NEURONAL MORPHOLOGY AND PLASTICITY IN THE *SCN1B* MOUSE MODEL OF DRAVET SYNDROME

Presented by Alisha Arnaz Ahmed

In partial fulfillment of the requirements for graduation with the Dean's Scholars Honors Degree in Neuroscience

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Dr. MacKenzie Howard
Supervising Professor

Date

_____ _____

Dr. George Pollak
Honors Advisor in Neuroscience

Date

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Department: Neuroscience

Alisha Ahmed

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Signature Date

Dr. MacKenzie Howard

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I would like to thank a number of individuals without whom this research, and my growth as a scientist, would not have been possible. First, I would like to thank Dr. MacKenzie Howard, for supervising me in this project, and for making me feel welcome in the Howard Lab as soon as I walked in. Dr. Howard has provided me with invaluable mentorship in exciting me about neuroscience, helping me with PhD applications, and pushing me to pursue new opportunities. Next, I would like to thank Dr. Jessica Chancey, who not only supervised me on this research, but has also made sure I have all of the skills needed to further my goals in graduate school. Dr. Chancey's mentorship has taught me how to be rigorous and balanced in my science, and has motivated me to become an independent scientist. I would also like to give a special thanks to both Dr. Howard and Dr. Chancey for constantly going back and forth on drafts of this thesis with me, and for teaching me the wonders of scientific writing and communication. Next, I would like to thank all members of the Brumback and Howard Labs, who have provided a safe space for me in science (as well as delicious snacks in weekly lab meetings). I would also like to thank the Plan II Honors Program, the Dean's Scholars Honors Program, and the UT Neuroscience Department for providing me with communities to tackle college with. Most importantly, none of this work would have been possible without guidance from my family and friends. Thank you to Ammu, Abbu, Arushi, and all of my friends who have motivated and inspired me to get where I am today, and have constantly supported me in my dreams of being a neuroscientist.

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Abstract

In order to selectively process their inputs and make outputs to other neurons, a neuron's physiology is tightly regulated by a variety of factors, including morphology and synaptic plasticity. The morphology, or structure, of a neuron can determine its intrinsic properties. Synaptic plasticity determines a neuron's ability to strengthen and weaken in response to inputs, influencing its effects in learning and memory. When any of these processes are impaired, it can cause severe neuronal dysfunction and broader cognitive deficits, leading to neurological disease. Our lab is interested in Dravet syndrome (DS), which is a severe genetic epilepsy that leads to prolonged seizures, developmental delays, and severe cognitive deficits. Mutations in the *SCN1B* gene, which encodes the protein β1, have been linked to DS. We use an *Scn1b* knockout mouse model, which models many aspects of DS including spontaneous seizures and early death, to study the neurophysiological changes underlying the disease. β1 is important in regulating neuronal excitability and physiology, and has also been shown to play a role in neuron growth and development. We have found that loss of β1 leads to abnormal intrinsic and synaptic physiology in one of the primary neuron types responsible for learning and memory: hippocampal pyramidal neurons. My research aims to examine how loss of β1 affects both neuronal morphology and synaptic plasticity, the selective strengthening (long-term potentiation) or weakening (long-term depression) of neuronal synapses, in hippocampal pyramidal neurons. Our lab has found that hippocampal pyramidal neurons are hyperexcitable, motivating me to hypothesize that dendritic arborization and spine density would be altered in *Scn1b* knockouts, because neuronal structure is tightly linked to neuronal physiology as it largely affects intrinsic properties of neurons. Our lab has additionally found that long-term potentiation is lost in *Scn1b* knockouts. This finding suggests that these knockout neurons may have reached an upper limit of plasticity, leading me to

hypothesize that long-term depression is increased in *Scn1b* knockout mice. To examine neuronal morphology, I analyzed dendritic branching patterns and spine density in *Scn1b* knockout neurons, and found no overall changes in morphology. To examine long-term depression, I analyzed local field potential recordings using a low-frequency stimulus, and found no difference in long-term depression between knockouts and wildtype littermates. My findings illustrate that the links between neuronal physiology, morphology, and plasticity are altered atypically in β1-deficient hippocampal neurons, suggesting a complex role for β1 in this brain region.

Background

The Basics of Neuron Anatomy and Physiology

Forming tens of thousands of connections with one another, our brains' neurons process information through complex integration of the electrical signals evoked at their synapses along their dendrites. Most neurons receive a collection of both excitatory and inhibitory synaptic inputs. Excitatory inputs change the voltage across the neuron's membrane by moving it closer to the voltage threshold for firing an action potential, while inhibitory synaptic inputs reduce the probability of the neuron firing. This integration of many different inputs requires the coordination of multiple aspects of a neuron's function, including passive membrane properties (its properties at rest) and active properties (for instance, when the neuron is driven to fire an action potential). Factors such as the ionic and molecular signaling occurring at synapses, the ion channel composition of a neuron's membrane, and the size and shape of the cell influence the neuron's excitability, or the ability of the neuron to respond to its inputs and create an output in the form of an action potential. Determining the relationships between these factors, neuronal excitability, and resulting circuit function is critical to our understanding of cognitive function.

The hippocampal CA1 pyramidal neuron

My research is focused on hippocampal CA1 pyramidal neurons (PNs), which are one of the primary neuron types responsible for neural processing, memory formation, and memory recall (Ishikawa et al. 2016). These neurons are distinctive in their physiology and morphology, with tens of thousands of synapses innervating long, branching apical dendrites and shorter basal dendrites (Figure 1). PNs are optimized for storing memories and filtering information, as they physically segregate inputs from various upstream brain areas to different types of dendrites or different locations along a dendrite. For example, hippocampal CA1 PNs receive inputs from CA2 onto their basal dendrites, from CA3 onto their proximal apical dendrites, and from the entorhinal cortex onto distal apical dendrites (Masurkar 2018), as seen in Figure 1. These types of dendrites can have fundamentally different physiological properties despite having similar structures, resulting in a complex integration of inputs within a single pyramidal neuron (Brzdak et al. 2019).

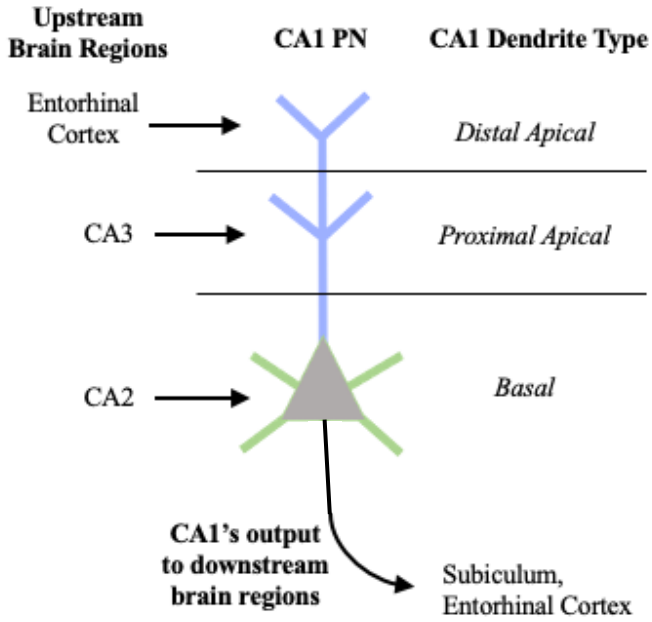

Figure 1: Schematic depicting structure, inputs, and outputs of hippocampal CA1 PN.

The influence of morphology on neuronal signaling

The CA1 PN's ability to integrate its many inputs depends largely on its morphology, or cellular structure. Each component of the neuron's structure can be represented as a functional unit with its own properties that affect the voltage (Chen et al. 2011). For example, each dendrite can be considered as a singular compartment that propagates voltage across the neuron's membrane uniquely. The lower the membrane resistance is, the more likely it is that an electrical signal will leak out of the membrane. The more membrane present along the neuron, the larger the membrane capacitance is, which causes the electrical signal to degrade in strength and speed. The resulting signal that reaches the cell body is thus influenced by the membrane properties of the dendrite it has travelled through, including its membrane resistance and capacitance. Changes in these properties in the dendrites or in the cell body can affect the neuron's overall excitability, as these intrinsic properties play a large role in regulating the neuron's membrane voltage. Similarly, the structure of a neuron's spines, or the compartments at which many synapses occur, greatly influence the voltage change when synapses on that spine are activated (Acker et al. 2016). The number of spines is correlated with the number of incoming excitatory synaptic connections. Spines are additionally critical for isolating synaptic transmission at synapses, as the neck resistance of each spine amplifies the input and allows pre and post-synaptic activity to remain local at the synapse (Yuste 2013).

The mechanisms of synaptic plasticity

A neuron's ability to learn and make memories is dependent upon its capacity to change and reorganize its synaptic connections through plasticity. Synaptic plasticity is the selective strengthening or weakening of synapses to prioritize specific inputs for memory storage and information processing. Interplay between synaptic inputs and the neuron's response causes

plasticity in either direction; cellular memory occurs as synaptic plasticity makes the connections between neurons stronger (potentiation) or weaker (depression). The canonical NMDAR-dependent forms of synaptic potentiation are dependent upon calcium (Ca^{2+}) and Calcium/Calmodulin-dependent Kinase II (CaMKII) activity (Lisman et al. 2012). During long-term potentiation (LTP), the synapse is activated by strong depolarization, high amounts of Ca^{2+} enter the neuron, and the CaMKII enzyme complex is activated and phosphorylates several postsynaptic proteins, resulting in elevated postsynaptic AMPA receptor currents. Increased AMPA receptor currents result in an increased neural response, essentially meaning the synapse has strengthened. Synapses also undergo canonical NMDAR-dependent long-term depression (LTD), which is dependent upon calcium and calcineurin activity. LTD induction requires repetitive synaptic activation that allows for low amounts of Ca^{2+} enter the neuron, The Ca^{2+} activates calcineurin, which dephosphorylates several postsynaptic proteins, resulting in reduced AMPA receptor currents. There is a decreased neural response, meaning the synapse has weakened (Morishita et al. 2005).

The mechanisms of LTP and LTD are triggered by specific patterns of activity between the presynaptic neuron and postsynaptic neuron. A strong presynaptic signal, such as a high frequency stimulus or theta burst stimulus, leads to strong depolarization and larger amounts of calcium entering the cell, triggering LTP. A weaker presynaptic signal, such as a low frequency stimulation, leads to lower amounts of calcium entering the neuron, triggering LTD (Christie et al. 1994). As such, synaptic plasticity requires a balance between input patterns, molecular signaling at synapses, and intrinsic properties of both presynaptic and postsynaptic neurons.

NMDAR-dependent plasticity is believed to be the cellular basis for memory encoding in hippocampal CA1 PNs. LTP and LTD have been studied extensively in the hippocampus, and

have shown to be important in spatial memory tasks in both rodents and humans, with multiple studies showing that disrupting plasticity interferes with memory tasks (Stevens et al. 1998, Martin et al. 2005, Pastalk et al. 2006). As such, determining the role of long-term synaptic plasticity in neurological disease can elucidate the cellular mechanisms underlying cognitive deficits associated with them.

What happens when things go awry?

When changes to any of a neuron's properties lead to hyperexcitability, or increased excitability, it can lead to severe neural dysfunction that manifests in neurological disease. This can occur due to a change in the balance between excitation and inhibition (Ben-Ari et al. 2012). Changes in neuron morphology and/or plasticity can also influence a neuron's excitability. For example, dendritic degeneration has been shown to be linked to hyperexcitability in Alzheimer's disease (Siskova et al. 2014). Additionally, impairments in both neuron morphological development (Bauman & Kemper 1985) as well as in synaptic plasticity (Bourgeron 2009) have been shown to be linked to Autism Spectrum Disease. Most notably, studies on a number of genetic epilepsies have shown links between abnormal dendritic morphology and epileptogenesis (Ma et al. 2013, Reid et al. 2014).

Dravet syndrome

Dravet syndrome (DS) is a genetic epileptic encephalopathy characterized by prolonged seizures, severe cognitive deficits, and developmental delays (Dravet 2011). DS is known to be linked to two genes, *SCN1A* and *SCN1B* (Marini et al. 2011). Most cases of DS are associated with loss of function mutations in *SCN1A*, which encodes the voltage-gated sodium channel $Na_v1.1$ (Zuberi et al. 2011). Mutations in *SCN1A* leads a reduction in the amount of $Na_v1.1$, which is more highly expressed in inhibitory interneurons in the brain (Yu et al. 2006). This leads to reduced

excitability of inhibitory cells and network hyperexcitability, resulting in epilepsy (Escayg et al. 2000, Patino et al. 2009, Ogiwara et al. 2012, Catterall et al. 2010). Mutations in *SCN1B* have also been linked to Dravet syndrome (Brackenbury & Isom 2011, Patino et al. 2009, Ogiwara et al. 2012). The focus of my work is to determine the cellular and circuit mechanisms by which this dysfunction occurs after disruption of the *SCN1B* gene.

The β1 protein

The *SCN1B* gene encodes the protein β1 (Isom et al. 1992, Brackenbury et al. 2008), which is critical for regulating a number of different cellular functions. β1 was first discovered to be an ion channel auxiliary subunit (Isom et al. 1992), regulating the subcellular localization and physiology of a number of different voltage-gated ion channels important for dendritic excitability, action potential initiation, and synaptic plasticity (Reid et al. 2014, Brackenbury & Isom, 2011). β1 subunits have specifically been shown to associate with sodium channels $Na_v1.1$, $Na_v1.2$, and $Na_v1.3$, and $K_v4.2$ potassium channels, and are expressed throughout the brain (Marionneau et al. 2012, Hull et al. 2020). β1 also has principal roles in regulating neuron development and neurite outgrowth (Brackenbury et al. 2008), as well as in cell adhesion (Chioni et al. 2009, Hull & Isom, 2017). β1 is highly expressed in excitatory neurons, such as in hippocampal pyramidal neurons.

The role of β1 in hippocampal pyramidal neurons

It is critical to determine how loss of β1 affects the physiology of CA1 PNs to understand how it may contribute to the complex phenotype of DS. It is possible that altered function of hippocampal pyramidal neurons underlies the deficits in learning and memory of DS. As such, researching changes in morphology and plasticity that result from mutations in *SCN1B* can shed light on the specific roles β1 has in regulating neuron function, allowing us to better understand the cellular mechanisms of diseases like DS. Additionally, looking specifically at pyramidal

neuron morphology and plasticity can tell us about structure function relationships within single neurons, providing insight on fundamental aspects of learning and memory.

Research Aims

To study the neurophysiological dysfunction underlying DS, our lab uses a *Scn1b* knockout (KO) mouse model. Like DS patients, *Scn1b* KO mice exhibit spontaneous seizures, ataxia, and early death (Chen et al. 2004, Lopez-Santiago et al. 2007), making this a valid model of DS. The goal of my work is to determine what structural and physiological changes are associated with loss of β1 at the level of an individual neuron. Specifically, my research aims to examine how loss of β1 affects aspects of both neuronal morphology and synaptic plasticity in hippocampal CA1 PNs.

1. How does loss of β1 affect neuron morphology?

Previous work in an *Scn1b* mutant DS model found that subicular pyramidal neurons lacking β1 exhibit increased excitability and decreased dendritic arborization (Reid et al. 2014), or a decrease in the complexity of their dendrites' branching patterns. Our lab has investigated intrinsic and synaptic properties of CA1 pyramidal neurons and found increased intrinsic excitability and action potential firing (Figure 2, adapted from Chancey et al. 2020). Figure 2A shows example responses to current steps from WT and KO slices. Figure 2B shows that KO PNs fire more spikes in response to current injections compared to WTs. Our lab has additionally found reduced capacitance, and increased synaptic integration in KO PNs (Chancey et al. 2020). These results motivated me to examine dendritic arborization and spine density to determine if β1 plays a role in regulating neuronal morphology in hippocampal CA1 PNs. I hypothesized that dendritic arborization would be reduced in *Scn1b* knockout pyramidal neurons, and that spine density would be increased in knockouts.

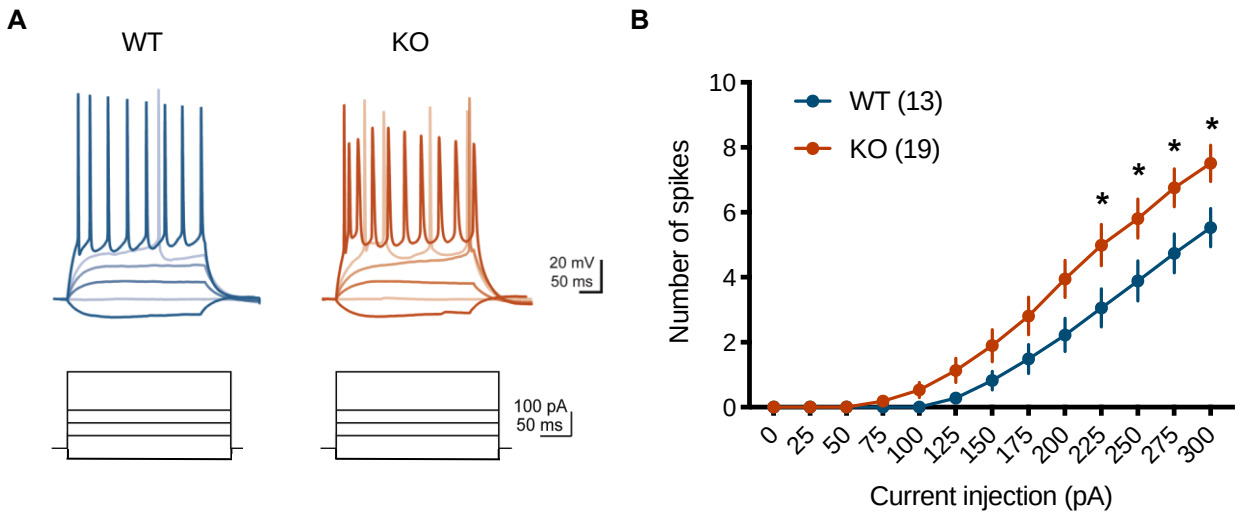

Figure 2: A) Example 250 ms current steps (-50, 0, 50, 100, 150, 300 pA) in CA1 PNs from Scn1b+/+ (WT; blue) and Scn1b-/- (KO; orange). B) Increased firing rate in KOs compared to littermate WT controls (main effect of current injection: $p < 0.001$; genotype: $p < 0.05$; interaction: $p < 0.001$; Repeated measures (RM) ANOVA. *$p < 0.05$, post-hoc Sidak's multiple comparisons test.

2. How does loss of β1 affect synaptic plasticity?

In addition to changes in intrinsic and synaptic properties, our lab has found changes in synaptic plasticity in Scn1b KO mice. We have seen a loss of LTP in *Scn1b* knockouts (Figure 3, adapted from McConnell et al. 2019), quantified as the change in normalized fEPSP (field excitatory post-synaptic potential) slope after an LTP-inducing stimulus, in wild-type slices compared to KOs, as shown in Figure 3B. The increased intrinsic excitability and loss of LTP in *Scn1b* knockouts lead to the hypothesis that this circuit is highly potentiated and could not be potentiated any further, and that this highly potentiated circuit would likely undergo a larger amplitude of synaptic depression. To test this we performed LTD experiments to determine if *Scn1b* knockouts exhibited global dysfunction of plasticity or only specific to particular input patterns.

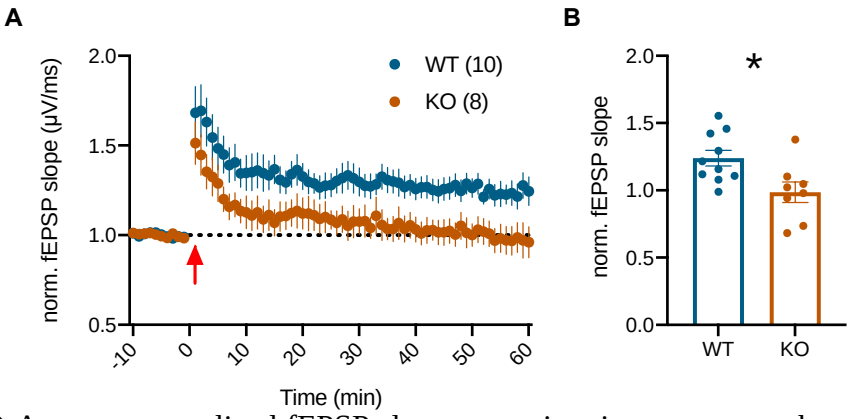

Figure 3: A) Average normalized fEPSP slopes over time in response to theta burst stimulus: 4 pulses at 100 Hz, 10 times with 200 ms interburst interval, repeated 5 times with 20 seconds in between. Theta burst stimulus was delivered at time 0 (red arrow). B) The change in normalized slope after theta burst stimulus was greater in WT than KO mice (* $p < 0.05$; unpaired t-test).

Methods

Hippocampal slice preparation

Studies were performed on *Scn1b* KO mice and WT littermate controls, ages postnatal day 15-20. A ketamine/xylazine solution (90 and 10 mg/kg) was given by intraperitoneal injection to anesthetize the mice. Then, the mice were perfused with ice cold, oxygenated cutting solution containing (in mM): 205 mM sucrose, 25 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 7 mM $MgCl_2$, 7 mM D-glucose, 3 mM $NaC_3H_3O_3$, 1.3 mM $C_6H_8O_6$ and 0.5 mM of 1M $CaCl_2$. The mice were then decapitated, and brains dissected and submerged in ice cold cutting solution. Acute hippocampal slices were prepared using a Leica vibratome, and were transferred to a holding chamber containing oxygenated holding solution that was heated to 37ºC and contained 125 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 12.5 mM D-glucose, 3 mM $NaC_3H_3O_3$, 1.3 mM $C_6H_8O_6$ and 2 mM $MgCl_2$, 2 mM $CaCl_2$, 1.3 mM ascorbic acid, and 3 mM Na-pyruvate for 30 min. Slices were then incubated in the holding solution at room temperature for 1-6 hours prior to plasticity experiments. Before field potential recordings, a cut was performed with a

scalpel between the CA1 and CA3 hippocampal regions, to prevent backpropagating action potentials from contaminating the recordings (Figure 4).

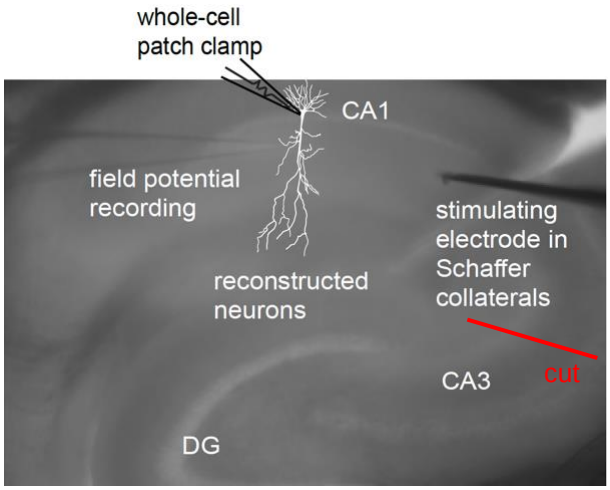

Figure 4: Schematic showing recording configuration of CA1 neurons in mouse hippocampus (adapted from Chancey et al. 2020).

Field potential recordings

Artificial cerebral spinal fluid (ACSF) was flowed over the brain slice at a rate of ~2mL/min. The ACSF solution consisted of 125 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 12.5 mM D-glucose, 1 mM $MgCl_2$ and 2 mM $CaCl_2$. A bipolar tungsten stimulating electrode was used to stimulate the Schaffer collateral axons, the projection from CA3 to CA1 PNs. Field excitatory postsynaptic potentials (fEPSPs) were recorded by placing a glass recording electrode in the stratum radiatum of the CA1 hippocampal region. Baseline fEPSPs were recorded using at the half maximum stimulus intensity using a paired pulse paradigm (100 ms inter-stimulus-interval) at 0.2 Hz. Then an input/output function was gathered by stimulating at the minimum stimulus, 25% of the maximum, the half-max stimulus (at which the baseline was recorded), 75% of the maximum, and at the maximum stimulus (the stimulus at which a population spike was detected, or the fEPSP no longer increased with increased stim intensity). After obtaining a stable baseline of at least 10 minutes, long-term depression was induced in knockout

and wild-type littermate control slices using a low-frequency stimulus (LFS) paradigm (1 Hz for 15 min). After the LFS induction protocol, a 60 minute post-conditioning period was recorded with the same 0.2 Hz paired pulse stimuli as the baseline.

Analysis of dendritic arborization

PNs from KO mice and WT littermates were filled with Neurobiotin during whole cell recordings (Figure 5A). PNs were stained using Vectastain ABC-HRP and DAB kits (Vector Labs) (Figure 5B) and reconstructed using the Neurolucida Tracing Program (MBF Bioscience) (Figure 5C). Sholl analyses were used to measure dendritic length and dendritic branching patterns along the dendrites of PNs. Concentric sholl circles were drawn 15 μm apart around the cell body of each neuron.

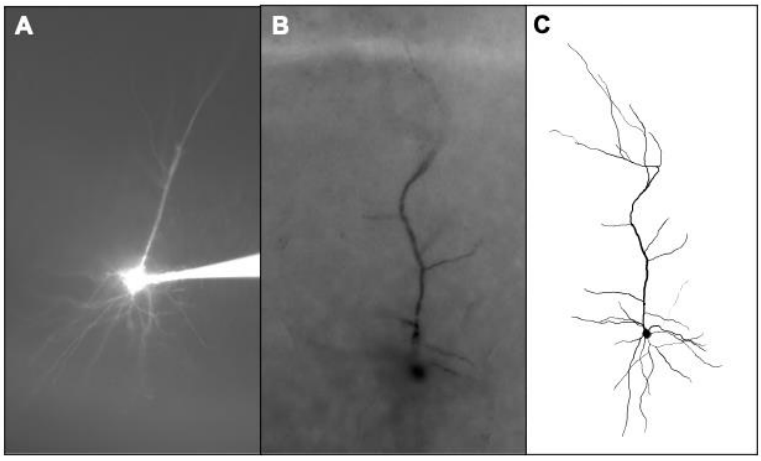

Figure 5: A single PN at each stage of the experimental process: A) the live neuron is patched, and filled with fluorescent dye and biocytin while physiological properties are recorded; B) after recording, the tissue is fixed and the neuron is stained with DAB; C) a reconstruction of the neuron is made in Neurolucida.

Analysis of spine density

KO and WT PNs were filled with a fluorescent dye, 40 mM Alexa-594, via the whole cell recording pipette. Z-stack images were taken of proximal (within 100 um of soma) and distal (in the apical tuft) dendritic branches off of the apical dendrite using a Leica 2-photon microscope.

Images were then analyzed using the cell counter plugin to count spines, and the Simple Neurite Tracer to measure dendritic length in ImageJ. Spine density was calculated as: number of spines / dendritic length (in microns).

Statistical Analyses

In all cases, I was blinded to the genotype when performing my analyses. Unpaired t-tests were done to compare total dendritic lengths and spine densities between WT and KO PNs. Repeated measures ANOVA (RM ANOVA) tests were done to compare Sholl intersections and Sholl lengths between genotypes. Unpaired t-tests were used to compare fEPSP slopes between genotypes, as well as to analyze paired-pulse ratio between groups. To compare the baseline and post-LFS fEPSP slopes, paired t-tests were done. Input/output functions were compared using a linear regression analysis. All data are presented as mean +/- standard error of the mean (SEM) with n values in parentheses.

Results

No change in dendritic arborization in knockout animals

Previous studies in a mutant model of *Scn1b* found that increased excitability was associated with reduced dendritic arborization in mutant *Scn1b* subicular pyramidal neurons (Reid et al. 2014). Furthermore, Scn1b KO mice have deficits in neurite outgrowth (Brackenbury et al. 2008). As such, I hypothesized that because our lab found hyperexcitability in *Scn1b* knockout CA1 PNs, I would see reduced dendritic arborization as well. After whole cell recordings were done in both *Scn1b* KO and WT hippocampal CA1 PNs, brain slices were processed for visualization of PNs using DAB staining. The neurons were then reconstructed using the Neurolucida tracing program. Figure 6 shows dendritic arbors of knockout and wild-type PNs

arranged by age from left to right. Both WT and KO PNs exhibit a large amount of heterogeneity, likely due to the young age of the mice during neuron recordings.

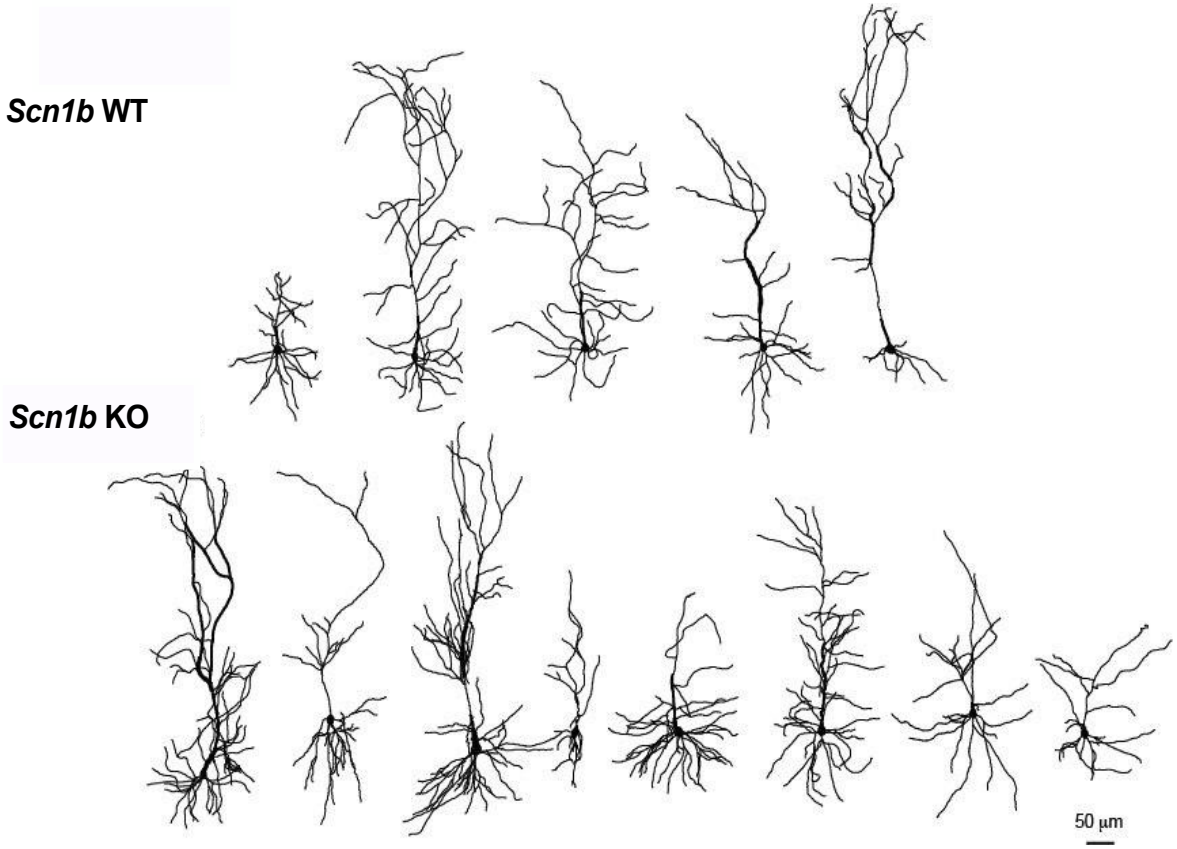

Figure 6: Example reconstructions of *Scn1b* WT (top) and KO (bottom) PNs.

To quantify the dendritic structures of both apical and basal dendrites of *Scn1b* WT and KO pyramidal neurons, Sholl analyses were used. First, total dendrite length was measured and compared between KO and WT CA1 PNs. Unexpectedly, total dendrite length was not different in *Scn1b* KO PNs compared to WT, as seen in Figure 7A. Sholl intersections and Sholl length were also unchanged, as seen in Figures 7A-D. These findings were surprising, as I hypothesized I

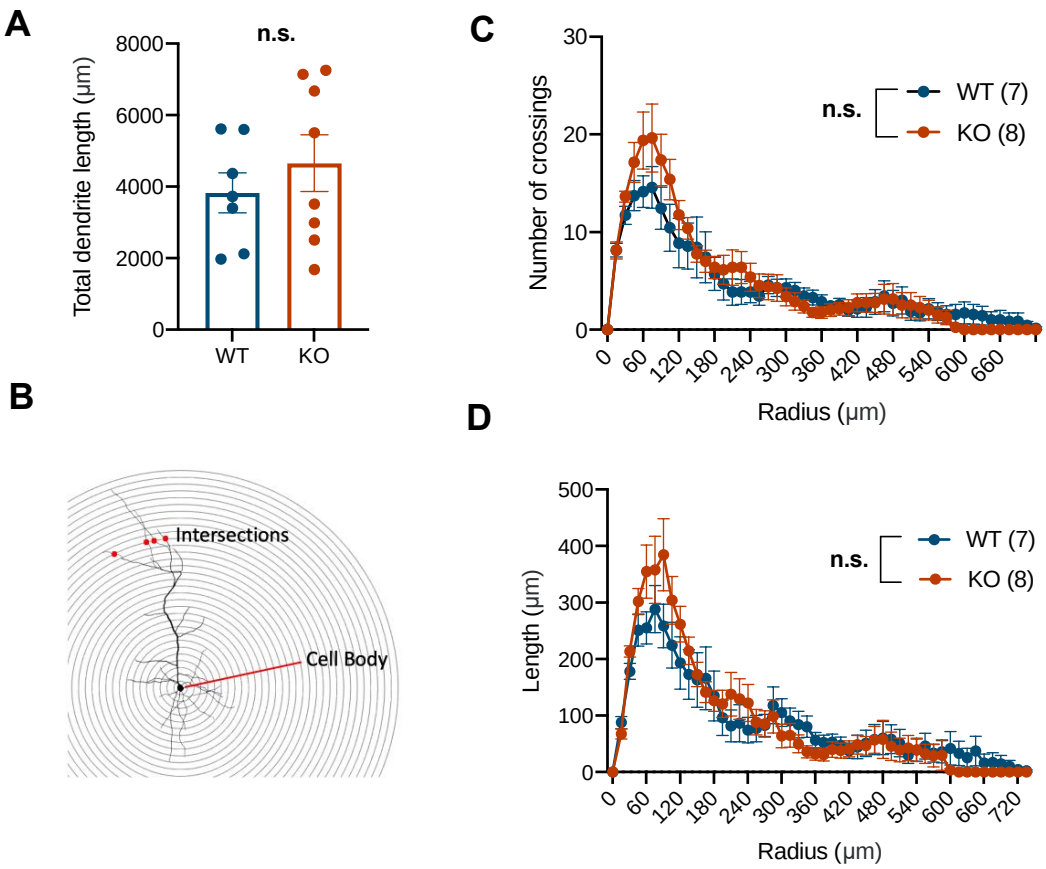

Figure 7: A) Unchanged total dendrite length in KO and WT CA1 PNs ($p > 0.05$, unpaired t-test). B) Dendritic structure was analyzed using a Sholl analyses, in which concentric circles 15 µm apart were drawn around the neuron, and intersections made by the neuron's dendrites (shown in red dots) as well as dendrite length within each circle was measured. C) No difference in sholl length between KO and WT CA1 PNs ($p > 0.05$, n.s.= not significant, repeated measures (RM) ANOVA). D) No difference in sholl crossings between KO and WT PNs ($p > 0.05$, n.s., RM ANOVA).

would see a change in dendritic morphology – changes in dendritic structure can lead to changes in a neuron's intrinsic properties, thus influencing its excitability and potentially contributing to the hyperexcitability we have seen.

Although overall dendritic arborization was unchanged in KO PNs, more nuanced differences in dendritic structure can be explored as a potential mechanism for changes in excitability. In particular, because apical and basal dendrites of pyramidal neurons receive inputs from different locations (see Introduction, Figure 1) and because of their differences in length and

shape, it is possible that differences in either apical or basal dendrites could be altered due to a loss of β1. As such, I analyzed dendritic arborization further by comparing apical and basal dendrites of KO PNs and their WT littermates, in order to determine if finer scale changes in morphology may underlie the complex phenotype of DS. Total dendrite length, number of intersections, and Sholl length were similar in apical and basal dendrites of KO and WT PNs, as seen in Figure 8.

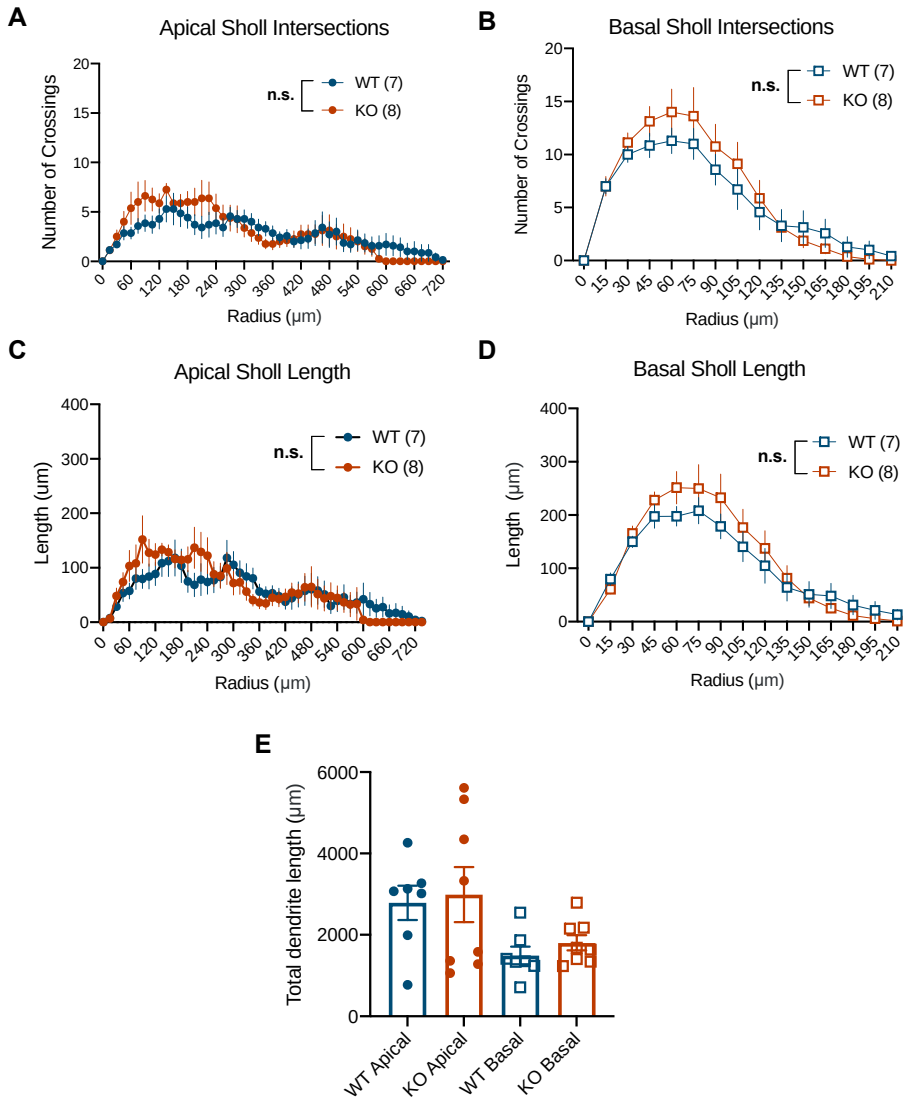

Figure 8: A) Sholl intersections are similar in apical dendrites of KO and WT CA1 PNs ($p > 0.05$; RM ANOVA). B) Sholl intersections are similar in basal dendrites of KO and WT CA1 PNs ($p > 0.05$; RM ANOVA). C) Sholl lengths are similar in apical dendrites of KO and WT CA1 PNs ($p > 0.05$, n.s.; RM ANOVA). D) Sholl lengths are similar in basal dendrites of KO and WT CA1 PNs ($p>0.05$, n.s.; RM ANOVA). E) Total dendrite length is unchanged in both apical and basal dendrites of KO PNs compared to WT PNs ($p > 0.05$, unpaired t-tests).

No change in spine density in knockout animals

Altered dendritic spine morphology is common in different human neurodevelopmental disorders, including autism (Nishiyama et al. 2019). Our lab has additionally found differences in physiology across the apical dendrites, as we have seen that the Schaffer collateral inputs to the proximal dendrites are more likely to drive the neurons to fire action potentials, while temporoammonic inputs to the distal dendrites are less likely to do so (Chancey and Howard, unpublished observation). As such, I measured dendritic spine density in both proximal and distal dendrites of *Scn1b* KO and WT PNs to determine if these changes in synaptic properties were due to changes in synapse number. Because proximal Schaffer collateral inputs were more likely to drive CA1 PNs to fire action potentials in KO PNs, I hypothesized that spine density would be increased in proximal apical dendrites of KO PNs. Figure 9A shows two-photon images of proximal and distal tuft apical dendritic spines. Dendritic length and number of spines were measured in distal and proximal dendrites of both KO and WT PNs, and average spine density was calculated. There was no difference between KO and WT PNs in proximal or distal tuft apical dendrites, as seen in Figures 9B and 9C. While this is a preliminary data set, our early results

suggest that spine density is unchanged in KO PNs, reducing the likelihood that changes in synapse number underlie altered synaptic integration associated with loss of β1.

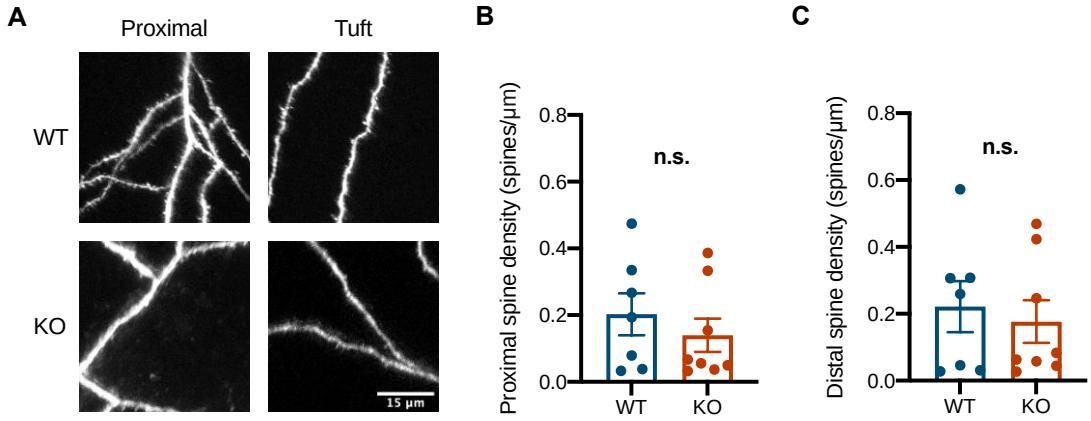

Figure 9: A) Example two-photon images of apical proximal (left; measured from primary branches < 100 µm from soma) and tuft (right) dendrites and spines of Scn1b WT (top) and KO (bottom) CA1 PNs. B) No difference in spine density in proximal apical dendrites comparing KO and WT CA1 PNs (n.s. = $p > 0.05$, Student's t-test). B) No difference in spine density in distal tuft dendrites between KO and WT CA1 PNs (n.s. = $p > 0.05$, Student's t-test).

No change in long-term depression in knockout animals

Our lab found loss of long-term potentiation in knockouts in response to a theta-burst stimulus at the Shaffer collateral to CA1 synapse (Figure 3, McConnell et al. 2019). As such, I hypothesized that the synapses have reached a limit of potentiation, and thus that I would see an increase in LTD in knockouts. LTD was induced in both KO and WT slices using a low-frequency stimulation (LFS), and local field potentials (fEPSPs) were recorded. Loss of β1 did not cause a change in long-term depression compared to WT littermate controls when using the LFS LTD-inducing paradigm (Figure 10). Figure 10A shows an example of the average of baseline fEPSP traces (solid line) overlaid with the average of the last 10 minutes of the post-LFS fEPSP traces (dotted line). In both WT and KO slices, there are noticeable decreases in magnitude of the post-conditioning traces. Figure 10B shows fEPSP responses of both KO and WT PNs as a function of time. There is an observable decrease in magnitude of fEPSP slope in both KO and WT PNs

compared to baseline. Figure 10C compares the average (bar) and individual slices (dots) of the last 10 minutes of the normalized fEPSP slopes post-LFS. There was no difference in normalized fEPSP slopes post-LFS between WT and KO slices (Figure 10C: $p > 0.05$; unpaired t-test). When comparing the average fEPSP slopes of the baseline recordings to the last 10 minutes of post-LFS recordings, there was a significant decrease in slopes for both WT slices (Figure 10D: * $p < 0.05$; paired t-test) and KO slices (Figure 10E: ** $p < 0.01$; paired t-test), indicating that both groups exhibit LTD. Figure 10F shows an input/output curve, which plots fEPSP slope as a function of fiber-volley amplitude. There was no significant difference in input/output curves between WT and KO slices (Figure 10F: $p = 0.54$; linear regression), suggesting there was no difference in postsynaptic response for the same given input. Next, we measured paired-pulse ratios in slices to determine if there were any changes in neurotransmitter release probability after plasticity induction, to account for short-term plasticity. Figure 10G shows the baseline paired pulse ratios of WT and KO slices ($p > 0.05$; n.s. t-test) were similar. Figures 10H and 10I compare the baseline paired pulse ratio to the post-LFS paired pulse ratio for both WT and KO slices, respectively. Paired pulse-ratios for both WT (Figure 10H) and KO (Figure 10I) slices did not change following

LTD induction ($p > 0.05$; paired t-test), suggesting there was no change in vesicle release probability, providing support for a postsynaptic mechanism of LTD.

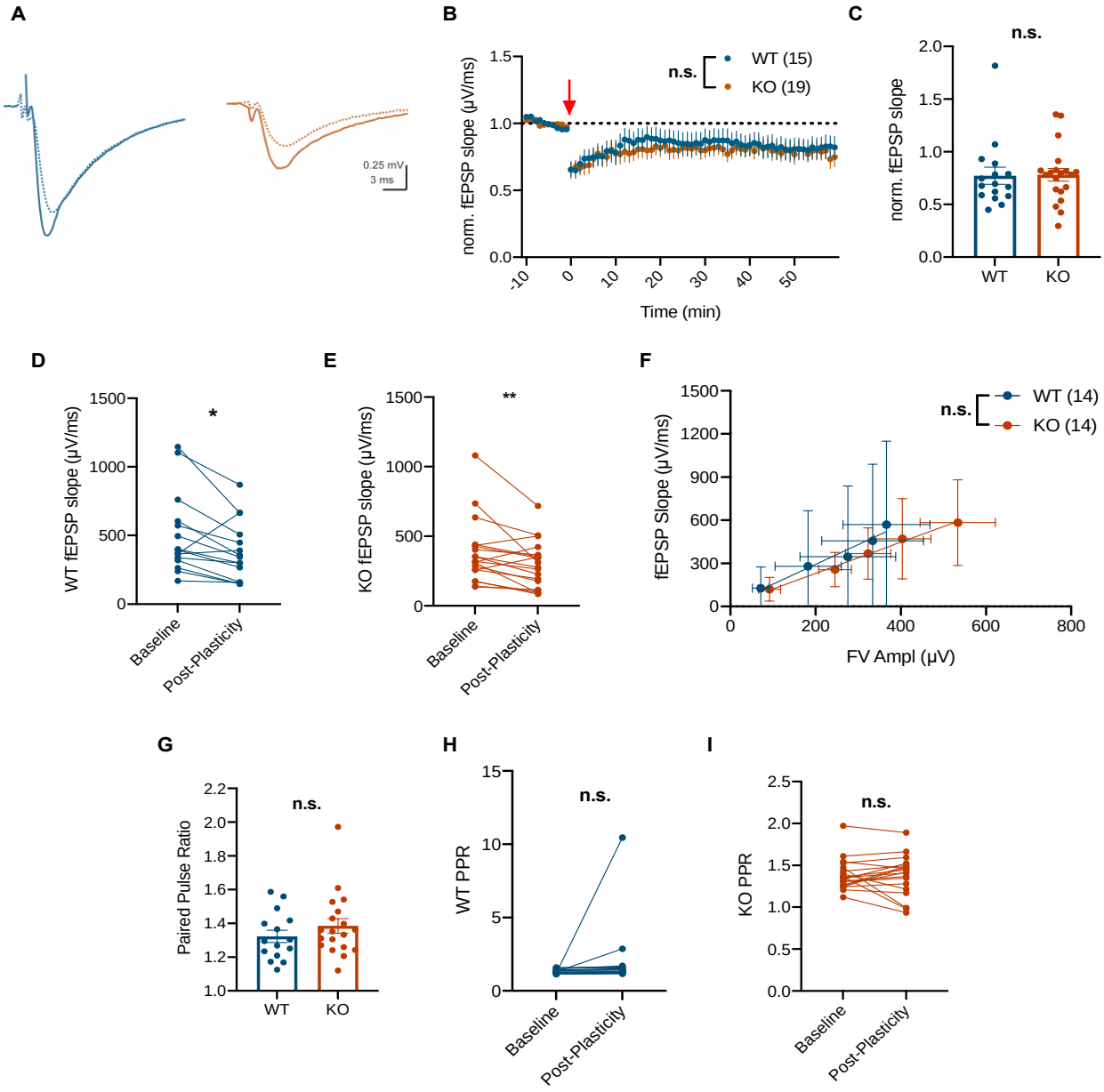

Figure 10: Low frequency stimulus (LFS) induced LTD. A) Example traces of fEPSPs recorded at baseline (solid) and post-LFS (dotted line) in response stimulation of Schaffer collateral axons with a 100 ms interstimulus interval. B) Average normalized fEPSP slopes over time. The LFS paradigm was delivered at time 0 (red arrow; 1 Hz for 15 min). C) There was no change in normalized slope in KO compared to WT mice ($p > 0.05$; unpaired t-test). D) LFS induced induce significant LTD in WT mice (* $p < 0.05$; paired t-test). E) LFS induced significant LTD in KO mice (** $p < 0.01$; paired t-test). F) No change in input/output curves between WT and KO ($p = 0.54$; linear regression). G) Similar baseline PPR in WT and KO mice ($p > 0.05$; t-test). H) No significant change in paired-pulse ratio in WT mice or KO mice (I) following LFS ($p > 0.05$; t-test).

Discussion

Mutations in the gene *SCN1B* cause severe neural dysfunction, leading to network hyperexcitability and Dravet syndrome, but the cellular mechanisms of this are not fully understood. My research aims to uncover cellular mechanisms, looking specifically at how single neuron morphology and synaptic plasticity are altered in CA1 hippocampal pyramidal neurons to potentially cause broader neurological deficits. My results show that dendritic arborization is unchanged in *Scn1b* KO PNs compared to WT PNs. Similarly, my preliminary results on dendritic spine density suggest that spine density is also unchanged in knockouts. These results together suggest that factors beyond single neuron morphology underlie hyperexcitability and changes in synaptic function of CA1 KO PNs. Despite this, our research shows that synaptic plasticity is fundamentally altered in *Scn1b* KO PNs. While LTP is lost in KO PNs (McConnell et al. 2019), LTD appears normal.

Dendritic structure and neurological dysfunction

Similar studies on genetic-based neurological disorders have shown links between altered neuronal morphology and hyperexcitability. Genetic mutations linked to Amyotrophic Lateral Sclerosis (ALS) have been shown to cause progressive loss of neuronal projections, loss of axonal transport, as well as hyperexcitability in Drosophila sensory neurons (Machamer et al 2018). Deletion of the Kv10.2 potassium channel leads to increased dendritic arborization, increased synaptic currents and hyperexcitability in rat CA3 hippocampal neurons (Liu et al. 2020). Most notably, studies in mutant *Scn1b* models of Dravet syndrome have found that hyperexcitability in subicular pyramidal neurons was accompanied by reductions in dendritic arborization (Reid et al. 2014). Additionally, loss of beta subunits in voltage-gated sodium channels have been found to lead to disruptions in neurite outgrowth and migration (Brackenbury et al. 2008).

Because of these significant links between neuron physiology and morphology in the context of neurological disease, I hypothesized that in the *Scn1b* knockout model of Dravet syndrome, I would see altered neuronal morphology in hippocampal CA1 pyramidal neurons correlated with the hyperexcitability and changes in synaptic activity we see. Instead, I found no difference in dendritic structure between WT and KO PNs, suggesting that changes in overall dendritic structure do not underlie the hyperexcitability of CA1 KO PNs. There are multiple mechanisms that could be at play instead of changes in morphology that cause hyperexcitability. Loss of β1 likely affects the gating and trafficking of sodium channels (Baroni et al. 2018) and some potassium channels (Marionneau et al. 2012), thus altering the intrinsic excitability of the pyramidal neurons themselves. Our lab has also found decreases in sag voltage (Chancey et al. 2020), which suggests a change in HCN channel activity. Increased activation of HCN channels can lead to increased depolarization in the neuron's membrane, causing hyperexcitability (Nolan et al. 2004). Overall, our results on dendritic arborization suggest that loss of β1 likely affects pyramidal neuron morphology differently in varying brain regions, as we saw no change in CA1 pyramidal neuron morphology, whereas altered morphology has been seen in subiculum PNs in another *Scn1b* mouse model of Dravet (Reid et al. 2014).

In addition to changes in excitability, our lab has found changed synaptic integration in CA1 pyramidal neurons (Chancey et al. 2020). Specifically, we found increased temporal summation in CA1 neurons in response to theta-burst synaptic stimulation of Schaffer collateral axons. I hypothesized that this increased synaptic response was a result of increased spine number (i.e., synapse number), and as such, quantified spine density across CA1 pyramidal neuron dendrites. Unexpectedly, my data suggests that spine density is not changed with the loss of β1, suggesting that changes in spine or synapse number do not underlie these changes in synaptic

integration. One possible explanation for the changes in synaptic integration we have seen may be because of an alteration in inhibitory GABAergic inputs. Development of GABAergic signaling has been found to be altered in both *Scn1b* and *Scn1a* mouse models (Yuan et al. 2019). Additionally, dendritic arborization was found to be reduced in GABAergic interneurons in an *Scn1a* model of Dravet syndrome (Tiraboschi et al. 2020), suggesting that altered inhibition to CA1 inputs may be causing the changes in synaptic integration and excitability we have seen.

Synaptic plasticity and neurological dysfunction

In addition to pyramidal neuron morphology, my research involved investigations of long-term synaptic plasticity. Because our lab previously found that long-term potentiation at the Schaffer collateral to CA1 synapse was lost in *Scn1b* knockouts, I hypothesized that these neurons had already reached a limit of potentiation (i.e., saturation), suggesting that the only direction for the synapses to go in terms of plasticity would be to depress. As such, I predicted that I would see increased long-term depression. Instead, I found no difference in LTD in knockouts compared to wild-types. The altered LTP and unaltered LTD could be explained by an alteration in molecular signaling pathways of plasticity, that the molecular signals governing LTP, such as CaMKII signaling, are altered as a result of loss of β1, while the molecular signal pathways governing LTD, such as calcineurin signaling pathways, are not altered. This disparity between LTP and LTD could also be due to differences in local calcium entry – differences in calcium channel gating and mitochondrial and endoplasmic reticulum calcium homeostasis (Gleichmann & Mattson 2011) could affect calcium levels at different locations along the neuron, causing a disparity in synaptic plasticity.

Future directions

Our next immediate task in this work is to complete the spine density data set by adding more cells to increase the power in our study. Further studies on pyramidal neuron morphology in this model could involve analysis on cell body size and shape, as this could have a large influence on intrinsic properties of the neurons, as well as analysis of spine shape and size. Because the mechanisms of synaptic plasticity in this model remain unclear, future studies could involve looking at molecular signaling at synapses during plasticity induction. Specifically, we could measure calcium signaling, CaMKII enzymatic signaling, and calcineurin signaling at synapses to determine if these pathways underlie the complex changes in synaptic plasticity we have seen. It is additionally worthwhile to investigate physiology in apical versus basal dendrites, as it is possible that mechanisms of synaptic and dendritic integration differ between the two types of dendrites. Lastly, behavioral experiments testing for changes in learning and memory in *Scn1b* knockouts can give us more insight on the cognitive deficits resulting from a loss of β1.

Another avenue to explore would be to repeat all studies using adult mice. Our studies use mice ages postnatal day 15 through day 20, making developmental state and developmental deficits confounding variables, as many of the neurons we are studying are still growing and forming synapses. As a result of the developmental stage of these neurons, the dendritic structures of both knockout and wild-type neurons we studied varied greatly. To account for this in future experiments, we can use siRNA interference to do a viral knockdown of the *Scn1b* gene in adult mice. Additionally, we can use an *Scn1b* floxed conditional mouse model in future experiments to knockout *Scn1b* in certain populations of neurons or specific brain areas at various development stages or in adult mice. Using these methods, we can further investigate if the neural dysfunction we have observed a result of cell autonomous loss of β1 itself or developmental deficits.

Conclusion

My unexpected results show that despite changes in hyperexcitability, synaptic integration, and long-term potentiation, overall dendritic morphology and long-term depression are unchanged in *Scn1b* knockout pyramidal neurons. Because dendritic arborization and spine density are not different between KO and WT PNs, it can be inferred that dendritic structure does not underlie the hyperexcitability of CA1 KO PNs in our model, nor does the hyperexcitability inherent in this epileptic model induce marked structural plasticity. Although our hypotheses were unsupported by our work, our results impel us to investigate other factors mechanisms beyond overall dendritic morphology and synaptic plasticity as underlying causes of the neural dysfunction of Dravet syndrome.

Broadly, this research will shed light on the relationship between structure and function in neurons that will ultimately reveal the workings of learning and memory in the context of a disease model. While preliminary examinations of neuron physiology and structure have been made in disparate brain regions, no one has yet correlated the physiological and morphological changes occurring within single neurons in the context of neurodegenerative disease. Determining the correlations between neuronal morphology and neurophysiology can help us understand how such changes might interact to produce the neural dysfunction and complex symptoms underlying Dravet syndrome. Additionally, by analyzing both neurophysiology and neuron morphology in hippocampal pyramidal neurons, my results provide insight into mechanisms of neural circuitry and function in learning and memory.

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