2015.09.008

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

Ramlackhansingh, A. F., Brooks, D. J., Greenwood, R. J., Bose, S. K., Turkheimer, F. E., Kinnunen, K. M., Gentleman, S., Heckemann, R. A., Gunanayagam, K., Gelosa, G., & Sharp, D. J. (2011). Inflammation after trauma: Microglial activation and traumatic brain injury. *Annals of Neurology*, *70*(3), 374-383. https://doi.org/10.1002/ana.22455

Reichelt, A. C., Lemieux, C. A., Princz-Lebel, O., Singh, A., Bussey, T. J., & Saksida, L. M. (2021). Age-dependent and region-specific alteration of parvalbumin neurons, perineuronal nets and microglia in the mouse prefrontal cortex and hippocampus following obesogenic diet consumption. *Scientific Reports*, *11*(1), 5593. https://doi.org/10.1038/s41598-021-85092-x

Rivera, P., Pérez-Martín, M., Pavón, F. J., Serrano, A., Crespillo, A., Cifuentes, M., López-Ávalos, M. D., Grondona, J. M., Vida, M., Fernández-Llebrez, P., de Fonseca, F. R., & Suárez, J. (2013). Pharmacological administration of the isoflavone daidzein enhances cell proliferation and reduces high fat diet-induced apoptosis and gliosis in the rat hippocampus. *PLoS ONE*, *8*(5), e64750. https://doi.org/10.1371/journal.pone.0064750

Schartz, N. D., Herr, S. A., Madsen, L., Butts, S. J., Torres, C., Mendez, L. B., & Brewster, A. L. (2016). Spatiotemporal profile of Map2 and microglial changes in the hippocampal CA1 region following pilocarpine-induced status epilepticus. *Scientific Reports*, *6*(1), 1–12. https://doi.org/10.1038/srep24988

Schartz, N. D., Wyatt-Johnson, S. K., Price, L. R., Colin, S. A., & Brewster, A. L. (2018). Status epilepticus triggers long-lasting activation of complement C1q-C3 signaling in the hippocampus that correlates with seizure frequency in experimental epilepsy. *Neurobiology of Disease*, *109*(October 2017), 163-173. https://doi.org/10.1016/j.nbd.2017.10.012

Schwarz, J. M., & Bilbo, S. D. (2011). LPS elicits a much larger and broader inflammatory response than Escherichia coli infection within the hippocampus of neonatal rats. *Neuroscience Letters*, *497*(2), 110-115. https://doi.org/10.1016/j.neulet.2011.04.042

Shapiro, L. A., Wang, L., & Ribak, C. E. (2008). Rapid astrocyte and microglial activation following pilocarpine-induced seizures in rats. *Epilepsia*, *49*(SUPPL. 2), 33-41. https://doi.org/10.1111/j.1528-1167.2008.01491.x

Sharma, S. (2021). High fat diet and its effects on cognitive health: alterations of neuronal and vascular components of brain. *Physiology & Behavior*, *240*(15).

Sierra, A., Abiega, O., Shahraz, A., & Neumann, H. (2013). Janus-faced microglia: Beneficial and detrimental consequences of microglial phagocytosis. *Frontiers in Cellular Neuroscience*, 7(JANUARY), 6. https://doi.org/10.3389/fncel.2013.00006

Swissa, E., Serlin, Y., Vazana, U., Prager, O., & Friedman, A. (2019). Blood-brain barrier dysfunction in status epileptics: Mechanisms and role in epileptogenesis. https://doi.org/10.1016/j.yebeh.2019.04.038

Swithers, S. E. (2000). Effects of metabolic inhibitors on ingestive behavior and physiology in preweanling rat pups. *Appetite*, *35*(1), 9-25. https://doi.org/10.1006/appe.2000.0330

Swithers, S. E., Melendez, R. I., Watkins, B. A., & Davis, R. J. (2001). Metabolic and behavioral responses in pre-weanling rats following alteration of maternal diet. *Physiology and Behavior*, *72*(1-2), 147-157. https://doi.org/10.1016/S0031-9384(00)00385-1

Thaler, J., Yi, C., Schur, E., Guyenet, S., Hwang, B., Dietrich, M., Zhao, X., Sarruf, D., Izgur, V., Maravilla, K., Nguyen, H., Fischer, J., Matsen, M., Wisse, B., Morton, G., Horvarth, T., Baskin, D., Tshop, M., & Schwartz, M. (2011). Obesity is associated with hipothalamic injury in rodents and humans. *Journal of Clinical Investiga*, *122*(1), 153. https://doi.org/10.1172/JCI59660.adjacent

Tsan, L., Décarie-Spain, L., Noble, E. E., & Kanoski, S. E. (2021). Western diet consumption during development: Setting the stage for neurocognitive dysfunction. *Frontiers in Neuroscience*, *15*. https://doi.org/10.3389/fnins.2021.632312

Turski, L., Ikonomidou, C., Turski, W. A., Bortolotto, Z. A., & Cavalheiro, E. A. (1989). Review: Cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: A novel experimental model of intractable epilepsy. *Synapse*, *3*(2), 154-171. https://doi.org/10.1002/syn.890030207

Turski, W. A., Cavalheiro, E. A., Schwarz, M., Czuczwar, S. J., Kleinrok, Z., & Turski, L. (1983). Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study. *Behavioural Brain Research*, *9*(3), 315-335. https://doi.org/10.1016/0166-4328(83)90136-5

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

Vinuesa, A., Bentivegna, M., Calfa, G., Filipello, F., Pomilio, C., Bonaventura, M. M., Lux-Lantos, V., Matzkin, M. E., Gregosa, A., Presa, J., Matteoli, M., Beauquis, J., & Saravia, F. (2019a). Early exposure to a high-fat diet impacts on hippocampal plasticity: implication of microglia-derived exosome-like etracellular vesicles. *Molecular Neurobiology*, *56*(7), 5075-5094. https://doi.org/10.1007/s12035-018-1435-8

Vinuesa, A., Pomilio, C., Menafra, M., Bonaventura, M. M., Garay, L., Mercogliano, M. F., Schillaci, R., Lux Lantos, V., Brites, F., Beauquis, J., & Saravia, F. (2016). Juvenile exposure to a high fat diet promotes behavioral and limbic alterations in the absence of obesity. *Psychoneuroendocrinology*, *72*, 22-33. https://doi.org/10.1016/j.psyneuen.2016.06.004

White, C. L., Pistell, P. J., Purpera, M. N., Gupta, S., Fernandez-Kim, S. O., Hise, T. L., Keller, J. N., Ingram, D. K., Morrison, C. D., & Bruce-Keller, A. J. (2009). Effects of high fat diet on Morris maze performance, oxidative stress, and inflammation in rats: Contributions of maternal diet. *Neurobiology of Disease*, *35*(1), 3-13. https://doi.org/10.1016/j.nbd.2009.04.002

Wieser, H. G. (2004). Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia*, *45*(6), 695-714. https://doi.org/10.1111/j.0013-9580.2004.09004.x

Wolff, R. L., & Sébédio, J. L. (2003). Trans-18:1 isomers in rat milk fat as effective biomarkers for the determination of individual isomeric trans-18:1 acids in the Dams' diet. *Lipids*, *38*(11), 1143-1148. https://doi.org/10.1007/s11745-003-1172-z

Wyatt, S. K., Witt, T., Barbaro, N. M., Cohen-Gadol, A. A., & Brewster, A. L. (2017). Enhanced classical complement pathway activation and altered phagocytosis signaling molecules in human epilepsy. *Experimental Neurology*, *295*, 184-193. https://doi.org/10.1016/j.expneurol.2017.06.009

Wyatt-Johnson, S. K., & Brewster, A. L. (2020). Emerging roles for microglial phagocytic signaling in epilepsy. *Epilepsy Currents*, *2*0(1), 33-38. https://doi.org/10.1177/1535759719890336

Wyatt-Johnson, S. K., Herr, S. A., & Brewster, A. L. (2017). Status epilepticus triggers time-dependent alterations in microglia abundance and morphological phenotypes in the hippocampus. *Frontiers in Neurology*, *8*(DEC), 1-10. https://doi.org/10.3389/fneur.2017.00700

Yu, S., Zhang, H., Hei, Y., Yi, X., Baskys, A., Liu, W., & Long, Q. (2019). High mobility group box-1 (HMGB1) antagonist BoxA suppresses status epilepticus-induced neuroinflammatory responses associated with Toll-like receptor 2/4 down-regulation in rats. *Brain Research*, *1717*, 44-51. https://doi.org/10.1016/j.brainres.2019.04.007

Zack, M. M., & Kobau, R. (2015). Morbidity and mortality weekly report national and state estimates of the numbers of adults and children with active epilepsy-United States, 2015. *Morbidity and Mortality Weekly Report*, *66*(31).

Zarnowska, I. M. (2020). Therapeutic use of the ketogenic diet in refractory epilepsy: What we know and what still needs to be learned. *Nutrients*, *12*(9). https://doi.org/10.3390/nu12092616

Zhao, X. F., Liao, Y., Alam, M. M., Mathur, R., Feustel, P., Mazurkiewicz, J. E., Adamo, M. A., Zhu, X. C., & Huang, Y. (2020). Microglial mTOR is neuronal protective and antiepileptogenic in the pilocarpine model of temporal lobe epilepsy. *Journal of Neuroscience*, *40*(40), 7593-7608. https://doi.org/10.1523/JNEUROSCI.2754-19.2020