

Role of Nav1.7 in Electrical Excitability of Rat Subfornical Organ Neurons

by

Nathan Ayano

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Department of Biological Sciences

University of Manitoba

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Abstract

The subfornical organ (SFO) is a key sensory circumventricular organ involved in homeostatic regulation including drinking behaviour, salt appetite, energy balance, and cardiovascular function. Voltage-gated sodium channels (VGSC), particularly the persistent sodium current, have been implicated in shaping SFO neuronal excitability, and thus plays a central role in the ability of SFO neurons to carry out their role. The VGSC isoforms $\text{Na}_v1.1$, 1.2 , 1.3 , 1.7 and possibly 1.6 have been demonstrated to be expressed in the SFO, yet the specific contribution of $\text{Na}_v1.7$ remains unclear. In this study, we examined the effects of the selective $\text{Na}_v1.7$ antagonist ProToxin-II on dissociated rat SFO neurons using whole-cell voltage-clamp, current-clamp, and cell-attached recordings. ProToxin-II produced hyperpolarizing shifts in the voltage dependence of activation and inactivation, accelerated fast inactivation, slowed recovery from inactivation, and preferentially inhibited persistent sodium current (~28% reduction relative to transient current). These biophysical changes were associated with decreased evoked action potential output despite a modest hyperpolarizing shift in action potential threshold. Spontaneous firing showed heterogeneous, bidirectional modulation, with ProToxin-II shifting neurons between tonic and burst-like firing patterns. Computational modeling using a Hodgkin-Huxley-type mathematical model of an SFO neuron confirmed that $\text{Na}_v1.7$ conductance, particularly its persistent current component, strongly promotes high-frequency tonic firing. Together, these results indicate that $\text{Na}_v1.7$ functions as a significant contributor to persistent sodium current in SFO neurons and plays a context-dependent role in amplifying subthreshold signals and supporting sustained excitability critical for homeostatic regulation.

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1. Introduction

1.1 The Subfornical Organ

The subfornical organ (SFO) is a sensory forebrain structure located in the anteroventral third ventricle (AV3V) region. It plays a central role in the regulation of homeostatic functions, including hydromineral balance, cardiovascular control, immune regulation, reproduction, and energy balance (see Smith and Ferguson, 2010 for review). Unlike most brain regions, the SFO lacks a blood–brain barrier (BBB), placing it in a privileged position to detect circulating signals and relay systemic information to homeostatic control centers (Murabe et al., 1981; Akert, 1989; Smith and Ferguson, 2010). Early studies established a key role of the SFO in thirst generation: microinjection of as little as 0.1 ng angiotensin II (ANG II) into the SFO rapidly elicited drinking in water-satiated rats, whereas SFO lesions abolished this effect (Simpson and Routtenberg, 1973). In mice, distinct populations of excitatory and inhibitory SFO neurons have been shown to promote or suppress drinking behaviour, respectively, although it remains unclear whether an analogous circuit organization exists in rats (Oka et al., 2015). SFO neurons projecting to the magnocellular neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) stimulate vasopressin release to enhance water reabsorption during dehydration or hypovolemia (Matsuda et al., 2017; Wang et al., 2023).

In addition to its role in thirst and water balance, the SFO is also a major site for regulating salt intake behaviour and sodium (Na^+) sensing, mediated in part by the atypical voltage-gated sodium channel Na_x (Hiyama et al., 2004a; Noda and Hiyama, 2015). Infusion of hypertonic Na^+ solution into cerebral ventricle induces water intake and salt aversion in wild-type mice but not in Na_x -KO mice (Hiyama et al., 2004b). However, introduction of Na_x cDNA

into knock-out mice, recovers salt-avoiding behavior under dehydrated conditions (Hiyama et al., 2004b).

Evidence also supports a key role for the SFO in regulation of energy balance: SFO neurons have been observed to be responsive to insulin (Lakhi et al., 2013), and more recently, Lakhi et al. (2024) examined the effects of fasting on Na⁺ channel expression in the rat SFO. Following a 48 h fast, Na_v1.3 mRNA transcripts were reduced, accompanied by depolarizing shifts in voltage-dependence of activation and inactivation. Neurons of the SFO sense numerous circulating metabolic/energy factors such as insulin, leptin, ghrelin, amylin, neuropeptide Y and peptide YY. Transcriptomic changes during fasting alter SFO excitability, integrating metabolic and cardiovascular cues to influence feeding, glucose regulation and energy expenditure (Hindmarch and Ferguson, 2015; Cancelliere and Ferguson, 2017; Kwon Jeong et al., 2019; Kim et al., 2024). Additionally, the SFO also plays important roles in cardiovascular/autonomic control by sensing ANG II, proinflammatory cytokines (e.g., IL-1 β , TNF- α) and other signals to modulate sympathetic output, blood pressure, heart rate and baroreflex function. Lesions or receptor knockdowns in the SFO attenuate sympathetic activation and hypertension in models like renovascular or cytokine-induced states, while projections to parvocellular PVN and rostral ventrolateral medulla (RVLM) support tonic sympathetic drive (Wei et al., 2013; Cancelliere and Ferguson, 2017; Rossi et al., 2019; Griffiths et al., 2021).

Reproductively, the SFO senses changes in circulating hormones associated with pregnancy, such as relaxin which acts to stimulate water drinking, inhibit vasopressin release and influence gestation and parturition timing in rats; ablation of SFO abolishes these effects (Summerlee and Wilson, 1994; Sunn et al., 2002). SFO is also suggested to be involved in

regulating hypersecretion of follicle-stimulating hormone (FSH) during proestrus and modulating estrous cyclicity (Limonta et al., 1981).

Since changes in circulating signals are communicated to homeostatic control centres via changes in action potential activity of SFO neurons, recent work has begun to characterize the intrinsic electrophysiological properties of SFO neurons and suggests that voltage-gated Na^+ currents—particularly the persistent Na^+ current (I_{NaP})—are critical determinants of neuronal excitability. SFO neurons exhibit at least two predominant firing patterns, bursting and tonic firing (Huang et al., 2019). Using a Hodgkin-Huxley-type mathematical model SFO neuron, Medlock et al. (2018) reported that burst initiation is facilitated by a depolarizing afterpotential (DAP), which can be supported by I_{NaP} , whereas burst maintenance depends on a calcium (Ca^{2+}) current. In the same model, increasing either Na^+ or potassium (K^+) conductance was sufficient to shift firing from bursting to tonic. Many SFO neurons fire action potentials spontaneously; during quiescent periods, these neurons exhibit subthreshold membrane potential oscillations (Fry and Ferguson, 2007). Notably, the activation range of these oscillations closely matches that of I_{NaP} , suggesting that I_{NaP} -dependent oscillatory dynamics may contribute to spontaneous firing and help maintain baseline neuronal tone.

Anatomically, the SFO contains two regions—core (cSFO) and shell (sSFO)—distinguished by calbindin (CalB) and calretinin (CalR) expression, respectively (Huang et al., 2019). Neurons in these regions display different firing phenotypes: cSFO neurons exhibit more low-frequency bursting, whereas sSFO neurons more often display higher-frequency tonic firing (Huang et al., 2019). Core and shell populations also project to distinct downstream targets and may drive different physiological outcomes. Core projections include the paraventricular nucleus (PVN), rostral bed nucleus of the stria terminalis (BNST), and the organum vasculosum of the

lamina terminalis (OVLt) (Saper and Levisohn, 1983; Swanson and Lind, 1986; Kawano and Masuko, 2010), whereas shell neurons project to the median preoptic nucleus (MnPO) and magnocellular neurons of the SON and PVN (Swanson and Lind, 1986). Both regions project to PVN, but to different subregions: core neurons preferentially target parvocellular PVN (pPVN), linked to cardiac output, whereas shell neurons preferentially target magnocellular PVN (mPVN), linked to fluid homeostasis (ML et al., 1996; Kawano and Masuko, 2010; Hicks et al., 2021).

1.2 Voltage-gated Sodium Channels

Voltage-gated sodium channels (VGSCs) are essential for electrical signaling in excitable cells and are the primary drivers of the rapid depolarizing (rising) phase of the action potential. They are widely expressed throughout the nervous system and are activated by changes in membrane potential, enabling rapid Na^+ influx during spike initiation. Extensive research has characterized VGSC structure and function, in part because altered channel expression or biophysical properties have been implicated in a broad range of neurological and neurodevelopmental disorders, including autism spectrum disorders, ataxia, paroxysmal extreme pain disorder (PEPD), epilepsy syndromes, and erythromelalgia (Abou-Khalil et al., 2001; Dib-Hajj et al., 2005; Lampert et al., 2009; Estacion et al., 2011; Volkers et al., 2011; Nwebube et al., 2021; Espino et al., 2022). An understanding of how VGSC structure influences channel gating, how altered gating shapes neuronal excitability, and how these cellular changes translate to circuit- and system-level dysfunction is critical for improving existing treatment strategies and for developing novel therapeutics.

1.3 Channel Structure

The VGSC family comprises nine pore-forming isoforms (Na_v1.1–Na_v1.9), each encoded by one of nine genes (*SCN1A–SCN5A*, *SCN8A–SCN11A*) (De Lera Ruiz and Kraus, 2015). A tenth sodium-channel-related protein, Na_x, is considered atypical because it is not voltage-gated; instead, it is sensitive to extracellular Na⁺ concentration (Hiyama et al., 2002, 2004b; Grob et al., 2004). As such, Na_x functions as a Na⁺ sensor and contributes to osmoregulatory control. However, localization of Na_x differs between rats and mice. Within mice, Na_x is generally expressed in glial cells, while rats exhibit broader expression in both glial cells and neurons (Nehmé et al., 2012).

Each VGSC consists of a pore-forming α subunit and auxiliary β subunits (Bouza and Isom, 2018). The α subunit is a single polypeptide containing four homologous domains (DI–DIV), and each domain contains six membrane-spanning segments (S1–S6) (Nguyen and Yarov-Yarovoy, 2022). The S5 and S6 segments form the central pore region (Chakka et al., 2012), while the P-loop between S5 and S6 forms the outer mouth of the pore and contributes to Na⁺ selectivity (Catterall et al., 2020). Tetrodotoxin (TTX), a potent neurotoxin first detected in puffer fish (Lorentz et al., 2016), blocks VGSCs from the extracellular side and prevents Na⁺ influx (Narahashi, 2008a). Although TTX sensitivity differs across isoforms. Na_v1.1–Na_v1.3, Na_v1.6, and Na_v1.7 are inhibited by sub-micromolar TTX concentrations and are therefore considered TTX-sensitive (TTX_s), whereas Na_v1.5, Na_v1.8, and Na_v1.9 require substantially higher concentrations and are considered TTX-resistant (TTX_r) (Farmer et al., 2008; Narahashi, 2008a).

The S4 segment serves as the voltage sensor; its distinctive structure enables it to respond to changes in membrane potential and initiate channel opening. S4 contains positively charged

lysine and arginine residues at approximately every third position, and upon depolarization, outward movement of these charges promotes channel opening (Noda et al., 1984; Yang and Horn, 1995). Within ~1–2 ms of opening, VGSCs rapidly inactivate through a mechanism mediated by the intracellular loop linking DIII and DIV. This loop acts as a “hinged lid,” and a conserved IFM motif (isoleucine–phenylalanine–methionine) occludes the IC mouth of the pore to block ion conduction (West et al., 1992).

Four β subunits are encoded by four genes (SCN1B–SCN4B), with SCN1B producing $\beta 1$ and its splice variant $\beta 1B$ (O’Malley and Isom, 2015). All β subunits contain an extracellular immunoglobulin (Ig) domain, and all except $\beta 1B$ possess a single membrane-spanning segment (O’Malley and Isom, 2015). Although β subunits are not required for α subunits to form functional channels, they modulate multiple properties, including gating kinetics (e.g., voltage dependence of activation/inactivation, inactivation rate, and persistent Na^+ current), channel localization, and cell adhesion (via the Ig domain) (De Lera Ruiz and Kraus, 2015; O’Malley and Isom, 2015).

1.4 $\text{Na}_v1.7$

$\text{Na}_v1.7$ is encoded by the SCN9A gene (De Lera Ruiz and Kraus, 2015). This isoform has been reported to exhibit rapid activation and inactivation and relatively slow recovery from inactivation (De Lera Ruiz and Kraus, 2015). Compared with other VGSC isoforms, $\text{Na}_v1.7$ displays a slightly more hyperpolarized voltage dependence of activation (V-ACT) (Yang et al., 2016a). $\text{Na}_v1.7$ also generates prominent ramp currents in response to small depolarizations, enabling spike initiation in response to subthreshold inputs; for this reason, $\text{Na}_v1.7$ has been described as a “threshold channel” (De Lera Ruiz and Kraus, 2015). In terms of neuronal

excitability, this property increases the probability of action potential generation by boosting small depolarizing inputs that might otherwise fail to reach threshold.

Table 1. Biophysical properties of Nav1.7 currents reported in the literature across different species and expression systems

Species	Expression System	$V_{1/2}$ -ACT (mV)	$V_{1/2}$ -INACT (mV)	Inactivation Rate (ms)	Recovery (ms)	References
Human	HEK293	-28.9	-77.6	0.6	30.6	(Jarecki et al., 2008)
Human	HEK293	-17.2 – -18.6	-71.6 – -72.4		6.48 – 7.76	(Laedermann et al., 2013a)
Human	DRG neurons	-27.3	-71.3		72	(Herzog et al., 2003)
Human	HEK293	-16.1 – -22.3	-65.7 – -70.9		6.19 – 7.55	(Laedermann et al., 2013b)
Rat	<i>Xenopus</i> Oocytes	-24.5	-59.9	6.6		(Tan and Soderlund, 2011)
Rat	<i>Xenopus</i> Oocytes	-22.4 – -27.7	-68.2 – -69.8	1.8 – 19.8	6.6 – 19.6	(Vijayaragavan et al., 2001)
Rat	Nodose ganglia	-28.16	-41.68			(Loya-López et al., 2022)
Rat	<i>Xenopus</i> Oocytes	-25.56	-68.38	14.42		(Chevrier et al., 2004)

Mutations in the VGSC $\text{Na}_v1.7$ (encoded by *SCN9A*) are strongly linked to diverse pain phenotypes. Gain-of-function variants, such as N395K, I848T, and L858H, cause painful disorders like inherited erythromelalgia (characterized by burning pain and erythema in extremities), paroxysmal extreme pain disorder, and small fiber neuropathy by shifting activation to more hyperpolarized potentials, slowing deactivation, impairing inactivation, or enhancing ramp currents, leading to neuronal hyperexcitability (Cummins et al., 2004; Sheets et al., 2007; Bennett and Woods, 2014; Yang et al., 2016b). In contrast, loss-of-function mutations (e.g., S459X, I767X, W897X) result in painless syndromes such as congenital insensitivity to pain (CIP), where individuals cannot perceive painful stimuli despite intact detection of other sensations, a phenotype mirrored in global $\text{Na}_v1.7$ knockout mice (Cox et al., 2006; Gingras et al., 2014). $\text{Na}_v1.7$ also contributes to injury-induced hypersensitivity, as shown in burn models where wild-type mice exhibit lowered heat-pain thresholds alongside hyperpolarized activation shifts and increased $\text{Na}_v1.7$ current density in dorsal root ganglion and spinal cord tissue, effects absent in conditional $\text{Na}_v1.7$ knockout mice that fail to develop heat hypersensitivity (Shields et al., 2012).

1.5 $\text{Na}_v1.7$ in the Central Nervous System

Most research investigating roles of $\text{Na}_v1.7$ have focused on its expression in the peripheral nervous system (PNS) and its role in pain pathways. However, $\text{Na}_v1.7$ is also expressed at lower levels in select central nervous system (CNS) regions. Using *in situ* hybridization (ISH), Morinville et al. (2007) identified several brain structures expressing $\text{Na}_v1.7$. Importantly, the areas expressing $\text{Na}_v1.7$ are established homeostatic control centres, these include including prominent expression in the SON, PVN, arcuate nucleus (ARC) and SFO.

Osmoregulation, a key aspect of homeostasis, relies on the SON, where magnocellular neurons produce and release vasopressin to promote water reabsorption in the kidneys (Pauža et al., 2021; Yu and Das, 2023). Studies show that salt loading increases expression of $\text{Na}_v1.7$ (along with $\text{Na}_v1.2$ and $\text{Na}_v1.6$), in the SON, and increases Na^+ current density in MNCs (Tanaka et al., 1999; Voisin and Bourque, 2002; Black et al., 2013). These changes may support the phasic burst firing pattern that drives vasopressin release from magnocellular neurons (Andrew and Dudek, 1983), as $\text{Na}_v1.7$'s biophysical properties—such as hyperpolarized activation, rapid kinetics, and substantial ramp currents—help lower spike threshold and amplify small depolarizations, thereby boosting neuronal excitability in response to osmotic challenges (Voisin and Bourque, 2002; De Lera Ruiz and Kraus, 2015; Yang et al., 2016a).

The ARC plays a central role in long-term appetite regulation and energy homeostasis through two opposing neuronal populations: orexigenic AgRP/NPY neurons that promote feeding and anorexigenic POMC neurons that suppress it (Sohn, 2015; Branco et al., 2016). These neurons function as efficient synaptic integrators with a low input-to-output ratio, characterized by prolonged excitatory postsynaptic potentials (EPSPs) that enable temporal summation and gradual depolarization over extended timescales, supported by a persistent Na^+ current that is TTX-sensitive (Branco et al., 2016; Li et al., 2020). This integration relies critically on $\text{Na}_v1.7$ (encoded by *Scn9a*), which is highly expressed in both AgRP and POMC neurons; pharmacological blockade (e.g., with ProToxin-II) or genetic knockdown/deletion of *Scn9a* reduces I_{NaP} , shortens EPSP duration, impairs synaptic integration, and alters body weight—leading to reduced weight gain when deleted in AgRP neurons and increased weight when deleted in POMC neurons (Schmalhofer et al., 2008a; Branco et al., 2016).

1.6 Voltage-gated Na⁺ Currents in the Subfornical Organ

The SFO expresses a broad complement of ionic currents, including I_{Na} , I_{NaP} , I_K , I_A , I_{Ca} , I_{NSCC} , I_{KS} , and I_L (Medlock et al., 2018). There is substantial interest in defining how these channels shape intrinsic SFO excitability, particularly VGSCs, as voltage-gated Na⁺ currents underlie multiple electrical features of SFO neurons and may therefore influence homeostatic control. Fry and Ferguson (2007) observed subthreshold membrane potential oscillations in SFO neurons that were eliminated by hyperpolarization and abolished by TTX, indicating a dependence on voltage-gated Na⁺ current. They further showed that I_{NaP} contributes to these oscillations, as the voltage dependence of oscillation onset closely matched the activation range of I_{NaP} (Fry and Ferguson, 2007). Consistent with this, Medlock et al. (2018) demonstrated that I_{NaP} is important for burst initiation and that reducing I_{NaP} abolished spontaneous activity. As mentioned earlier, many SFO neurons (40-50%) fire action potentials spontaneously (Washburn et al., 2000; Huang et al., 2019; Shute and Fry, 2025). A proposed role of spontaneous firing is in maintaining synaptic tone or a steady release of neurotransmitter on synaptic targets. This constant low-level excitatory tone may provide more nuanced bidirectional modulation than a simple “on/off” mode of activation (Kavalali, 2014).

As previously noted, the SFO is composed to two distinct subregions, core and shell; notably, Huang et al. (2019) reported that CalB[−] neurons exhibited a more depolarized resting membrane potential and higher I_{Na} density than CalB⁺ neurons. A more depolarized baseline membrane potential could facilitate Na⁺-dependent subthreshold oscillations, while increased I_{Na} density could enhance spike initiation. Medlock et al. (2018) developed a single-compartment Hodgkin–Huxley model incorporating these SFO ionic currents and reproduced SFO-like action potentials and firing behaviours, including bursting and tonic firing. However, the relative

contributions of specific Na_v isoforms expressed in the SFO were not resolved, and contribution of all VGSCs is lumped into a single I_{Na} conductance.

1.7 Objectives and Hypotheses

In the present study, our objective is to elucidate specific roles of $\text{Na}_v1.7$ in the electrical excitability of rat SFO neurons. The I_{Na} of SFO neurons comes from $\text{Na}_v1.1$, 1.2, 1.3, 1.6 and 1.7. To isolate and assess the contribution of the $\text{Na}_v1.7$ current, we used the selective $\text{Na}_v1.7$ antagonist ProToxin-II. Voltage-clamp will be used to observe effects of ProToxin-II on the intrinsic electrical properties of SFO neurons, while current-clamp experiments will be used to observe effects of ProToxin-II on action potential firing. Results from voltage-clamp experiments can then be categorized into 2 sets of currents; ProToxin-II-sensitive (i.e. the current that was blocked by ProToxin-II – $\text{Na}_v1.7$ -specific) and ProToxin-II-resistant (i.e. current that was unaffected by ProToxin-II). Hodgkin-Huxley (HH) parameters for both sets of currents can then be extracted. Using a HH-type mathematical model of an SFO neuron, previously constructed by Medlock et al. (2018), HH parameters can be used to model current clamp data.

Hypothesis 1: ProToxin-II will alter HH parameters of Na^+ current from SFO neurons. Specifically, we expect ProToxin-II to (1) cause depolarizing shift in voltage-dependence of activation, thereby requiring stronger depolarization to initiate channel opening, (2) slow kinetics of inactivation, prolonging channel open state, (3) accelerate channel recovery from inactivation, allowing higher frequency firing, and (4) proportionally decrease persistent current. In order to test this, whole-cell voltage-clamp recordings in acutely dissociated rat SFO neurons under control conditions and following application of ProToxin-II, we quantified the voltage dependence of activation and inactivation, time constants of inactivation, time-dependent

recovery from inactivation, and the amplitude and relative contribution of persistent Na^+ current measured during slow depolarizing voltage ramps. These parameters were then directly compared between control and ProToxin-II conditions to determine the specific effects of $\text{Na}_v1.7$ blockade on channel gating and kinetics.

Hypothesis 2: Current clamp data can be modeled using previously extracted HH parameters and a mathematical single-compartment model of an SFO neuron. In order to test this, HH parameters specific to the ProToxin-II-sensitive current (i.e. $\text{Na}_v1.7$) can be derived from voltage-clamp recordings. These experimentally extracted parameters will be used to modify the HH-type mathematical model constructed by Medlock et al. (2018), enabling simulation of action potential firing behavior. This will allow us to determine how the unique biophysical properties of $\text{Na}_v1.7$ influence spontaneous firing frequency, regularity (coefficient of variation), and overall excitability in the SFO.

2. Materials and Methods

2.1 Animals and Primary Neuronal Culture

All animal procedures were approved by the University of Manitoba Animal Care Committee and conducted in accordance with Canadian Council on Animal Care (CCAC) guidelines. Male Sprague–Dawley rats (150–175 g) were housed in a temperature- and light-controlled facility with ad libitum access to standard chow and water. Rats were euthanized by rapid decapitation, and brains were immediately removed into ice-cold, oxygenated (95% O_2 /5% CO_2) artificial cerebrospinal fluid (aCSF; in mM: 126 NaCl, 2.5 KCl, 1.2 NaH_2PO_4 , 11 D-glucose, 25 NaHCO_3 , 1.2 MgCl_2 , and 2.4 CaCl_2) for ~2 min. Brains were then sectioned using a

vibratome to obtain a 1-mm-thick coronal slice containing the SFO. The SFO was micro dissected and incubated in Hibernate-A medium (Gibco) containing papain (2 mg/mL; Worthington Biochemical, Lakewood, NJ) for 30 min at 30°C. Tissue was subsequently washed in Hibernate-A supplemented with 1× B27 (Gibco, Thermo Fisher Scientific, Grand Island, NY) and gently triturated to dissociate cells. The cell suspension was centrifuged at $240 \times g$ for 5 min, and the resulting pellet was resuspended in Neurobasal-A medium (Gibco, Thermo Fisher Scientific, Grand Island, NY) supplemented with 1× B27 and 1× GlutaMAX (Gibco, Thermo Fisher Scientific, Grand Island, NY). Cells (50 μ L) were plated onto 35-mm glass-bottom culture dishes (MatTek, Ashland, MA) and incubated (37°C/5% CO₂) for 2 h to allow adhesion. An additional 2 mL of supplemented Neurobasal-A medium was then added, and cultures were maintained at 37°C. Half of the medium was replaced with fresh supplemented Neurobasal-A ~24 h after plating and every 2 days thereafter.

2.2 Electrophysiology

All experiments were conducted within 1-5 days of culturing. Whole cell patch clamp in voltage clamp was used to measure Na⁺ current. Glass electrodes were pulled from borosilicate glass (Sutter Instrument, Novato, CA). Electrodes had resistance of 2-4 M Ω when filled with internal pipette solution containing (mM): 3 NaCl, 5 CsCl, 120 CsMeSO₄, 1 MgCl₂, 10 EGTA, 10 HEPES and 2 Na₂ATP (pH 7.2 and 290 mOsM). External bath solution contained (mM): 25 NaCl, 125 TEA-Cl, 1 MgCl₂, 1 CaCl₂, 1 CsCl, 1 BaCl₂, 10 HEPES and 10 Glucose (pH 7.3 and 300 mOsM). To record spontaneous activity of SFO neurons, cell-attached voltage-clamp was used. Both the external bath and internal pipette solutions contained (mM): 140 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES and 10 Glucose (pH 7.4 and 300mOsM). Whole-Cell Current-Clamp was used to record action potentials. External bath solution contained (mM): 140 NaCl, 4 KCl, 2

CaCl₂, 1 MgCl₂, 10 HEPES and 10 Glucose (pH 7.4 and 300mOsM). Internal pipette solution contained (mM): 130 K-Gluconate, 10 KCl, 0.1 CaCl₂, 1 MgCl₂, 10 HEPES, 10 EGTA, 4 Na₂ATP and 0.3 Na₂GTP (pH 7.3 and 290 mOsM). To evaluate the impact of Na_v1.7, cells were recorded in the presence of 200 nM, 100 nM or 20 nM ProToxin-II (Tocris Bioscience, Bristol, UK). Data were acquired using a HEKA EPC 10 amplifier and PatchMaster software.

For activation protocols, neurons were held at -100 mV for 20 ms and then subjected to a series of 40-ms depolarizing voltage steps from -60 mV to $+70$ mV in 10-mV increments. Time constants of inactivation were quantified by fitting biexponential functions to the decay of Na⁺ currents evoked by voltage steps, allowing separation of fast and slow components of inactivation. For steady-state inactivation protocols, cells were held at -60 mV and subjected to 250-ms conditioning pre-pulses ranging from -120 mV to -10 mV (in 10-mV increments), followed by a 10-ms test pulse to 0 mV. Time-dependent recovery from inactivation (TDRI) was assessed using a paired-pulse protocol: neurons were held at -120 mV, depolarized to a test pulse of 0 mV (20 ms duration), and then allowed to recover (at -120 mV) for varying inter-pulse intervals (approximately 15–1000 ms) before a second identical test pulse to 0 mV. Finally, persistent sodium current was measured using slow depolarizing voltage-ramp protocols from -100 mV to $+60$ mV over 250 ms.

Cell-attached voltage-clamp recordings (without rupturing the membrane) were used to monitor spontaneous firing activity. In this configuration neurons were held at 0 mV to record action current frequency and regularity before and after ProToxin-II application. This minimally invasive approach allowed assessment of how sodium channel modulation influences baseline neuronal activity.

Whole-cell current-clamp recordings were performed to evaluate the effects of ProToxin-II on action potential generation and excitability. Neurons were held at approximately -70 mV and subjected to a series of 2-second depolarizing current injections in 2 pA or 5 pA increments (2 pA steps were used for higher input-resistance cells and 5 pA steps for lower input-resistance cells). In addition, slow current-ramp protocols were applied from a holding potential of approximately -70 mV, consisting of a linear ramp from -10 pA to $+10$ pA over 100 ms, to determine action potential threshold (defined as the membrane potential at which the rate of rise, dV/dt , exceeded 10 mV/ms; Platkiewicz and Brette, 2010).

2.3 Mathematical Modeling

A single-compartment Hodgkin-Huxley mathematical model of SFO neuron has previously been published by Medlock et al. (2018). This model includes two Na^+ conductances (I_{NaT} and I_{NaP}) to represent all voltage-gated Na^+ currents in a single neuron. In this study, we have generated two additional Na^+ conductances based on the properties of the ProToxin-II-sensitive currents and incorporated them into the model. Experimentally derived Hodgkin-Huxley gating parameters for voltage-gated Na^+ currents were obtained from whole-cell voltage-clamp recordings performed in this study (see Table 3 for summary of parameters). Specifically, control conditions and ProToxin-II-sensitive ($\text{Na}_v1.7$) currents were characterized for voltage dependence of activation and inactivation, and fast inactivation time constant (τ_h). Persistent Na^+ current was incorporated as a slowly inactivating component scaled to the experimentally observed fraction of peak transient Na^+ current.

To simulate the functional role of $\text{Na}_v1.7$, four modeling experiments were performed:

1. Control model: Simulations were run using the original (Medlock et al., 2018) parameters to reproduce baseline spontaneous tonic firing.
2. Reduced conductance: A fraction of the total voltage-gated Na^+ conductance (approximately 12% of I_{NaT} and 25% of I_{NaP} , based on the mean block observed with 20 nM ProToxin-II) was removed to mimic $\text{Na}_v1.7$ -specific Na^+ current reduction.
3. $\text{Na}_v1.7$ replacement: The same fractional conductances removed in experiment 2 were replaced with $\text{Na}_v1.7$ conductance using our experimentally derived gating parameters (including $V_{1/2}\text{-ACT} \approx -32.5$ mV, $\tau_h \approx 1$ ms, and a prominent persistent component). The value of -32.5 mV was obtained by mathematical subtraction of the ProToxin-II-resistant current. However, because our experimental data indicated that $\text{Na}_v1.7$ activates at more depolarized potentials in SFO neurons, we also modeled $\text{Na}_v1.7$ conductance with a $V_{1/2}\text{-ACT}$ of -29.5 mV to assess sensitivity to activation voltage.
4. Slowed inactivation: $\text{Na}_v1.7$ conductance from experiment 3 was further modified by increasing τ_h from 1 ms to 1.1 ms, and in one simulation to 3 ms, to evaluate the impact of inactivation kinetics on firing behavior.

2.4 Statistical Analysis

All data were analyzed using OriginLab software (OriginLab Corp., Northampton, MA, USA) unless otherwise specified. Spontaneous action potential activity recorded in the cell-attached configuration was analyzed using Spike2 software (Cambridge Electronic Design, Cambridge, UK). Data are reported as mean $\pm$ standard error of the mean (SEM) unless otherwise noted.

Normality of data distributions was assessed using the Shapiro–Wilk test. For normally distributed paired data (e.g., control vs. ProToxin-II conditions within the same neuron), statistical comparisons were performed using two-tailed paired Student's t-tests. When multiple concentrations of ProToxin-II were compared against control (dose-response experiments), separate paired t-tests were conducted for each concentration, with Bonferroni correction applied to adjust for multiple comparisons (adjusted $\alpha = 0.0167$ for three concentrations).

For the computational modeling experiments, differences in mean firing frequency and coefficient of variation across multiple conditions were evaluated using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's multiple comparison tests. Paired t-tests were used for direct two-condition comparisons within the modeling dataset (e.g., effects of inactivation time constant).

Number of evoked action potentials as a function of injected current were compared descriptively between control and ProToxin-II conditions, with data presented as mean $\pm$ SEM for each current step. Action potential threshold values were compared using paired t-tests.

A significance level of $p < 0.05$ was considered statistically significant for all tests unless otherwise adjusted for multiple comparisons. Exact p-values are reported throughout the Results section.

3. Results

To investigate the role of $\text{Na}_v1.7$ in the intrinsic excitability of rat SFO neurons, we performed whole-cell voltage-clamp and current-clamp recordings, as well as cell-attached recordings in acutely dissociated SFO neurons before and after application of the selective

Nav1.7 antagonist ProToxin-II. ProToxin-II reduced overall Na⁺ current amplitude, produced hyperpolarizing shifts in the voltage dependence of both activation and steady-state inactivation, accelerated fast inactivation and slowed recovery from inactivation. Additionally, ProToxin-II caused a proportionally greater reduction in persistent Na⁺ current relative to peak transient Na⁺ current. Current-clamp recordings revealed decreased evoked action potential output across a range of depolarizing current injections, despite a hyperpolarization in action potential threshold. Spontaneous firing activity recorded in the cell-attached configuration showed heterogeneous, bidirectional modulation, with ProToxin-II shifting individual neurons between high-frequency tonic and lower-frequency burst patterns. Together, these findings reveal complex effects of Nav1.7 blockade on SFO neuronal excitability.

3.1 Dose-dependent Block of Nav1.7

To establish an appropriate concentration of ProToxin-II for use in subsequent experiments, we first examined its concentration-dependent effects on voltage-gated Na⁺ currents. Whole-cell voltage-clamp recordings were performed under control conditions and in the presence of ProToxin-II at 200 nM, 100 nM, or 20 nM (Fig. 1). Mean ($\pm$ SEM) percent decrease in peak Na⁺ currents were $55.4 \pm 4.15\%$ at 200 nM ($n = 8$), $41.6 \pm 5.31\%$ at 100 nM ($n = 10$), and $14.6 \pm 4.83\%$ at 20 nM ($n = 12$) (Fig. 1B). Paired t-tests confirmed that each concentration significantly reduced Na⁺ currents compared with control (200 nM: $p < 0.0001$; 100 nM: $p < 0.001$; 20 nM: $p = 0.012$). Based on these results, we selected 20 nM ProToxin-II as the working concentration for all subsequent experiments, as it provided consistent blockade of Na⁺ currents consistent with what we expect from the literature, while minimizing potential off-target effects on other VGSC subtypes. This concentration aligns closely with the range of reported IC₅₀ values for ProToxin-II against rat Nav1.7 in the literature (see Table 2).

Table 2. ProToxin-II subtype selectivity between rat and human Na_v channels. Shaded cells represent Na_v channels not expressed in SFO (Peterson, 2018).

Na _v Subtype	Human IC ₅₀ (nM) (Gonçalves et al., 2018)	Rat IC ₅₀ (nM) (Park et al., 2014; Fouillet et al., 2017)
Na _v 1.1	16	n.a.
Na _v 1.2	41	105
Na _v 1.3	102	n.a.
Na _v 1.4	39	n.a.
Na _v 1.5	79-398	n.a.
Na _v 1.6	26	n.a.
Na _v 1.7	0.3	72
Na _v 1.8	146	n.a.
Na _v 1.9	n.s.	n.a.

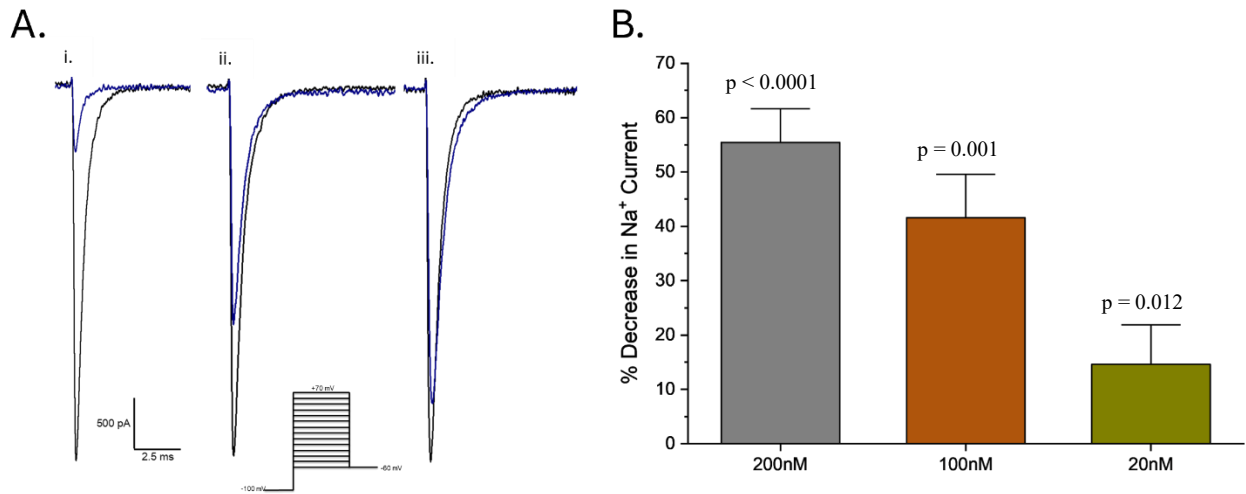


Figure 1. Dose-dependent block of Nav1.7 by ProToxin-II. (A) Representative whole-cell transient Na⁺ currents (I_{NaT}) recorded from SFO neurons. Currents were elicited by holding cells at -100 mV for 10 ms followed by depolarizing steps from -60 to $+70$ mV in 10-mV increments. Black traces represent control currents, and blue traces show currents following application of ProToxin-II at (i) 200 nM, (ii) 100 nM, and (iii) 20 nM. (B) Mean percent decrease in Na⁺ current at each ProToxin-II concentration. Data presented as mean $\pm$ SEM. Mean current reduction was $55.4 \pm 4.15\%$ at 200 nM ($n = 8$), $41.6 \pm 5.31\%$ at 100 nM ($n = 10$), and $14.26 \pm 4.83\%$ at 20 nM ($n = 12$). Statistical significance was assessed using paired t-tests for each concentration. 200 nM ($p < 0.0001$), 100 nM ($p = 0.001$) and 20 nM ($p = 0.012$). These effects remained significant after Bonferroni correction for multiple comparisons (adjusted $\alpha = 0.0167$).

3.2a ProToxin-II Causes Hyperpolarizing Shift in Voltage of Activation & Inactivation

To investigate the effects of ProToxin-II on activation and inactivation gating, we employed voltage-clamp activation and inactivation protocols. ProToxin-II produced a hyperpolarizing shift in $V_{1/2}$ -ACT from -18.9 ± 0.41 mV (control) to -21.5 ± 0.40 mV (20 nM ProToxin-II, paired t-test, $p = 3.87 \times 10^{-4}$, $n = 15$; Fig 2C). $V_{1/2}$ -INACT also significantly shifted from -49.1 ± 1.99 mV (control) to -54.2 ± 1.62 mV (20 nM ProToxin-II, paired t-test, $p = 5.77 \times 10^{-4}$, $n = 10$; Fig 2D). These results indicate that $Na_v1.7$ has a depolarized voltage of activation and inactivation. Notably, this apparent depolarized voltage of activation suggests that $Na_v1.7$ channels require more depolarization to activate, which is unlikely to increase neuronal excitability and may instead reduce it by raising the threshold for action potential initiation. This contrasts with the literature on wild-type or gain-of-function mutant $Na_v1.7$ channels, where hyperpolarized shifts in activation promote hyperexcitability in neurons by allowing channels to open more readily (Cummins et al., 2004; Sheets et al., 2007; Bennett and Woods, 2014; Yang et al., 2016b).

3.2b ProToxin-II Accelerates Fast Inactivation

Next, we next asked whether ProToxin-II alters the rate of Na^+ channel inactivation. To address this, we quantified the fast and slow components inactivation by fitting biexponential functions to whole-cell Na^+ current decay. The fit used the equation $I(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + C$ where τ_1 and τ_2 are the fast and slow inactivation time constants, A_1 and A_2 are their respective amplitudes, and C is the steady-state offset (Fig. 3A). ProToxin-II significantly accelerates τ_1 from 1.01 ± 0.09 ms (control) to 0.76 ± 0.06 ms (20 nM ProToxin-II, paired t-test, $p = 9.49 \times 10^{-4}$, $n = 15$; Fig. 3B). Similarly, τ_2 decreased from 5.59 ± 0.71 ms (control) to $5.27 \pm$

0.74 ms (20 nM ProToxin-II), however, this was not statistically significant (paired t-test, $p = 0.53$, $n = 14$; Fig. 3C). This data suggests $\text{Na}_v1.7$ exhibits *slower* kinetics of inactivation (i.e. takes longer to inactivate), which may allow it to remain in a conducting state for slightly longer.

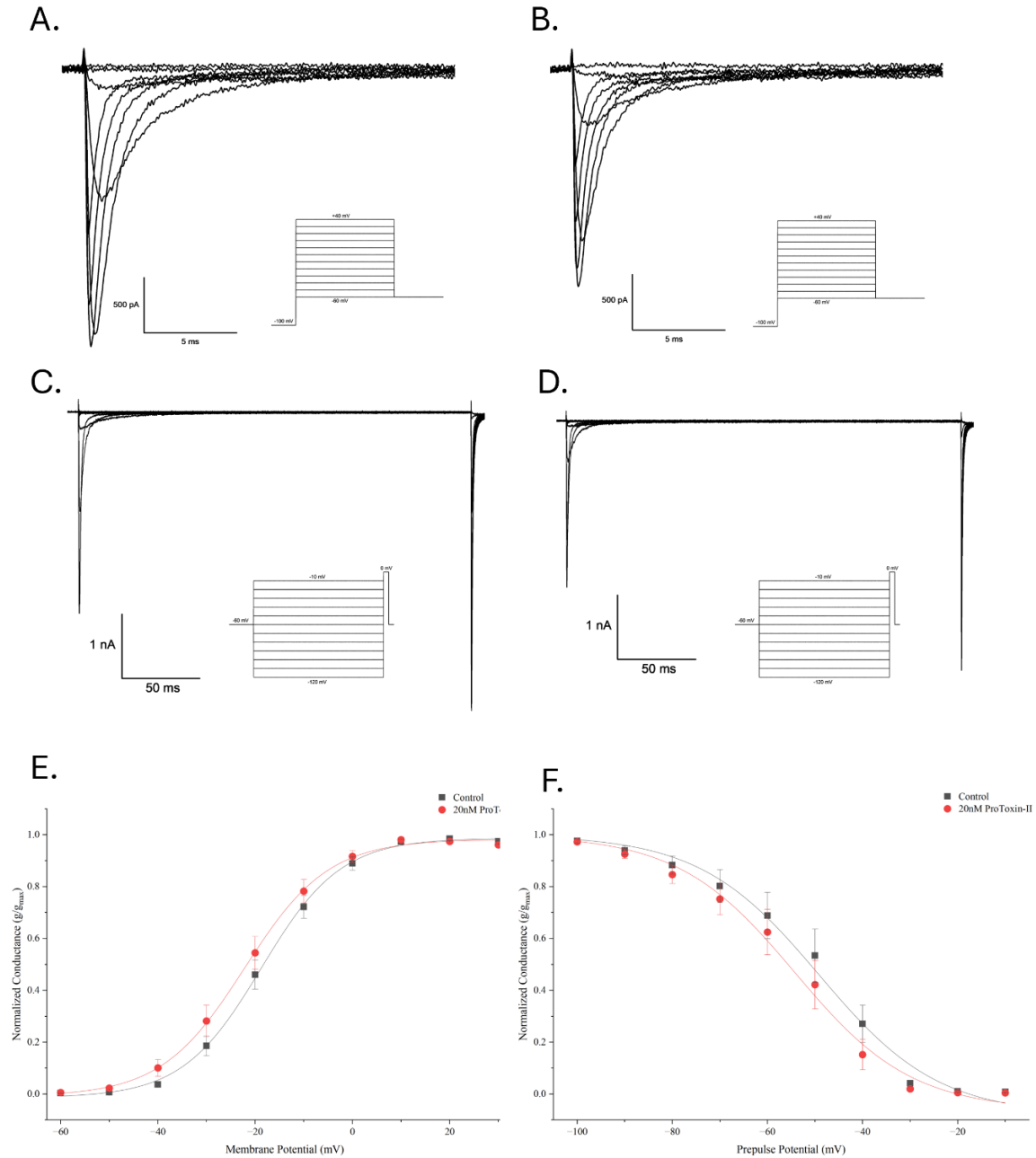


Figure 2. Effects of 20 nM ProToxin-II on the gating properties of voltage-gated Na⁺ currents in SFO neurons. (A-D) Representative Na⁺ current traces recorded from single neuron during activation (A, B) and inactivation (C, D) protocols before and after application of 20 nM ProToxin-II. Peak Na⁺ current decreases from 2.46 nA (A, control) to 1.95 nA (B, 20 nM ProToxin-II) (~20% decrease). (E, F) Analysis of voltage-dependence of activation and inactivation. (E) Mean shift in half-activation ($V_{1/2}$ -ACT) shifted from -18.9 ± 0.41 mV (control, $n = 15$) to -21.5 ± 0.40 mV (20 nM ProToxin-II). (F) Mean shift in half-inactivation ($V_{1/2}$ -INACT) shifted from -49.1 ± 1.99 mV (control, $n = 10$) to -54.2 ± 1.62 mV (20 nM ProToxin-II). Paired t-test revealed mean shifts in $V_{1/2}$ -ACT and $V_{1/2}$ -INACT were statistically significant ($p = 3.87 \times 10^{-4}$ and $p = 5.77 \times 10^{-4}$, respectively)

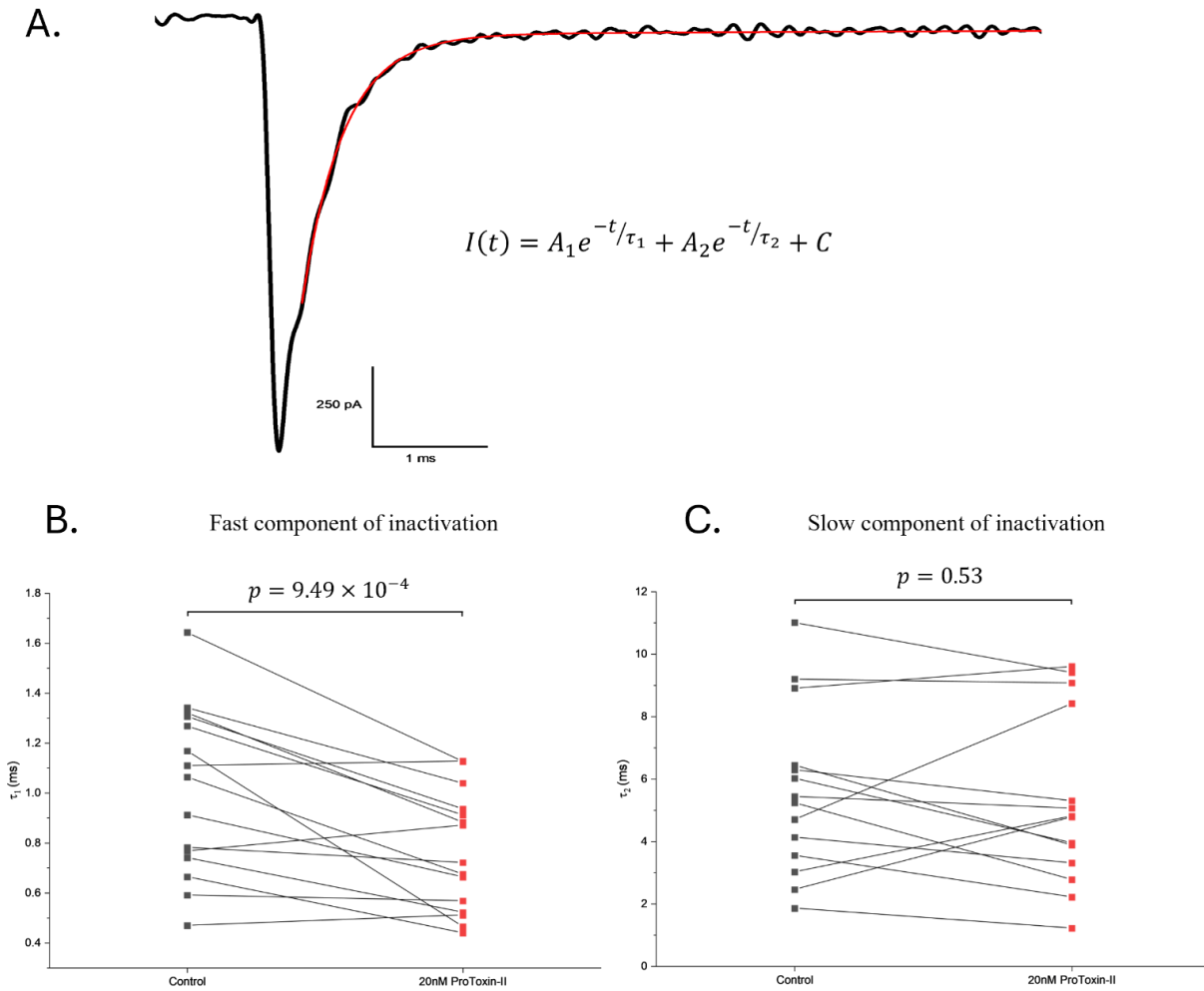


Figure 3. Effect of ProToxin-II on inactivation kinetics of voltage-gated Na^+ current in SFO neurons. (A) Representative current trace (black) evoked by a depolarizing step, with biexponential fit to the decay phase (red) overlaid. The fit used the equation $I(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + C$ where τ_1 and τ_2 are the fast and slow inactivation time constants, A_1 and A_2 are their respective amplitudes, and C is the steady-state offset. (B) τ_1 decreased significantly from 1.01 ± 0.09 ms (control) to 0.76 ± 0.06 ms (20 nM ProToxin-II, paired t-test, $p = 9.49 \times 10^{-4}$, $n = 15$). (C) τ_2 decreased from 5.59 ± 0.71 ms (control) to 5.27 ± 0.74 ms (20 nM ProToxin-II), however, the change was not statistically significant (paired t-test, $p = 0.53$, $n = 14$)

3.2c Time-Dependent Recovery from Inactivation is Prolonged

Time-dependent recovery from inactivation was examined using a two-pulse voltage-clamp protocol with variable inter-pulse intervals (Fig. 4A). Fractional recovery was quantified by fitting biexponential functions to recovery time courses obtained from individual neurons under control conditions and following application of 20 nM ProToxin-II (Fig. 4B), yielding fast (τ_1) and slow (τ_2) recovery components. ProToxin-II prolonged the fast recovery component, with τ_1 increasing from 5.4 ± 0.38 ms in control to 15.7 ± 4.9 ms after treatment; however, this effect narrowly failed to reach statistical significance (paired t -test, $p = 0.0507$, $n = 12$). In contrast, ProToxin-II significantly slowed the slow component of recovery, with τ_2 increasing from 144.7 ± 16.2 ms under control conditions to 200.6 ± 26.3 ms following ProToxin-II application (paired t -test, $p = 0.0045$, $n = 12$). Recovery from inactivation (repriming) is an important factor limiting neuronal firing frequency. Together, these results demonstrate $\text{Na}_v1.7$ displays a relatively fast recovery from inactivation.

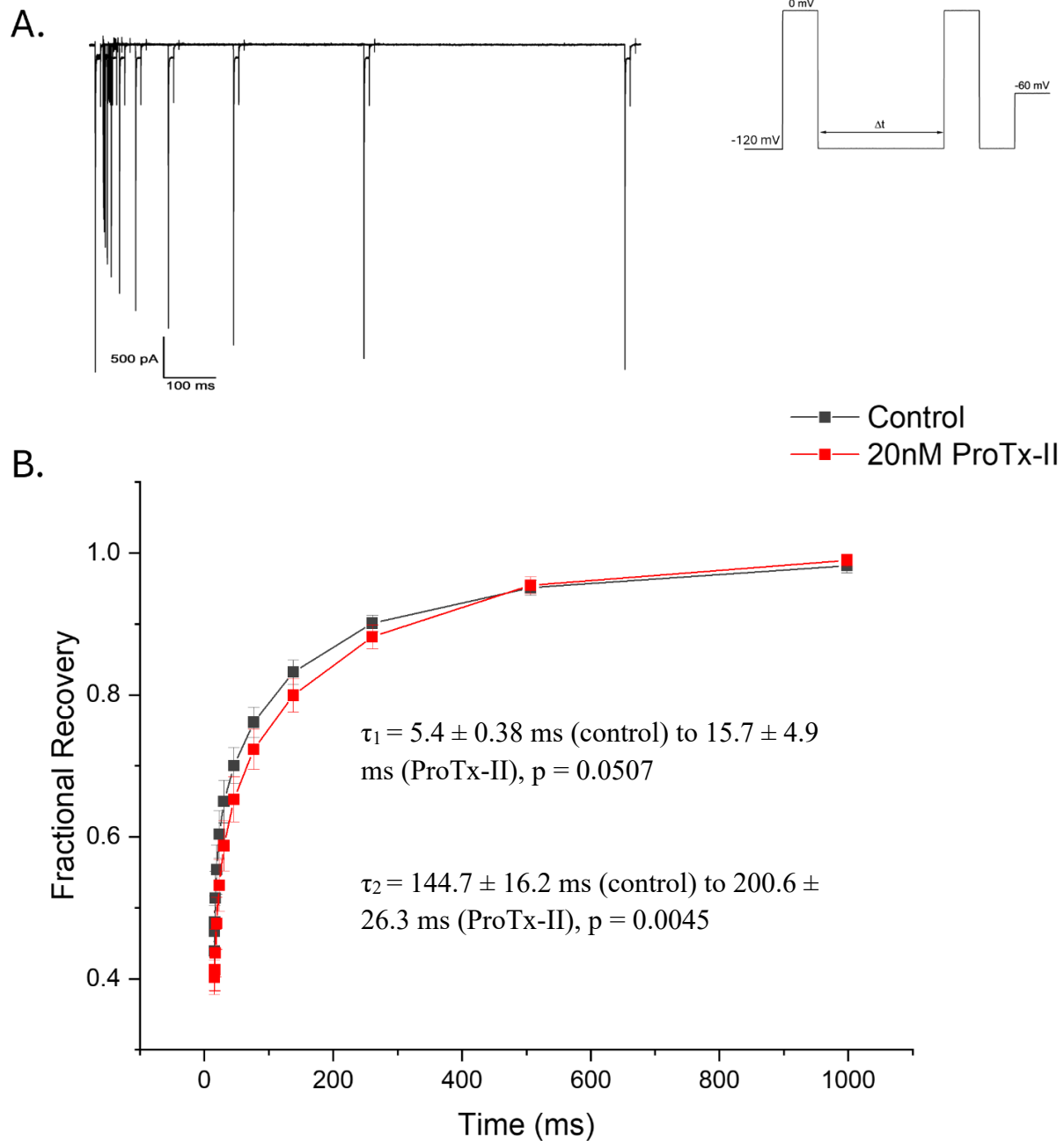


Figure 4. ProToxin-II slows recovery from inactivation. (A) Representative trace of SFO neuron subjected to two 0 mV pulses with varying time between pulses (15-998 ms). (B) Recovery from inactivation was quantified by fitting biexponential functions to recovery time courses obtained from individual neurons before and after ProToxin-II application, yielding fast (τ_1) and slow (τ_2) recovery time constants. τ_1 increased from 5.4 ± 0.38 ms under control conditions to 15.7 ± 4.9 ms following ProToxin-II application ($n = 12$). Although τ_1 tended to increase, this difference narrowly failed to reach statistical significance (paired t-test, $p = 0.0507$). τ_2 increased from 144.7 ± 16.2 ms in control to 200.6 ± 26.3 ms after treatment (paired t-test, $p = 0.0045$, $n = 12$).

3.2d ProToxin-II Blocks Proportionally More Persistent Current

Persistent Na^+ current was assessed to determine whether ProToxin-II preferentially affects sustained channel activity in addition to transient Na^+ currents. Representative traces illustrate a sustained inward Na^+ current during a slow depolarizing ramp under control conditions (Fig. 5A). Mean amplitude of I_{NaP} decreased from 141 ± 23.2 pA (control) to 87 ± 18.6 pA (20 nM ProToxin-II), paired t-test, $p < 0.0001$, $n = 18$; Fig. 5B). To quantify ProToxin-II's effect independently of cell-to-cell variability in peak current amplitude, I_{NaP} was measured as a percentage of the peak transient Na^+ current (I_{NaT}). Under control conditions, I_{NaP} accounted for $4.48 \pm 0.58\%$ of I_{NaT} whereas application of 20 nM ProToxin-II significantly reduced this fraction to $3.21 \pm 0.51\%$ (paired t-test, $p < 0.0001$, $n = 18$; Fig. 5C), representing a ~28% decrease in I_{NaP} . These data indicate that $\text{Na}_v1.7$ contributes a significant amount of I_{NaP} .

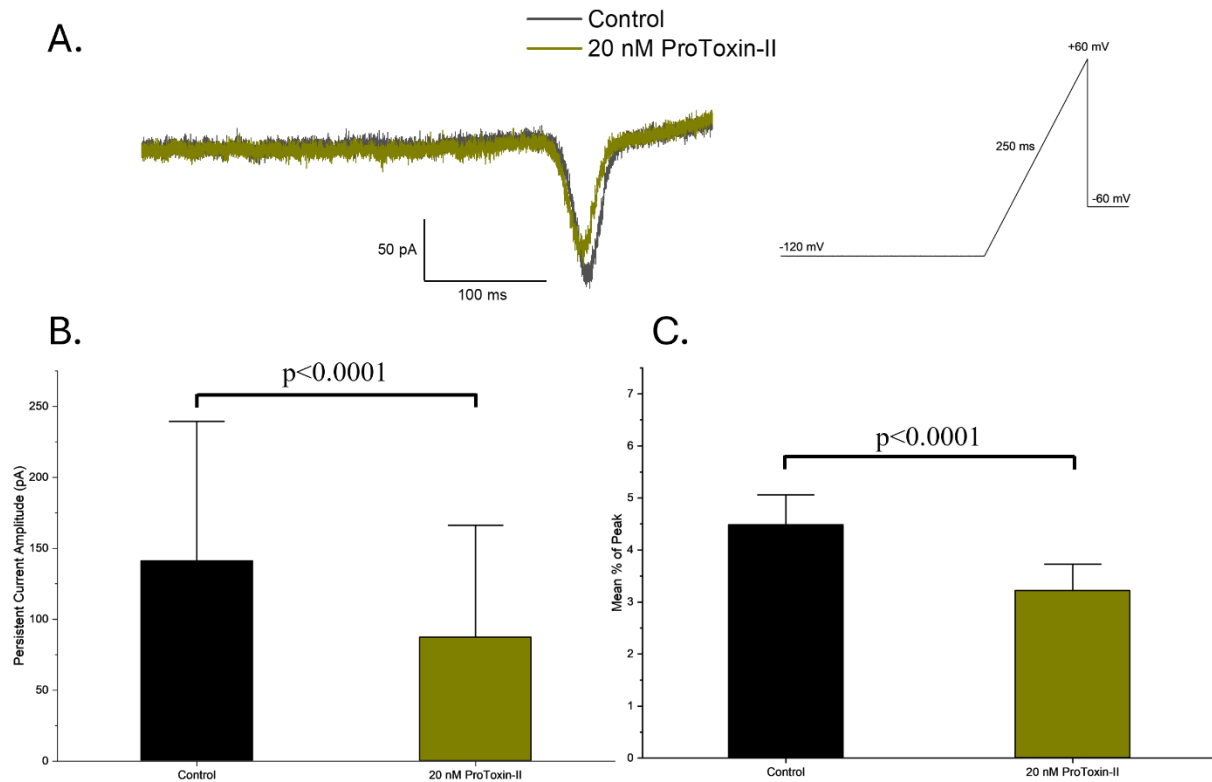


Figure 5. ProToxin-II causes proportional block of persistent Na^+ current. (A) Representative trace of I_{NaP} before and after ProToxin-II treatment. (B) Mean amplitude of I_{NaP} decreased from 141 ± 23.2 pA (control) to 87 ± 18.6 pA (20 nM ProToxin-II), paired t-test, $p < 0.0001$, $n=18$. (C) I_{NaP} was quantified as a percentage of peak transient sodium current (I_{NaT}). Under control conditions, I_{NaP} accounted for $4.48 \pm 0.58\%$ of peak I_{NaT} . Application of 20 nM ProToxin-II significantly reduced I_{NaP} to $3.21 \pm 0.51\%$ of peak I_{NaT} (paired t-test, $p < 0.0001$, $n = 18$).

3.3 Heterogenous Modulation of Spontaneous Activity

To determine whether ProToxin-II alters spontaneous firing properties of SFO neurons, we examined both firing rate and firing regularity before and after toxin application (Fig. 6). Representative traces illustrate spontaneous neuronal activity under control conditions and following exposure to 20 nM ProToxin-II in bursty neurons (Fig. 6A) and tonically firing neurons (Fig. 6B). To quantify these changes, we examined the relationship between the change in action potential frequency (Δ firing rate, x-axis) and the change in firing irregularity, measured as the change in coefficient of variation (Δ CV, y-axis) across 16 neurons (Fig. 6C). Positive values on the x-axis indicate increased firing rate after ProToxin-II, while positive values on the y-axis indicate increased irregularity (higher CV). Interestingly, the data revealed two apparent subpopulations: one group of neurons exhibited increased firing rates accompanied by more regular spiking (negative Δ CV), whereas another showed decreased firing rates with more irregular spiking (positive Δ CV). These findings demonstrate that 20 nM ProToxin-II elicits heterogeneous modulation of spontaneous excitability in SFO neurons, with bidirectional effects on both firing frequency and spiking regularity.

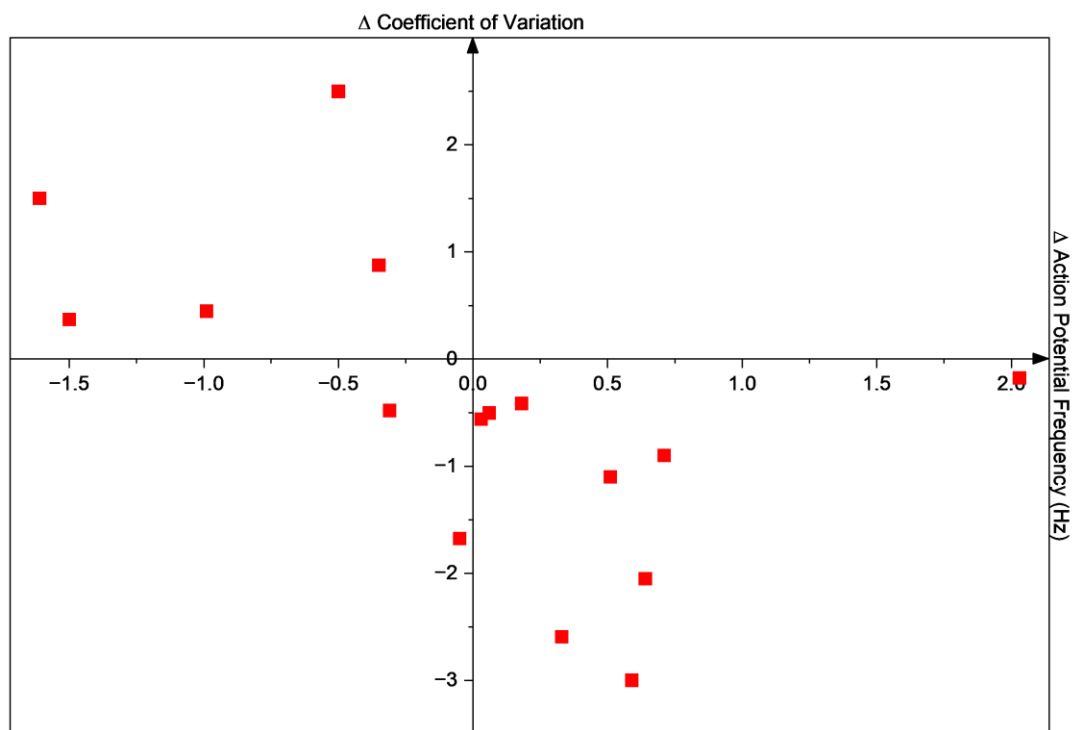
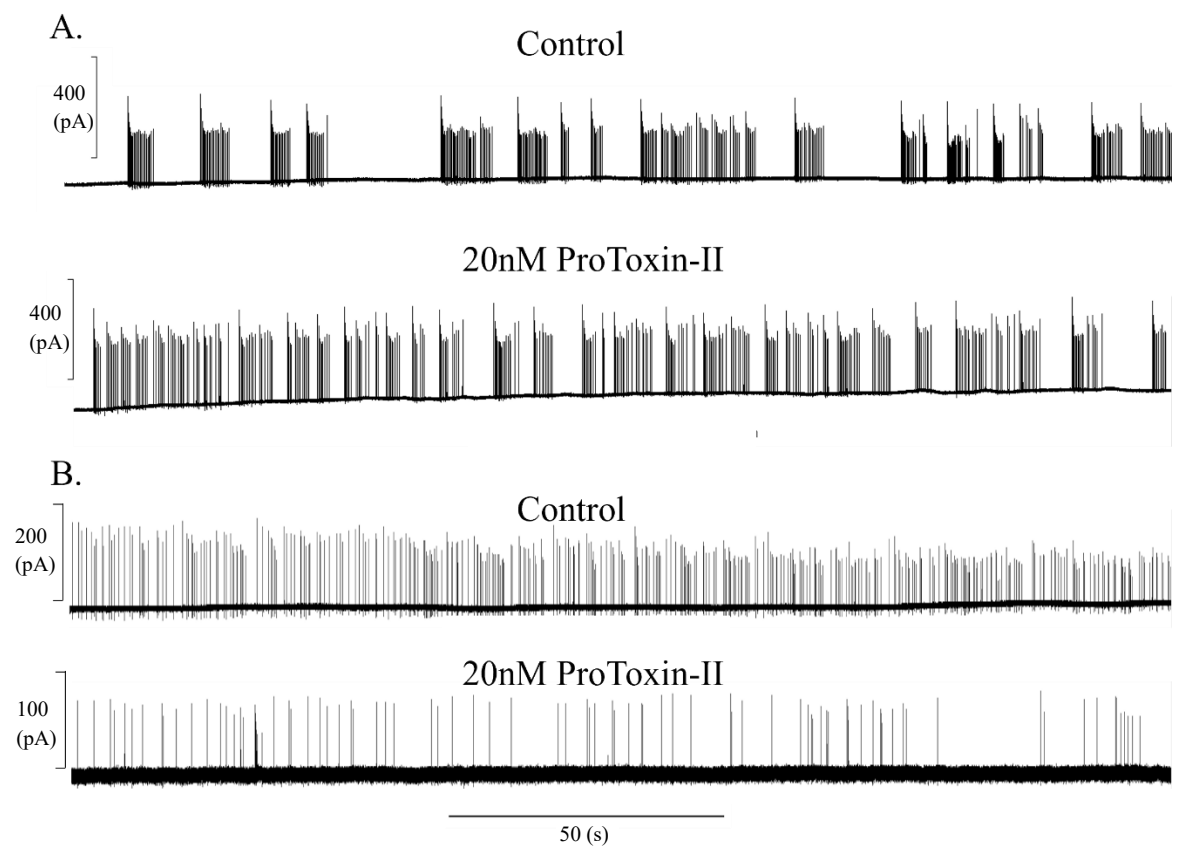


Figure 6. ProToxin-II induces heterogeneous changes in spontaneous action potential firing in cell-attached recordings of SFO neurons. (A, B) Representative cell-attached voltage-clamp traces showing spontaneous action potentials. (C) Scatter plot of the change in action potential frequency (x-axis) versus the change in coefficient of variation (ΔCV , y-axis) across 16 neurons. Positive values on x-axis indicate increased firing rate after ProToxin-II; positive values on y-axis indicate increased irregularity (higher CV). Data reveals two apparent populations: one showing increased firing rate with more regular spiking (negative ΔCV), and the other showing decreased firing rate with more irregular spiking (positive ΔCV). Data points represent individual neurons.

3.4 ProToxin-II Decreases Number of Evoked Action Potentials

To determine how ProToxin-II affects neuronal firing behavior, we examined action potential generation in SFO neurons. Whole-cell current-clamp recordings from SFO neurons held at -70 mV demonstrated that application of 20 nM ProToxin-II modified the response to depolarizing current injections (Fig. 7A). Under control conditions, small depolarizing current steps (e.g., +5 pA) elicited multiple action potentials, whereas in the presence of ProToxin-II, slightly larger currents were required (+7 pA) and evoked fewer spikes (Fig. 7A).

To quantify these effects, input-output relationships were constructed by plotting the number of elicited action potentials as a function of injected current amplitude. Current was injected in increments of either 2 pA (for neurons with higher input resistance) or 5 pA (for neurons with lower input resistance). In experiments using 2 pA current increments ($n = 7$ neurons), ProToxin-II reduced the number of action potentials across most tested current levels (Fig. 7B). Similarly, with 5 pA increments ($n = 11$ neurons), the input-output curve showed a comparable reduction in spike number for a given current injection (Fig. 7C). Values are presented as mean $\pm$ SEM. These observations indicate that 20 nM ProToxin-II decreases the excitability of SFO neurons.

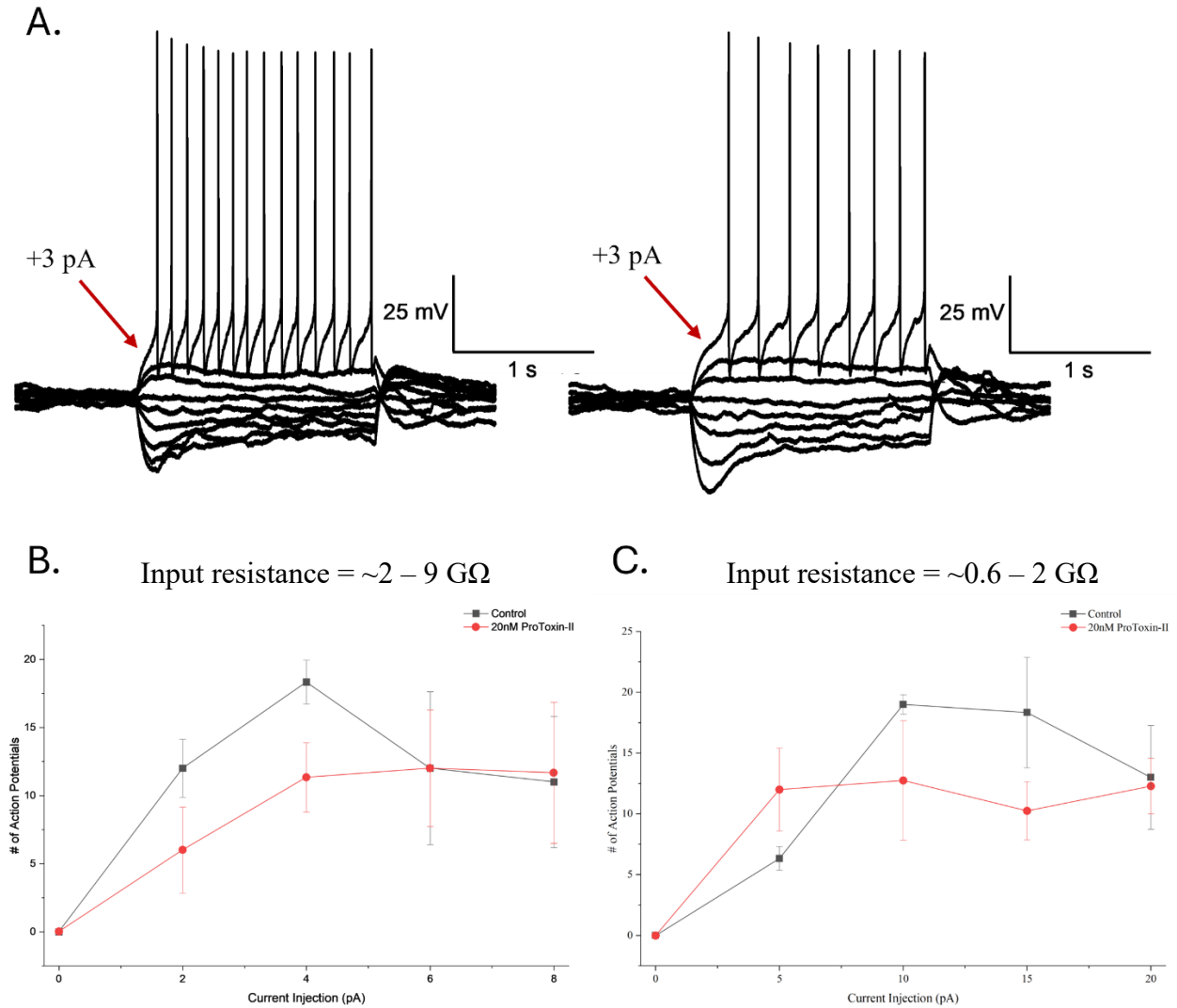


Figure 7. ProToxin-II decreases number of evoked action potentials in SFO neurons. Effects differ slightly based on input resistance. (A) Representative whole-cell current-clamp recordings from SFO neurons under control conditions (left) and in the presence of 20 nM ProToxin-II (right). Neurons were held at -70 mV and injected with series of depolarizing current steps. At same amount of injected current, neuron evoked fewer action potentials after ProToxin-II (control = 14, ProToxin-II = 8). (B, C) Input-output relationships showing the number of action potentials elicited as a function of injected current under control conditions (black squares) and in the presence of 20 nM ProToxin-II (red circles). Data in (B) used 2 pA current increments ($n=7$ cells); data in (C) used 5 pA increments ($n=11$ cells). Values are presented as mean $\pm$ SEM.

3.5 ProToxin-II Causes Hyperpolarizing Shift in Action Potential Threshold

To assess the impact of 20 nM ProToxin-II on action potential initiation, we employed a current-clamp ramp protocol in which neurons were injected with slow depolarizing current ramps (from -10 pA to +10 pA over 100 ms) from a holding potential of -70 mV. Representative voltage traces showed that ProToxin-II application resulted in action potentials firing at more negative membrane potentials compared to control conditions (Fig. 8A). Action potential threshold was operationally defined as the membrane potential at which the rate of rise (dV/dt) exceeded 10 mV/ms during the ramp depolarization. Paired comparison across 16 neurons revealed a significant hyperpolarizing shift in threshold following ProToxin-II application, from -35.7 ± 1.77 mV under control conditions to -38.0 ± 1.50 mV in the presence of 20 nM ProToxin-II (Figure 8B; paired t-test, $p = 0.023$, $n = 16$; Fig. 8B). The results are consistent with our earlier results (Fig 2A), suggesting $Na_v1.7$ activates at more depolarized potentials.

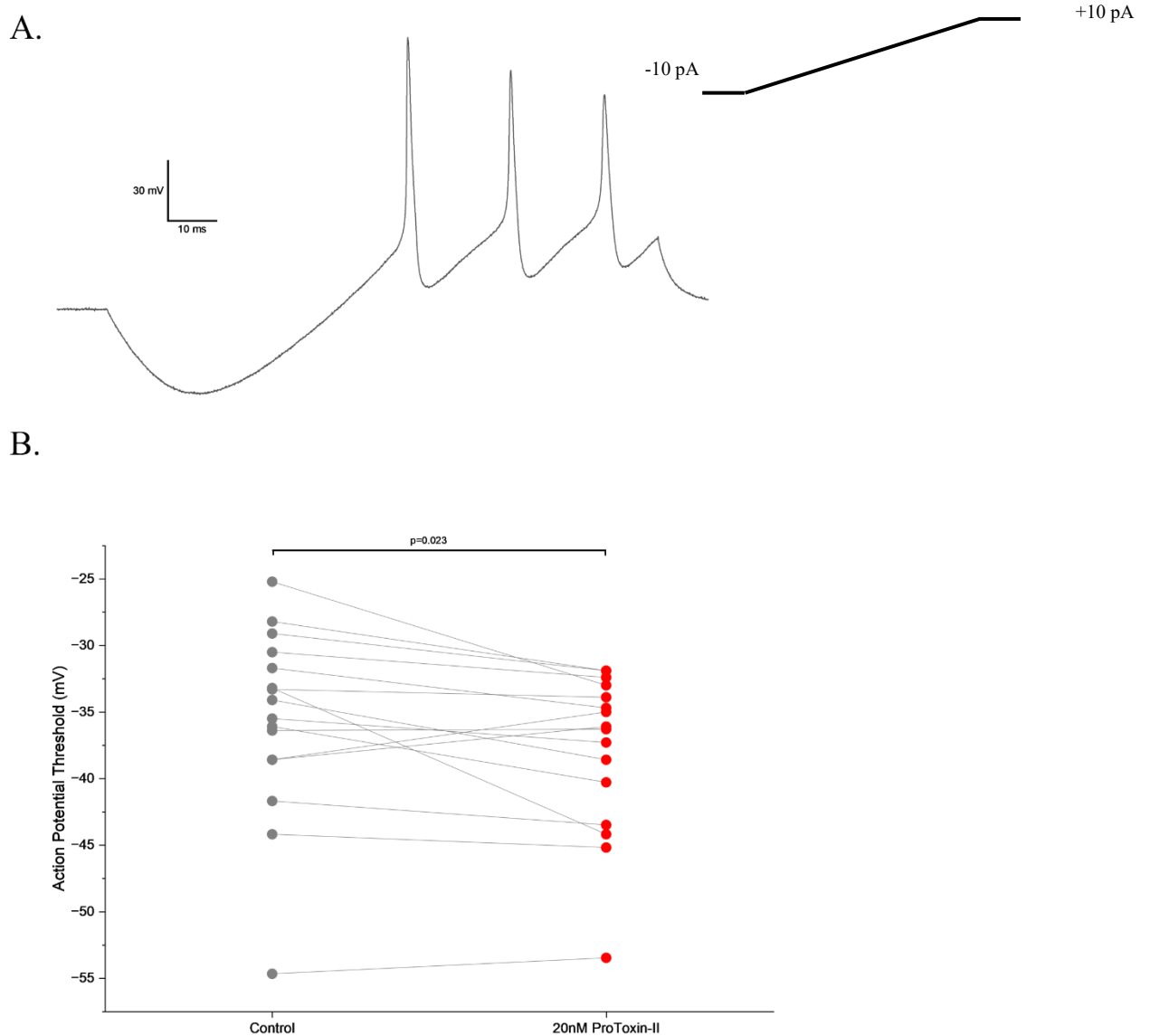


Figure 8. ProToxin-II causes hyperpolarizing shift in action potential threshold. (A) Representative voltage trace of action potentials elicited by current clamp ramp protocol. Neuron subjected to depolarizing current injection from -10 to +10 pA over 100 ms. Action potential threshold was defined as membrane potential at which $\Delta V/\Delta t$ exceeded 10 mV/ms during ramp depolarization. (B) Paired comparison of action potential threshold measured during current ramp protocols. Each line represents an individual neuron. ProToxin-II significantly hyperpolarized action potential threshold from -35.7 ± 1.77 mV to -38.0 ± 1.50 mV (paired t-test, $p = 0.023$, $n = 16$).

3.6 Mathematical Modeling: I_{NaP} is Major Driver of Electrical Excitability

Medlock et al. (2018) investigated the ionic mechanisms driving tonic and burst firing behaviours observed in SFO neurons. Using patch clamp data collected from dissociated rat SFO neurons, they were able to construct a single-compartment Hodgkin-Huxley-type mathematical model of an SFO neuron, which incorporated the following currents: transient (I_{Na}) and persistent (I_{NaP}) Na^+ , delayed-rectifier (I_K) and transient (I_A) K^+ , N-type Ca^{2+} (I_{Ca}), slow-activating K^+ (I_{KS}), nonselective cationic (I_{NSCC}), leak (I_L), applied (I_{app}), and noise (I_{noise}) currents. The model reproduces both firing regimes: burst firing requires I_{NSCC} for depolarization to I_{NaP} threshold, I_{Ca} for plateau maintenance, and I_{KS} for termination via slow activation/deactivation dynamics; burst firing could be transitioned to tonic firing by increasing I_{Na} or I_K conductances, which enhance afterhyperpolarization to prevent bursts.

Computational modeling of $Na_v1.7$ properties in the Medlock et al. (2018) single-compartment Hodgkin-Huxley-type SFO neuron model markedly altered spontaneous tonic firing behavior. Representative 60-second voltage traces showed infrequent, irregular spiking in the unmodified control condition (i), complete elimination of activity following reduction of I_{NaT} and I_{NaP} conductances by 12% and 25%, respectively (ii), and robust, high-frequency regular tonic firing upon replacement of the same fractional conductances with $Na_v1.7$ conductance (iii), with similar high-frequency activity when the $Na_v1.7$ conductance incorporated a slightly depolarized $V_{1/2-ACT}$ (iv). One-way ANOVA revealed significant differences in mean firing frequency among groups. Post-hoc Tukey test indicated that control firing frequency (0.358 ± 0.028 Hz) was significantly lower than both $Na_v1.7$ replacement conditions (Experiment 2: 3.517 ± 0.037 Hz; Experiment 3: 3.425 ± 0.021 Hz; $p < 0.0001$), while the two $Na_v1.7$ conditions did not differ from each other. Similarly, one-way ANOVA showed significant group differences in

the CV. Post-hoc Tukey test demonstrated that control CV (0.814 ± 0.072) was significantly higher than in both $\text{Na}_v1.7$ replacement conditions (Experiment 2: 0.580 ± 0.014 ; Experiment 3: 0.631 ± 0.010 ; $p < 0.05$), with no significant difference between the two $\text{Na}_v1.7$ conditions. All data are presented as mean $\pm$ SEM ($n = 6$ simulations per condition). These findings indicate that introduction of $\text{Na}_v1.7$ conductance promotes sustained, high-frequency, regular tonic firing in the SFO neuron model.

Table 3. Experimentally derived Hodgkin-Huxley parameters for voltage-gated Na⁺ current in SFO neurons

	$V_{1/2\text{-ACT}}$ (mV)	k_{ACT}	τ_m (ms)	$V_{1/2\text{-INACT}}$ (mV)	k_{INACT}	τ_h (ms)
Medlock Model	-31	6.1	0.1	-62	-6.2	0.8
Control	-18.9	8.2	n.d.	-49.1	12.6	1.01
ProToxin-II	-21.5	8.9	n.d.	-54.2	12.3	0.76
Na _v 1.7 parameters (used in model)	-32.5	4.9	n.d.	-61.5	-6.7	1.1

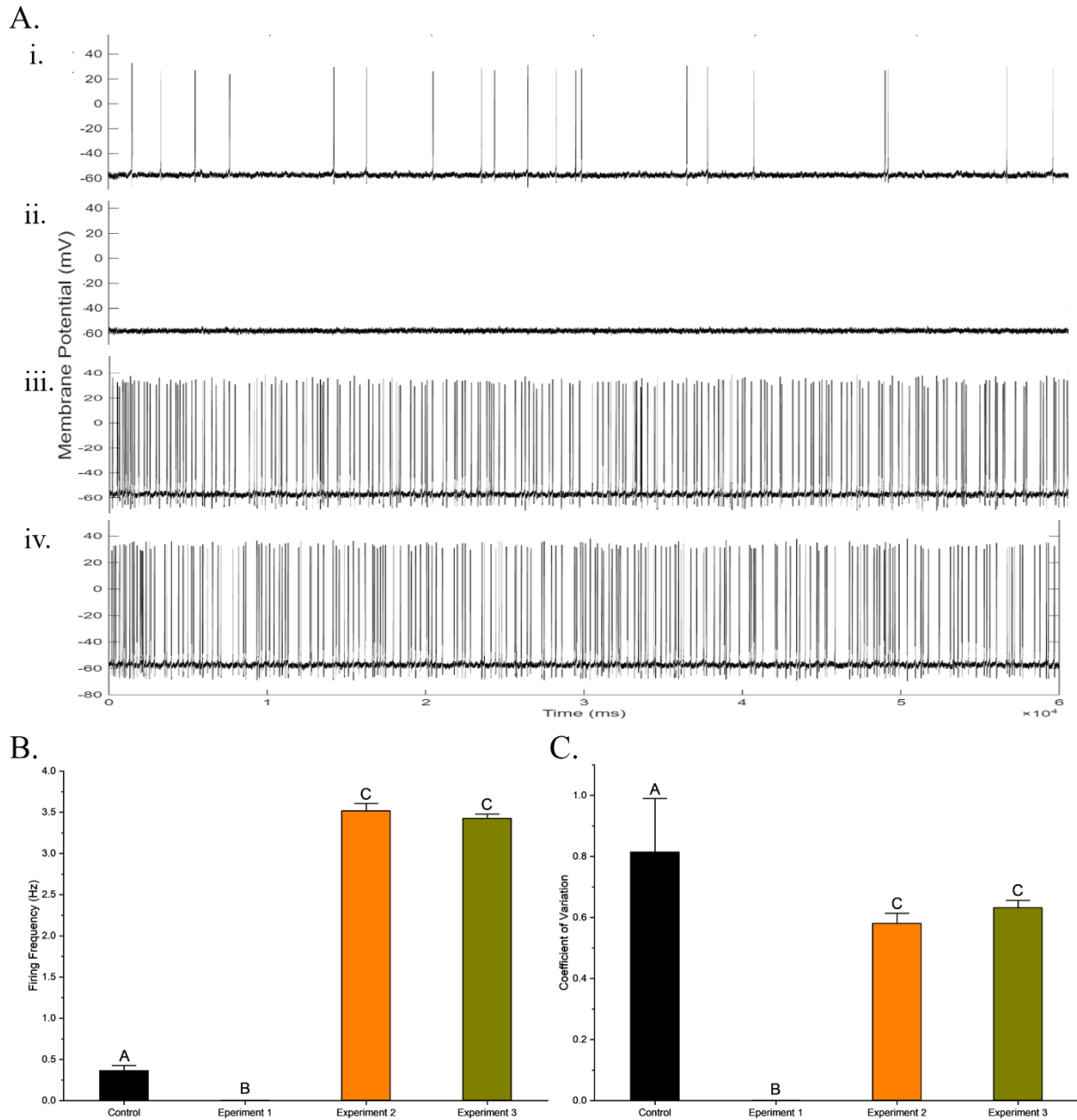


Figure 9. Computational modeling of Nav1.7 properties on spontaneous tonic firing using Hodgkin-Huxley-type mathematical model of SFO neuron. Representative 60-second voltage traces of spontaneous tonic firing are shown under four conditions: (i) unmodified control model; (ii) I_{NaT} and I_{NaP} conductance reduced by 12% and 25%, respectively; (iii) replacement of 12% and 25% of model I_{NaT} of I_{NaP} with Nav1.7 Na^+ conductance; (iv) same as (iii) but with Nav1.7 conductance modeled using a slightly depolarized voltage of half-activation ($V_{1/2-ACT}$) relative to control. (B) One-way ANOVA revealed significant differences in mean firing frequency among groups. Post-hoc Tukey tests indicated that control (0.358 ± 0.028 Hz) differed significantly from Experiment 1 (0 Hz), Experiment 2 (3.517 ± 0.037 Hz) and Experiment 3

(3.425 ± 0.021 Hz; $p < 0.0001$). Experiments 2 and 3 did differ from Experiment 1 ($p < 0.0001$) but did not differ from each other. (C) Coefficient of variation significantly differed among groups. Post-hoc Tukey tests showed that control (0.814 ± 0.072) had significantly higher CV than Experiment 1 (0), Experiment 2 (0.580 ± 0.014) and Experiment 3 (0.631 ± 0.010 ; $p < 0.05$). Experiments 2 and 3 did differ from Experiment 1 ($p < 0.0001$) but did not differ significantly from each other. Means not sharing the same letter are significantly different. Data presented as mean $\pm$ SEM, $n = 6$ for all experimental groups.

To examine whether slowing the inactivation kinetics of $\text{Na}_v1.7$ conductance influences model tonic firing, we compared the baseline $\text{Na}_v1.7$ replacement condition (identical to Figure 9A iii, with inactivation time constant $\tau_h = 1$ ms) to a modified condition in which inactivation was slowed by increasing τ_h to 1.1 ms (Experiment 4). Representative 60-second voltage traces showed robust, high-frequency tonic firing in both conditions, with no obvious qualitative differences in frequency or regularity (Fig. 10A). Quantitative analysis of mean firing frequency revealed no significant change between the baseline ($\tau_h = 1$ ms: 3.517 ± 0.037 Hz) and slowed inactivation conditions ($\tau_h = 1.1$ ms: 3.425 ± 0.021 Hz; paired t-test, $p = 0.48$, $n = 6$; Fig. 10B). Mean CV was minimally reduced with slower inactivation ($\tau_h = 1$ ms: 0.663 ± 0.010 ; $\tau_h = 1.1$ ms: 0.634 ± 0.010 ; paired t-test, $p = 0.017$, $n = 6$; Fig. 10C). An additional single-trial simulation with a substantially slower inactivation rate ($\tau_h = 3$ ms) produced firing patterns qualitatively similar to those at $\tau_h = 1$ ms and 1.1 ms, with no evident further changes in frequency or regularity. Together, the results of Figures 9 and 10 indicate that I_{NaP} is the major driver of spontaneous tonic firing activity in this model, as neither alterations in inactivation time constants (Fig. 10) nor modest shifts in half-activation voltage (Fig. 9A iv) substantially altered firing frequency or regularity.

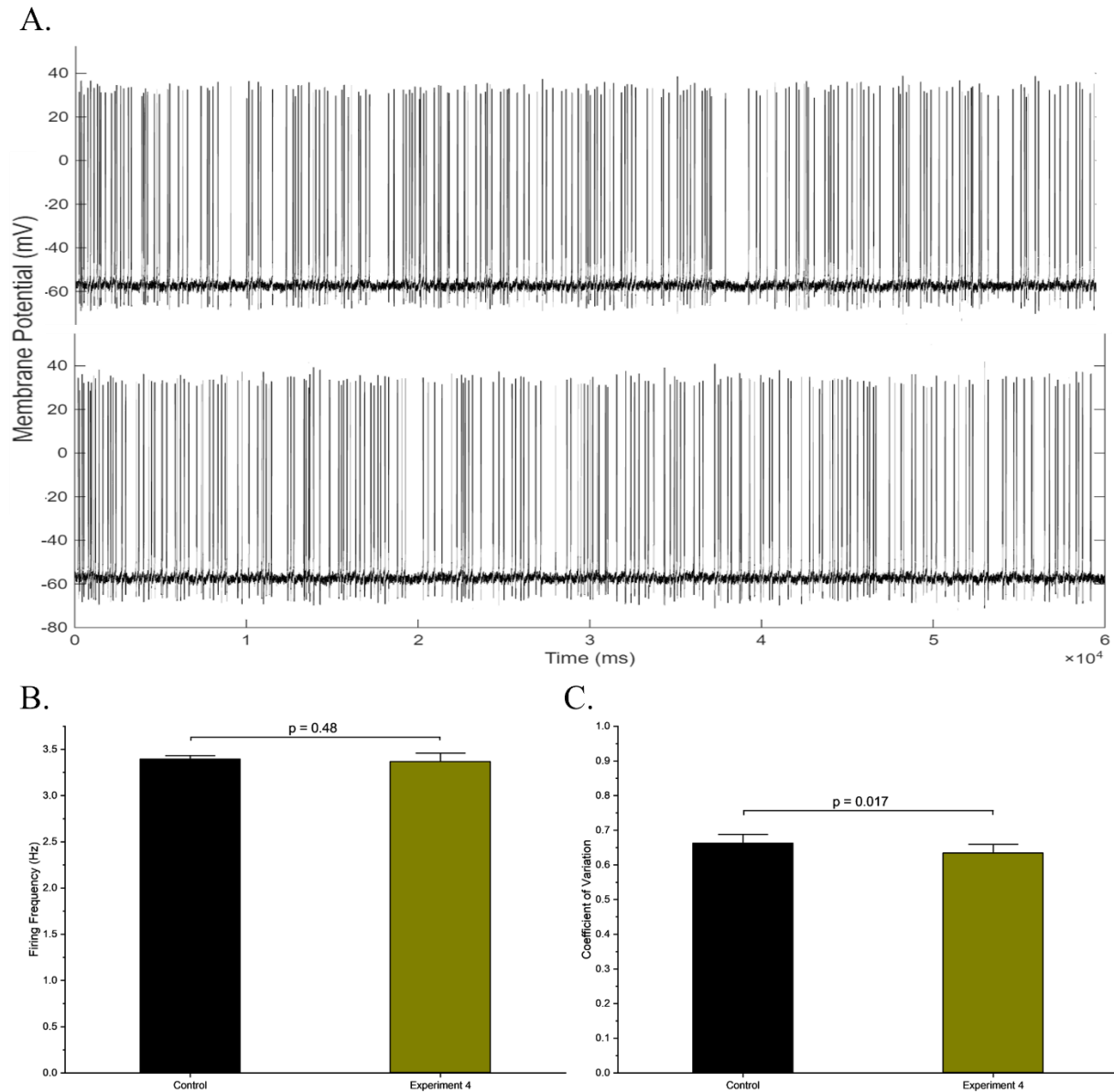


Figure 10. Changing Nav1.7 rate of inactivation has no considerable effect on spontaneous firing. Figure compares the baseline $\text{Na}_v1.7$ replacement condition (identical to Figure 9A iii) with a modified condition in which the inactivation time constant (τ_h) was slowed by increasing τ_h from 1 to 1.1 ms (Experiment 4). (A) Representative 60-second voltage traces of spontaneous tonic firing. Top trace: $\tau_h = 1$ ms (control $\text{Na}_v1.7$ condition); bottom trace: $\tau_h = 1.1$ ms (Experiment 4). (B) Paired t-test showed no significant difference in mean firing frequency between $\tau_h = 1$ ms (3.517 ± 0.037 Hz) and $\tau_h = 1.1$ ms (3.425 ± 0.021 Hz; $p = 0.48$, $n = 6$). (C) Paired t-test revealed a small but statistically significant reduction in mean CV with a slower inactivation time constant ($\tau_h = 1$ ms: 0.663 ± 0.010 ; $\tau_h = 1.1$ ms: 0.634 ± 0.010 ; $p = 0.017$, $n = 6$).

4. Discussion

Voltage-gated Na^+ channels are critical determinants of neuronal excitability, and subtle changes in their gating or kinetics can profoundly change firing behavior. $\text{Na}_v1.7$ is well characterized in the PNS for its role in pain signaling but is limited in expression within the CNS, being mainly found in key homeostatic regulatory centres where it plays critical roles in the electrical activity of these centres. Among these regulatory centres expressing $\text{Na}_v1.7$ is the SFO, however, the role of $\text{Na}_v1.7$ has not been studied in SFO neurons. This study attempted to address this question by testing the effects of the $\text{Na}_v1.7$ -selective toxin ProToxin-II on SFO neurons, in order to elucidate the properties of the ProToxin-II-sensitive current (i.e. $\text{Na}_v1.7$). We show that ProToxin-II alters voltage-gated Na^+ current kinetics and excitability in a manner that is partially consistent with a functional contribution of $\text{Na}_v1.7$. While voltage-dependence of activation and inactivation was significantly altered, ProToxin-II produced a hyperpolarizing shift, implying $\text{Na}_v1.7$ has a depolarized voltage of activation which is not consistent with the described literature. Additionally, the accelerated kinetics of inactivation and prolonged recovery from inactivation suggest that the $\text{Na}_v1.7$ current has slow kinetics of inactivation and fast recovery from inactivation, both of which again do not conform to the known literature. Lastly, ProToxin-II proportionately blocked more persistent current than transient, indicating the $\text{Na}_v1.7$ current in SFO neurons carries a significant amount of persistent current, which is consistent with the literature and what we expected. Current-clamp recordings revealed a consistent decrease in excitability despite a lowering of action potential threshold.

4.1 ProToxin-II: Mechanism of Inhibition, Na_v1.7 Specificity and Working

Concentration

An important consideration in interpreting the present findings is the concentration of ProToxin-II used. Derived from tarantula venom, ProToxin-II is one of the most potent gating-modifier toxins of Na_v1.7, with an IC₅₀ of ~0.3 nM in heterologously expressed human Na_v1.7 (Schmalhofer et al., 2008b). Unlike pore blockers like TTX, which occlude the channel pore and physically prevent Na⁺ passage, ProToxin-II alters gating kinetics by interacting with the voltage-sensing domain (VSD) of VGSCs (Narahashi, 2008b; Sokolov et al., 2008). In particular, ProToxin-II binds with the voltage sensor of domain II (VSDII) trapping it in a closed state, though it may also interact with VSDIV to alter fast inactivation (Sokolov et al., 2008; Xiao et al., 2010). Because ProToxin-II acts as a voltage-sensor–targeting gating modifier rather than a pore blocker, VGSCs can remain conductive while the toxin is bound, with its effects arising from altered gating kinetics and channel availability rather than physical occlusion. ProToxin-II binds Na_v1.7 through a defined receptor site within the VSDII S3–S4 loop, particularly the LFLAD motif (L812–F813–L814–A815–D816), which constitutes a major binding hotspot (Xu et al., 2019). Because toxin affinity is determined by precise side-chain interactions within these interfaces, even subtle sequence differences in the VSDII S3–S4 loop between species are expected to alter binding energetics. Consistent with this, rodent Na_v1.7 channels exhibit reduced apparent sensitivity to ProToxin-II compared with human Na_v1.7, necessitating higher concentrations to achieve partial inhibition in native rat neurons. Despite this, ProToxin-II still provides sufficient selectivity for Na_v1.7 for our experiments. We observed a graded, concentration-dependent reduction of voltage-gated Na⁺ current in SFO neurons, with 20 nM ProToxin-II producing a clear but incomplete inhibition of the Na⁺ current (Fig. 1). We therefore

selected 20 nM as an effective working concentration that allowed robust modulation of Na⁺ channel function while avoiding near-complete current block. This concentration also conforms well to previously reported IC₅₀ values for rat Na_v1.7 (see Table 2). This intermediate level of inhibition was critical for resolving kinetic effects on inactivation, recovery from inactivation, and persistent current that would be obscured at higher concentrations, where extensive channel block could increase the likelihood of off-target effects.

4.2 Hyperpolarizing Shifts in Activation and Inactivation with Net Inhibitory Effect

With an established working concentration, we then assessed the effects of ProToxin-II on Na_v1.7 gating. 20 nM ProToxin-II produced significant hyperpolarizing shifts in the voltage dependence of both activation and inactivation, as well as a hyperpolarizing shift in action potential threshold (Fig. 2 and Fig. 8). These changes indicate that Na_v1.7 current exhibits a relatively depolarized activation threshold and inactivation profile compared to the remaining (non-blocked) Na⁺ conductance. This depolarized gating would raise the depolarization required to activate Na_v1.7, thereby limiting its contribution to subthreshold excitability and action potential initiation under normal conditions. This contrasts with the well-documented hyperpolarized activation of Na_v1.7 in many native nociceptive neurons and heterologous systems; activation profiles become even more hyperpolarized in gain-of-function mutants associated with numerous painful neuropathies (Lampert et al., 2006; Estacion et al., 2008). The apparent depolarized gating of the Na_v1.7 component observed here may reflect “cell-type-specific” modulation of Na_v1.7 in SFO neurons. This could arise from differences in the complement of auxiliary β-subunits, which are known to shift the voltage dependence of activation and steady-state inactivation in a subunit-specific manner (Vijayaragavan et al., 2001; Laedermann et al., 2013b). Additional contributing factors may include alternative splice variants

of SCN9A (Farmer et al., 2012), post-translational modifications (phosphorylation, glycosylation, ubiquitylation, etc.; Chatelier et al., 2008; Laedermann et al., 2013a, 2013b), or distinct local lipid environments and intracellular signaling pathways (Li et al., 2018; Albani et al., 2024) that may differ between SFO neurons and the more commonly studied small-diameter DRG nociceptor neurons. Such context-dependent regulation may explain why the $\text{Na}_v1.7$ current in SFO neurons displays a more depolarized activation threshold and inactivation profile compared with the hyperpolarized gating typically reported for $\text{Na}_v1.7$ in heterologous expression systems and native nociceptive neurons.

Despite the hyperpolarized activation and threshold of the residual Na^+ current following ProToxin-II application (Figs. 2 and 8), whole-cell current-clamp recordings revealed decreased excitability, with fewer evoked action potentials across a range of depolarizing current injections (Fig. 7). This reduction in firing capability occurred despite the remaining current activating more readily. ProToxin-II blocked nearly 30% of total I_{NaP} (Fig. 5C), a key depolarizing drive that amplifies subthreshold depolarizations and supports repetitive or sustained firing in SFO neurons. Although ProToxin-II also induced hyperpolarizing shifts in the voltage dependence of both activation and inactivation (Fig. 2 and Fig. 8)—a change that would normally be expected to increase excitability by facilitating channel opening—the primary mode of action for decreased activity is the net reduction in total Na^+ conductance, particularly the substantial loss of I_{NaP} . Consequently, although the residual (non- $\text{Na}_v1.7$) Na^+ current displayed more excitable gating properties overall (Fig. 2 and Fig. 8), the dominant impact of I_{NaP} suppression resulted in fewer action potentials (Fig. 7). This interpretation that I_{NaP} is the main driver of excitability is supported by our later modeling experiments (see Discussion section 4.6).

4.3 Altered Kinetics of Inactivation

Assessment of ProToxin-II's effects on the kinetics of inactivation revealed significantly accelerated fast inactivation (Fig. 3) and markedly prolonged recovery from inactivation (Fig. 4). These effects indicate that $\text{Na}_v1.7$ in SFO neurons exhibits relatively slow inactivation kinetics together with comparatively fast recovery/repriming. This profile generally contrasts with the canonical description of $\text{Na}_v1.7$ in much of the literature, which typically characterizes the channel as having rapid inactivation and slower recovery from inactivation (Vijayaragavan et al., 2001). While this difference was not entirely expected, it is also not surprising given the complexity of biological systems and the strong influence of cellular context. As noted earlier regarding the shifts in activation/inactivation gating, $\text{Na}_v1.7$ gating can be substantially modulated by cell-type-specific factors such as alternative splicing of the *SCN9A* gene, association with different auxiliary β -subunits, and various post-translational modifications. Importantly, the apparent properties of $\text{Na}_v1.7$ observed here—slower inactivation and faster repriming—would be expected to facilitate neuronal excitability in the SFO. Slower inactivation kinetics would allow channels to remain in the open, conducting state for longer, thereby permitting greater sodium influx and enhanced depolarization. Concurrently, faster recovery from inactivation would enable channels to become available for reactivation more rapidly, supporting repetitive and higher-frequency firing.

4.4 $\text{Na}_v1.7$ as a Major Contributor to I_{NaP} in SFO Neurons

Evaluation of I_{NaP} revealed ProToxin-II blocks proportionally more I_{NaP} than I_{NaT} (nearly 30% decrease). This suggests that $\text{Na}_v1.7$ carries a substantial portion of the persistent sodium conductance in this neuronal population. This finding is functionally important because persistent sodium current acts as a critical subthreshold amplifier, providing a steady inward

drive that facilitates the transition from small depolarizations to action potential initiation (Schwindt and Crill, 1995; Stuart and Sakmann, 1995). The reduction in I_{NaP} may therefore help explain the suppression of excitability observed despite the hyperpolarizing shifts in activation gating produced by ProToxin-II (see Discussion section 4.2). The loss of this persistent component could diminish the depolarizing drive keeping membrane potential closer to threshold, thereby reducing the probability of sustained or high-frequency firing. Such effects highlight how even modest alterations in the persistent fraction of Na^+ current can exert a disproportionately large influence on neuronal output in the SFO.

4.5 $Na_v1.7$ Stabilizes Bursting and Tonic Firing Behaviour

ProToxin-II produced variable effects on spontaneous firing activity in SFO neurons (Fig. 6). Plotting the change in firing frequency against the change in coefficient of variation revealed two apparent subpopulations of neurons: one characterized by a shift to higher-frequency tonic firing and another by a shift to lower-frequency bursting behavior. Rather than uniformly suppressing excitability, ProToxin-II tended to shift neurons between these groups. High-frequency tonic neurons under control conditions often transitioned toward a lower-frequency, more burst-like pattern following ProToxin-II application, while some lower-frequency burst neurons shifted in the opposite direction. This bidirectional shift resulted in an overall reduction in mean firing rate but highlighted the heterogeneous nature of SFO neuronal populations.

These observations are reminiscent of the two distinct firing phenotypes—tonic and burst—previously described in SFO neurons by Huang et al. (2019), who reported that neurons in core versus shell regions of the SFO differ in their propensity for bursting versus tonic activity and spontaneous firing rates. One possible explanation for the observed bidirectional shifts is that

$\text{Na}_v1.7$ -mediated I_{NaP} may contribute differently depending on the intrinsic properties of each SFO neuronal subpopulation. In high frequency tonic neurons, I_{NaP} could provide a background subthreshold depolarizing drive that helps maintain the inter-spike membrane potential closer to action potential threshold, thereby facilitating rapid and regular spiking. In contrast, in burst prone neurons the same persistent current might promote the generation of depolarizing afterpotentials that allow spike summation into bursts (Washburn et al., 2000b; Medlock et al., 2018). These opposing yet complementary actions of the same conductance explain why selective $\text{Na}_v1.7$ blockade can bidirectionally shift neurons between firing modes—disrupting the tonic drive in one subpopulation while unmasking or enhancing burst-like behavior in the other. Such heterogeneity is consistent with the regional and intrinsic differences (core vs. shell SFO neurons) previously reported by Huang et al. (2019) and with the detailed ionic mechanisms described by Medlock et al. (2018).

4.6 I_{NaP} is Major Driver of Electrical Excitability

Our computational modeling demonstrates that introducing $\text{Na}_v1.7$ channel properties into the Medlock et al. (2018) model profoundly enhances spontaneous tonic firing activity. Replacement of a modest fraction of the model's native Na^+ conductance with $\text{Na}_v1.7$ conductance produced robust, high-frequency (~ 3.4 – 3.5 Hz), and highly regular tonic firing, in contrast to the sparse, irregular spiking observed in the unmodified control model and the complete loss of activity following simple reduction of native I_{NaT} and I_{NaP} conductances. These findings suggest that the unique biophysical properties of $\text{Na}_v1.7$ —particularly its substantial persistent sodium current component—play a dominant role in driving sustained depolarizing drive and repetitive firing in this model system.

A key observation is that the persistent current appears to be the primary determinant electrical excitability. In the reduced-conductance condition (modeling experiment 1), lowering both I_{NaT} and I_{NaP} eliminated spontaneous activity and caused a small but statistically significant hyperpolarization of the resting membrane potential (~ 0.82 mV), consistent with loss of a tonic depolarizing influence from persistent sodium influx. In contrast, selective replacement with $Na_v1.7$ conductance—which preserves a significant persistent current fraction—fully rescued and even amplified high-frequency tonic firing. Moreover, neither modest shifts in half-activation voltage nor substantial slowing of inactivation kinetics produced meaningful changes in firing frequency or pattern. This relative insensitivity to alterations in activation threshold and inactivation rate strongly implicates the persistent Na^+ current as the critical feature enabling sustained tonic activity, rather than fast transient current dynamics or voltage-dependent gating shifts within the tested range.

These modeling results align with established roles of $Na_v1.7$ in neuronal excitability. $Na_v1.7$ is known to produce a relatively large persistent sodium current compared to other VGSC isoforms, which contributes to subthreshold depolarization, amplification of generator potentials, and support for repetitive firing in sensory neurons. Gain-of-function mutations in SCN9A (encoding $Na_v1.7$) that increase persistent current or hyperpolarize activation lead to hyperexcitability and severe pain disorders (e.g., primary erythromelalgia, paroxysmal extreme pain disorder), whereas loss-of-function or pharmacological inhibition reduces excitability. The present simulations extend this concept to the SFO neuron context, where $Na_v1.7$ properties appear sufficient to convert a low-activity, irregular firing phenotype into a high-frequency, regular tonic pattern—potentially reflecting a mechanism by which $Na_v1.7$ could modulate autonomic or homeostatic circuits involving the SFO.

The small but significant reduction in the coefficient of variation with slower inactivation is noteworthy but minor in magnitude and did not translate to changes in mean firing rate. This suggests that, while inactivation kinetics may fine-tune spike timing precision in this model, they are not a primary driver of overall firing frequency or pattern. The lack of further effect at $\tau_h = 3$ ms (albeit $n = 1$) reinforces that inactivation rate is not rate-limiting for tonic activity here.

4.7 Physiological Implications

The present findings highlight the functional significance of $\text{Na}_v1.7$ in modulating the intrinsic excitability of SFO neurons, a critical sensory organ that senses circulating signals such as angiotensin II, hypertonic sodium, and metabolic hormones to orchestrate homeostatic responses including thirst elicitation, salt aversion, metabolic regulation and sympathetic outflow for cardiovascular regulation (Hiyama et al., 2004b; Smith and Ferguson, 2010; Wei et al., 2013; Hindmarch and Ferguson, 2015; Noda and Hiyama, 2015; Cancelliere and Ferguson, 2017). By preferentially contributing to persistent sodium currents and fine-tuning subthreshold dynamics, $\text{Na}_v1.7$ appears to enhance the SFO's capacity for sustained neuronal activation during prolonged physiological challenges, thereby facilitating adaptive integration of systemic cues into autonomic and behavioral outputs; disruptions in this mechanism could potentially exacerbate conditions like dehydration, hypertension, or metabolic imbalances. Understanding $\text{Na}_v1.7$'s role in the SFO context is particularly vital given the extensive pharmaceutical efforts to develop hyper-specific $\text{Na}_v1.7$ inhibitors as analgesics for neuropathic pain syndromes. This raises concerns about potential off-target disruption of SFO-dependent homeostasis (e.g., thirst, vasopressin secretion, salt appetite, metabolic and cardiovascular regulation) and underscores the importance of thorough preclinical assessment to minimize unanticipated CNS consequences.

Alternatively, Nav1.7 inhibitors may have potential as novel pharmacological strategies for the treatment of conditions such as hypertension, obesity and diabetes.

4.8 Limitations & Future Directions

A primary limitation of the present study is the lack of a highly selective antagonist for rat Nav1.7. ProToxin-II, while one of the most potent gating-modifier toxins targeting Nav1.7, exhibits reduced potency and selectivity in rodent channels compared with the human isoform, largely due to sequence differences in the domain II voltage sensor. Although we selected a working concentration of 20 nM that produced consistent blockade of Nav1.7 while minimizing overt off-target effects on other VGSC subtypes expressed in the SFO (e.g., Nav1.1, 1.2, 1.3, 1.6), some degree of non-specific inhibition of non-Nav1.7 currents cannot be entirely excluded. This inherent non-specificity may have contributed modestly to the observed changes in Na⁺ current kinetics and excitability. Despite this limitation, use of a rat model was deliberately chosen as much of the organization and function of SFO circuitry are considerably better characterized in rats than in mice. Future studies could address this limitation through the development or use of more selective rat-optimized Nav1.7 inhibitors (e.g., engineered ProToxin-II analogues with improved rodent affinity).

Another notable limitation is that recordings were performed on dissociated SFO neurons without distinguishing between core and shell subpopulations. Previous work has demonstrated that these two anatomically and functionally distinct regions exhibit different firing phenotypes, with core neurons preferentially displaying low-frequency bursting and shell neurons showing higher-frequency tonic activity (Huang et al., 2019). Because core and shell SFO neurons also differ in resting membrane potential and sodium current density, pooling data across both

populations may have masked subpopulation-specific effects of $\text{Na}_v1.7$ blockade. Future studies could overcome this limitation by selectively targeting core versus shell neurons. This could be achieved using molecular markers such as calbindin (CalB, exclusive to core) and calretinin (CalR, exclusive to shell). Approaches including viral vectors expressing GFP under the control of CalB or CalR promoters, or the use of transgenic reporter lines, would enable targeted electrophysiological recordings and allow direct comparison of $\text{Na}_v1.7$ -dependent properties between these functionally relevant subpopulations. We conducted preliminary experiments using AAVs expressing fluorescent reporters under calbindin or calretinin promoters confirmed that promoter-driven labeling of SFO neurons is feasible and established a foundation for further refinement of this strategy to distinguish core and shell populations.

An additional limitation concerns the computational model employed in this study. The Hodgkin-Huxley-type SFO neuron model developed by Medlock et al. (2018) does not incorporate independent parameters for recovery from inactivation. Instead, inactivation and recovery kinetics are mathematically coupled, with repriming rate simply assumed to be the inverse of the inactivation rate. This simplification may have underestimated the functional impact of the observed changes in recovery time constants following ProToxin-II application. Future modeling efforts should incorporate separate voltage- and time-dependent parameters for recovery from inactivation to more accurately reflect the distinct effects of $\text{Na}_v1.7$ on fast and slow recovery components and better predict their consequences for neuronal firing patterns.

Additional future directions include combining $\text{Na}_v1.7$ -specific pharmacological or genetic tools with in vivo recordings or behavioral assays to determine how alterations in SFO $\text{Na}_v1.7$ function translate to changes in thirst, salt appetite, vasopressin release, or sympathetic

outflow. Such integrative approaches will be essential for fully elucidating the physiological role of $\text{Na}_v1.7$ in SFO-mediated homeostatic regulation.

5. Conclusion

Our study demonstrates that the ProToxin-II-sensitive component of voltage-gated Na^+ current (presumably to be $\text{Na}_v1.7$) contributes meaningfully to the electrical excitability of rat SFO neurons, primarily through its substantial contribution to persistent Na^+ current and its influence on subthreshold membrane dynamics. Although ProToxin-II produced unexpected hyperpolarizing shifts in activation and inactivation, as well as accelerated fast inactivation and slowed recovery from inactivation, the net functional outcome was a reduction in evoked excitability, driven largely by the loss of persistent Na^+ influx. Computational modeling further supported I_{NaP} as the dominant driver of sustained tonic firing. These findings may indicate that $\text{Na}_v1.7$ enhances the capacity of SFO neurons to integrate and respond to prolonged circulating homeostatic signals such as hormones including ANG II, extracellular Na^+ concentration, and other metabolic cues. By facilitating subthreshold amplification and repetitive firing, $\text{Na}_v1.7$ likely plays a state-dependent role in enabling adaptive autonomic and behavioral responses critical for fluid, electrolyte, and cardiovascular homeostasis. Future studies combining isoform-specific genetic approaches, anatomical subregion identification, and in vivo physiological correlates will be essential to fully define $\text{Na}_v1.7$'s contributions to SFO-mediated homeostatic regulation.

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