EFFECTS OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS ON PROPERTIES AND FUNCTION OF THE MOUSE MAJOR PELVIC GANGLION NEURONS

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

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Dedicated to the Memory of my mother, Emily Jean Henderson

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To my committee:

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Abstract

Experimental autoimmune encephalomyelitis (EAE) serves as a valuable mouse model for studying the pathophysiology of multiple sclerosis (MS), a chronic autoimmune disease characterized by inflammation and demyelination of the central nervous system (CNS). While EAE mimics many aspects of MS, its effects on the lower urinary tract (LUT) remain poorly understood. This study investigated alterations in lower urinary tract output, excitability of major pelvic ganglion (MPG) neurons, and expression profiles of MPG neurons in EAE. Adult female SJL/J mice were induced with EAE, and clinical scores were monitored daily. Real-time quantitative polymerase chain reaction (qPCR) was employed to analyze gene expression in MPGs, while functional bladder output and electrophysiological measurements of MPG neurons were conducted to evaluate lower urinary tract output and MPG neuron excitability. The results revealed significant changes in lower urinary tract output, with decreased urine output observed in EAE mice compared to controls.

Furthermore, MPG neuron excitability was altered, with changes in passive membrane properties and action potential characteristics observed in EAE-induced animals. Analysis of gene expression profiles in MPG neurons revealed significant alterations in the mRNA levels of ion channels and receptors implicated in bladder control mechanisms. These findings provide valuable insights into the pathophysiological mechanisms underlying lower urinary tract dysfunction in EAE, suggesting potential targets for therapeutic intervention in MS-related bladder dysfunction. Further research is warranted to elucidate the complex interplay between immune-mediated CNS damage and LUT dysfunction in MS and related conditions.

CHAPTER 1: Neurogenic Bladder and Lower Urinary Tract Dysfunction in Multiple Sclerosis

Lower Urinary Tract (LUT) Function and Dysfunction

The Lower Urinary Tract (LUT) consists of the bladder and the urethra (as well as the prostate in males) and is responsible for the storage and voiding of urine collected from the kidneys (Fashemi & Mysorekar, 2018; Fowler et al., 2008a; Hou et al., 2016). The autonomic nervous system is ultimately responsible for the control of the LUT. The sympathetic nervous system has a role in maintaining urinary continence (Fowler et al., 2008b). Activation of the sympathetic nervous system typically results in the relaxation of the bladder wall and constriction of the urethra, leading to the collection or “storage” phase of a voiding cycle. When the bladder is full, the parasympathetic nervous system stimulates bladder contractions and relaxation of the urethra, leading to the “voiding” phase and the elimination of urine and emptying of the bladder (also known as micturition).

In a healthy individual, the coordination between these two divisions of the autonomic nervous system ensures proper storage and emptying functions. LUT dysfunction (LUTD) refers to a range of problems that affect the normal coordination and functioning of the bladder and urethra. This can include conditions such as urinary incontinence (UI), overactive bladder (OAB), underactive bladder (UAB), and various types of voiding dysfunction (VD). However, in cases of LUTD like OAB, UAB, UI, and VD, this coordination can be disrupted (K. E. Andersson et al., 2021; K.-E. Andersson & Arner, 2004; Steers, 2002; Steers & Tuttle, n.d.). In OAB, the bladder muscles can contract involuntarily and excessively, leading to a sudden and strong urge to urinate even when the

bladder is not full. In UAB, the bladder muscles may not contract adequately during voiding, leading to incomplete emptying. UI refers to involuntary urine leakage and can be caused by various factors, including weakened pelvic floor muscles, improper bladder-urethra coordination, or problems with autonomic nervous system regulation. VD can also be caused by autonomic regulation dysfunction, causing difficulties initiating or maintaining urination. The treatment of LUTD often involves a combination of lifestyle changes, behavioral therapies, medications, and sometimes surgical intervention. It is crucial to assess the underlying cause of the dysfunction and address any disruptions in autonomic regulation to manage the condition effectively. However, a lack of understanding of neural mechanisms underlying healthy LUT function makes treatment difficult and increases the risk of iatrogenic injury (Fowler et al., 2008a; Miyazato et al., 2013, 2017a; Tornic & Panicker, 2018).

Neurogenic Lower Urinary Tract Dysfunction (LUTD)

Neurogenic LUTD is a complex set of medical conditions that arise when there is a dysfunction in the communication between the nervous system and the lower urinary tract (LUT) that is the underlying cause of bladder dysfunction (Dorsher & McIntosh, 2012; Kirby Ma Frcs, 1988; Mastri, 1980) Neurogenic LUTD is a common complication of a wide variety of nervous system injuries and diseases, including spinal cord injury (Taweel & Seyam, 2015), Parkinson's disease (Hajebrahimi et al., 2019a), stroke (Chou et al., 2013), multiple sclerosis (MS) (Sadiq & Brucker, 2015a), and many other neurological disorders (Kirby Ma Frcs, 1988; Powell, n.d.; Tudor et al., 2016) The condition disrupts the coordination

between the detrusor smooth muscle of the bladder and the internal and external urethral sphincters. The underlying cause of this dysfunction can occur at various levels of the nervous system, from the brain to the spinal cord, as well as the peripheral nerves (Sadiq & Brucker, 2015b).

Patients suffering from neurogenic LUTD may experience a range of symptoms that can significantly impact their quality of life. These symptoms include urinary incontinence, frequent urination, urgency, difficulty initiating or completing urination, nocturia, and urinary tract infections (UTIs) (Tractenberg et al., 2023). The severity of symptoms varies depending on the underlying cause and extent of nervous system damage. Diagnosing neurogenic LUTD involves a comprehensive evaluation of the patient's medical history, physical examination, and urodynamic tests (Dorsher & McIntosh, 2012). Urodynamic studies are essential in assessing LUT function and involve bladder pressure measurements and flow rates during filling and emptying. Imaging techniques such as ultrasound, MRI, and CT scans can help identify neurological lesions (Miyazato et al., 2017a).

The management of neurogenic LUTD is tailored to the underlying cause, the severity of symptoms, and patient preferences (Tractenberg et al., 2023). Treatment strategies aim to optimize LUT function, prevent complications, and improve patients' QOL scores. Conservative management involves lifestyle modifications, such as timed voiding, fluid management, and pelvic floor exercises, which can help manage milder cases. Various medications are frequently prescribed, including anticholinergics for OAB and alpha blockers for UAB.

Intermittent catheterization can prevent retention and subsequent infections for patients with voiding dysfunction. In severe cases, surgical options like bladder augmentation or the creation of an artificial urinary sphincter might be considered. Techniques like sacral neuromodulation involve electrical stimulation of nerves to regulate LUT function and improve symptoms. Botulinum toxin injections into the detrusor can help relax OAB and reduce stress incontinence. Nerve or spinal cord stimulation and urinary diversion surgeries are often considered in complex cases (Hu et al., 2016; Panicker et al., 2015; Tornic & Panicker, 2018). Neurogenic LUTD can significantly impact a patient's quality of life, leading to social isolation, psychological distress, and physical pain (Khalaf et al., 2016). The unpredictable nature of urinary symptoms may restrict patients' activities and hinder their participation in daily life. Adequate management focuses on symptom relief and addresses emotional and psychological aspects.

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic autoimmune disease affecting the CNS, characterized by inflammation, demyelination, and neurodegeneration. This condition typically manifests between the ages of 20 and 40, with a higher prevalence in women than men, 3:1 (Huang et al., 2017; Popescu et al., 2013). MS presents with a wide range of symptoms and variable disease progression, making it a complex condition to diagnose and manage. There are four types of MS:

1. **Relapsing-Remitting MS (RRMS):** This is the most common form of MS, characterized by episodes of neurological symptoms followed by partial or

complete recovery (remission). New symptoms may arise during relapses, or existing ones may worsen, but these typically improve over time. Between relapses, individuals may experience periods of stability.

2. **Secondary Progressive MS (SPMS):** In SPMS, which often develops after years of RRMS, neurological function gradually worsens with or without relapses and remissions. Individuals with SPMS may experience a steady accumulation of disability over time.

3. **Primary Progressive MS (PPMS):** PPMS is characterized by steadily worsening neurological function from the onset, without distinct relapses or remissions. Symptoms may progress gradually or in occasional step-like deterioration. PPMS tends to have a later onset than RRMS and is less common overall.

4. **Progressive-Relapsing MS (PRMS):** This subtype is characterized by a steady worsening of neurological function from the onset, with distinct relapses and periods of remission. PRMS is relatively rare compared to other subtypes.

MS progression varies widely among individuals and can be influenced by subtype, genetics, environmental factors, and treatment. The progression of MS is typically measured by the accumulation of disability over time, assessed through standardized scales such as the Expanded Disability Status Scale (EDSS). A higher EDSS score is often associated with an unfavorable urologic

course (Betts et al., 1993; de Groat et al., 2010; de Medeiros Junior et al., 2020).

Immunological Underpinnings of Multiple Sclerosis

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS), affecting millions of people worldwide. It is characterized by the immune system attacking the myelin sheath, the protective covering of nerve fibers, leading to impaired communication between the brain and the rest of the body. This section looks into the immunological mechanisms that underpin MS, providing insights into its pathogenesis and potential therapeutic approaches. MS is an autoimmune disorder where the body's immune system mistakenly targets its own tissues. The exact cause of MS remains unknown, but it is believed to result from a combination of genetic predisposition, environmental factors, and infections. There are several forms of MS, including relapsing-remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive-relapsing MS (PRMS).

Immunological Mechanisms in MS

1. Genetic Susceptibility

Genetic factors play a significant role in MS. The human leukocyte antigen (HLA) region on chromosome 6, particularly the HLA-DRB1*15:01 allele, has been strongly associated with increased MS risk. Other genetic loci involved in immune regulation also contribute to susceptibility (Irizar et al., 2012).

2. Environmental Triggers

Environmental factors such as vitamin D deficiency, smoking, and viral infections (e.g., Epstein-Barr virus) are implicated in MS development. These factors may trigger an abnormal immune response in genetically susceptible individuals (Bar-Or et al., 2020).

3. Autoimmune Response Activation

The autoimmune response in MS involves several key players:

Antigen-Presenting Cells (APCs): Dendritic cells and macrophages process myelin antigens and present them to T cells, initiating the autoimmune cascade.

T Cells: CD4+ T helper cells, particularly Th1 and Th17 subsets, are central to MS pathogenesis. Th1 cells secrete pro-inflammatory cytokines like IFN-γ, while Th17 cells produce IL-17, both contributing to inflammation and myelin damage (Markovic-Plese et al., 2004).

B Cells: B cells produce antibodies against myelin components, forming immune complexes that enhance inflammation and demyelination.

4. Blood-Brain Barrier Disruption

In MS, the blood-brain barrier (BBB), which normally protects the CNS from peripheral immune cells, becomes compromised. Pro-inflammatory cytokines and matrix metalloproteinases (MMPs) degrade the BBB, allowing immune cells to infiltrate the CNS.

5. CNS Inflammation and Demyelination

Once inside the CNS, autoreactive T cells, B cells, and macrophages attack myelin. This leads to the formation of demyelinating lesions or plaques, disrupting nerve signal transmission. The inflammatory environment is sustained by cytokines such as TNF-α, IL-1β, and IL-6, exacerbating tissue damage. MS is not solely a demyelinating disease; it also involves significant neurodegenerative processes. Axonal damage occurs due to inflammatory mediators and loss of myelin's protective support, leading to irreversible neurological deficits over time.

Immunoregulatory Mechanisms

Despite the chronic nature of MS, periods of remission can occur, especially in RRMS. These remissions are attributed to various immunoregulatory mechanisms. Regulatory T Cells (Tregs) suppress autoreactive T cells and promote immune tolerance, helping to resolve inflammation. Meanwhile, anti-inflammatory cytokines like IL-10 and TGF-β play a role in dampening inflammation and facilitating tissue repair. In this environment remyelination can occur. Oligodendrocytes can sometimes remyelinate damaged axons, contributing to functional recovery, although this process is often incomplete (Steinman, 2014).

MS is a complex autoimmune disease driven by intricate immunological mechanisms. The interplay between genetic predisposition, environmental triggers, and immune dysregulation leads to the characteristic demyelination and neurodegeneration seen in MS. Advances in understanding these processes continue to inform the development of targeted therapies, offering hope for improved management and outcomes for individuals with MS.

Neurogenic LUTD in MS

Neurogenic LUTD is the most common complication of MS that directly results from lesions in the spinal cord and brain (Kirby Ma Frcs, 1988; Powell, n.d.). The pathophysiology of NLUTD in MS involves a complex interplay of neurological, muscular, and reflex mechanisms. In MS, the immune system attacks myelin, causing demyelination. The loss of myelin disrupts the standard transmission of neural signals. Neural pathways involved in bladder control are often affected, including those responsible for voluntary inhibition and reflex activation.

Neurological mechanisms of NLUTD in MS include the interruption of the neural signals that regulate detrusor activity. This can lead to detrusor overactivity, causing urgency, frequency, and urge incontinence.

Normally, LUT function relies on the coordination of intricate neural reflexes. Reflex mechanisms contribute to NLUTD in MS because dysfunctional neural pathways controlling LUT sphincters can lead to inadequate relaxation during voiding, causing urinary retention or difficulty initiating urination. LUT function relies on the coordination of intricate neural reflexes. However, in NLUTD, these reflexes are damaged due to the loss of inhibitory signal. During remissions it in humans it is speculated that there can be improvements in bladder function due to remyelination (Sungur et al., 2019).

In MS, lower motor neuron lesions below the S2-S4 spinal cord level generally manifest as OAB, whereas lower motor neuron lesions or peripheral nerve demyelination manifest as an areflexic bladder. Lesions above spinal cord level S2-S4 are typically more common in MS and present in urodynamic studies as neurogenic detrusor overactivity (NDO). NDO increases bladder pressure, urgency, urge incontinence, and frequency. Suprapontine lesions can lead to urge incontinence by disinhibiting the micturition reflex. Combining upper motor neurons and peripheral lesions can cause detrusor sphincter dyssynergia. The dyssynergia between these structures can lead to retention, elevated post-void residual volumes, and vesicoureteral reflux. Those affected by MS can show storage and voiding dysfunction simultaneously or independently.

Additionally, as relapses and progression are hallmarks of MS, the type of NLUTD can change with time (Sadiq & Brucker, 2015b; Tornic & Panicker, 2018). Furthermore, prolonged NLUTD can lead to structural changes in the bladder and urethra. The bladder walls can become thicker and less compliant, reducing the bladder's ability to stretch and accommodate urine, which could further exacerbate urinary retention and overactivity. NLUTD in MS is a multifaceted condition arising from the complex interplay of demyelination, disrupted neural pathways, structural changes, and impaired reflex mechanisms. During remissions bladder function can change due to remyelination, but this area is largely understudied.

Neural Control of LUT Function

To better understand how MS disrupts neural control of LUT function, it is necessary to first understand how that control normally works. The LUT consists of the bladder, urethra, associated muscles, ganglia, and nerves responsible for storing and eliminating urine. The neural regulation of LUT function involves both central and autonomic pathways. The central pathways involve the brain and spinal cord, primarily areas responsible for sensory perception, decision-making, and voluntary control. The cerebral cortex, specifically the medial frontal cortex, plays a role in conscious awareness and voluntary control of micturition. The brainstem's pontine micturition center (PMC) is involved in the switch between bladder storage and voiding phases. The PMC receives afferent signals from the bladder regarding bladder fullness and sends efferent signals to the spinal cord to

initiate or inhibit voiding (de Groat, 2006; de Groat & Yoshimura, 2006; Drake, 2018).

The midbrain periaqueductal gray (PAG) is connected to the PMC and can modulate reflexive aspects of voiding. These reflexes are mediated by the brainstem and spinal circuits, providing rapid responses to bladder filling and emptying. In the bladder filling reflex, as the bladder fills with urine, stretch receptors in the bladder wall create low-level afferent signals to the pontine storage center (PSC), sending inhibitory signals to the detrusor via the sympathetic nervous system to maintain bladder storage. When the bladder reaches a certain level of distension, stretch receptors trigger the voiding reflex(Stoffel, 2016). This reflex involves parasympathetic mediated bladder detrusor muscle contraction and relaxation of the internal urethral sphincter to initiate the voiding process. Bladder fullness causes the rapid firing of bladder afferent signals that travel via afferent pathways to the PAG to the PMC, where the switch from storage to voiding occurs (de Groat et al., 2015a; de Groat & Yoshimura, 2012). LUT function is a complex interplay between central and autonomic pathways. These pathways cooperate to ensure proper control and coordination of bladder storage and voiding phases.

Autonomic innervation of the LUT

The autonomic nervous system plays a vital role in maintaining the balance of bladder storage and voiding functions. The coordination between the sympathetic and parasympathetic pathways helps ensure effective urinary control and the proper elimination of urine. The sympathetic axons of the hypogastric

nerve originate from the thoracolumbar region of the spinal cord T10-L2 in humans and L1-L2 in rodents (Figure 3) (Inskip et al., 2009). This nerve releases norepinephrine as the primary neurotransmitter. The sympathetic innervation of the lower urinary tract primarily serves to maintain urinary continence. Continence is achieved through sympathetic relaxation of the bladder's detrusor muscle, contraction of the internal urethral sphincter, and inhibition of the external urethral sphincter. Norepinephrine release to the detrusor inhibits the bladder's involuntary contractions to prevent premature emptying. Norepinephrine release to the internal urethral sphincter promotes contraction.

Parasympathetic innervation of the lower urinary tract is responsible for promoting bladder emptying. Furthermore, while the external urethral sphincter is under voluntary control, the hypogastric nerve helps prevent premature relaxation. The parasympathetic pelvic nerves arise from the sacral region of the spinal cord S2-S4 (humans) and L6-S1 (rats) and release acetylcholine as their primary neurotransmitter (Inskip et al., 2009). When the bladder reaches capacity, parasympathetic nerve activity is dominant, and mechanosensitive afferents (i.e., Aδ, C) send signals about bladder distension. Emptying is achieved through the contraction of the detrusor muscle and relaxation of the internal urethral sphincter. The detrusor muscle contracts under the influence of acetylcholine, expelling urine from the bladder. As the bladder contracts and pushes urine toward the urethra, the internal urethra sphincter relaxes, allowing the proper passage of urine.

Additionally, somatic pudendal nerves under voluntary control innervate the external urethral sphincter. The pudendal nerves originate from spinal cord S3 -S4 in humans and L6-S1 in rats(de Groat et al., 2015a). Both sympathetic and parasympathetic divisions must work synergistically and coordinate to control LUT function.

The Major Pelvic Ganglia in LUT Function

The major pelvic ganglia (MPGs) play a crucial role in the LUT function of rodents. MPGs are mixed ganglia containing predominantly parasympathetic neurons innervating the pelvic viscera(Hamill et al., 2012). These ganglia are located near the pelvic region's bladder base and comprise cholinergic, adrenergic, and small, intensely fluorescent cells (Kanjhan et al., 2003a; Keast, 2006). Most pelvic ganglia cells in rodents are monopolar and have sparse short dendrites compared to those in higher mammalian species(Arellano et al., 2019; Keast, 2006). The inputs to the MPG include that of the pelvic, hypogastric, and pudendal nerves (Figure 3) (Girard et al., 2013). The MPG sends out post-ganglionic fibers that innervate the smooth muscles of the bladder and urethra. The ganglia provide the necessary motor signals for these muscles to contract and relax in a coordinated manner, allowing urinary flow control. MPGs act as integration centers (Félix et al., 1998; Kyi, 2022), where signals from higher brain centers and sensory nerves are integrated to facilitate appropriate LUT responses. The ganglia then relay these signals to indicate when pressure increases within the bladder and when the expulsion of urine is necessary. The MPGs are also

involved in various reflex pathways that regulate LUT function. The MPGs help regulate urethral control and coordinate the contraction of the bladder in a synchronized manner. MPGs are essential components of the autonomic nervous system that aid in coordinating LUT function in rodents, and these neurons are the primary focus of the studies in our work.

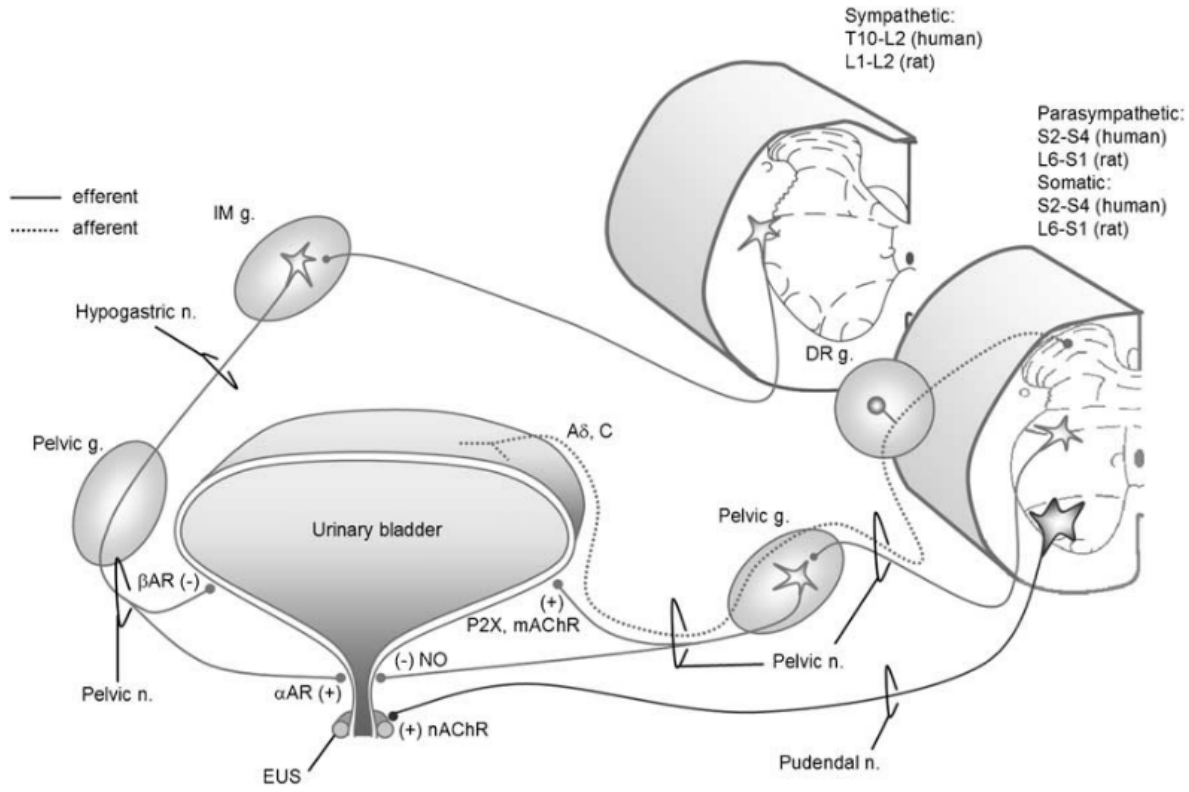

Figure 1.1 LUT Innervation

Schematic of LUT showing bladder, urethra, and urethral sphincters innervated by hypogastric, pelvic, and pudendal nerves. The hypogastric nerve arises from the spinal cord between L1 and L2 in rodents. This nerve uses alpha-adrenergic receptors to excite the trigone, bladder neck, and urethra during storage, and beta-adrenergic receptors of the bladder dome inhibit contraction. The pelvic nerve arises from the spinal cord between L6 and S1. This nerve uses cholinergic muscarinic receptors to mediate micturition. The pudendal nerve arises from the spinal cord between L6 and S1. This nerve provides somatic innervation to the external urethral sphincter. In addition to the efferent function, each nerve carries afferent signals from the LUT. AR, adrenergic receptors; DR g, dorsal root ganglion; EUS, external urethral sphincter; g, ganglion; IM g, inferior mesenteric ganglion; L, lumbar spinal cord; mAChR, muscarinic cholinergic receptors; nAChR, nicotinic cholinergic receptors; n, nerve; NO, nitric oxide; P2X, purinergic receptors; S, sacral spinal cord; + denotes excitatory synapses while – denotes inhibitory synapses. (Inskip et al., 2009)

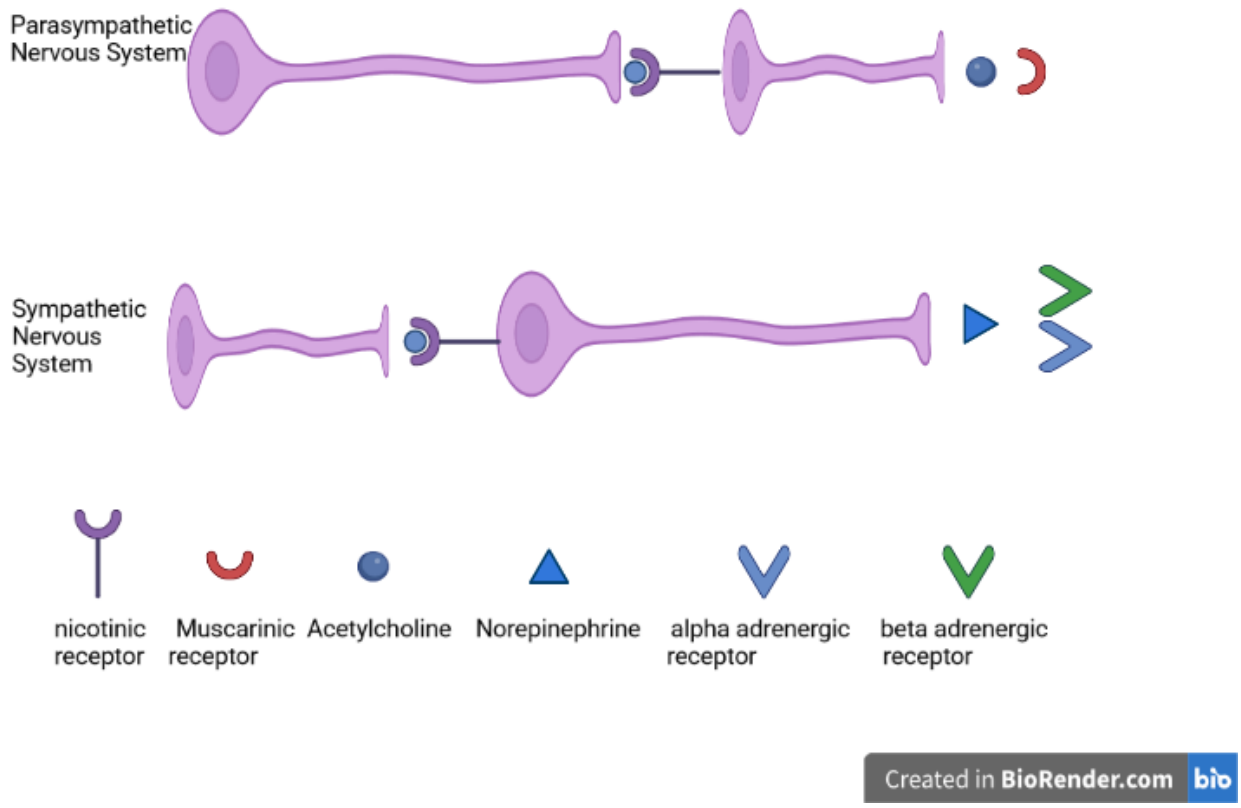

Figure 1.2 Efferent arm of autonomic nervous system

Pre-ganglionic sympathetic neurons have short axons, while sympathetic post-ganglionic axons are long as sympathetic ganglia lie close to the spinal cord. Conversely, parasympathetic neurons have ganglia closer to target organs, necessitating long pre-ganglionic and short post-ganglionic fibers. Both sympathetic and parasympathetic divisions rely on pre-ganglionic acetylcholine transmission but on post-ganglionic norepinephrine and acetylcholine transmission.

Experimental Autoimmune Encephalomyelitis
Induction of EAE:

EAE is a model for multiple sclerosis. EAE is typically induced in mice or laboratory animals by immunization with CNS-derived antigens combined with an adjuvant to enhance the immune response. The activated immune cells then migrate to the CNS, initiating an inflammatory cascade leading to demyelination and neurodegeneration, similar to MS (Bjelobaba et al., 2018). EAE models recapitulate key clinical and pathological features of MS, including motor deficits, sensory disturbances, inflammation, demyelination, and neurodegeneration within the CNS. The severity and course of EAE can vary depending on factors such as the mouse strain, antigen used, and experimental protocol (Lassmann & Bradl, 2017a).

Mouse Strains and Antigens Used in EAE Models:

C57BL/6 and SJL/J Mice:

Antigen Used: Myelin oligodendrocyte glycoprotein (MOG)

Modeling RRMS and SPMS: Immunization of C57BL/6 mice with MOG peptide emulsified in adjuvant typically induces a relapsing-remitting or secondary progressive EAE phenotype, characterized by relapses followed by periods of remission or gradual neurological decline.

Biozzi ABH Mice:

Antigen Used: Myelin basic protein (MBP)

Modeling PPMS: Biozzi ABH mice immunized with MBP in adjuvant develop a primary progressive EAE phenotype, resembling the progressive course of MS without distinct relapses or remissions.

PL/J and SJL/J Mice:

Antigen Used: Proteolipid protein (PLP)

Modeling Relapsing-Remitting and Progressive Forms of MS: Immunization of PL/J or SJL/J mice with PLP peptide induces a progressive EAE and relapsing-remitting phenotype, resembling the chronic progression observed in PPMS and RRMS.

Table1.1 Comparison of relapsing remitting Multiple Sclerosis and PLP mouse Model

Aspect	RRMS	PLP Mouse Model
Disease Type	Spontaneous autoimmune disorder affecting CNS	Induced autoimmune disorder affecting CNS
Pathogenesis	Immune system attacks multiple antigens in myelin in CNS	Immune system attacks myelin proteolipid protein (PLP) in CNS
Clinical Presentation	Periods of relapse and remissions	Variable course that can include relapses and remissions
Symptoms	Vision problems, fatigue, motor dysfunction, paralysis, tremors	Visual alterations, motor dysfunction, paralysis, tremors
Histopathology	Inflammation, demyelination, axonal damage	Inflammation, demyelination, axonal damage
Causes	Genetic susceptibility combined with environmental triggers	Induced autoimmunity in genetically susceptible mouse strains
Treatment Strategies	Disease-modifying therapies, steroids, immunosupressants	steroids, immunosupressants
Research Utility	Insight into natural MS pathogenesis, testing of treatments	Insight into disease progression and therapeutic response in controlled setting

(Gold et al., 2006; Lassmann & Bradl, 2017b)

Table1.2 Comparison of Human and Mouse cystometry in Multiple Sclerosis

Parameter	Human Cystometry in MS	Mouse Cystometry in MS Models
Bladder Capacity	Often reduced; may exhibit urgency and frequency	Often reduced; similar symptoms modeled
Compliance	May be decreased, indicating a stiffer bladder wall	Measured similarly; decrease indicates fibrosis or stiffness
Detrusor Overactivity	Common, leading to urgency and incontinence	Induced in EAE models to mimic human detrusor overactivity
Voiding Pressure	Increased due to detrusor overactivity or sphincter dyssynergia	Increased, reflecting detrusor-sphincter dyssynergia in EAE models
Residual Volume	Often increased, indicating incomplete bladder emptying	Measured to assess voiding efficiency
Sphincter Activity	Dyssynergia common, leading to poor coordination	Similar patterns induced in mouse models for comparison
Frequency of Voiding	Increased due to urgency and detrusor overactivity	Increased frequency observed in EAE models

(Altuntas et al., 2012a; Babović et al., 2014; Horowitz et al., n.d.; Torad et al., 2020)

Experimental Autoimmune Encephalomyelitis (EAE) is a widely utilized animal model of MS (Glatigny & Bettelli, 2018; William Lindsey, 2005a). EAE was discovered accidentally by Dr. Thomas Rivers in the late 1930s when working on developing a treatment for rabies (Baxter, 2007a; Gold et al., 2006). This treatment entailed the injection of rabbit nervous tissue into patients to attenuate the virus. Some individuals developed neurological symptoms similar to MS, such as paralysis and loss of coordination. This observation piqued interest in understanding the immune system's role in CNS-related diseases. Later, in the 1940s and 1950s, researchers continued investigating EAE and its relationship to autoimmune processes (’t Hart et al., n.d.; William Lindsey, 2005b). Dr. Michael

Heite published a study in 1949 demonstrating that EAE could be induced in guinea pigs, highlighting the potential of EAE as a model for studying autoimmune responses targeting the nervous system. The 60s and 70s saw significant advancements in understanding the mechanisms underlying EAE. Whereas previously whole nervous tissue extracts were used. Dr. Thomas Miller and his colleagues demonstrated that EAE could be induced in rodents using myelin basic protein (MBP) to deteriorate the myelin sheath. This finding suggested that autoimmune responses targeting myelin were responsible for the development of EAE.

As researchers gained a deeper understanding of EAE, its relevance to MS became increasingly apparent. EAE mirrors many aspects of MS, including CNS demyelination, CNS inflammation, and the resulting neurological symptoms (’t Hart et al., n.d.; William Lindsey, 2005a). This led to the widespread adoption of EAE as a model for studying the mechanisms of MS and testing potential therapeutic interventions. Throughout the 80s and 90s, scientists used EAE to investigate different aspects of autoimmune responses and potential treatment strategies. The model allowed them to explore the role of different immune cells, cytokines, and genetic factors in developing and progressing CNS autoimmune diseases. EAE was instrumental in the development of immunomodulatory therapies for MS, such as interferon-beta and glatiramer acetate, which were tested and refined in this model before entering clinical trials (Denic et al., 2011; Lassmann & Bradl, 2017a).

Over time, scientists developed various strains and variants of EAE, each with unique characteristics and applications. These models allowed researchers to mimic different forms of MS and study specific aspects of the disease, such as relapses, remissions, and chronic progression. Researchers also developed models that target specific myelin proteins like proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), further expanding the versatility of the EAE model. However, while EAE provided valuable insights into MS and autoimmune processes, it also faced criticism due to its limitations. Some argued that the model did not fully replicate the complexity of human MS, particularly the chronic and progressive phases of the disease. Technological advancements like functional imaging and molecular profiling allowed scientists to better understand the underlying mechanisms of EAE. These insights contributed to a deeper understanding of MS and provided potential avenues for developing more targeted therapies. The model continues to evolve alongside advances in immunology, genetics, and neuroscience, ensuring its ongoing relevance in studying autoimmune disorders and developing novel therapies (Baxter, 2007b).

Experimental Goals and Hypotheses

Understanding the neurogenic bladder dysfunction observed in multiple sclerosis (MS) requires delving into the intricate workings of the nervous system, particularly its autonomic component. Neurons, the fundamental units of the nervous system, exhibit diverse structures and functions yet share conserved molecular mechanisms for generating electrical signals. Excitability, a hallmark of

neurons, stems from the opening of ion channels, which facilitate the flow of ions down electrochemical gradients, altering cellular voltage. When this voltage reaches a threshold, action potentials are generated, propagating along axons to transmit signals to postsynaptic neurons, thereby conveying information throughout the nervous system. The CNS acts as an integrative hub, receiving sensory input from the periphery and orchestrating responses via effectors, such as muscles and organs, through the autonomic nervous system (ANS). The ANS, a component of the peripheral nervous system, regulates involuntary functions of vital systems like the cardiovascular, respiratory, and digestive systems. However, the impact of decentralization or CNS injury on the input delivered by the autonomic system's peripheral networks remains a pivotal question.

Studies have shown that the properties of MPG neurons change in states of spinal cord injury (SCI) (Kyi, 2022) and disease (Gray et al., 2019a). The study by Kyi characterized the synaptic properties of major pelvic ganglia (MPG) neurons and compared these properties between control and spinal cord injury (SCI) mice. Pelvic nerve stimulation elicited excitatory postsynaptic potentials (EPSPs) in MPG neurons. Results showed that single stimulus pulses to the pelvic nerve induced fast synaptic responses, resulting in action potentials in 48% of MPG neurons, while 52% responded with EPSPs. Repeated stimulation led to synaptic depression and action potential (AP) filtering, with EPSP amplitudes decreasing with each subsequent pulse and fewer stimuli, resulting in AP generation at higher frequencies. The study also used pharmacological inhibitors to investigate cholinergic receptor expression in MPG neurons, revealing a significant reduction

in EPSP amplitude with nonselective and specific cholinergic receptor blockers. There were significant changes in EPSP characteristics in SCI animals, including amplitude, rise and decay times, and EPSP area, particularly in chronic SCI states. Additionally, synaptic depression was altered in SCI animals, with chronic SCI animals showing less synaptic depression compared to control and acute SCI animals. These findings provide insights into the synaptic properties of MPG neurons and how they are altered by SCI, suggesting potential mechanisms underlying bladder dysfunction in this condition.

In the context of MS-associated neurogenic bladder dysfunction, investigations by Dr. Cindy Kyi in the lab of Dr. David Schulz have shed light on how loss of central inputs in spinal cord injury (SCI) induces plastic changes in the major pelvic ganglion (MPG), a key autonomic center regulating lower urinary tract (LUT) function. Dr. Kyi's work demonstrated that complete loss of supraspinal inputs renders MPG neurons hypoexcitable, contributing to the urinary retention phenotype observed in SCI mice (Kyi, 2022). Building upon this paradigm, Dr. Schulz's lab employs a parallel model in experimental autoimmune encephalomyelitis (EAE), which mimics aspects of MS, to investigate the efferent activity of autonomic neurons in the MPG following partial and reversible loss of central inputs.

By elucidating how decentralization alters autonomic efferent activity and modifies LUT function in EAE, these studies provide crucial insights into the pathophysiology of neurogenic bladder dysfunction in MS. Ultimately; this research

may inform the development of targeted therapeutic interventions aimed at ameliorating urinary symptoms and improving quality of life for individuals living with MS. We hypothesize that neurogenic bladder dysfunction arises from alterations in autonomic efferent activity due to decentralization induced by central nervous system (CNS) injury, leading to hypoexcitability of neurons in the major pelvic ganglion (MPG) and subsequent impairment of lower urinary tract (LUT) function.

Chapter 2: Experimental Autoimmune Encephalomyelitis affects Lower Urinary Tract Function

INTRODUCTION

Multiple Sclerosis (MS) is a multifaceted neurodegenerative condition that impacts the central nervous system. MS commonly manifests between the ages of 20 and 40, with more women affected than men (Popescu et al., 2013). It is onset is characterized by muscle weakness, sensory disturbances, and neurogenic lower urinary tract dysfunction (Miyazato et al., 2017b; Sadiq & Brucker, 2015a). Neurogenic lower urinary tract dysfunction or neurogenic bladder is a frequent autonomic dysfunction associated with multiple sclerosis (MS). Neurogenic bladder is a common and often distressing symptom in individuals with MS(Araki et al., 2002; Tudor et al., 2016; Wyndaele, 2016).

Experimental autoimmune encephalomyelitis (EAE) is a common animal model for studying MS (’t Hart et al., n.d.). EAE provides a valuable tool for researchers to investigate the immunological and pathological mechanisms involved in MS and to test potential treatments. EAE is typically induced in rodents (mice or rats) by immunization with myelin-derived antigens, such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or proteolipid protein (PLP) (Pahan, 2010). This process triggers an autoimmune response, producing autoreactive T cells and antibodies that target the CNS. EAE replicates many aspects of MS pathology. Both diseases involve the infiltration of immune cells into the CNS, including the brain and spinal cord. In EAE, this immune response leads to demyelination, also a hallmark of MS (Kuerten & Angelov, 2008). The

inflammation and tissue damage observed in EAE models parallels the inflammatory lesions seen in MS patients.

Urethane anesthetized cystometry in rodent models is widely used to study bladder function and voiding patterns (Smith & Kuchel, 2010). Cystometry is the measurement of intravesical pressure and is commonly used to assess various aspects of urinary bladder function, including bladder capacity, voiding frequency, and the evaluation of detrusor muscle activity. The void spot assay (VSA) is another widely used technique for evaluating lower urinary tract function. This non-invasive technique involves capturing urine patterns of awake and behaving mice. Both techniques evaluate muro-neuro-urodynamics (Ito et al., 2017). In 2008, bladder dysfunction was reported in EAE models, and the model quickly became established for studying neurogenic bladder dysfunction in MS (Altuntas et al., 2008, 2012b; Horowitz et al., n.d.). However, relatively few datasets still utilize cystometry and VSA together to examine the impacts of MS on bladder output and function in the EAE model. This study adds to this body of knowledge in the EAE model in 10-12-week-old female SJL mice to further characterize how lower urinary tract function is altered. Our findings reveal decreased micturition volume and area and increased micturition pressure. These results suggest the presence of bladder outlet obstruction (BOO) by way of detrusor sphincter dyssynergia (DSD) in EAE (Drake, 2018; Gajewski & Drake, 2018; Stoffel, 2016, 2017).

METHODS

Animals

All experimental procedures were approved by the University of Missouri Institutional Animal Care and Use Committee and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were kept in groups on a 12:12 hour light/dark cycle with food and water ad libitum. Adult female SJL/J were obtained from Jackson Laboratory Farmington, CT, USA, at the age of 6 weeks. We acclimated these animals to the animal facility for seven days before beginning any experiments. Data were collected on two different cohorts of animals, induced six months apart. A total of 45 animals were used in this study. Mice were placed into different experimental groups based on the maximum clinical score achieved approximately two weeks following EAE induction (see below).

Induction of experimental autoimmune encephalomyelitis by active immunization

EAE was induced in female SJL/J mice 9 to 10 weeks (about two and a half months) of age by subcutaneous injection of 200uL of .5mg/mL proteolipid protein (PLP139-151) in complete Freund's adjuvant (CFA) and intraperitoneal injection of pertussis toxin (Ptx) obtained from Hooke Laboratories Lawrence, MA. Mice underwent clinical observation and were scored daily to assess the progression of

EAE. Assessments were reported using a standardized rating scale for the evaluation of motor deficits: 0 no deficit; 0.5 tip of the tail is limp; 1 limp tail; 1.5 limp tail and hind leg inhibition; 2 limp tail and weakness of hind legs; 2.5 limp tail and dragging of hind legs; 3 limp tail and complete paralysis of hind legs; 3.5 limp tail and legs are together on one side of the body; 4 limp tail, complete hind leg and partial front leg paralysis; 4.5 complete hind leg, and partial front leg paralysis with no movement around cage; 5 moribunds. EAE in SJL mice follows a relapsing-remitting time course of paralysis. In EAE induction by active immunization with $PLP_{139-151,}$ mice typically score 3 to 4.5 10-16 days post-induction ("Hooke - Protocols - EAE Induction by Active Immunization in SJL Mice" n.d.). In this study, mice were monitored for signs of urinary dysfunction and motor deficits starting eight days post-induction and harvested at scores of 2 or more (Fig 1). Control animals were unmanipulated animals that were age-matched to the EAE cohorts. Data were obtained from N=25 control and n=20 EAE animals in this study.

Void spot assay (VSA)

Testing was performed in the vivarium where mice were housed. Individual mice were placed in chromatography paper-lined enclosures daily for 2 hours between 2 pm and 4 pm CST. Enclosures consisted of inverted polycarbonate mouse cages placed on a rack in the same animal facility room where the mice were housed. The mice could walk directly on the filter paper within the enclosure. The mice did not have access to food and water during testing. Seven-week-old mice were acclimated to testing by performing the VSAs for ten days before induction with

EAE. Testing then was resumed four days post-induction and continued until peak illness (approximately 10-15 days).

The filter paper was imaged under ultraviolet light (Figure 1) using an iPhone 13 camera and UV light source that consisted of a uvBeast version 1 flashlight. Filter paper that had been chewed thoroughly or had visibly overlapping spots was excluded. A standard curve was uploaded to Void Whizzard so that the pictures could be analyzed. The data we obtained from the VSAs included void area, number of voids, and void volume.

Cystometry

Mice were anesthetized by intraperitoneal injection of 1.2g/kg urethane. Supplemental doses were given to maintain an adequate plane of anesthesia. Mice were placed on a heating pad to maintain body temperature. Surgical scissors made a midline incision of about 1 cm in the lower abdominal area. The bladder was exposed carefully without damage to the surrounding tissues. A hole was placed in the dome of the bladder using a 25 gauge needle. A catheter was placed within the dome of the bladder and held in place using a purse string suture. The catheter was connected to a T-shaped stopcock, an infusion pump NE-300 Infusion Syringe P, and a pressure transducer Edwards Lifesciences 600I. 0.9% NaCl solution was infused into the bladder at a rate of 20 µL/min for one hour as the mouse acclimated to the catheter (Figure 1). Data were acquired using an axoclamp 900A molecular devices amplifier and digitized via molecular devices Digidata 1440A to be recorded in Clampex 10.7 Parameters assessed included: i)

Rate 1 (the initial rate of infusion to induce a voiding contraction), ii) Constant Rate (the rate of infusion maintained throughout the experiment), iii) baseline pressure (average pressure between voids), iv) maximum pressure (peak pressure during cystometrogram), v) micturition pressure (pressure during voiding contraction minus baseline), vi) void duration (time of one complete void cycle), and vii) intercontraction interval. Mice with areflexic bladder (Kaplan et al., 1995; Light et al., 1985) or where holes were discovered in their bladders upon infusion were excluded from this study.

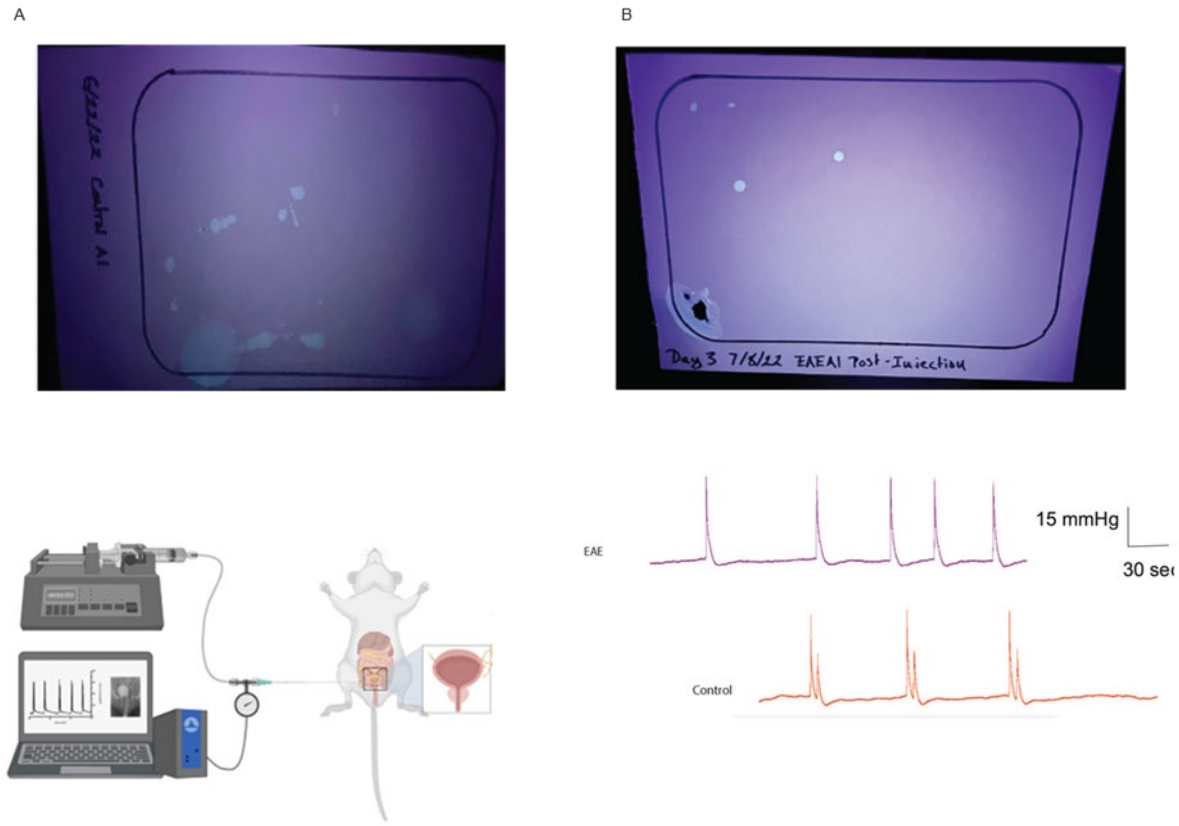

Figure 2.1 Top: A representative sample of void spot assays for a control mouse (top; panel A) and a mouse post-EAE induction (top; panel B) under UV light. Bottom: Schematic showing the cystometry setup

Top: A representative sample of void spot assays for a control mouse (top; panel A) and a mouse post-EAE induction (top; panel B) under UV light. Bottom: Schematic showing the cystometry setup. A catheter is inserted through the bladder wall and connected by a three-way stopcock to a pressure transducer and infusion pump. The output of the pressure transducer is amplified and recorded through a computerized data acquisition system (see methods). Representative traces of 5 minutes of bladder pressure recordings in a control and EAE mouse are provided for reference.

Statistics: Data were stored and organized in Microsoft Excel. All statistics and graphs were made in R 4.2.1 and formatted in Adobe Illustrator. A Shapiro-Wilk test was performed on all data sets to assess for normality. Upon determination of non-normally distributed data (Shapiro-Wilk P-value <0.05), pairwise comparisons between experimental groups were made using the Wilcoxon Rank Sum test. Repeated measures of ANCOVA were performed on the VSA data to interrogate day x group interaction differences among control and EAE-induced animals. Because of the relatively low sample sizes we obtained for EAE animals for this study, we combined all EAE animals with a clinical score of 2.0 or greater into a single experimental group.

RESULTS

EAE decreases urine output as measured by Void Spot Assay: VSAs were performed to determine if EAE altered the output of the lower urinary tract. We found that compared to controls, EAE mice produced a significantly lower number of urine spots on the VSA ($p= 0.0021$; Figure 2). Additionally, we found that the total volume of urine recorded via VSA was significantly reduced in EAE mice ($p= 0.002$; Figure 2). EAE mice had lower urinary output overall than controls (figure 3A-B).

In addition, we analyzed whether there is a difference in the trajectory of VSA results over time post-induction for the EAE animals relative to control. EAE animals show an overall negative trajectory of VSA output measures with time post-induction (Figures 3 and 4). However, there were no significant interaction

effects for the experimental group versus day post-induction relative to controls for the total number of spots or urine volume recorded via VSA (Figures 3 and 4).

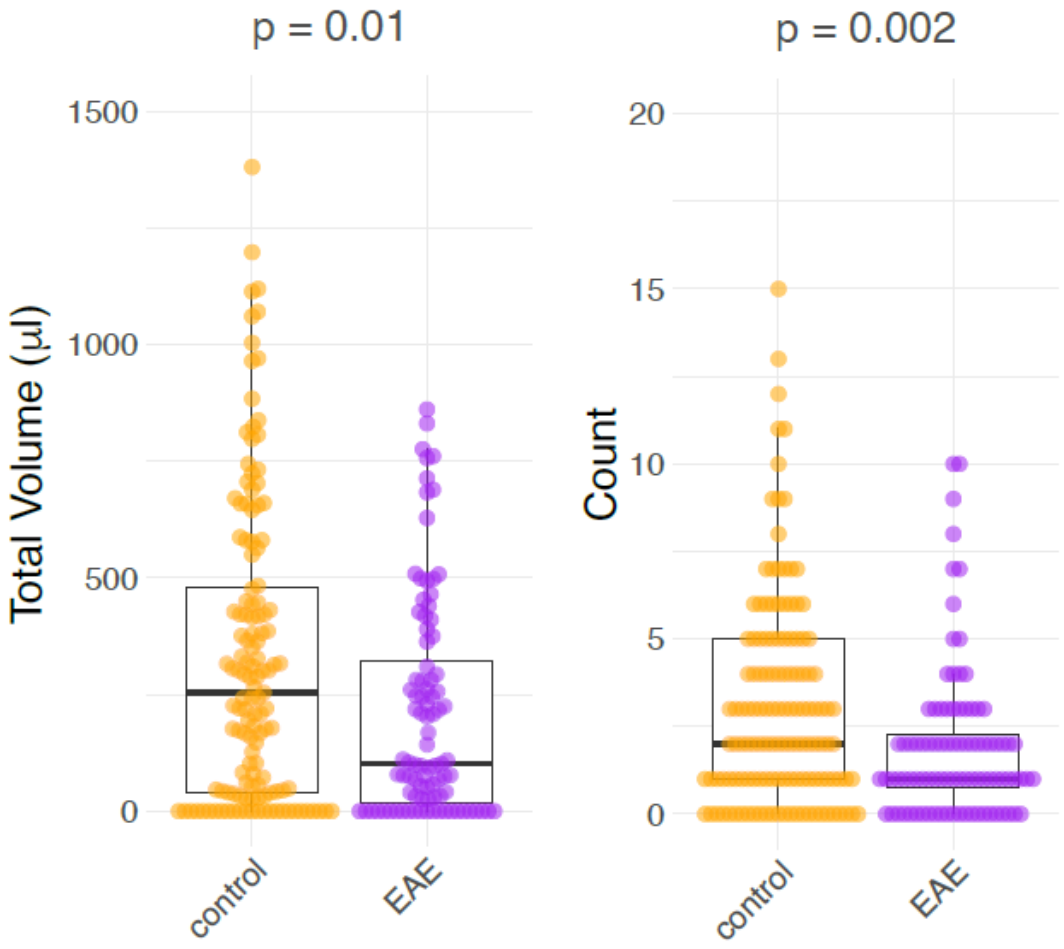

Figure 2.2 ***Boxplots of volume and urine spot count***

Each data point is a urine spot. Colors represent denote control and EAE groups. Data are shown as median and interquartile ranges. a. Mice induced with EAE produced fewer void spots during the assay period than control mice. b. EAE mice produced a lower volume of urine than control mice

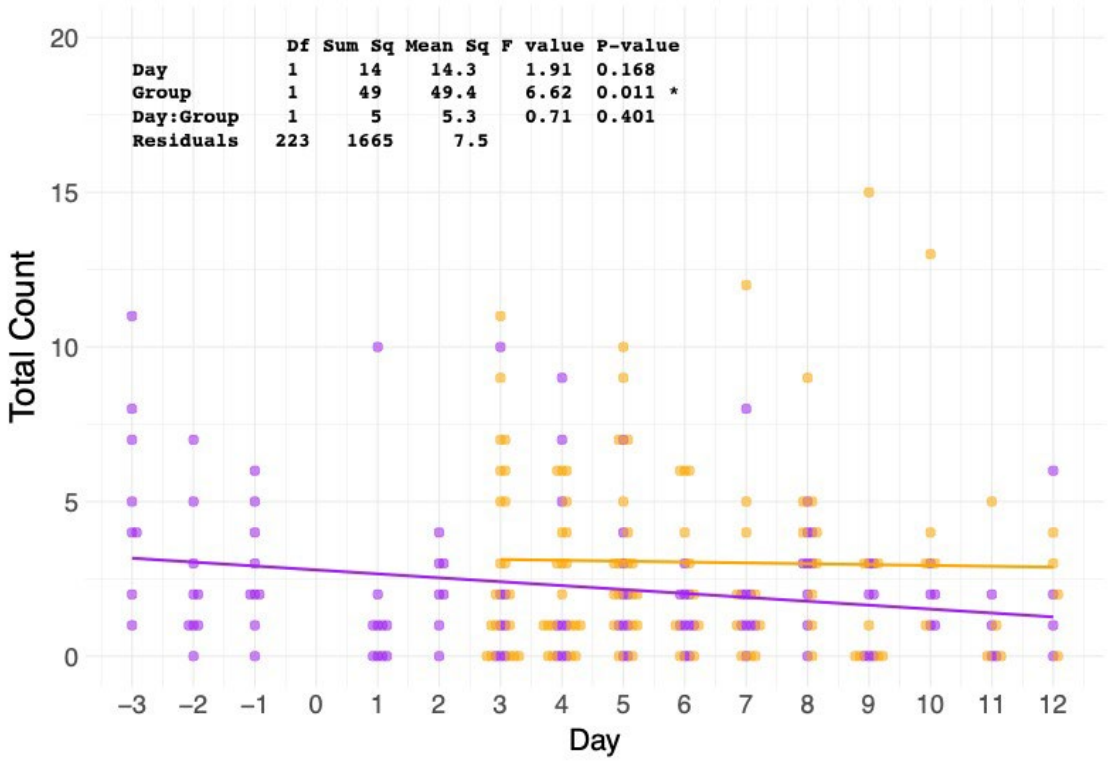

Figure 2.3 ANCOVA results for spot count

Total count of urine spots measured by VSA each day in control and EAE animals. For EAE animals, the induction and 4-day post-induction period with no testing is represented by the lack of data labeled as “Day 0.” Days -3 to -1 represent the three days prior to induction, while days 1 to 12 represent the 12 days of VSA data collected after the induction period. Control data are shown for ten days labeled as days 3 through 12. For both EAE and control animals, the first two days of VSAs were not included (corresponding to days -5 and -4 in EAE and days 1 and 2 in control) to familiarize the animals with the assay conditions. ANCOVA analysis of void spot assay data reveals no significant interaction effect between day and group. However, the group has a statistically significant effect on the number of urine spots.

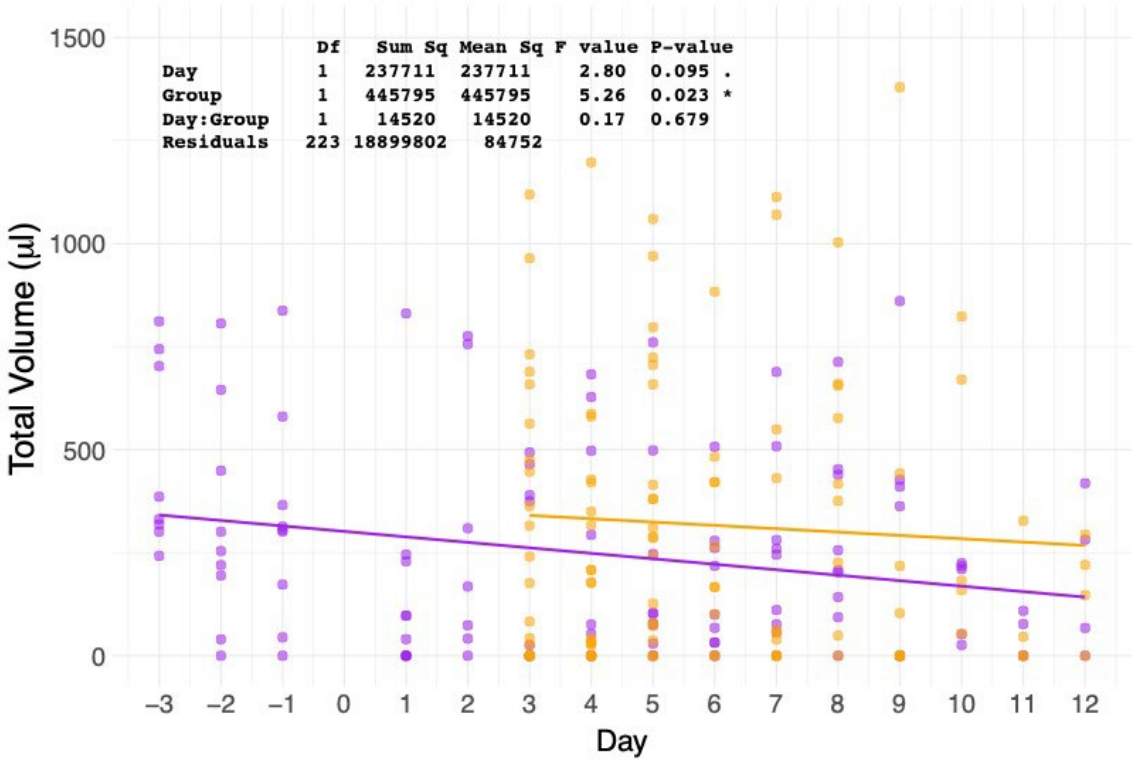

Figure 2.4 ANCOVA results for volume of urine spots

The total volume of urine spots was measured by VSA each day in control and EAE animals—data as described in Figure 3.

EAE impacts urodynamics as measured by cystometry: We conducted end point cystometry on 25 mice to investigate urinary voiding dynamics in Experimental Autoimmune Encephalomyelitis (EAE), a mouse model of Multiple Sclerosis. Seven mice were excluded from the analysis due to bladder perforations or areflexia. The remaining mice underwent ten micturition cycles, with the last five cycles subjected to detailed analysis. Our findings revealed no significant differences between control and EAE mice in Rate 1 (Figure 5a), constant rate (Figure 5b), baseline pressure (Figure 5c), and maximum pressure (Figure 5d). However, micturition pressure was significantly elevated in EAE mice compared to controls (Figure 2e), indicating increased contractile force during voiding. Void duration (Figure 2f) and intercontraction interval (Figure 2g) showed no significant differences between the two groups, although both trended higher in the EAE animals than the controls.

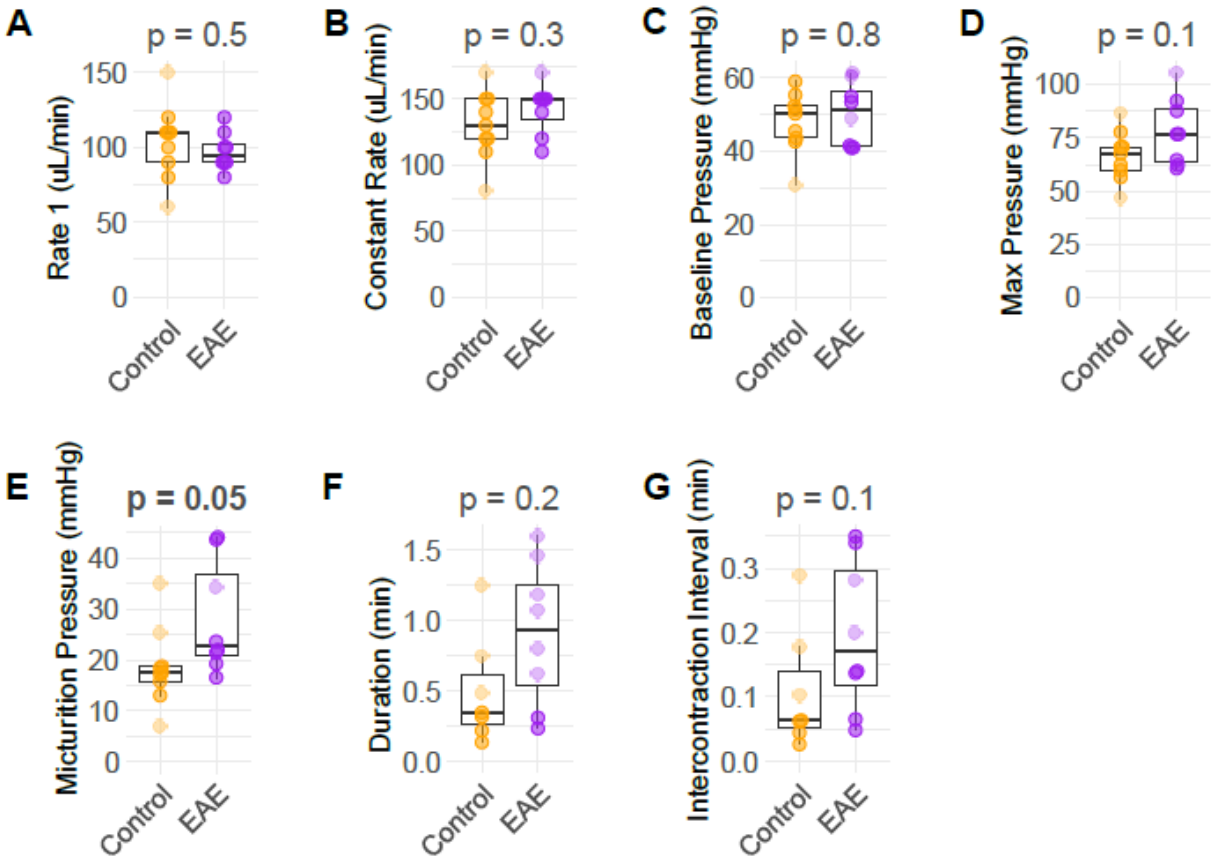

Figure 2.5 Boxplots of cystometrogram parameters

Each data point is a cystometrogram. Colors represent the group. Data are shown as median and interquartile ranges. A. EAE mice and control mice show no significant difference in the rate that produced the first voiding contraction. B. EAE mice and controls show no difference in the rate used to perform the cystometry. C. EAE and control mice show no difference in baseline pressure. D. EAE and control mice show no difference in maximum pressure. E. Control mice have significantly lower micturition pressure. F. EAE and control mice have no difference in void duration. G. EAE and control mice have no difference in intercontractile interval.

DISCUSSION

Neurogenic bladder is a common complication of MS that affects the bladder's normal function due to damage to the neural pathways involved in micturition. The damage caused by MS disrupts the communication between the CNS and the bladder, leading to various phenotypes of bladder dysfunction. The main types of neurogenic bladder include overactive bladder (OAB), underactive bladder (UAB), and detrusor sphincter dyssynergia (DSD) (Panicker et al., 2015). In 2008, it was reported that EAE, a common MS model, produces neurogenic bladder symptoms comparable to DSD. Most individuals diagnosed with multiple sclerosis (MS) experience issues with bladder control, such as a sudden urge to urinate, urinary leakage, frequent urination, and difficulty fully emptying the bladder. Approximately 60% of MS patients exhibit a condition known as detrusor-sphincter dyssynergia, where there is impaired coordination between the muscles controlling urinary flow, leading to obstruction and retention of urine (Altuntas et al., 2008). We hypothesized that changes to the input of autonomic neurons alter them in a way that causes neurogenic bladder symptoms.

Previous studies report increased micturition frequency, basal pressure, and average pressure and decreased bladder capacity and micturition pressure in EAE mice (Altuntas et al., 2008; Franken et al., 2016). The present study found that compared to control mice, EAE mice displayed a significantly lower number of urine spots on the VSA and a reduced total volume of urine recorded via VSA. Thus, EAE mice have decreased urinary output compared to controls. Furthermore, the overall trend of VSA output measures over time post-induction

showed a negative trajectory for EAE animals, indicating a worsening of urinary dysfunction as the disease progresses. However, there were no significant interaction effects between the experimental group and days post-induction relative to controls, suggesting that the decline in urinary output in EAE mice is consistent over time without significant fluctuations.

Additionally, endpoint cystometry was conducted to investigate urinary voiding dynamics in EAE mice compared to controls in this study. Seven mice were excluded due to bladder perforations or areflexia. There were no significant differences between control and EAE mice in Rate 1, constant rate, baseline pressure, and maximum pressure. This indicates that the basic parameters of bladder function, such as the rate of urine flow and pressure, were similar between the two groups. However, micturition pressure was significantly elevated in EAE mice compared to controls, suggesting increased contractile force during voiding. This could be a compensatory mechanism to overcome LUT dysfunction (Wu et al., 2016). Void duration and intercontraction interval showed no significant differences between the two groups, although both trended higher in EAE animals than controls. This suggests a tendency towards longer voiding durations and increased intervals between voiding contractions in EAE mice, indicating possible bladder dysfunction.

Together these results suggest BOO by way of DSD. BOO is the term for obstruction during voiding characterized by increased micturition or detrusor pressure and reduced urine flow or output (Gajewski & Drake, 2018). BOO can be

caused by poor coordination between the bladder and the external urethral sphincter, which is termed DSD. During urine storage, sensory nerves transmit information about bladder wall pressure (A fibers) and bladder pain/temperature (C fibers) via the pelvic/hypogastric/pudendal nerves to the lumbosacral spinal cord. This information then ascends through the spinal cord's spinothalamic tracts to the midbrain periaqueductal gray region (Arya & Weissbart, 2017). Feedback from the limbic system and prefrontal cortex influences the midbrain, promoting further bladder storage or initiating micturition (Stoffel, 2016). This study shows decreased urine output, likely due to a lack of coordination preventing the proper passage of urine. This is further substantiated by increased micturition pressures as the bladder will contract harder to produce the same flow as controls. When detrusor-sphincter dyssynergia (DSD) occurs, the detrusor muscle contracts against a closed bladder outlet because of involuntary contraction of the urinary sphincter.

A potential limitation of this study is the use of urethane anesthesia. Urethane has a relatively short duration of action compared to other anesthetics. The short duration of action sometimes necessitated supplemental doses to maintain adequate anesthesia. In doses above 1.2 g/kg, urethane can impact murine voiding by delaying voiding pressure thresholds (Abdelkhalek et al., 2021). However, the amplitudes of voiding contractions are not affected as the afferent limb of the the micturition reflex pathway has higher sensitivity to urethane than the efferent limb (Smith & Kuchel, 2010).

Chapter 3: Excitability of Major Pelvic Ganglion Neurons

INTRODUCTION

Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) and one of the most prevalent autoimmune diseases (Goldenberg, 2012; Huang et al., 2017; Wallin et al., 2019). This disease is characterized by CNS demyelination and is accompanied by neurocognitive, motor, sensory, and autonomic deficits. The most widespread autonomic deficit is lower urinary tract dysfunction, as it impacts over 90% of people suffering from MS (Tornic & Panicker, 2018; Wallin et al., 2019). In MS, this bladder dysfunction is associated with central nervous system demyelination, as peripheral demyelination only occurs in about 5% of cases, likely due to epitope spreading (Misawa et al., 2008). This dysfunction is correlated with lower quality of life scores and tertiary insults like bladder and urinary tract infections, accounting for 50 to 80% of all MS hospitalizations (Khan et al., 2022; Medeiros Junior et al., 2020). This decreased quality of life and high hospitalization rate necessitates studying these bladder deficits, including those that are likely the result of neurogenic etiology.

A neurogenic bladder is a name for any condition impacting bladder control due to brain, spinal cord, or nerve-related pathologies (Drake et al., 2016; Hu et al., 2016). In addition to MS, the neurogenic bladder is a common occurrence in individuals with spinal cord injury (Best et al., 2017; Taweel & Seyam, 2015), diabetes (Mastri, 1980; Powell, n.d.), and Parkinson's Disease (Hajebrahimi et al.,

2019; Kirby Ma Frcs, 1988). Neurogenic bladder can manifest as issues with storage or voiding of urine and can result in a range of impacts from overactive to underactive bladder, incontinence, or nocturia (Dorsher & McIntosh, 2012). Overactive bladder (storage) symptoms include urgency, frequent urination, leakage, and incontinence while voiding symptoms (detrusor-sphincter dyssynergia), incomplete bladder emptying, retention, and straining to produce urine.

While the loss of central control of the bladder remains a primary consideration for neurogenic bladder, more recently, evidence suggests that the peripheral autonomic inputs to the bladder may also be impacted in neurogenic bladder conditions. In the mouse, the major pelvic ganglion (MPG) neurons are a major source of parasympathetic input to genitourinary organs (de Groat, 2006; de Groat et al., 2015). The properties of these MPG neurons are known to change in response to physiological and pathological conditions (Keast, 2006). For example, in conditions of urinary dysfunction or pelvic organ disorders, the MPG may undergo functional changes due to the altered neural input or to adapt to the pathological state(Chung, 2012). These changes can affect the overall function of the MPG and its regulation of pelvic organ activities. In a mouse model of type II diabetes, the passive and firing properties, such as input resistance and afterhyperpolarization duration of MPG neurons, decrease (Gray et al., 2019a). While in spinal cord injured (SCI) mice, the synaptic properties of MPG neurons are altered where animals with acute SCI exhibited a general rise in excitatory post synaptic potential (EPSP) area and a longer duration to reach a peak, attributed to

enhancements in both rise time and decay time. In contrast, animals with chronic SCI displayed the opposite trend: notably reduced rise and decay time constants, resulting in a shorter time to peak (Kyi, 2022). Both models are associated with bladder function deficits (Kim et al., n.d.; Saito et al., 2021; Wada et al., 2017).

Given that other conditions that result in the neurogenic bladder are associated with changes to MPG neurons, it is pertinent that these neurons are studied in MS. We hypothesize that axon loss in the central neuraxis modifies the input to the major pelvic ganglion (MPG) autonomic neurons, resulting in modifications of their intrinsic properties, potentially contributing to LUT dysfunction. In this investigation, we utilize the Experimental Autoimmune Encephalomyelitis (EAE) model of MS in 11-12-week-old female SJL mice to explore the active and passive properties of MPG neurons through sharp electrode current clamp recordings. Based on observed urine staining, we hypothesized that the neurons contributing to the parasympathetic input to the bladder would exhibit hyperexcitability. Contrary to our expectations, our findings reveal that during the most severe illness, the half-width of action potentials is increased while the maximum firing frequency of tonic cells is decreased. Our results imply that the input to the bladder undergoes alterations in EAE, potentially impacting its output.

METHODS

Animals: All experimental procedures were approved by the University of Missouri Institutional Animal Care and Use Committee and followed the National Institutes of Health Guide for the Care and Use of Laboratory animals. The animals were kept in groups on a 12:12 hour light/dark cycle with food and water ad libitum. Adult

female SJL/J were obtained from Jackson Laboratory. These adult female SJL/J were placed into three groups: control, EAE score 2, and EAE score 3.5.

Induction of experimental autoimmune encephalomyelitis by active immunization: EAE was induced in female SJL/J 9 to 10 weeks (about two and a half months) of age by subcutaneous injection of 200uL proteolipid protein (PLP139-151) in complete Freund's adjuvant (CFA) and IP injection of pertussis toxin (Ptx) obtained from Hooke Laboratories. Mice underwent clinical observation and were scored daily to assess the progression of EAE. Assessments were reported using a standardized rating scale for the evaluation of motor deficits: 0 no deficit; 0.5 tip of the tail is limp; 1 limp tail; 1.5 limp tail and hind leg inhibition; 2 limp tail and weakness of hind legs; 2.5 limp tail and dragging of hind legs; 3 limp tail and complete paralysis of hind legs; 3.5 limp tail and legs are together on one side of the body; 4 limp tail, complete hind leg and partial front leg paralysis; 4.5 complete hind leg, and partial front leg paralysis with no movement around cage; 5 moribunds. EAE in SJL mice follows a relapsing-remitting time course of paralysis. In EAE induction by active immunization with $PLP_{139-151,}$ mice typically score 3 to 4.5 10-16 days post-induction ("Hooke - Protocols - EAE Induction by Active Immunization in SJL Mice" n.d.). In this study, mice were monitored for signs of urinary dysfunction and motor deficits starting eight days post-induction and harvested at scores of 2 or more (Fig 1). 45 control and EAE animals were used.

Tissue isolation for electrophysiology recordings: Mice were euthanized with an overdose of isoflurane by inhalation, and their MPGs were dissected. The MPGs

were pinned down in a Sylgard dish containing oxygenated physiological saline solution composed of (in mM) NaCl, 146; KCl, 4.7; $MgSO_4$, 0.6; $NaHCO_3$, 1.6; NaH_2PO_4, 0.13; $CaCL_2$, 2.5; Glucose, 7.8; HEPES, 20, adjusted to a pH of 7.3 (Jobling and Lim 2008). The saline was continuously perfused into the dish for in-vitro intracellular recordings.

Electrophysiology recordings: Dissections and experiments were performed in oxygenated physiological saline at room temperature. The MPGs from 10–12-week-old female SJL/J were dissected and pinned in a Sylgard dish. The preparations were then de-sheathed by removing fat and connective tissue. Action potential properties, passive properties, and firing rate were calculated using the auto-statistics package of the Clampfit program from the pClamp 10.3 suite of software (Molecular Devices, Sunnyvale, Ca) and Spike2 (Cambridge Electronic Design Limited, Cambridge, England). Electrodes pulled on a P-97 microelectrode puller (Sutter, Novato, CA) were filled with 500 mM KCl and had resistances of 130 to 200 MΩ. Recordings were obtained using an Axoclamp 900A (Molecular Devices, Sunnyvale, CA) amplifier and digitized at 10 kHz using a Digidata 1440 (Molecular Devices, Sunnyvale, CA). Ground wires and silver electrodes were chlorinated in household bleach (Clorox, Oakland, CA).

Passive, excitability, and action potential properties: Resting membrane potential, resistance, membrane time constant, membrane capacitance, rheobase, negative rheobase, max firing rate, halfwidth, anti peak, peak, max rise slope, max decay slope, rise slope, and decay slope were estimated from step protocols -300pA to

400pA by steps of 50pA or -500pA to 700pA by steps of 100pA current injections having a duration of 1000 ms.

Inclusion criteria: Because sharp electrode recordings cause impalement damage, we set criteria for the cells' resting membrane potential and input resistance in this study. All neurons must have a resting membrane potential below -25mV. The neurons included in this study must also have an input resistance greater than 30 MΩ.

Statistical analysis: Data were stored and organized in Microsoft Excel. All statistics and graphs were made in R and formatted in Adobe Illustrator. A Shapiro-Wilk test was performed to assess for normality. A Kruskal-Wallis was performed, and pairwise comparisons were followed using the Wilcoxon rank sum test. If the P value is not stated *P<.05, **P<.01, ***P<.001

RESULTS

Experimental Autoimmune Encephalomyelitis: Experimental autoimmune encephalomyelitis (EAE) is a T-cell mediated autoimmune disease characterized by primary demyelination of axonal tracts in the central neuraxis. EAE is currently the most studied model of Multiple Sclerosis (MS) (Procaccini et al. 2015). The SJL $PLP_{139-151}$ model is known to develop neurogenic bladder (Altuntas et al. 2012). We compared animals with moderate to severe disease severity. Animals were monitored daily, and when they did not show any further increase in clinical score for 2 to 5 days, they were harvested for experimental analysis. This yielded animals over a range of scores from 0.5 to 4.5. We focused on two-time points for differing levels of disease severity: 2 and 3.5. Animals were harvested in two batches, with

the disease onset occurring 9-19 days following injection. A clinical time course can be seen in Figure 1.

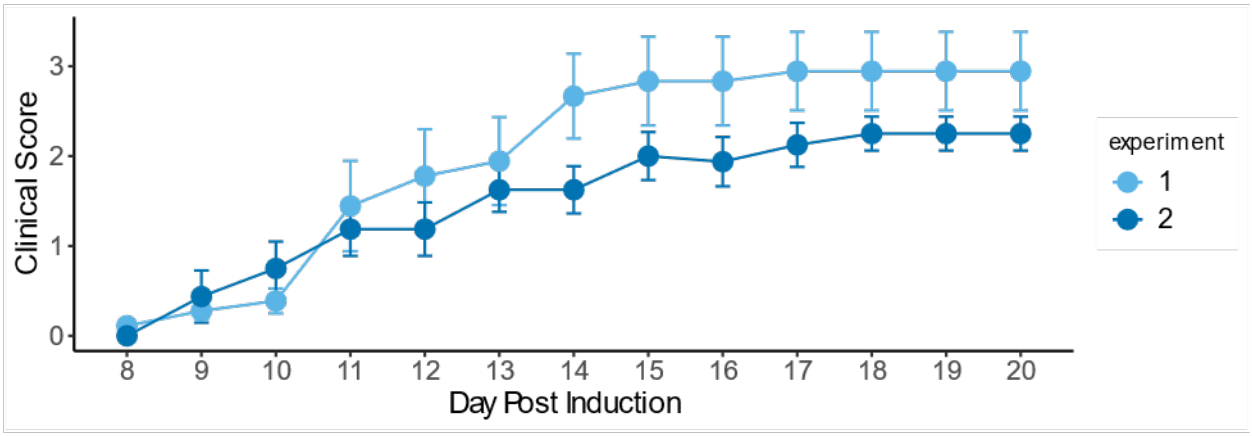

Figure 3.1 EAE timecourse

Mice were induced in two batches over 3 to 5 days. Both experimental batches show similar progression in clinical score, but animals from experiment 1 reached a higher peak severity. Clinical scores were used to separate animals into groups for data analysis.

Passive properties of MPG neurons in control and EAE mice: This study investigates the impact of EAE on the passive properties, excitability, and action potential characteristics of MPG neurons in mice. Intracellular recordings from 75 neurons were obtained, categorized based on clinical scores (0,2 and 3.5), and analyzed for resting membrane potential (RMP), input resistance, membrane time constant, and membrane capacitance. We estimated these properties by injecting depolarizing current from -300pA to 400pA in increments of 50pA or from -500pA to 700pA in increments of 100pA. The analysis of passive properties revealed no significant differences in resting membrane potential, input resistance, or membrane time constant among the experimental groups (table 2 $p>0.05$). Similarly, the results of membrane capacitance were not statistically significant.

EAE impacts the excitability and action potential properties of MPG neurons: To assess the impact of EAE on MPG neuron excitability, firing properties were characterized using current clamp protocols. No significant differences in rheobase were observed among the clinical score groups. However, a significant decrease in negative rheobase was noted in animals with a clinical score of 3.5 compared to 0 ($p=0.042$). Maximum firing rate exhibited an overall significant change among the clinical score groups (Kruskal-Wallis $p=0.0307$). Although pairwise comparisons did not reveal statistical significance, a decreased maximum firing rate trend was observed in the clinical score 2.5-4.5 group ($p=0.07$). Examination of action potential properties demonstrated no differences in peak, anti-peak, or

rise slope (p> 0.05). However, halfwidth increased with disease severity (p=0.003). Furthermore, increasing EAE severity (p= and respectively) altered max rise and decay slopes.

Table 2 Passive properties parameters

Table 2. EAE associated passive properties parameters. Combined represents the pooled phasic, tonic, and undefined cell types. Pairwise Wilcoxon tests were run after the Kruskal-Wallis analysis, and p-values for each pairwise comparison were reported.

Property (unit)	Clinical Score	Cell Type	n	Mean ± SD	Statistics	
					Kruskal-Wallis	Wilcoxon Pairwise Comparisons
RMP (mV)	0	Combined	23	-31.1 ± 7.8	H =0.7527; (p =0.686)	-
		Phasic	13	-30.2 ± 6.6		
		Tonic	7	-34.1 ± 10.4		
	2	Combined	29	-31.2 ± 7.7		P = 1 (vs. 0) P = 1 (vs. 3.5)
		Phasic	17	-30.7 ± 7.1		
		Tonic	9	-32.2 ± 9.3		
	3.5	Combined	23	-29.5 ± 5.6		P = 1 (vs. 0) P = 1 (vs. 2)
		Phasic	x	-30.1 ± 6.6		
		Tonic	x	-29.3 ± 5.2		
Input resistance (MW)	0	Combined	23	82.2 ± 49.5	H = 0.2475; (p =0.884)	-
		Phasic	13	80.4 ± 44.5		

		Tonic	7	96.9 ± 64.3		
	2	Combined	29	83.6 ± 41.4		P 1 (vs. 0) P = 1 (vs. 3.5)
		Phasic	17	71.1 ± 25.0		
		Tonic	9	99.8 ± 64.3		
	3.5	Combined	23	82.8 ± 47.2		P = 1 (vs. 0) P = 1 (vs. 2)
		Phasic	15	89.1 ± 46.9		
		Tonic	2	96.2 ± 82.7		
Tau (ms)	0	Combined	23	3.8 ± 1.5	H = 0.0331; (p =0.9836)	-
		Phasic	13	3.3 ± 0.7		
		Tonic	7	5.0 ± 1.8		
	2	Combined	29	3.7 ± 1.5		P = 1 (vs. 0) P = 1 (vs. 3.5)
		Phasic	17	3.4 ± 1.5		
		Tonic	9	4.3 ± 1.5		
	3.5	Combined	23	3.8 ± 1.5		P = 1 (vs. 0) P = 1 (vs. 2)
		Phasic	15	3.8 ± 1.2		

		Tonic	2	2.7 ± 0.5		
Capacitance (mF)	0	Combined	23	0.06 ± 0.03	H = 0.4176 (p =0.81160)	-
		Phasic	13	0.06 ± 0.03		
		Tonic	7	0.07 ± 0.04		
	2	Combined	29	0.05 ± 0.03		P = 1 (vs. 0) P = 1 (vs. 3.5)
		Phasic	17	0.05 ± 0.03		
		Tonic	9	0.06± 0.03		
	3.5	Combined	23	0.06 ± 0.04		P = 1 (vs. 0) P = 1 (vs. 2)
		Phasic	15	0.05 ± 0.03		
		Tonic	2	0.05 ± 0.05		

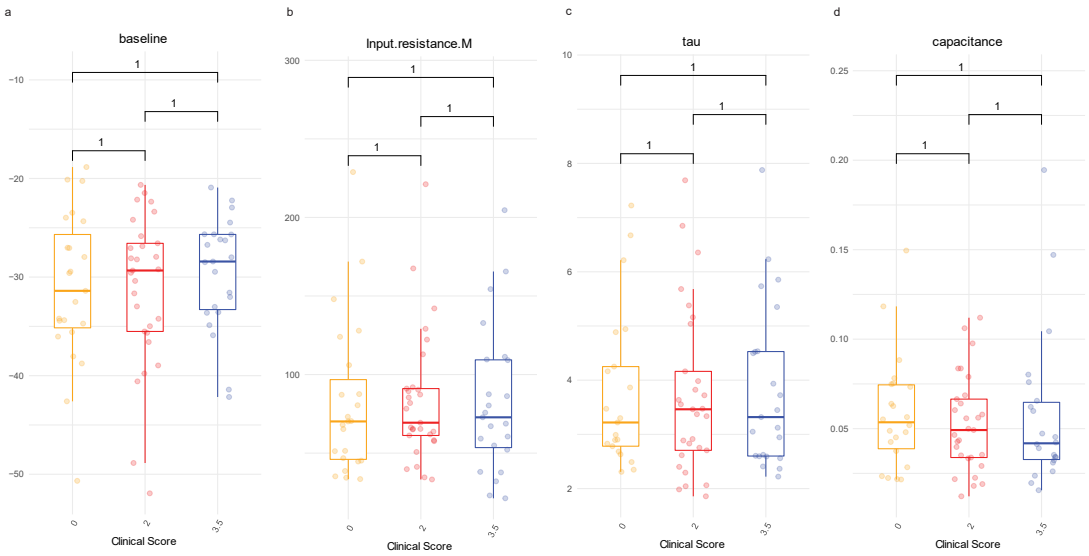

Figure 3.2 Boxplots of passive properties

Passive properties of central pelvic ganglion (MPG) neurons in control (Score 0) and EAE mice with a clinical score of 2 and 3.5. Each data point represents a single cell. Data are shown as median and interquartile ranges. There were no significant differences in **A**) Resting membrane potential, **B**) Input resistance, **C**) Membrane time constant, or **D**) Membrane capacitance of MPG neurons across EAE scores. Results of Kruskal-Wallis Paired Wilcoxon rank sum tests are provided in Table 2.

Table 3 Excitability properties parameters

Table 3. EAE associated properties. Combined represents the pooled phasic, tonic, and undefined cell types. Pairwise Wilcoxon tests were run after the Kruskal-Wallis analysis, and p-values for each pairwise comparison were reported.

Property (unit)	Clinical Score	Cell Type	n	Mean ± SD	Statistics	
					Kruskal-Wallis	Wilcoxon Pairwise Comparisons
Rheobase (pA)	0	Combined	23	177.5 ± 117.5	H =4.1837; (p =0.1235)	-
		Phasic	13	226.9 ± 118.4		
		Tonic	7	85.7 ± 24.4		
	2	Combined	29	169.2 ± 107.8		P = .79 (vs. 0) P = .22 (vs. 3.5)
		Phasic	17	211.8 ± 111.1		
		Tonic	9	88.9 ± 22.0		
	3.5	Combined	23	111.8 ± 57.4		P = .22 (vs. 0) P = .22 (vs. 2)
		Phasic	15	-120 ± 56.1		
		Tonic	2	50 ± 0.0		
Negative Rheobase (pA)	0	Combined	23	-202.2 ± 93.5	H = 6.0446; (p =0.0486)	-
		Phasic	13	-226.9 ± 88.1		
		Tonic	7	-142.9 ± 93.2		
	2	Combined	29	-177.6 ± 102.3		P =0.312 (vs. 0) P = 0.312(vs. 3.5)
		Phasic	17	-211.8 ± 105.4		
		Tonic	9	-88.9 ± 22.1		
	3.5	Combined	23	-137.0 ± 78.7		P = 0.042 (vs. 0) P = 0.312 (vs. 2)
		Phasic	15	-106.7 ± 32.0		
		Tonic	2	-50 ± 0.0		

Halfwidth (ms)	0	Combined	23	4.0 ± 1.3	H = 10.905; (p =0.0043)	-
		Phasic	13	4.2 ± 1.6		
		Tonic	7	3.6 ± 0.6		
	2	Combined	29	3.6± 1.2		P = 0.217 (vs. 0) P = 0.003(vs. 3.5)
		Phasic	17	3.7 ± 1.3		
		Tonic	9	3.2 ± 1.1		
	3.5	Combined	23	5.0 ± 1.6		P = 0.066 (vs. 0) P = 0.003 (vs. 2)
		Phasic	15	4.9 ± 1.7		
		Tonic	2	3.5 ± 0.2		
Max Frequency (Hz)	0-0.5	Combined	23		H = 6.9676; (p =0.0307)	-
		Phasic	13			
		Tonic	7	39.3±11.4		
	1.5-2	Combined	29			P = .671 (vs. 0-0.5) P = 0.068 (vs. 2.5-4.5)
		Phasic	17			
		Tonic	9	37.9±10.5		
	2.5-4.5	Combined	23			P = 0.068 (vs. 0-0.5) P = 0.068 (vs. 1.5-2)
		Phasic	15			
		Tonic	2	22.3±5.8		

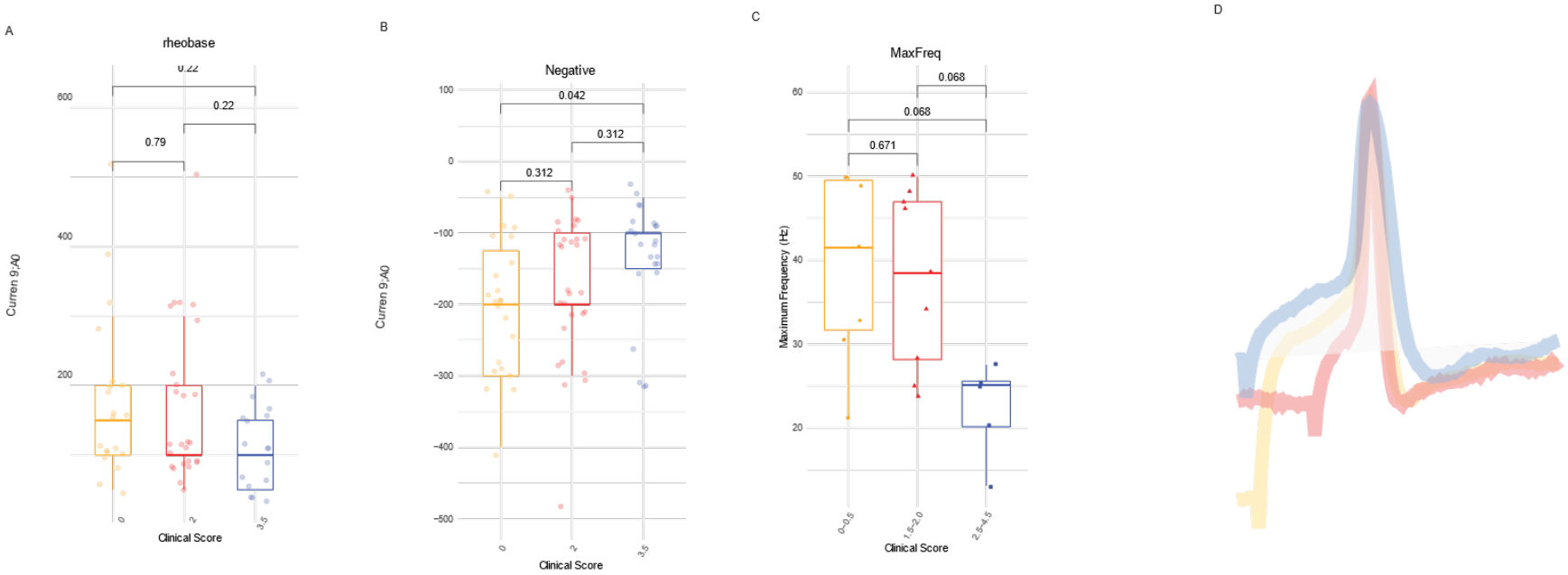

Figure 3.3 Boxplots of excitability properties and half-width traces

Effect of EAE on excitability properties of MPG neurons. A) Rheobase of MPG neurons decreases with the severity of the condition. **B**) The amount of negative current needed to produce a rebound action potential increases with the severity of the illness. **C**) Maximum firing rate decreases with severity of illness as halfwidth of action potentials is altered with illness (*left traces*). Each data point represents a single cell. Data are shown as median and interquartile ranges.

Table 4 Action potential properties parameters

Table 4. EAE associated properties. Combined represents the pooled phasic, tonic, and undefined cell types. Pairwise Wilcoxon tests were run after the Kruskal-Wallis analysis, and p-values for each pairwise comparison were reported.

Property (unit)	Clinical Score	Cell Type	n	Mean ± SD	Statistics	
					Kruskal-Wallis	Wilcoxon Pairwise Comparisons
Antipeak	0	Combined	23	-8.18±6.10	H=0.123 (p=0.9404)	
		Phasic	13	-8.19±7.11		
		Tonic	7	-9.82±4.41		
	2	Combined	29	-7.77±3.07		P = 1 (vs. 0) P = 1(vs.3.5)
		Phasic	17	-6.69±3.22		
		Tonic	9	-9.87±1.77		
	3.5	Combined	23	-8.10±3.65		P = 1 (vs. 0) P = 1 (vs. 2)
		Phasic	15	-9.32±2.61		
		Tonic	2	-10.69±0.0		
Decay Slope	0	Combined	23	-9.97±5.91	H=6.9723 (p=0.0306)	
		Phasic	13	-8.67±6.16		
		Tonic	7	-13.93±4.83		
	2	Combined	29	-10.03±5.0		P = .90 (vs. 0) P =.03(vs.3.5)
		Phasic	17	-10.22±5.58		
		Tonic	9	-10.61±4.59		
	3.5	Combined	23	-6.53±3.55		P = .09 (vs. 0) P = .03 (vs. 2)
		Phasic	15	-7.05±3.76		
		Tonic	2	-9.61±0.63		
Rise Slope	0	Combined	23	15.42±15.74	H=2.0808 (p=0.3533)	
		Phasic	13	14.99±16.68		
		Tonic	7	17.99±18.14		

	2	Combined	29	20.64±16.47		P = .62 (vs. 0) P = .62(vs.3.5)
		Phasic	17	25.83±16.57		
		Tonic	9	12.46±14.63		
	3.5	Combined	23	14.51±14.70		P = .95 (vs. 0) P = .62(vs. 2)
		Phasic	15	16.73±16.25		
		Tonic	2	24.93±3.56		
Peak	0	Combined	23	31.82±117.51	H=0.0176 (p=0.9912)	
		Phasic	13	29.44±13.52		
		Tonic	7	38.57±12.70		
	2	Combined	29	31.09±11.14		P = 1 (vs. 0) P = 1(vs.3.5)
		Phasic	17	34.09±10.51		
		Tonic	9	26.39±11.73		
	3.5	Combined	23	30.54±14.70		P = 1 (vs. 0) P = 1 (vs. 2)
		Phasic	15	32.44±11.68		
		Tonic	2	35.63±11.75		
Max Rise Slope	0	Combined	23	28.24±16.39	H=6.3479 (p=0.04184)	
		Phasic	13	27.49±17.05		
		Tonic	7	34.80±16.13		
	2	Combined	29	36.07±18.77		P = .27 (vs. 0) P = .04(vs.3.5)
		Phasic	17	39.57±20.92		
		Tonic	9	30.45±16.13		
	3.5	Combined	23	23.95±15.18		P = .46 (vs. 0) P = .04 (vs. 2)
		Phasic	15	26.84±16.28		
		Tonic	2	35.41±3.45		
Max Decay Slope	0	Combined	23	-14.04±6.49	H=8.5225 (p=0.0141)	
		Phasic	13	-12.66±6.25		
		Tonic	7	-18.67±5.95		
	2	Combined	29	-14.27±5.57		P = .65 (vs. 0) P = .02(vs.3.5)
		Phasic	17	-14.74±6.17		

		Tonic	9	-14.45±5.23		
	3.5	Combined	23	-9.85±3.56		P = .06 (vs. 0) P = .02 (vs. 2)
		Phasic	15	-10.23±3.85		
		Tonic	2	-12.96±0.64		

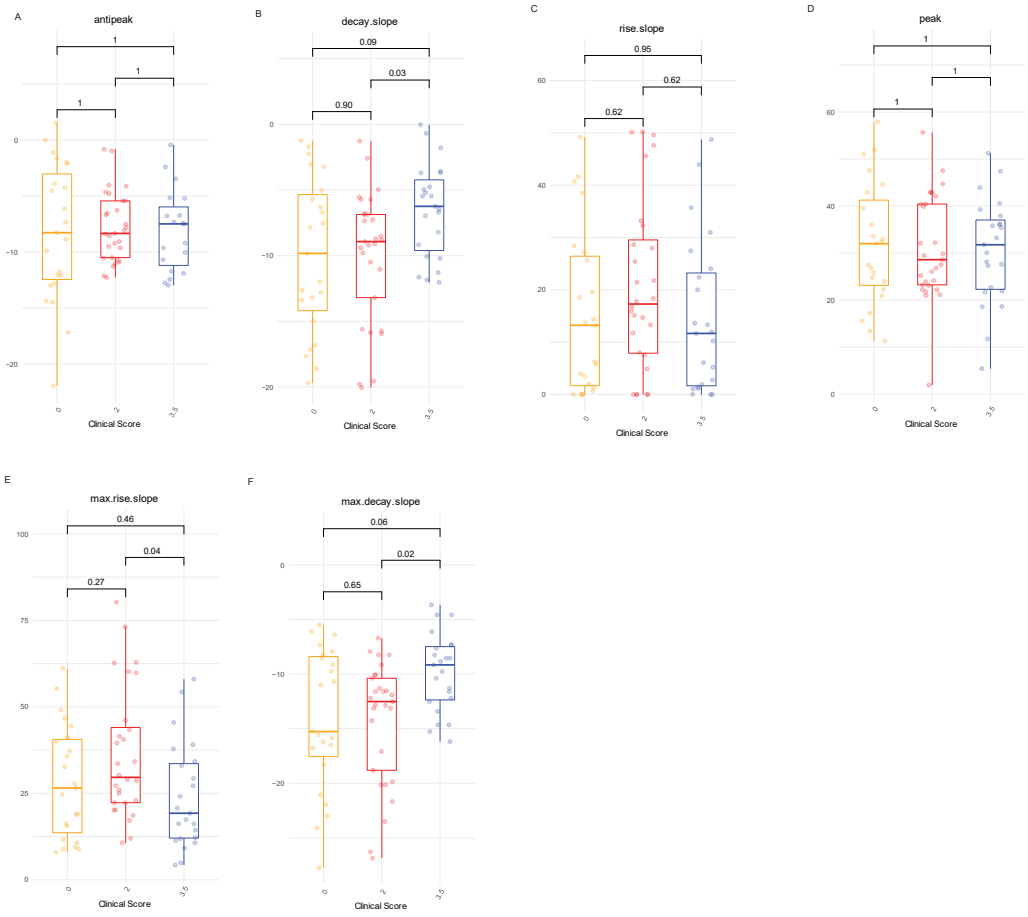

Figure 3.4 Boxplots of action potential properties

Effect of EAE on Action Potential Properties a. antipeak does not change across scores b. decay slope in score 3.5 significantly differs from a score of 2 c. rise slope does not change across scores d. peak does not change across scores. The max rise slope is significantly different between scores 2 and 3.5 f. The max decay slope is significantly different between scores 3.5 and 2.

DISCUSSION

More than 80% of Multiple sclerosis (MS) patients experience lower urinary tract (LUT) dysfunction within the first eight years of diagnosis (Tornic and Panicker, 2018). As the disease progresses, the prevalence of LUT symptoms increases to 100% in ten years (Tornic and Panicker, 2018; McCombe, Gordon, and Jackson, 2009). Experimental autoimmune encephalomyelitis replicates the urodynamic changes seen in Multiple Sclerosis in SJL/J mice immunized with $PLP_{139-151}$ and C57Bl/6J mice immunized with MOG_{35-55}(Altuntas et al. 2012; Franken et al. 2016). However, unlike other injuries and illnesses that affect the LUT, no one has investigated the effect of EAE on the autonomic neurons directly innervating the LUT. Therefore, this study attempts to identify the impacts of EAE on MPG neurons for the first time. We hypothesized that EAE would impact the passive and active properties of MPG neurons. Furthermore, we hypothesized that these changes would vary with the severity of the illness.

The complexity of the neural control of the lower urinary tract makes it susceptible to injuries and diseases like spinal cord injury, diabetes, and multiple sclerosis, which result in the development of an assortment of dysfunctional urinary phenotypes (Golbidi & Laher, 2010; Panicker et al., 2015). Plasticity of the neural pathways controlling the lower urinary tract in injury and disease is partially responsible for forming LUT dysfunction. This plasticity is associated with changes in ion channel and receptor profiles and changes to the excitability, active, and passive properties of neurons (de Groat & Yoshimura, 2012; Gray et al., 2019b; Miyazato et al., 2017a; Tompkins et al., 2013; Vignes et al., 2007). In conditions

with the sequela of LUT dysfunction, alterations in the passive properties of efferent MPG neurons are implied by changes in neuronal morphology (Marwaha et al., 2020; Miyazato et al., 2017a; Steers, 2002) and are rarely directly examined. The studies that directly attempt to document how LUT dysfunction impacts the passive properties of MPG neurons are in models of diabetes. In 2013, Tompkins et al. determined that some passive membrane properties of MPG neurons were altered in the db/db diabetic model. Their findings indicate that db/db mice have a significantly depolarized resting membrane potential and reduced input resistance compared to controls with no difference in capacitance across groups (Tompkins et al., 2013). Another study finds no difference in resting membrane potential, reduced input resistance, reduced time constant, and a transient increase in capacitance of db/db mice compared to controls (Gray et al., 2019b). Conflicting with these studies, we find that the passive properties of MPG neurons, specifically RMP, input resistance, capacitance, and tau, are not altered in EAE. This suggests no changes to the morphology of MPG cells, but changes to the input of these cells are likely occurring, as evident from the changes in excitability and action potential properties.

In the current study, we used rheobase, negative rheobase, and max firing frequency (tonic cells) as excitability measures. No significant differences were detected in rheobase across scores. Some studies report that parasympathetic MPG neurons have mostly phasic firing patterns, while sympathetic neurons display a mostly tonic firing pattern in response to depolarizing current injection (J. H. Lee et al., 2002; K.-S. Park et al., 2006). In contrast, a study by Kanjhan et al.

concluded that parasympathetic and sympathetic MPG neurons could have tonic and phasic firing phenotypes (Kanjhan et al., 2003b). Based on the latter study, we decided to group MPG neurons into a single population. In another study using male rats, Tan found that MPG neurons show significant differences in rheobase when divided into subpopulations by firing pattern (Tan et al., 2007). If the principles in the Tan study hold in female mice, grouping the subpopulations in this study could obscure significant differences in rheobase across groups.

In contrast, negative rheobase significantly increased the EAE score, meaning the severity of EAE decreased the hyperpolarizing current injection needed to produce rebound spikes in MPG neurons. Needing less current to generate rebound spikes indicates that neurons in more diseased animals possess a form of augmented excitability. Lee and colleagues demonstrated rebound spikes in the tonic cells of the rat MPG (J. H. Lee et al., 2002). However, this study reports rebound spikes in tonic and phasic MPG neurons. The ability for both firing types to produce rebound spikes at the end of hyperpolarizing current pulses is corroborated in a 2007 study by Tan et al., which demonstrated 59.3% of phasic cells and all tonic cells firing rebound spikes. Variations in stimulus strength can cause alterations in the frequency of action potential firing patterns in tonic cells. We report that the severity of illness decreases the firing frequency in tonic cells, indicating decreased excitability with higher disease scores. This demonstrates that MPG neurons in EAE need greater stimulus strength to produce the same output as healthy controls. Together, these findings suggest that the severity of EAE alters MPG neurons' excitability. In SCI, the balance of intrinsic and synaptic

properties of efferent neurons are altered both above and below the injury site. These neurons undergo morphological changes that lead to decreased rheobase and increased input resistance following SCI. Such changes have been linked to hyperreflexia in SCI (Jean-Xavier et al., 2018). Similarly, with EAE, we see decreases in rheobase, negative rheobase, and maximum firing rate of MPG neurons. It takes less current for these neurons to fire action potentials, but the maximum firing rate could be limited by the action potentials halfwidth, which decreases then increases with disease severity.

In some cases of SCI, efferent neurons can increase excitability during the acute phase. This hyperexcitability is associated with losing inhibitory input from damaged interneurons and sensory afferents (de Groat & Yoshimura, 2006). Without proper inhibition, efferent neurons can fire more easily, leading to uncontrolled muscle spasms or spasticity. Such changes within the LUT could lead to OAB. We found that halfwidth, decay slope, max rise slope, max decay slope, and decay slope are all altered in EAE. In mice with scores of 3.5, halfwidth is shown to increase, while the halfwidth of mice with two scores is no different from controls. Halfwidth is altered due to the increase in decay slope with the severity of illness, leading to a prolonged action potential. The duration of an action potential can impact the duration of muscle contractions in the lower urinary tract. Prolonged action potentials may result in sustained muscle contractions, leading to incomplete emptying and high postvoid residual volumes. Changes in the action potential properties of neurons in the LUT can profoundly affect its function. These changes can result in a range of urinary symptoms, including overactive bladder,

urinary retention, incontinence, and altered perception of bladder fullness and urination. These findings suggest that EAE induces changes in the passive properties, excitability, and action potential characteristics of MPG neurons. Understanding these neurophysiological alterations may contribute to unraveling the complex mechanisms underlying the neurological manifestations associated with EAE and MS.

Chapter 4: EAE Alters Expression of Selection of Channels and Receptors of MPG Neurons

INTRODUCTION

Multiple Sclerosis (MS) is a chronic autoimmune disorder characterized by inflammatory demyelination of the central nervous system (CNS), leading to a diverse array of neurological deficits. Despite extensive research, the precise etiology of MS remains elusive, with a complex interplay of genetic predisposition, environmental factors, and dysregulated immune responses implicated in disease onset and progression (Huang et al., 2017). While the destruction of myelin sheaths surrounding axons is a hallmark of MS, emerging evidence suggests that molecular alterations beyond myelin loss contribute significantly to disease pathogenesis (Goldenberg, 2012). Understanding the molecular underpinnings of MS is crucial for deciphering its intricate mechanisms and developing targeted therapies to halt or reverse disease progression. Recent advancements in molecular biology techniques have provided unprecedented insights into the cellular and molecular processes underlying MS pathophysiology (Bar-Or et al., 1999; Ciccarelli et al., 2014; Lassmann & Van Horssen, 2011), offering new avenues for therapeutic intervention.

The Major Pelvic Ganglion (MPG) serves as a vital component of the autonomic nervous system (ANS) in rodents, particularly in regulating the lower urinary tract (LUT) (Kanjhan et al., 2003b; Keast, 2006). Comprising clusters of neurons and ganglionic cells, the MPG primarily consists of postganglionic sympathetic and parasympathetic neurons. These neurons play a pivotal role in

coordinating bladder function through autonomic innervation, with sympathetic fibers from the lumbar spinal cord facilitating bladder storage by regulating detrusor muscle relaxation and bladder neck constriction. In contrast, parasympathetic fibers from the sacral spinal cord regulate bladder emptying by promoting detrusor muscle contraction and inhibiting bladder-neck constriction.

In exploring how altered inputs affect network output, intrinsic properties of neurons, regulated by ion channel expression, emerge as crucial determinants. Studies in invertebrate systems, such as the stomatogastric ganglion of Cancer borealis, have demonstrated how loss of modulatory input alters ion channel expression profiles, emphasizing voltage regulation as a primary factor (Santin & Schulz, 2019; Temporal et al., 2012). Within the MPG, select ion channel and receptor subunits associated with autonomic ganglia were measured, including voltage-gated calcium channel subunits, nicotinic acetylcholine receptor (nAChR) subunits, sodium, and potassium channel subunits.

Calcium influx through voltage-dependent calcium channels is a possible candidate mechanism for axonal degeneration in inflammatory demyelinating disorders (Kornek et al., 2001). Furthermore, changes to the intrinsic function of these channels and their position within the active zone can profoundly alter the timing and strength of synaptic output (Dolphin & Lee, 2020; Katz & Miledi, 1967). To this end, we examined P/Q, N, R, and L-type calcium channels of MPG neurons. We also examined nAChRs in the CNS that possess ameliorating properties in EAE (Nizri et al., 2006; Simard et al., 2013). Among the different alterations

witnessed in the CNS in pathological conditions such as MS, ion channel disturbances were observed in different cell types; these alterations were similarly observed in experimental models like EAE, which mimic the disease (Bagheri et al., 2021; ELBini & Neili, 2023; Schattling et al., 2014). This necessitated the study of potassium and sodium channels.

MS profoundly impacts the LUT, resulting in a spectrum of urinary symptoms and dysfunction, with the neurogenic bladder being a hallmark feature. Neurogenic bladder arises from disruptions in neural pathways controlling bladder function, leading to detrusor overactivity, detrusor hypo-contractility, detrusor-sphincter dyssynergia, and sensory disturbances, significantly impacting patients' quality of life (Betts et al., 1993; Panicker et al., 2015). Experimental Autoimmune Encephalomyelitis (EAE), an established animal model of MS, closely mimics MS-related LUT dysfunction, providing insights into molecular changes within the MPG. EAE induction in rodents mirrors key aspects of MS, including neurogenic bladder dysfunction, offering a valuable platform to investigate molecular alterations in the MPG and their impact on LUT function. This study aims to elucidate the molecular changes occurring in MS within the MPG, hypothesizing that these changes modulate neuronal properties, thereby influencing LUT function.

METHODS

Animals: All experimental procedures were approved by the University of Missouri Institutional Animal Care and Use Committee and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were kept in groups on a 12:12 hour light/dark cycle with food and water ad libitum. Adult female SJL/J were obtained from Jackson Laboratory. These adult female SJL/J were placed into three groups: control, EAE score 2, and EAE score 3.5.

Induction of experimental autoimmune encephalomyelitis by active immunization: EAE was induced in female SJL/J 9 to 10 weeks (about two and a half months) of age by subcutaneous injection of 200uL proteolipid protein (PLP139-151) in complete Freund's adjuvant (CFA) and IP injection of pertussis toxin (Ptx) obtained from Hooke Laboratories. Mice underwent clinical observation and were scored daily to assess the progression of EAE. Assessments were reported using a standardized rating scale for the evaluation of motor deficits: 0 no deficit; 0.5 tip of the tail is limp; 1 limp tail; 1.5 limp tail and hind leg inhibition; 2 limp tail and weakness of hind legs; 2.5 limp tail and dragging of hind legs; 3 limp tail and complete paralysis of hind legs; 3.5 limp tail and legs are together on one side of the body; 4 limp tail, complete hind leg and partial front leg paralysis; 4.5 complete hind leg, and partial front leg paralysis with no movement around cage; 5 moribunds. EAE in SJL mice follows a relapsing-remitting time course of paralysis. In EAE induction by active immunization with PLP139-151, mice typically score 3 to 4.5 10-16 days post-induction ("Hooke - Protocols - EAE Induction by Active Immunization in SJL Mice" n.d.). In this study, mice were monitored for signs of

urinary dysfunction and motor deficits starting eight days post-induction and harvested at scores of 2 or more (Fig 1). 45 control and EAE animals were used.

Real-time quantitative polymerase chain reactions (qPCR): Following electrophysiology experiments, whole MPGs from each animal's left and right side were collected in 750µl TRIzol (Invitrogen) for RNA extraction. Total RNA was isolated from MPGs according to the manufacturer's protocol (Invitrogen). cDNA was generated from 100 ng total RNA primed with a mixture of oligo-dT and random hexamers that was reverse transcribed using qScript reverse transcriptase (QuantaBio). The final volume of the reverse transcription reaction was 20 µl and contained a final concentration of 2.5 ng/µl random hexamers, 2.5 µM oligo-dT, 40 U of RNase inhibitor, and 200 U of reverse transcriptase. Following heat inactivation of the enzyme, samples were diluted in ultrapure water to a final volume of 175 µl before this template was used in qPCR analyses.

cDNA was used in qRT-PCR reactions to generate copy numbers for nine distinct genes of interest from the mouse (see Table 5). Primer sets were previously validated in this system (Garcia et al. 2014, 2018; Gray et al. 2019). qPCR reactions consisted of primer pairs at a final concentration of 2.5 µM, cDNA template, and SsoAdvanced SYBR master mix (BioRad) according to the manufacturer's instructions. Reactions were carried out on a CFXConnect (BioRad) machine with a three-step cycle of 95ºC-15s, 58ºC-20s, and 72ºC-20s, followed by a melt curve ramp from 65ºC to 95ºC. Data were acquired during the 72ºC step and every 0.5ºC of the melt curve. All reactions were run in triplicate, and the average Ct (cycle threshold) was used for interpolation with the standard

curve to generate copy number for a given reaction. The units with which we express all of the qPCR data in this study are “normalized copy number per ng total RNA,” described in our previous work (Garcia et al. 2014, 2018) through normalization.

Table 5 Gene identification and primer sets used for realtime PCR

Table 5. Gene identification and primer sets used for realtime PCR reactions. Accession numbers provided for each gene of interest. Primer sets were obtained from PrimerBank (Spandidos et al., 2010) or generated *de novo* using PRIMER3 software. All primers sets were validated before use (See Garcia et al. 2014).

Gene name	Accession #	Gene Function	Forward primer (5' to 3')	Reverse Primer (5' to 3')
CHRNA2	NM_144803	Nicotinic α2	TTATCTCTGGTGTCTGCTTCTGA	CCCAGCGATTGTAGCCTCC
CHRNA3	NM_145129	Nicotinic α3	TCCAGTTTGAGGTGTCTATGTCT	TGGTAGTCAGAGGGTTTCCATTT
CHRNA4	NM_015730	Nicotinic α4	CTAGCAGCCACATAGAGACCC	GACAAGCCAAAGCGGACAAG
CHRNA5	NM_176844	Nicotinic α5	ATCCTCTGCTGCAAAACATGA	TCCACGTCCACTAACTGAGAT
CHRNA6	NM_021369	Nicotinic α6	TAAAGGCAGTACAGGCTGTGA	AAAATGCACCGTGACGGGAT
CHRNA7	NM_007390	Nicotinic α7	CACATTCCACACCAACGTCTT	AAAAGGGAACCAGCGTACATC
CHRM2	NM_203491	Muscarinic M2	TGTGATTGGCTACTGGCCTTT	GGGTAGGTTAGAGGTTTTGTGAC
CHRM4	NM_007699	Muscarinic M4	AGATGGTGTTCATTGCGACAG	GAGAACGCCCCTATGATGAGA
CACNA1A	NM_007578	P/Q-type Ca^{2+} ($Ca_V2.1$)	AAAGGCTCCTACCTGAGGAAT	CTCAGTGTCCGTAGGTCAAAC
CACNA1B	NM_007579	N-type Ca^{2+} ($Ca_V2.2$)	ACAACGTCGTCCGCAAATAC	CAGGGCCAGAACAATGCAGT
CACNA1C	NM_001159534	L-type Ca^{2+} ($Ca_V1.2$)	GGAGGGCGTGCATAAGCATT	AGGAAGAGATACTCCACTCGTTC
CACNA1D	NM_001083616	L-type Ca^{2+} ($Ca_V1.3$)	GCTTACGTTAGGAATGGATGGAA	GAAGTGGTCTTAACACTCGGAAG
CACNA1E	NM_009782	R-type Ca^{2+} ($Ca_V2.1$)	CAGTCCGGCAGAACTGTTTCA	GAGACATTGGGGTCTTGTCATC
CACNA1G	NM_001112813	T-type Ca^{2+} ($Ca_V3.1$)	AGAGTCCGCTGACCATGAAAT	CCGGAATGTGTCGCAGATCA
KCNN1	NM_032397	$K_{Ca}2.1$	CAAGCGGGTCAAAAATGCTG	GAAGGAACTTACGCTGGTGTTT

KCNN2	NM_080465	$K_{Ca}2.2$	AGCCGGAGCACAACAATTCTA	CCCAGCTTGTAGCCGATGT
KCNN3	NM_080466	$K_{Ca}2.3$	CTCGGAGAAACCTTATCGAGGC	GGTTGTGGGTAGCGTTGGG
KCNMA1	NM_010610	$K_{Ca}1.1$	CAGGCAGATGGTACTCTCAAGC	TTGGGTTTGACGAGTCTATGAAG
KCNA1	NM_010595	$K_V1.1$	CCCCTCCTACCCCTCTTC	CTGCCGCATTCAGACTCT
KCNA2	NM_008417	$K_V1.2$	GTCTGAGCTTCTGCAGGAAA	CCTATTTGTGTATCTGTGCCATC
KCNA3	NM_008418	$K_V1.3$	CTGCCCATTACCTTGTCGTT	CAGTAAAGCCACCTTCTCCA
KCNA4	NM_021275	$K_V1.4$	GAAAGCAGGAAATGAAGAGCATC	GTTGCAGCGTGGAAAAGG
KCNA5	NM_145983	$K_V1.5$	GCAGAGTCTCCAAGCAGAAG	TCTTCGAATACCCAGAAAGCTC
KCNA6	NM_013568	$K_V1.6$	CAGAGAAGTTCAAGATCGGGTA	ATTTCTGCTTGGGATGAGGAC
KCNB1	NM_008420	$K_V2.1$	GGTGGAGAGGACAATGAACA	GAGTTCGACAACACGTGCT
KCNB2	NM_001098528	$K_V2.2$	AAGTGTGTTGAGAGACAGAGC	TTGCTGGAGAAACCTAACTCG
KCNC1	NM_001112739	$K_V3.1$	TTCGGTCTTGTTCACGATGG	CCCTACTCATCCCGCTACG
KCNC2	NM_001025581	$K_V3.2$	TTGTGTCTCCTTTAGTCTGTGC	AACGTGTTTCCTGTTGACGA
KCNC3	NM_008422	$K_V3.3$	CACAATGCTGCTCAGGCT	GAAGACAAGAGCCCAATCACT
KCNC4	NM_145922	$K_V3.4$	CTTGGCAGGTCTCTGTGTT	GGACTATGCCTGTGCTGATG
KCND2	NM_019697	$K_V4.2$	CTGTGGTCACGTAAGGTTGT	GTGCAAGAACTCAGTACAATTCAG
KCND3	NM_001039347	$K_V.4.3$	ATCGAGCTCTCCATGCAG	CAAGACCACCTCACTCATCG
ACTB	NM_007393	Beta-actin	GATGACCCAGATCATGTTTGAGACC	AGATGGGCACAGTGTGGGTGA
GAPDH	NM_008084	Glyceraldehyde 3-phosphate dehydrogenase	TGCACCACCACCTGCTTAGC	GGCATGGACTGTGGTCATGAG

RESULTS

EAE does not alter calcium channel expression: This study investigates molecular changes to MPG neurons in EAE and control mice. Ganglia from 45 animals were obtained and categorized by a score of 0 (control), 2, or 3.5. We estimated the abundance of mRNA for a select group of genes using whole ganglia qPCR. Calcium channels play a crucial role in various cellular functions. There are several types of calcium channels, including P/Q, N, R, and L type calcium channels. P/Q-type channels are high voltage-activated channels involved in the release of neurotransmitters at synaptic terminals. Another kind of high voltage-activated channel, N-type channels, also aids in synaptic transmission. R-type channels are involved in regulating neuronal excitability. Similar to the R-type, the L-type channel is also a regulator of neuronal excitability and smooth muscle contraction (Dolphin, 2006, 2009; Perez-Reyes & Schneider, 1994). Given their contributions to the diverse roles of calcium signaling in physiological and pathological processes, we measured the mRNA abundance of a few calcium channel subunits. We found that EAE did not alter P/Q, N, and R-type calcium channels. However, it was observed that L type calcium channel subunit decreased expression from a score of 0 to a score of 3.5 ($p < .05$ Figure 1c).

EAE alters nicotinic subunit expression: We then examined the expression of nicotinic receptor subunits. Nicotinic acetylcholine receptors (nAChRs) are a type of ionotropic receptor found in the central and peripheral nervous systems of vertebrates. nAChRs are pentameric complexes consisting of five subunits

arranged symmetrically around a central ion channel. These subunits can be homomeric or heteromeric. There are multiple subtypes of nAChRs depending on the combination of subunits(Hurst et al., 2013; Lindstrom, 1997). In mammals, the most common subtypes include α2-α10 and β2-β4 subunits. nAChRs are found in both the CNS and the peripheral nervous system (PNS). In the CNS, they are primarily located in areas associated with cognitive function, such as the hippocampus and cortex. In the PNS, they are located at neuromuscular junctions, where they mediate the transmission of nerve impulses to skeletal muscles, as well as autonomic ganglia.

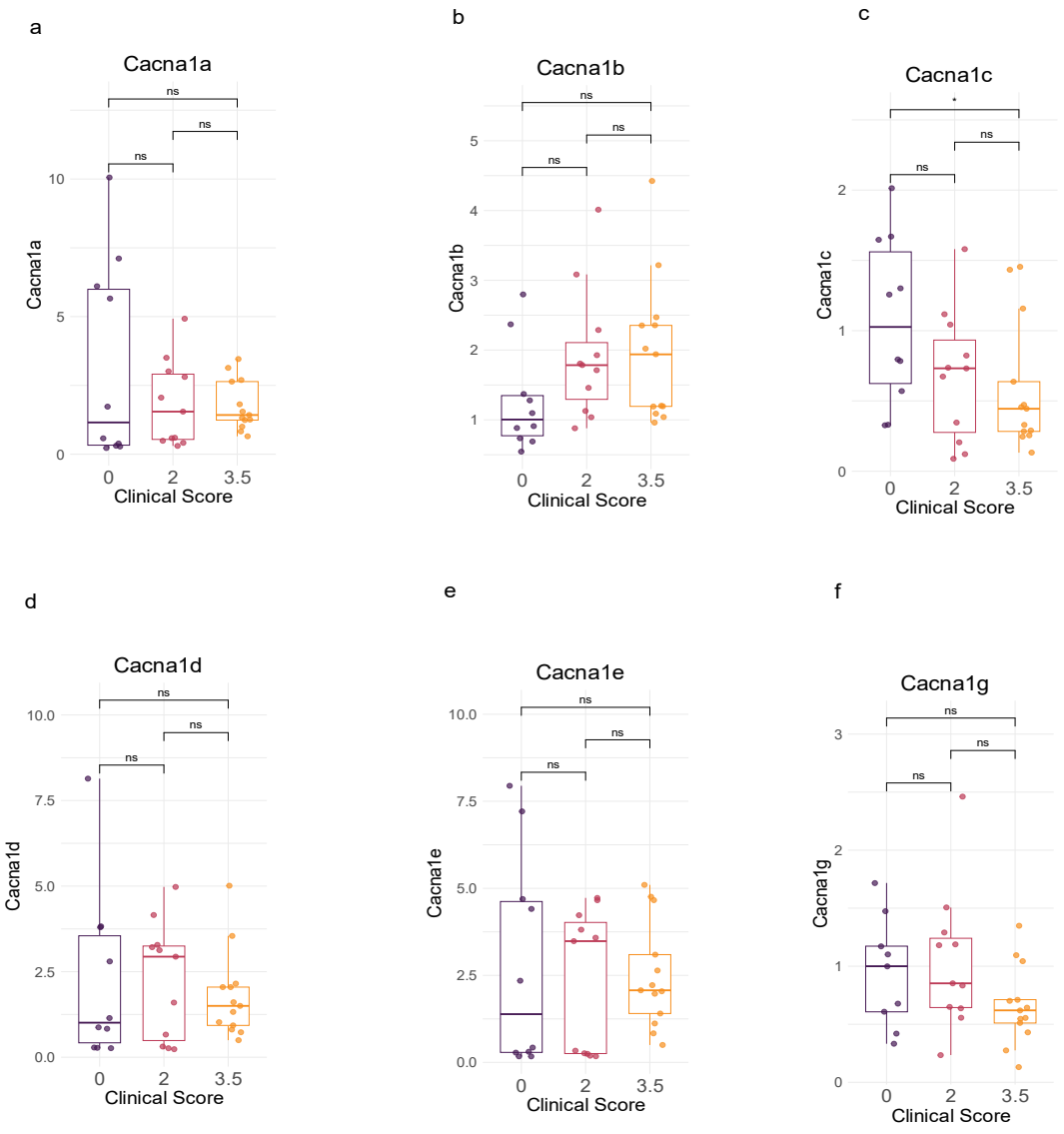

Figure 4.1 Boxplots of Calcium channel subunit expression

Expression of Calcium channel subunits in control (Score 0) and EAE mice with a clinical score of 2 and 3.5.

Each data point represents a pair of major pelvic ganglia (MPGs). Data are shown as median and interquartile ranges. a) No significant changes in Cacna1a or P/Q type calcium channel 2.1. b) No significant changes to cacna1b or N-type calcium channel 2.2. c) expression of Cacna1c or the L type calcium channel 1.2 significantly decreases between score 0 and score 3.5. d) No significant changes in Cacna1d or L-type calcium channel 1.3. e) No significant changes in Cacna1e

or R-type calcium channel 2.1. f) No significant changes in Cacna1g or T-type calcium channel 3.1.

The main subunits found in autonomic ganglia are α3, α4, α5, α7, β3, and β4. α3β4 nAchR is the predominant receptor subtype of the MPG, followed by α4β2 (Albuquerque et al., 2009; De Biasi, 2002; Kyi et al., 2022; Lindstrom, 1997). We found a significant decrease in α2 subunits between 0 score and 3.5 as well as 2 and 3.5 (figure 2a $p<.01$ and $p<.05$ respectively). Similarly, we observed a decrease in the expression of β4 subunits, with 3.5 scores having significantly lower expression than 0 and 2 scores (figure 2d $p<.001$ and $p<.01$). No changes in expression were found for α4 and β2 subunits (figure 2b and c). The mRNA copy numbers for all the subunits were plotted against each other to inspect if nAchRs were undergoing remodeling. There were no changes to the correlated expression of subunits in EAE (figure 3).

EAE alters voltage-gated potassium channel and calcium-activated potassium channel expression: Voltage-gated potassium channels (Kv) and calcium-activated potassium channels (KCa) play critical roles in regulating neuronal excitability by controlling membrane potential dynamics and action potential firing (Pongs, 1999, 2007). Their coordinated activity ensures proper neuronal function and contributes to the fine-tuning of neuronal circuits underlying sensory processing, motor control, and cognitive functions. Dysregulation of these channels can lead to neuronal hyperexcitability, contributing to the pathophysiology of various neurological disorders (Johnston et al., 2010; Yost, 1999). Therefore, understanding the changes in expression in EAE is essential. In this study, we found that there are decreases in expression from score 0 to 3.5 and

between score 2 and 3.5 for Kv1.1 (figure 4a p<.05). Likewise, the expression also decreases from score 0 to 3.5 and 2 to 3.5 for Kv1.2 (figure 4b p<.05 and p<.001). No changes in expression were discovered for Kv 2.1 and 2.2 (figure 4c and 4d, respectively). The expression of the large conductance potassium channel 1.1 remains unchanged (figure 5a). There is no alteration in the expression of the small conductance calcium-gated potassium channel 2.1 (figure 5b). The expression of the small conductance calcium-activated potassium channel 2.2 shows no change (figure 5c). Expression of the small conductance calcium-activated potassium channel 2.3 decreases between scores 0 and 3.5, as well as between scores 2 and 3.5 (figure 5d p<.05 and p<.01).

EAE alters the expression of voltage-gated sodium channels: Voltage-gated sodium channels play a pivotal role in neuronal signaling by mediating the initiation and propagation of action potentials. Their rapid activation and subsequent inactivation contribute to the generation of electrical impulses that underlie neuronal communication, enabling processes such as sensory perception, motor control, and cognitive function in the nervous system (Catterall, 1992; Catterall & Catterall, 2012). This study found the expression of voltage-gated sodium channel type 2 α1 decreases from score 0 to score 2, as well as from score 0 to score 3.5 (figure 6a p<.01 and p<.05). Similarly, the expression of voltage-gated sodium channel type 3α decreases from score 0 to 2 and again from score 0 to 3.5 (figure 6b p<.01 and p<.05). There are no changes in expression observed for voltage-gated sodium channel type 7α (figure 6c).

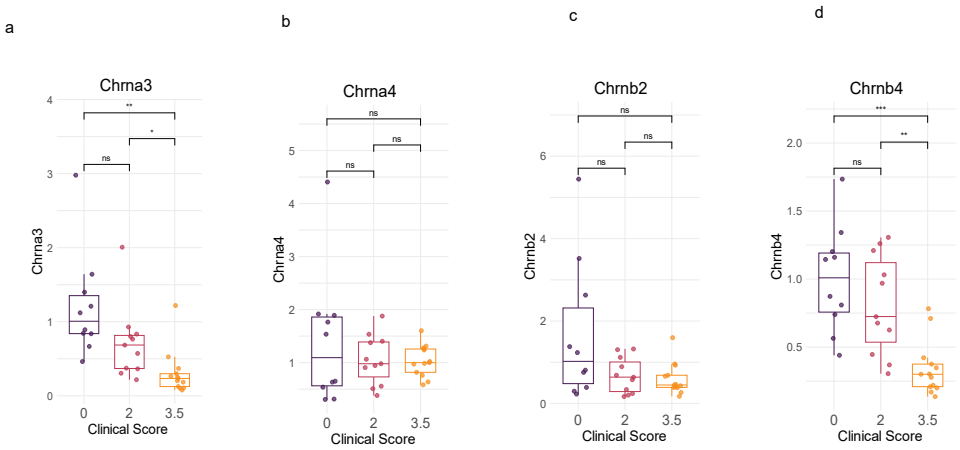

Figure 4.2 Boxplots of nicotinic receptor subunit expression

Expression of nicotinic receptor subunits in control (Score 0) and EAE mice of score 2 and 3.5. Each data point represents a pair of major pelvic ganglia (MPGs). Data are shown as median and interquartile ranges. a) there is a significant decrease in the expression of nicotinic subunit α3 between control and score 3.5 as well as between scores 2 and 3.5. b) No significant changes in nicotinic subunit α4. c) No significant changes to the expression of β2 subunits. d) Significant decreases in then expression of β4 subunits from control to score 3.5 and score 2 to 3.5.

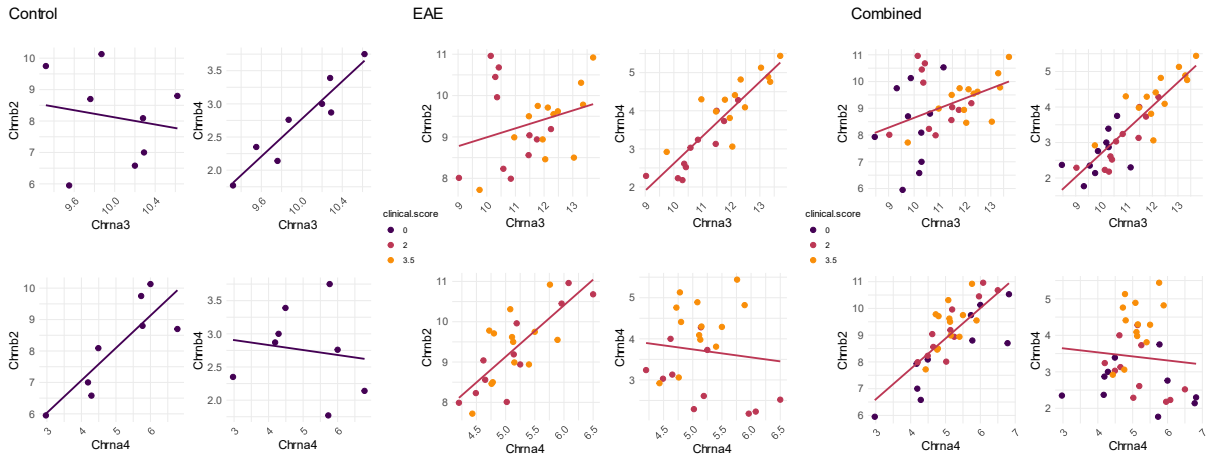

Figure 4.3 Correlated Expression of nicotinic receptor subunits

Correlated expression of subunits α3β2 α3β4 α4β2 and α4β4. Each point represents a pair of MPGs. The correlated expression of subunits does not change from control to EAE for α3β4 α4β2 and α4β4. α3β2 subunits go from being negatively correlated to positive correlation.

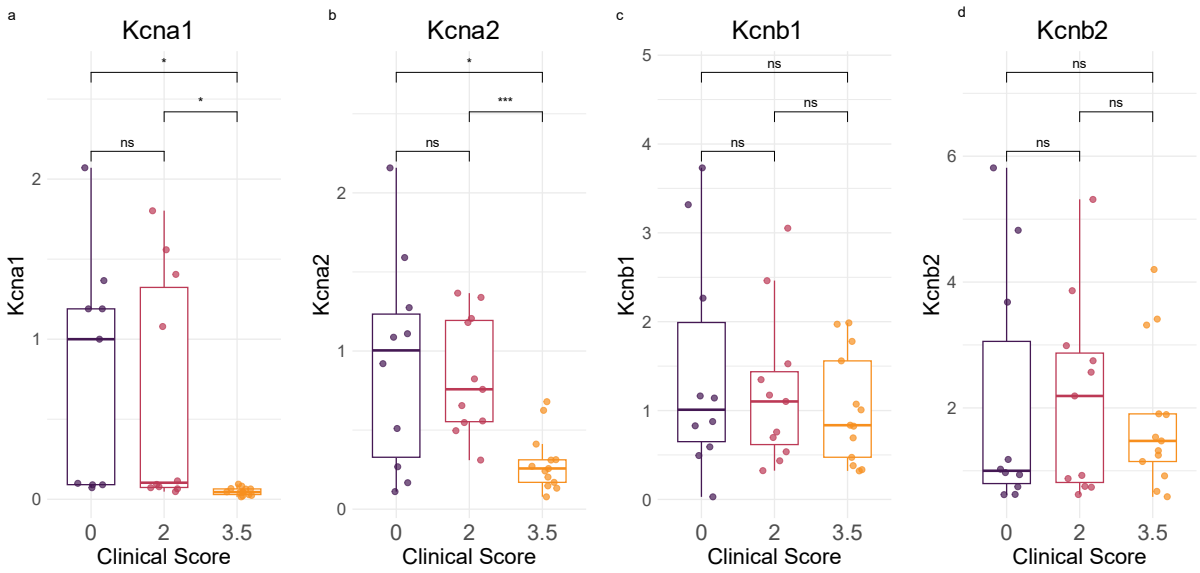

Figure 4.4 Boxplots of voltage-gated potassium channel subunits

Expression of voltage-gated potassium channel subunits in control (Score 0) and EAE mice of scores 2 and 3.5. Each data point represents a pair of major pelvic ganglia (MPGs). Data are shown as median and interquartile ranges. a) There are decreases in expression from score 0 to 3.5 and between score 2 and 3.5 for potassium channel 1.1. b) Expression also decreases from score 0 to 3.5 and 2 to 3.5 for potassium channel 1.2. c&d) no changes in expression for potassium channels 2.1 and 2.2, respectively.

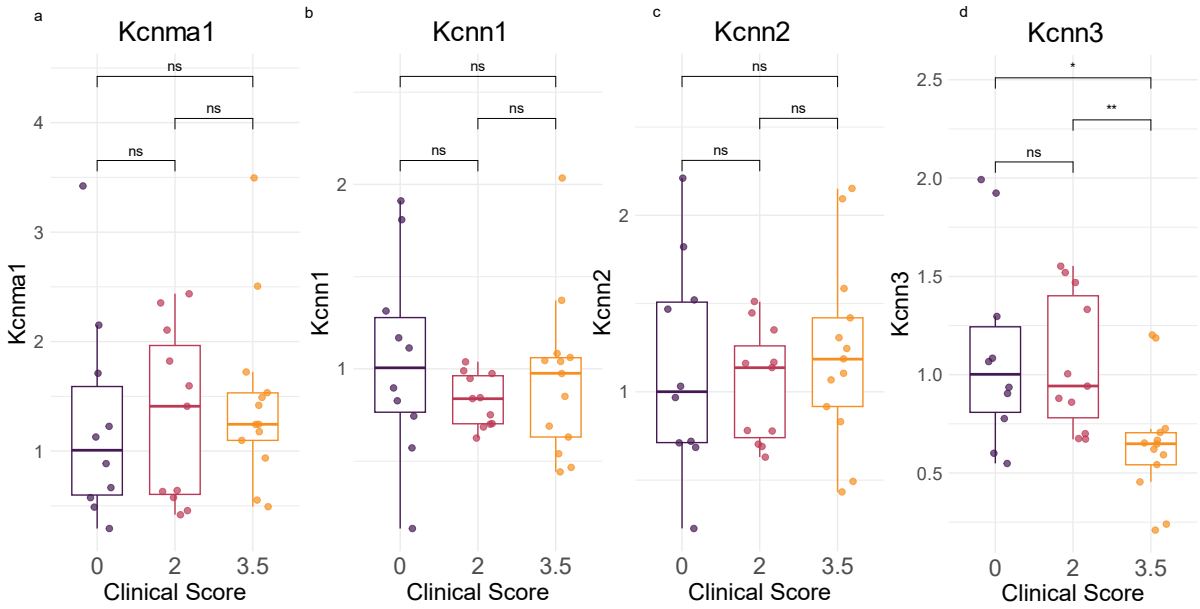

Figure 4.5 Boxplots of calcium-gated potassium channel subunit expression

Expression of calcium-gated potassium channel subunits in control (Score 0) and EAE mice of scores 2 and 3.5. Each data point represents a pair of major pelvic ganglia (MPGs). Data are shown as median and interquartile ranges. a) There is no change to the expression of large conductance potassium channel 1.1. b) There is no change to the expression of small conductance calcium-gated potassium channel 2.1. c) There is no change in the expression of small conductance calcium-activated potassium channel 2.2. D Expression decreases between score 0 and 3.5 and score 2 to 3.5 for small conductance calcium-activated potassium channel 2.3.

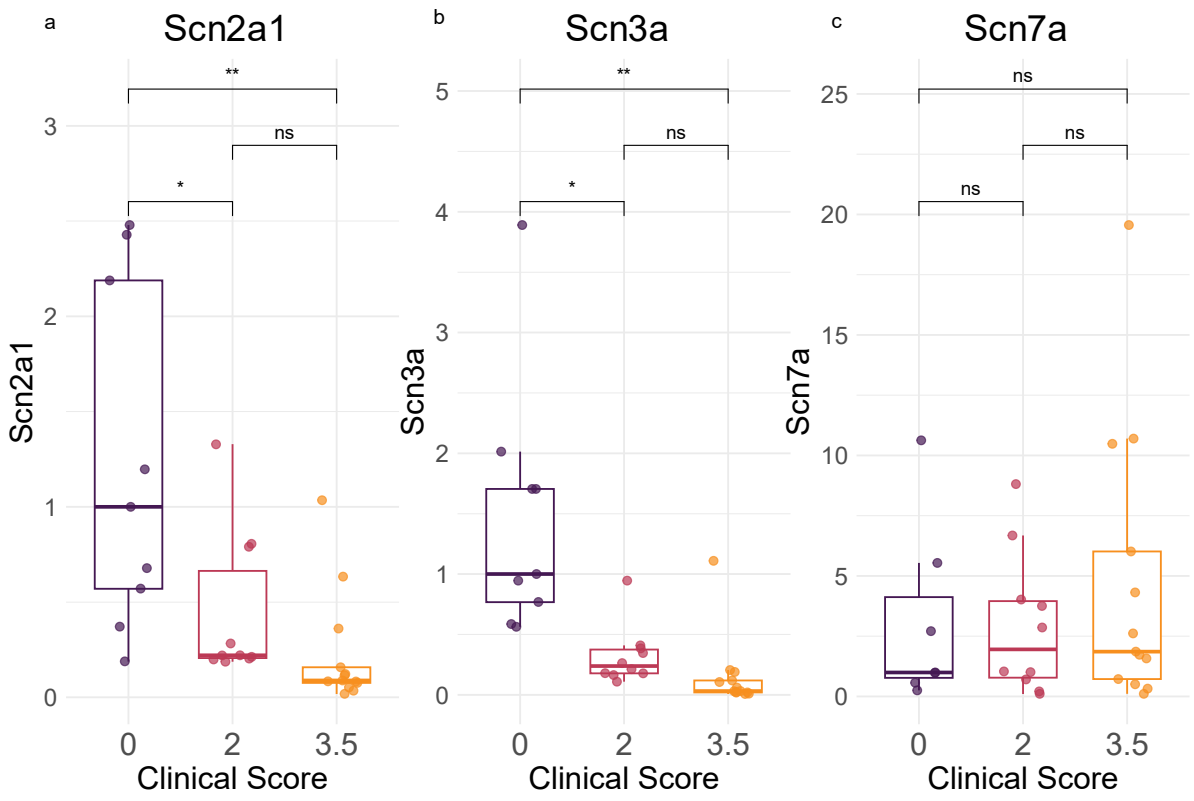

Figure 4.6 Boxplots of voltage-gated sodium channel subunits

Expression of a subset of voltage-gated sodium channel subunits subunits in control (Score 0) and EAE mice of score 2 and 3.5. Each data point represents a pair of major pelvic ganglia (MPGs). Data are shown as median and interquartile ranges. a) Expression of voltage-gated sodium channel type 2 α1 decreases from score 0 to score two and score 0 to score 3.5. b) Expression of voltage-gated sodium channel type 3α decreases from score 0 to 2 and again from score 0 to 3.5. c) There are no changes in expression to voltage-gated sodium channel type 7α.

DISCUSSION

The incidence of lower urinary tract (LUT) dysfunction in multiple sclerosis (MS) patients underscores the importance of understanding the neural control and expression profiles of the urinary system in neurological diseases. While experimental autoimmune encephalomyelitis (EAE) serves as a model for studying MS, the direct impact of EAE on autonomic neurons innervating the LUT remains unexplored. This study aims to elucidate the effects of EAE on major pelvic ganglia (MPG) neurons for the first time, hypothesizing alterations in passive properties, action potential characteristics, gene expression, and overall excitability, with variations corresponding to disease severity. The intricate neural control of the LUT renders it susceptible to dysfunction in various pathological conditions, including spinal cord injury, diabetes, and MS (Gray et al., 2019a; Kyi, 2022). This dysfunction often results from plasticity within neural pathways controlling the LUT, characterized by changes in ion channel and receptor profiles, as well as alterations in neuronal excitability and passive properties. Prior studies in models of diabetes have revealed alterations in passive membrane properties of MPG neurons, suggesting potential changes in morphology and function. However, conflicting findings regarding the passive properties of MPG neurons in diabetes prompted further investigation.

In a previous study, we examine rheobase, negative rheobase, and maximum firing frequency as measures of excitability. While rheobase remains unchanged across disease severity, negative rheobase increases with EAE

severity, indicating augmented excitability in more diseased animals. Additionally, the firing frequency decreases in tonic cells with higher disease scores, suggesting decreased excitability. These findings parallel observations in spinal cord injury (SCI), where alterations in intrinsic and synaptic properties of efferent neurons result in decreased rheobase and increased input resistance, leading to hyperreflexia. Similarly, EAE induces decreases in rheobase, negative rheobase, and maximum firing rate of MPG neurons, indicative of heightened excitability. However, the maximum firing rate may be constrained by changes in action potential characteristics, such as half-width.

Neurogenic bladder, a common complication of multiple sclerosis (MS), arises from damage to the neural pathways controlling bladder function due to MS-related lesions in the central nervous system (CNS). This disruption in communication between the CNS and the bladder manifests as various phenotypes of bladder dysfunction, including overactive bladder (OAB), underactive bladder (UAB), and detrusor sphincter dyssynergia (DSD) (Dorsher & McIntosh, 2012; Kirby Ma Frcs, 1988; Mastri, 1980; Powell, n.d.). Studies utilizing experimental autoimmune encephalomyelitis (EAE), a widely used MS model, have shown that it can reproduce neurogenic bladder symptoms. The hypothesis posited in this study suggests that alterations in the input of autonomic neurons contribute to these symptoms. Previous research has reported increased micturition frequency, basal pressure, and average pressure, along with decreased bladder capacity and micturition pressure in EAE mice. Our previous study contributes additional insights, revealing decreased urinary output, increased

micturition pressure, and voiding frequency. This aligns with findings from previous research conducted by the Schulz laboratory, which highlighted changes in the properties of autonomic neurons controlling the lower urinary tract in other conditions such as spinal cord injury (SCI) and diabetes.

Bladder outlet obstruction (BOO) is a condition characterized by obstruction during voiding, often resulting from poor coordination between the bladder and the external urethral sphincter, known as detrusor-sphincter dyssynergia (DSD). Sensory nerves convey information about bladder wall pressure and pain/temperature to the lumbosacral spinal cord, which then ascends to the midbrain periaqueductal gray region. Feedback from the limbic system and prefrontal cortex influences the midbrain, either promoting bladder storage or initiating micturition (T. Lee et al., 2008; M. G. Park et al., 2010; SUGAYA & DE GROAT, 2009). This study's findings suggest decreased urine output due to coordination issues, leading to increased micturition pressures as the bladder contracts harder against a closed outlet. DSD, characterized by involuntary contraction of the urinary sphincter during detrusor contractions, likely indicates a disruption in the spinobulbospinal tract, resulting in elevated urethral closure pressures. A potential limitation of the study is the use of urethane anesthesia, which may impact voiding patterns in mice. However, the study's findings provide valuable insights into the pathophysiology of neurogenic bladder in MS and highlight the need for further research to understand better and manage bladder dysfunction in MS patients.

The present study investigates molecular changes in major pelvic ganglia (MPG) neurons in experimental autoimmune encephalomyelitis (EAE), a commonly used model for multiple sclerosis (MS). Specifically, it explores alterations in the expression of various ion channels, including calcium channels, nicotinic acetylcholine receptor (nAChR) subunits, voltage-gated potassium channels (Kv), voltage-gated sodium channels, and calcium-activated potassium channels (KCa), aiming to elucidate the pathophysiological mechanisms underlying neurogenic bladder in MS. Here, I will discuss the findings and their implications in the context of neurogenic bladder.

Calcium Channel Expression in EAE: In this study, we measured subunits from P/Q, N, R, and L-type calcium channels. We found that Cacna1c, the L-type calcium channel subunit, decreased in EAE. This contrasts with a previous study by Gray et al. that showed no change to Cacna1c in diabetes. L-type calcium channels are key regulators of neurotransmitter release at presynaptic terminals. The calcium influx triggers the fusion of synaptic vesicles containing neurotransmitters with the presynaptic membrane, facilitating their release into the synaptic cleft and subsequent activation of postsynaptic receptors on the target neuron (Lipscombe et al., 2004). Therefore, in EAE, since L-type calcium channels are downregulated, we expect less synaptic release, resulting in decreased LUT output. This corroborates the results of Chapter 2, where we see decreased urine output in EAE mice as measured by void spot assay. In EAE, it was found that α1B, the pore-forming subunit of N-type calcium channels, was accumulated within axons and axonal spheroids of actively demyelinating lesions

(Kornek et al., 2001). The data from this study suggests that calcium influx could mediate axonal degeneration in EAE. In this study, we do not see an alteration in N-type calcium channel expression in MPG neurons.

Nicotinic Acetylcholine Receptor Subunit Expression in EAE: We assessed the abundance of four distinct nicotinic subunits, recognized as predominant subtypes in autonomic ganglia (Albuquerque et al., 2009; M. Skok, 2022; V. I. Skok, 2002) and acknowledged for their altered expression following spinal cord injury (Kyi, 2022). Our investigation revealed significant decreases in the levels of both CHRNA3 and CHRNB4 with increasing severity of Experimental Autoimmune Encephalomyelitis (EAE), while CHRNA4 and CHRNB2 levels remained unchanged in abundance within the EAE model. Notably, CHRNA3 and CHRNB4 encode the subunits for the alpha-3 beta-4 nicotinic receptor subtype, which is widely recognized as the most prevalent and predominant subunit in many autonomic ganglia (V. I. Skok, 2002). The marked decrease in levels of these subunits implies a reduction in synaptic signaling originating from pre-ganglionic inputs, potentially indicating diminished drive from the spinal cord. This observation aligns with the decreased urine output observed in our EAE animals, as measured by VSA, alongside the documented increases in micturition pressures and intercontractile intervals, all indicative of reduced bladder activity/drive. This contrasts with the changes observed in spinal cord injury, where CHRNA3 expression decreased but was accompanied by an increase in CHRNA4 (Kyi, 2022).

Furthermore, correlations of these subunits did not change as they did in SCI, indicating that there is no remodeling of subunits in EAE. nAChRs are involved in mediating synaptic transmission in both the central and peripheral nervous systems, including autonomic ganglia (Albuquerque et al., 2009). The altered expression of nAChR subunits suggests disruptions in cholinergic signaling pathways, which are essential for regulating bladder function. Dysregulation of cholinergic transmission may lead to aberrant bladder contractions or impaired bladder emptying, contributing to neurogenic bladder symptoms.

Voltage-Gated Potassium Channel and Calcium-Activated Potassium Channel Expression in EAE: The study observed decreases in expression of Kv1.1 and Kv1.2, indicating potential dysregulation of potassium channel activity and dopamine regulation of voiding in EAE. Voltage-gated potassium channels regulate membrane potential and action potential duration, influencing neuronal excitability. Similarly, decreased expression of calcium-activated potassium channel 2.3 suggests altered potassium channel-mediated repolarization mechanisms in EAE. In Chapter 3, we found the decreased halfwidth and decay slopes, which could be attributed to the loss of expression of potassium channels. Dysfunctional potassium channels can disrupt neuronal firing patterns, affecting bladder control circuits and contributing to neurogenic bladder symptoms.

Voltage-Gated Sodium Channel Expression in EAE: The study found decreases in the expression of voltage-gated sodium channel type 2 α1 and type 3α in EAE, indicating potential disruptions in action potential generation and propagation. Previously, we observed decreased halfwidth and rise slopes, which

can be credited to the decreased expression of voltage-gated sodium channels. Voltage-gated sodium channels are critical for initiating and propagating action potentials in neurons. Altered expression of sodium channels may lead to abnormal neuronal excitability and neurotransmission, affecting bladder control mechanisms and contributing to neurogenic bladder symptoms in MS.

Overall, the findings suggest complex alterations in ion channel expression in EAE, which may contribute to the pathophysiology of neurogenic bladder in MS. Dysregulation of calcium, potassium, and sodium channels in autonomic neurons could disrupt bladder control mechanisms, leading to aberrant bladder function observed in MS patients. Understanding the molecular mechanisms underlying neurogenic bladder in MS is crucial for developing targeted therapeutic interventions to alleviate bladder symptoms and improve the quality of life for MS patients. In conclusion, EAE alters the excitability of MPG neurons, akin to changes observed in SCI. These findings shed light on the pathophysiology of LUT dysfunction in neurological diseases, providing insights into potential therapeutic targets for managing urinary symptoms in MS and related conditions.

Chapter 5: Synthesis and Future Directions

In the collected work of this thesis, I have demonstrated that experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, is associated with significant alterations in bladder function and MPG neuron activity. In previous chapters we found mice with EAE exhibit decreased urinary output and greater pressures during voiding, indicating a compromised bladder function. While EAE does not alter the passive properties of MPG neurons, it significantly impacts their excitability. In severe illness, MPG neurons have a lower rheobase, making them more likely to fire action potentials in response to smaller stimuli, although their maximum firing rate is reduced. Changes in action potential properties are evident, with action potentials becoming "wider" due to decreased rise and decay slopes, which limits the rate of action potential firing. Molecularly we see decreases in voltage-gated sodium and potassium channel expression which could contribute to the widening of the action potential. Fewer channels decreases the density of currents contributing to the rise (sodium) and decay (potassium) slopes of the action potential. Additionally, EAE is associated with decreased expression of major autonomic nicotinic acetylcholine receptors at the MPG and increased purinergic receptor mRNAs at the bladder, indicating a shift in neurotransmitter profiles. This reduced density of acetylcholine currents may decrease the drive to the bladder, while the altered receptor profiles suggest neurogenic mechanisms contributing to bladder dysfunction. As such, increased excitability of MPG neurons coupled with a lower firing frequency could lead to an overactive bladder, characterized by increased contractility, and incomplete

voiding, resulting in decreased void volumes and fewer voiding episodes but higher micturition volumes.

Multiple Sclerosis (MS) is a complex neurological condition affecting the brain and spinal cord, marked by symptoms like muscle weakness, sensory issues, and urinary problems. Among these, lower urinary tract dysfunction is prevalent, affecting a significant majority of MS patients. While bladder issues in MS are linked to damage in the central nervous system, peripheral nerve damage is relatively rare. However, these bladder problems are associated with reduced quality of life and frequent hospitalizations due to complications like infections. Understanding these bladder deficits, particularly those stemming from spinal cord injury-related causes, is crucial. Research from the Schulz laboratory suggests alterations in the activity of autonomic efferent neurons controlling the bladder in rodents, leading to decreased bladder function in MS. This study aims to investigate how changes in the spinal cord affect the input to these neurons, ultimately contributing to neurogenic lower urinary tract dysfunction.

Bladder dysfunction affects over 90% of Multiple Sclerosis (MS) patients as the disease progresses, leading to issues like incontinence and urinary retention. These complications are common among neurological manifestations of MS, with prevalent symptoms including post-micturition dribble, urinary urgency, and feeling of incomplete emptying. Such symptoms likely stem from various underlying bladder pathologies, such as detrusor overactivity, bladder hypo-contractility, and detrusor sphincter dyssynergia. Understanding these peripheral impacts of MS is crucial due to their significant impact on patients' quality of life. Despite

advancements in urodynamic measures, the mechanistic effects of MS on lower urinary tract neurons remain poorly understood. Similar conditions like spinal cord injury and diabetic neuropathy affect bladder-innervating neurons, highlighting the importance of investigating these neural mechanisms in MS. This proposal aims to correlate spinal cord or brainstem lesions with motor and bladder dysfunction to understand how central demyelination affects bladder-innervating neurons. We will utilize two mouse models of experimental autoimmune encephalomyelitis (EAE), which is known to cause different CNS lesions and bladder function impacts, to achieve this goal. Through this research, we aim to enhance collaboration between investigators and generate preliminary data for future externally funded projects. Leveraging this research, we plan to secure funding from organizations like the National Institutes of Health, the National Multiple Sclerosis Society, and the Department of Defense Multiple Sclerosis Research Program. Our collaborative efforts and diverse skill sets will ensure high-quality research with the potential to make significant contributions to understanding and managing MS-related bladder dysfunction.

Specific Aims: Bladder dysfunction is a prevalent issue affecting a majority of Multiple Sclerosis (MS) patients, significantly impacting their quality of life. The underlying neural mechanisms linking central demyelination in MS to bladder dysfunction remain poorly understood. This proposal aims to investigate the correlation between spinal cord or brainstem lesions and motor and bladder dysfunction to elucidate the effects of MS on bladder-innervating neurons.

Aim 1: Characterize Central Demyelination Effects on Bladder-Innervating Neurons

- Investigate the impact of spinal cord and brainstem lesions on lower urinary tract neurons in mouse models of Experimental Autoimmune Encephalomyelitis (EAE).

- Utilize electrophysiological methods to determine the changes in bladder-innervating neurons following central demyelination.

- Compare the neural alterations in bladder-innervating neurons between EAE mouse models with distinct CNS lesions.

Aim 2: Examine Functional Consequences of Neural Alterations on Bladder Function

- Evaluate the functional consequences of central demyelination on bladder function in EAE mouse models.

- Utilize urodynamic measures to assess bladder function parameters, including detrusor overactivity, bladder hypo-contractility, and detrusor sphincter dyssynergia.

- Correlate the observed bladder dysfunction with the extent of central demyelination and neural alterations in bladder-innervating neurons.

This project aims to provide valuable insights into the neural mechanisms underlying MS-related bladder dysfunction, paving the way for improved understanding and management of this common complication in MS patients. Additionally, by establishing collaborative efforts and leveraging diverse skill sets, this research has the potential to yield substantial contributions to the field of MS research and enhance patient care outcomes. The proposed research represents a significant step forward in understanding the neural mechanisms underlying bladder dysfunction in Multiple Sclerosis (MS). As we move forward, it is imperative to identify future directions that can expand upon the findings of this study and address remaining gaps in knowledge regarding MS-related bladder dysfunction. The following future directions outline potential avenues for further research and development in this field:

1. Investigation of Peripheral Nerve Pathology

While the proposed research primarily focuses on the impact of central demyelination on bladder-innervating neurons, future studies should also explore the role of peripheral nerve pathology in MS-related bladder dysfunction. Investigating the integrity of peripheral nerves innervating the bladder, including the pelvic nerves and pudendal nerves, could provide valuable insights into the mechanisms underlying bladder dysfunction in MS. Techniques such as nerve conduction studies, histological examination of nerve fibers, and molecular profiling of peripheral nerves could help elucidate the extent of peripheral nerve damage and its contribution to bladder dysfunction in MS. Subtle peripheral abnormalities in MS have been suggested through additional neurophysiological

techniques such as reduced supernormality (Boërio et al., 2007) and prolonged refractory periods (Hopf & Eysholdt, 1978), indicating that mild peripheral nerve demyelination may be more prevalent in MS than previously believed, often remaining subclinical.

The development of multiple excitability measurements using threshold tracking has provided insights into axonal biophysical properties (Burke et al., 2001). Demyelination exposes paranodal and internodal axonal membranes, leading to alterations in nodal Na+ and nodal/internodal K+ currents (Nodera et al., n.d.; Sung et al., 2004). Misawa et al. provide nerve conduction studies and multiple excitability measurements systematically in a consecutive series of MS patients to determine the frequency of association between MS and demyelinating neuropathy and to assess changes in peripheral axonal excitability in MS. Their findings indicated that 5% of 60 consecutive MS patients exhibited clear signs of demyelinating polyneuropathy. In contrast, excitability testing showed no abnormalities in these MS patients. These results imply that central and peripheral demyelination coexist only in a distinct subgroup of MS patients. Additionally, it was observed that MS patients with peripheral demyelination manifest multifocally in the intermediate nerve trunk segments, potentially linked to the breakdown of the blood–nerve barrier. This process may share a common pathophysiology with demyelination observed in MS.

2. Exploration of Non-neuronal Contributions

In addition to neuronal dysfunction, MS-related bladder dysfunction may also involve non-neuronal factors such as alterations in the bladder epithelium,

inflammation, and changes in the urinary microbiome (McCafferty et al., 2008; McCombe et al., 2009). Future research should investigate the contributions of these non-neuronal factors to bladder dysfunction in MS. Studies examining the integrity of the bladder epithelium, the presence of inflammatory markers in the bladder tissue, and the composition of the urinary microbiome in MS patients could provide valuable insights into the multifactorial nature of bladder dysfunction in MS. Some of this work was conducted by a promising undergraduate student, Cameron Duello, who investigated the expression of bladder purinergic receptors. The functional purinergic receptors on bladder smooth-muscle cells that mediate contraction are either P2X1/P2X2 heteromers or P2X1, P2X2, and P2X4 homomers. Purinergic signaling, mediated by the release and action of purines such as adenosine triphosphate (ATP) and its derivatives, plays a critical role in both the normal function and pathology of the lower urinary tract (LUT). This signaling system involves the activation of purinergic receptors located on various cell types within the urinary system, including smooth muscle cells, urothelial cells, nerve terminals, and interstitial cells.

In normal function of the lower urinary tract, purinergic signaling contributes to several physiological processes that are essential for normal urinary function. During bladder filling and emptying, ATP released from urothelial cells during bladder filling acts as a signaling molecule, promoting relaxation of the detrusor smooth muscle through activation of purinergic receptors (P2X and P2Y receptors) located on smooth muscle cells. This facilitates bladder filling and storage of urine. Within the micturition reflex during the voiding phase, ATP release from bladder

urothelial cells and nerve terminals activates purinergic receptors on sensory nerves, triggering the micturition reflex. This reflex involves the coordinated contraction of the detrusor muscle and relaxation of the urethral sphincters, leading to urine expulsion. In Neurotransmission, purinergic signaling modulates neurotransmission within the autonomic nervous system, influencing the excitability of bladder sensory and motor neurons. ATP acts as a co-transmitter with other neurotransmitters, such as acetylcholine and noradrenaline, regulating synaptic transmission at nerve terminals in the bladder.

In pathological conditions of the LUT, like overactive bladder (OAB) and bladder outlet obstruction (BOO), purinergic signaling has been implicated. Dysregulated purinergic signaling has been implicated in the pathophysiology of OAB, a condition characterized by urinary urgency, frequency, and nocturia. Excessive ATP release from urothelial cells or nerve terminals, coupled with alterations in purinergic receptor expression or function, may lead to bladder overactivity and involuntary contractions. Purinergic signaling contributes to the adaptive responses of the bladder to obstruction, such as hypertrophy and hyperactivity. ATP release from stretched urothelial cells and increased purinergic receptor activation in response to elevated intravesical pressure may contribute to detrusor overactivity and impaired bladder emptying in BOO. We found that EAE decreases the expression of p2x2 and p2y1 receptors (figure 1b and d).

Targeting purinergic signaling pathways represents a promising therapeutic strategy for managing lower urinary tract disorders. Pharmacological agents that modulate purinergic receptor activity, ATP release, or degradation have shown

efficacy in preclinical and clinical studies for treating OAB, IC/BPS, and other bladder dysfunctions. For example, purinergic receptor antagonists or modulators may help alleviate bladder overactivity and reduce urinary symptoms by blocking excessive ATP-mediated signaling. In summary, purinergic signaling plays a multifaceted role in regulating normal urinary function and contributing to the pathophysiology of various lower urinary tract disorders. Understanding the intricacies of purinergic signaling pathways in health and disease may lead to the development of novel therapeutic interventions for managing bladder dysfunction and improving patient outcomes.

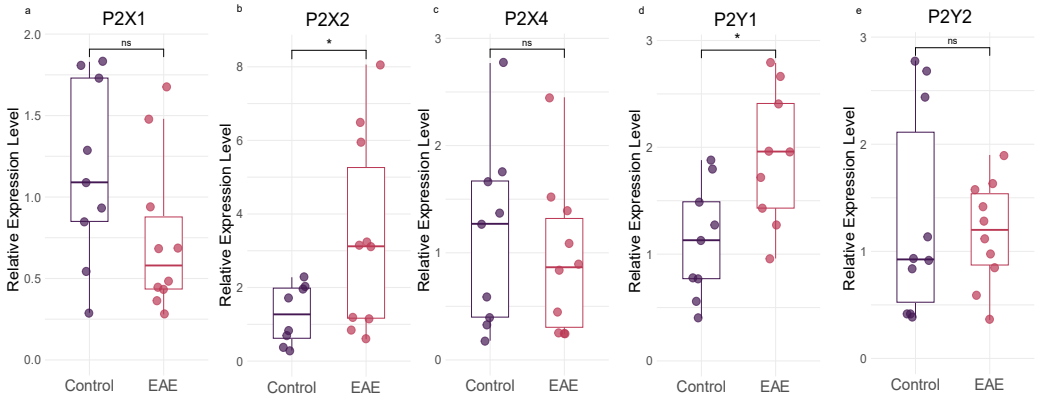

Figure 5.1 Boxplots of purinergic receptor expression

Expression of purinergic receptors in control and EAE mice. Each data point represents a pair of major pelvic ganglia (MPGs). Data are shown as median and interquartile ranges.

3. Identification of Biomarkers

The development of biomarkers for MS-related bladder dysfunction could facilitate early diagnosis, monitoring of disease progression, and evaluation of treatment efficacy. Future research should focus on identifying biomarkers, including molecular markers in urine or blood samples, neuroimaging markers, and electrophysiological markers, that are indicative of bladder dysfunction in MS (Cata et al., 2011; Cho & Kim, 2013; Fry et al., 2014; Khalil et al., 2018; Yu et al., 2023). Large-scale multicenter studies involving diverse cohorts of MS patients could help validate potential biomarkers and establish their clinical utility in the management of MS-related bladder dysfunction.

1. **Neurological Biomarkers:**

    - **Lesion Load and Localization:** Magnetic resonance imaging (MRI) can identify demyelinating lesions in the CNS, including areas involved in the neural control of the bladder, such as the brainstem, spinal cord, and periventricular regions. Lesion load and localization may correlate with the severity and type of LUTD symptoms in MS patients.
    - **Evoked Potentials:** Neurophysiological assessments such as urodynamic studies and somatosensory evoked potentials can evaluate the integrity of sensory and motor pathways involved in bladder control. Abnormalities in these evoked potentials may

indicate dysfunction within the neural circuitry regulating micturition and bladder function.

2. **Biomarkers of Inflammation and Neurodegeneration:**

    - **Cytokines and Chemokines:** Elevated levels of pro-inflammatory cytokines (e.g., interleukin-6, tumor necrosis factor-alpha) and chemokines in the CNS and peripheral circulation have been associated with neuroinflammation and disease progression in MS. These inflammatory mediators may contribute to neuronal damage and dysfunction in bladder control centers.

    - **Neurofilaments:** Increased levels of neurofilament proteins, which are released upon axonal injury and degeneration, have been detected in the cerebrospinal fluid (CSF) and blood of MS patients. Elevated neurofilament levels may reflect ongoing neurodegeneration within the CNS, including regions involved in bladder control.

3. **Biomarkers of Urothelial Dysfunction:**

    - **Urinary Biomarkers:** Analysis of urinary biomarkers such as cytokines, chemokines, and neurotrophic factors may provide insights into urothelial inflammation, barrier dysfunction, and sensory nerve activation in MS-related LUTD. Alterations in urinary biomarker profiles may correlate with the severity and progression of bladder symptoms in MS patients.

- **Urothelial Gene Expression:** Assessment of gene expression patterns in bladder urothelial cells may identify molecular signatures associated with urothelial dysfunction and neurogenic bladder in MS. Dysregulated expression of genes involved in inflammation, ion channel function, and sensory signaling pathways may contribute to bladder dysfunction in MS.

4. **Clinical Biomarkers:**

- **Bladder Function Tests:** Objective assessments of bladder function, such as urodynamic studies, voiding diaries, and symptom questionnaires, serve as important clinical biomarkers for evaluating LUTD severity and progression in MS patients. These tests provide quantitative measures of bladder storage and voiding function, detrusor overactivity, and urinary incontinence.

- **Biomarkers of Bladder Damage:** Biomarkers indicative of bladder wall integrity, such as urinary levels of bladder-specific proteins (e.g., uroplakins), may reflect urothelial damage and barrier dysfunction in MS-related LUTD. Detection of these biomarkers could aid in the early diagnosis and monitoring of bladder complications in MS patients.

4. Personalized Therapeutic Approaches

MS is a heterogeneous disease with considerable variability in disease course and symptomatology among patients. Future research should focus on developing personalized therapeutic approaches for managing bladder dysfunction in MS. This may involve stratifying patients based on clinical and molecular characteristics, such as disease subtype, severity of central demyelination, and presence of peripheral nerve pathology, to tailor treatment strategies to individual patient needs. Integrating patient-reported outcomes and quality-of-life measures into clinical trials could help assess the effectiveness of personalized therapeutic approaches in improving bladder function and overall well-being in MS patients.

5. Collaboration and Multidisciplinary Research

Collaboration between researchers from diverse disciplines, including neurology, urology, immunology, and molecular biology, is essential for advancing our understanding of MS-related bladder dysfunction. Future research should foster interdisciplinary collaborations to leverage complementary expertise and resources, accelerate progress, and maximize the impact of research efforts. Establishing consortia and research networks dedicated to studying MS-related bladder dysfunction could facilitate data sharing, standardization of methodologies, and the development of consensus guidelines for diagnosis and treatment.

Conclusions

The future directions outlined above offer promising opportunities for advancing our understanding and management of MS-related bladder dysfunction. By investigating peripheral nerve pathology, exploring non-neuronal contributions, conducting longitudinal studies in human patients, identifying biomarkers, developing personalized therapeutic approaches, and promoting collaboration and multidisciplinary research, we can make significant strides toward improving the quality of life for MS patients affected by bladder dysfunction. Continued research in this area holds the potential to transform clinical care and enhance outcomes for individuals living with MS.

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Vita

I was born in St. Louis, Missouri and raised in Jefferson city, Missouri. In 2009 I went to Xavier University of Louisiana to study Biology and Chemistry. At Xavier, I developed a fascination with the field of neurosciences and attained a position working at Tulane in the lab of Dr. Dohanich studying the effects of pregnancy, lactation, and motherhood on spatial memory. During my undergraduate career, I also spent two summers working with Dr. Meisel at the University of Minnesota studying the nucleus accumbens and striatum in addiction. I started my graduate career at the University of Missouri-Columbia in 2016 with a focus on spinal cord injury and Multiple Sclerosis. I plan to become a postdoctoral fellow and enter Academia.