Anxiety and dysautonomia symptoms in patients with a $Na_V1.7$ mutation and the potential benefits of low-dose short-acting guanfacine

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Abstract

Purpose: Guanfacine is a α_{2A}-adrenergic receptor agonist, FDA-approved to treat attention-deficit hyperactivity disorder and high blood pressure, typically as an extended-release formulation up to 7 mg/day. In our dysautonomia clinic, we observed that off-label use of short-acting guanfacine at 1 mg/day facilitated symptom relief in two families with multiple members presenting severe generalized anxiety. We also noted anecdotal improvements in associated dysautonomia symptoms such as hyperhidrosis, cognitive impairment, and palpitations. We postulated that a genetic deficit existed in these patients that might augment guanfacine susceptibility.

Methods: We used whole-exome sequencing to identify mutations in patients with shared generalized anxiety and dysautonomia symptoms. Guanfacine-induced changes in the function of voltage-gated Na^+ channels were investigated using voltage-clamp electrophysiology.

Results: Whole-exome sequencing uncovered the p.I739V mutation in *SCN9A* in the proband of two nonrelated families. Moreover, guanfacine inhibited ionic currents evoked by wild-type and mutant $Na_V1.7$ encoded by *SCN9A*, as well as other Na_V channel subtypes to a varying degree.

Conclusion: Our study provides further evidence for a possible pathophysiological role of $Na_V1.7$ in anxiety and dysautonomia. Combined with off-target effects on Na_V channel function, daily administration of 1 mg short-acting guanfacine may be sufficient to normalize Na_V channel mutation-induced changes in sympathetic activity, perhaps aided by partial inhibition of $Na_V1.7$ or other channel subtypes. In a broader context, expanding genetic and functional data about ion channel aberrations may enable the prospect of stratifying patients in which mutation-induced increased sympathetic tone normalization by guanfacine can support treatment strategies for anxiety and dysautonomia symptoms.

Keywords:

Dysautonomia, voltage-gated sodium channel, anxiety, $Na_V1.7$, guanfacine

Introduction

The α_{2A}-adrenergic receptor agonist guanfacine is FDA- and EMA-approved to treat hypertension and for use as a second-line treatment for attention-deficit hyperactivity disorder (ADHD) in children and adolescents [12, 43] under brand names such as Tenex®, Afken®, Estulic®, and the extended-release formulation Intuniv®. A meta-analysis of twelve randomized controlled trials involving both children and adults showed that guanfacine, if given short or long-term, was significantly superior to placebo in treating ADHD [82]. Guanfacine also reduces hyperactivity and attention deficit in animal models of ADHD [47, 55]. Moreover, it is well established that in rodents, monkeys, and humans, systemic administration of guanfacine improves cognitive function in the prefrontal cortex, an area of the brain that plays a key role in regulating attention, behavior, and emotion [8]. These findings correlate with the association between ADHD and genetic alterations that attenuate catecholamine signaling, particularly in the prefrontal cortex [4, 7]. Indeed, in neuropsychiatric disorders that involve dysfunction of the prefrontal cortex such as post-traumatic stress disorder, substance abuse, schizophrenia and autistic spectrum cognitive deficits, traumatic brain injury, emergence delirium, and Tourette Syndrome [6, 13, 38, 80], guanfacine has proven clinical efficacy and is therefore used off-label [8]. Anxiolytic effects of guanfacine are apparent from studies involving children and adolescents exhibiting different types of anxiety [14, 65]. Moreover, beneficial effects were also demonstrated in cases of anxiety related to individuals with drug-craving behaviors [23, 24, 42] and for an intensive-care patient with severe anxiety following cardiac surgery [63]. The neurological circuits involved in anxiety are complex and involve a variety of behavioral and autonomic components that can originate in multiple brain regions such as the amygdala, prefrontal cortex, hippocampus, anterior insula and lateral septum [69, 71]. Markedly, guanfacine can modulate synapses in many of these regions [1, 8, 57].

In general, activation of presynaptic α_{2A}-adrenergic receptors in the central nervous system decreases noradrenergic outflow through a negative feedback loop [8]. Potential central and peripheral side effects are consistent with the ample distribution of noradrenergic neurons and may include sedation, weakness, dizziness, bradycardia, fainting, headache, constipation, decreased appetite, dry mouth, and nausea. In the prefrontal cortex, postsynaptic α_{2A}-adrenergic receptor activation by guanfacine impedes cAMP production, which, in turn, closes Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels to augment network connectivity thus improving working memory and attention [73]. This ability of guanfacine to bolster prefrontal lobe connectivity is a possible basis of a report showing amelioration of Post-Acute Sequelae of SARS-CoV-2 (PASC)-associated 'brain fog' (*i.e.*,

cognitive impairment) in twelve patients who were also given high doses of N-acetyl cysteine, an immunomodulator that combats inflammation [22].

In our dysautonomia clinic, we observed that off-label use of guanfacine was effective in reducing generalized anxiety in two families with multiple members affected by similar symptoms that also included cognitive impairment, hyperhidrosis, and palpitations. In contrast to ADHD treatments that typically involve the use of an extended-release formulation up to 7 mg/day, we noted that short-acting guanfacine at only 1 mg/day facilitated long-term symptom relief in our patients without side effects. Therefore, we postulated that a particular genetic deficit existed in these patients that might underlie this heightened sensitivity to guanfacine.

Methods and Materials

5.1 Clinical patient assessment

Clinical data and survey results from the Hyperhidrosis Disease Severity Scale [61] and the COMPASS-31 Dysautonomia Score [59] were ascertained both during the clinic visit and self-reported by using the REDCap (Research Electronic Data Capture) tool [31, 32]. Study data were collected and managed using REDCap electronic data capture tools hosted at the Johns Hopkins University School of Medicine. REDCap is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources. The Institutional Review Board of Johns Hopkins University School of Medicine approved this study. Informed consent was obtained from each patient and any affected family member who underwent WES.

5.2 Whole exome sequencing of patients

Supported by the Baylor-Hopkins Center for Mendelian Genomics (BHCMG), we obtained Whole-Exome Sequencing (WES) data on two patients from two nonrelated families with a history of generalized anxiety and dysautonomia who presented to our Dysautonomia Clinic for treatment at the Johns Hopkins Hospital between 2018 and 2020. The WES procedure was previously described by Dhaheri and coworkers [3] using genomic DNA captured with the Agilent SureSelect Human All Exon V4 51MB Kit and sequenced on an Illumina HiSeq2000 platform. FASTQ files were aligned to the reference genome (GRCH38) with the Burrows-Wheeler Alignment (BWA 0.5.10) tool. Next, we performed multisample SNV and indel calling on the reduced-read BAM files with GATK's UnifiedGenotyper. Variant sites were filtered with GATK's Variant Quality Score Recalibration best practices and excluded heterozygous genotypes if they did not have at least 5 alternative allele reads. The annotated files (ANNOVAR) were analyzed using the PhenoDB Variant Analysis Tool [60] by selecting the rare (MAF <1%) functional (missense, nonsense, stop loss, splice site and indels) heterozygous and homozygous variants in each proband. We excluded variants with a MAF>0.01 in GnomAD or in our BHCMG sample. Next, each variant and gene was evaluated for their ClinVar, HGMD, OMIM, and mouse phenotype annotations. Finally, we selected for further investigation the gene variants that met the above criteria in heterozygous or homozygous states.

5.3 Electrophysiology on Xenopus laevis oocytes

The cDNA sequences of human voltage-gated Na^+ channel (Na_V) 1.1 (AF225985), Na_V1.2 (NP001035232), Na_V1.3 (AF225987), Na_V1.4 (NP000325), Na_V1.5 (AAI44622), Na_V1.6 (NP055006), wild type Na_V1.7

(NP002968) and the p.I739V mutant, $Na_V1.8$ (NP006505), and β1 (NP001028) (Origene, USA and Genscript, USA), were confirmed by automated Sanger sequencing. For oocyte experiments, RNA was synthesized using T7 polymerase (mMessage mMachine kit, Life Technologies, USA) after linearizing the DNA with an applicable restriction enzyme. Na_V channels were expressed with β1 in a 1:5 molar ratio by microinjecting RNA into defolliculated *Xenopus laevis* oocytes (toads obtained from Nasco®, USA) [26]. The use of toads complied with national and Flemish guidelines adhered to by the UGent University Animal Care and Use Committee. Oocytes (n = 5-8 per Na_V channel subtype; see figures) were incubated at 17°C in Barth's medium (in millimolar (mM): 96 NaCl, 2 KCl, 5 HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), 1 $MgCl_2$, and 1.8 $CaCl_2$, 50 µg/ml gentamycin, pH 7.6 with NaOH, chemicals from Sigma®, USA) and following 1–4 days incubation, studied using the two-electrode voltage-clamp recording technique (model OC-725C, Warner Instruments, USA) with a 150-µl recording chamber as previously described [26]. In brief, this established approach enables control of the transmembrane potential of cells and precise measurement of the ion flow across the cell membrane through voltage-gated ion channels as an electric current. Subsequently, it becomes possible to examine multiple biophysical gating parameters (*i.e.* opening/closing) of voltage-gated ion channels in response to changes in the transmembrane potential and investigate the influence of compounds on channel function. All data were filtered at 4 kHz (tunable active filter model 900, Frequency Devices Inc., USA) and digitized at 20 kHz using pClamp 10 software (Molecular Devices, USA). The external recording solution used was ND-100 (in mM: 100 NaCl, 5 HEPES, 1 $MgCl_2$, and 1.8 $CaCl_2$, pH 7.6 with NaOH, chemicals from Sigma®, USA) and microelectrode (Narishige® PC-10 vertical puller) resistances were 0.5 to 1.0 MΩ when backfilled with 3 M KCl. All experiments were performed at room temperature (~22°C). To avoid capacitance errors induced by the large membrane surface of *Xenopus laevis* oocytes and enable the detection of subtle biophysical events, maximum recorded current amplitudes were limited to ±1.5 µA by means of titrating injected RNA quantities. Leak and background conductances were identified and subtracted by blocking channels with tetrodotoxin (TTX; Alomone Labs, Israel), including a TTX-sensitive variant of $Na_V1.8$ [58]. Normalized conductance–voltage (G/G_{max}) and channel availability (I/I_{max}) relationships were obtained by measuring steady-state currents upon stepwise depolarizations for 50 ms from a holding potential of -90 mV in 5 mV increments and a single Boltzmann function was fitted to the data according to I/I_{max} (or G/G_{max}) $= [1 + \exp(-zF(V - V_{1/2})/(RT))]^{-1}$, where I/I_{max} is the normalized current amplitude, z is the equivalent charge, $V_{1/2}$ is the half-activation voltage, F is Faraday's constant, R is the gas constant, and T is temperature in kelvin. Recovery from inactivation was determined using a double-pulse protocol in which cells were kept at -90 mV between two 50-ms depolarization steps to 0 mV that were applied with a

varying interval between 0 ms and 1000 ms. Guanfacine hydrochloride was acquired from Sigma® (USA) and dissolved in ND-100 as a 1 mM stock solution from which a 100 µM working solution (86 µM effective drug concentration) was diluted for use with oocytes. Na_V channels were exposed to guanfacine through a gravity-fed perfusion system with a flow rate of 0.5 ml/minute. When adding guanfacine to the recording chamber, equilibration between channel and compound was monitored by means of depolarizations to $V_{1/2}$ of G/G_{max} at five-second intervals. Where needed, statistical differences were determined using the student's *t*-test ($p = 0.01$). Off-line data analysis was performed using Clampfit 10 (Molecular Devices, USA), Excel (Microsoft Office, USA) and Prism 8 (GraphPad, USA).

Results

Clinical description of the beneficial effects of guanfacine in patients with neuropsychiatric symptoms

We explored whether genetic abnormalities were present in patients belonging to two nonrelated families that visited our dysautonomia clinic because they reported suffering from generalized anxiety and concomitant sympathetic hyperarousal symptoms that ran in the family [25, 27, 51]. To assess dysautonomia, we used the COMPASS-31 validated questionnaire [59], a self-rating quantitative measure of autonomic symptoms evaluating orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor domains with our non-dysautonomia control group scoring 13 or less. Whole-exome sequencing (WES) analysis of the proband of each of the two examined families suggested the $Na_V1.7$ (gene: *SCN9A*) p.I739V channelopathy as likely clinically relevant (Fig. 1 and 2).

The first family (Maryland, USA) illustrates the treatment effect of guanfacine as it started with the case of a 20-year-old male (Fig. 1, proband), harboring the $Na_V1.7$ p.I739V variant, who presented to our outpatient clinic with hyperhidrosis denoted as intolerable on the Hyperhidrosis Disease Severity Scale (HDSS) [61] along with concurrent symptoms of generalized anxiety, cardiac palpitations, cognitive deficits such as forgetfulness and trouble concentrating – colloquially called 'brain fog' –, chronic fatigue, and insomnia. The COMPASS-31 score of this patient before treatment was 33.53. Due to his reduced adverse effect profile compared to clonidine, a α_{2A}-adrenergic receptor agonist used off-label to treat disabling hyperhidrosis and neuropsychiatric symptoms [5, 15, 39, 62, 68, 70], we opted to use short-acting guanfacine (Tenex®, 1 mg/day) as an adjunctive therapy. Six years later, the patient states that guanfacine continues to be well tolerated and highly effective in ameliorating his dysautonomia symptoms, especially his hyperhidrosis. He remarks that guanfacine seems so effective that whenever he misses a dose, his cognitive impairment, anxiety, and hyperhidrosis as well as other symptoms return. His self-reported post-treatment COMPASS-31 score was 27.87, substantiating a clinically relevant improvement in symptomatology but without complete resolution of all of his dysautonomia symptoms.

The second family is from South-East Asia and consists of three generations that exhibited extensive dysautonomia symptoms primarily as generalized anxiety, hyperhidrosis, neuropathic itch, orthostatic intolerance, and chronic fatigue (Fig. 2). The proband in this family with a COMPASS-31 score of 25.02, expressed the $Na_V1.7$ p.I739V variant and presented with such profound hyperhidrosis that she opted for surgical treatment (thoracic sympathectomy). Her maternal aunt also sought treatment in our clinic and was administered Tenex®, 1 mg/day. After three days, her symptoms of anxiety, cognitive impairment, cardiac palpitations, and hyperhidrosis lessened along with decreased paroxysmal hypertension. Two years later, the aunt states that she alternates daily between short-acting guanfacine

1 mg/day and 1.5 mg/day with continued amelioration of her symptoms, in particular her hyperhidrosis. Correspondingly, her self-reported COMPASS-31 score decreased from 53.01 to 34.77 after guanfacine treatment, and she feels that her dysautonomia symptoms are much more tolerable (Fig. 2).

To further substantiate the broad applicability and beneficial properties of low-dose guanfacine in the context of channelopathies, we also want to mention a third case of one member of a 30-year-old identical female twin who presented to our clinic with a chief complaint of non-painful facial erythromelalgia and generalized anxiety along with dysautonomia symptoms of chronic fatigue and irritable bowel syndrome. According to the patient, her erythromelalgia had become particularly pronounced shortly after a severe bout of mononucleosis. The erythromelalgia would occur almost daily and was associated with stressful social situations and not provoked by increased ambient temperature or exercise. She had been treated with Citalopram® and Escitalopram® without relief and was contemplating a bilateral thoracic sympathectomy. Her twin sister never contracted mononucleosis and never developed erythromelalgia but presented with other symptoms of dysautonomia including chronic fatigue, irritable bowel syndrome, generalized anxiety, cognitive impairment, orthostatic intolerance, and cardiac palpitations. Like the other patients mentioned above, this patient's erythromelalgia improved promptly with Tenex® 1 mg/day, and the frequency of erythromelalgia reduced to 1-3 times per year. Seven years later, the patient remains on Tenex® 1 mg/day with a durable response and no apparent side effects. This patient was treated empirically in the clinic and has not yet been enrolled to undergo WES for the presence of a Na_V channelopathy. It is worth noting that erythromelalgia has been associated with, at least in a subset of patients [84], gain-of-function mutations in $Na_V1.7$ [17, 20, 66, 74]. Unlike for neuropsychiatric symptoms, we are unaware of an established association between α_{2A}-adrenergic receptors and erythromelalgia in patients with Na_V channelopathies.

Guanfacine inhibits $Na_V1.7$ currents

Given its apparent low dose efficacy in our subset of studied patients, we explored whether guanfacine has potential off-target effects on $Na_V1.7$ channels. $Na_V1.7$ is expressed in sensory and sympathetic neurons that innervate all organs in the human body [28, 44, 49]. This channel subtype has also been found in brain subcortical structures, including the thalamus, amygdala, hypothalamus, axons of the olfactory epithelium [10, 37] and the locus coeruleus [36]. The $Na_V1.7$ p.I739V variant that we identified here, was previously found in patients with neuropathic pain and severe autonomic dysfunction including hyperhidrosis and palpitations [29, 30]. However, the proband in the first family of this study (Fig. 1) remarked that he had an unusually high tolerance to pain recalling that he had broken both arms without

feeling compelled to seek pain relief at a hospital. Although this symptomatic dichotomy is rather unusual, both gain- and loss-of-function mutations in $Na_V1.7$ have been associated with phenotypes ranging from a complete lack of pain to erythromelalgia, often with dysautonomia symptoms [28, 45, 81].

To test whether guanfacine alters channel function, we heterologously expressed wild type $Na_V1.7$ and the p.I739V mutant channel in *Xenopus laevis* oocytes [83] and measured resulting ionic currents by means of the two-electrode voltage-clamp technique before and after addition of 86 μM guanfacine. This concentration is rather high since oocytes are known to be less sensitive to compounds [74], up to one hundred times [36], compared to mammalian cells. As such, we are likely working with a saturating dose and the effective in vivo concentration should be much lower. When applying guanfacine to wild type $Na_V1.7$, we observed an inhibitory effect of 22% ± 5 on ionic currents whereas the conductance-voltage and channel availability relationship remained unaltered (Fig. 3, Table 1). Similarly, guanfacine reduces $Na_V1.7$ p.I739V currents by 25% ± 4 without altering its gating parameters (Fig. 3). The recovery from inactivation process plays an important role in regulating cellular spiking frequencies and can be assessed by employing a double-pulse protocol in which cells are kept at -90 mV between two depolarization steps to 0 mV with a varying interval between 0 ms and 1000 ms. Recovery from inactivation of wild type $Na_V1.7$ is virtually complete after 200 ms (Fig. 3) and no significant differences were observed between control and drug-exposed conditions. To test whether guanfacine prefers to interact with the inactivated state of the channel, we applied a test protocol consisting of a 5-s pulse to $V_{1/2}$ of channel availability (-40 mV, Table 1) to induce channel inactivation, followed by a 200-ms step to -90 mV for recovery from inactivation and a 50-ms test pulse to 0 mV. Such state-dependence has been shown to further increase *in vivo* affinity and selectivity over other ion channel families [2, 35]. We observed that under these conditions (Fig. 3), the inhibitory effect of guanfacine on ionic currents doubles from 21% ± 6 to 50% ± 6 indicating a degree of state-dependence. Altogether, these data illustrate the ability of guanfacine to reduce $Na_V1.7$ channel-mediated currents.

Effect of guanfacine on multiple Na_V channel subtypes

Since guanfacine inhibits both wild type and mutant $Na_V1.7$ to a similar extent, we tested for potential off-target effects on other Na_V channel subtypes ($Na_V1.1$-$Na_V1.8$). First, we report the effects of guanfacine on $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$, the commonly accepted predominant subtypes present within the brain [2, 41, 48]. To this end, we held the membrane at -90 mV and applied 50 ms depolarizing pulses to stepwise depolarize the membrane and activate channels before and after addition of guanfacine. Under these conditions, $Na_V1.1$- and $Na_V1.2$-mediated Na^+ currents were inhibited over the tested voltage range

by 23% and 27%, respectively (Fig. 4, Table 1). We did not observe significant changes in standard gating parameters associated with the conductance-voltage and channel availability (steady-state inactivation) relationships (Table 1). Similar results were seen when testing $Na_V1.3$ although current inhibition was reduced to 12%. Of these channel subtypes, $Na_V1.6$ was the least inhibited at 6%. Next, we tested whether Na_V channel subtypes associated with the skeletal ($Na_V1.4$) and cardiac ($Na_V1.5$) muscle were affected by guanfacine [11, 53, 76]. Like other Na_V channel subtypes, most gating parameters were unaltered (Fig. 5, Table 1). $Na_V1.4$ currents were inhibited but only by 12%. $Na_V1.5$ activity was hampered by a drug-induced current reduction of 31%. Finally, $Na_V1.8$ currents were inhibited by 20% and no other significant gating alterations were observed (Fig. 5). Combined, these data suggest that guanfacine can act as a non-selective Na_V channel inhibitor.

Discussion

We observed that off-label use of guanfacine seemed effective in reducing generalized anxiety and concomitant dysautonomia symptoms including cognitive impairment, hyperhidrosis, and palpitations in members of two nonrelated families. Strikingly, the short-acting guanfacine formulation at only 1 mg/day facilitated long-term symptom relief without apparent side effects. We hypothesized that a genetic deficit existed in these patients that might underlie, at least in part, this notable sensitivity to guanfacine. WES on a limited subset of patients that benefited from a low dose of guanfacine, identified the $Na_V1.7$ p.I739V gain-of-function variant [29, 30] as a possible hereditary pathology contributor (Fig. 1, 2). The causal link between this p.I739V substitution and resulting patient phenotypes has been challenging to ascertain [16, 29, 78]. Yet, this mutation is commonly observed as it represents about 1% of Na_V channel variants detected in patients with painful or non-painful peripheral neuropathy [72]. Moreover, intra-familial phenotypic variability has been reported with $Na_V1.7$ p.I739V [50] and the involvement of modifiers or interacting proteins has subsequently been proposed [2, 37]. In this study, patients with the $Na_V1.7$ p.I739V mutation reported symptom alleviation after being prescribed 1 mg/day of short-acting guanfacine. Moreover, this treatment had long-term benefits, up to six years, with few adverse effects. In patients with similar symptoms who were not sequenced, such as a patient with erythromelalgia, we also observed a durable, multiple year favorable response to low doses of guanfacine.

Our hypothesis that guanfacine may interact with other targets such as Na_V channels is consistent with our preclinical and clinical experience, and seems to align with literature reporting clinical efficacy in neuropsychiatric diseases associated with Na_V channelopathies [19, 46, 48, 52, 75]. Since the ability of prefrontal cortex neurons to retain working memory through excitatory networks relies on ion channels that help set the membrane potential, a Na_V channelopathy may dysregulate action potential firing producing cognitive deficits inherent to both ADHD and Postural Orthostatic Tachycardia Syndrome [21, 34, 67, 77]. A compound that helps regulate membrane potentials, through inhibition of $Na_V1.7$ or other ion channel subtypes, would be clinically valuable. As such, we examined pre-clinically whether guanfacine could inhibit Na_V channels and found that multiple subtypes, including $Na_V1.7$, were indeed partly inhibited (Fig. 3, 4, and 5). The guanfacine concentration used in our *in vitro* experiments seems rather high. However, all heterologous expression systems are limited in their ability to mimic human physiological conditions, and *Xenopus laevis* oocytes are no exception [36] as they can be up to one hundred times less sensitive to compounds [74] compared to mammalian cells. As such, *in vivo* effective concentrations of Na_V channel inhibition by guanfacine may be much lower [40]. Indeed, in cortical neurons, a tenfold lower guanfacine concentration was reported to inhibit Na^+ currents [48].

A conventional explanation of guanfacine efficacy in our patients involves its primary effect on α_{2A}-adrenergic receptors which would reduce the increased sympathetic tone induced by, for example, Na_V channel gain-of-function mutations. Such an effect could perhaps be complemented by a smaller, synergistic inhibition of Na_V channel subtypes expressed in the nervous system. Although a functional association between α_{2A}-adrenergic receptors and Na_V channels has yet to be established, hints supporting a link can be found in the literature. For example, α_{2A}-adrenergic receptors agonists such as clonidine and dexmedetomidine have clinically established analgesic properties, in part attributed to acute inhibition of multiple Na_V channel subtypes at similar or higher concentrations than tested here for guanfacine [33, 64]. Dexmedetomidine causes a concentration-dependent inhibition of Na^+ currents, an effect that can be prevented by yohimbine, a competitive α_{2A}-adrenergic receptor antagonist [33]. Moreover, dexmedetomidine-induced inhibition is blocked by intracellular perfusion of the G protein-coupled receptor (GPCR) inhibitor GDPβ-S. A functional coupling of the α_{2A}-adrenergic receptor to GPCRs opens up possible pathways to influence Na_V channel function [9, 56, 79]. Finally, it is worth noting that agonist-mediated activation of α_{2A}-adrenergic receptors was reported to be dependent on membrane voltage to the extent that negative membrane potentials promote agonist-mediated activation and downstream receptor signaling [54]. Therefore, membrane depolarization triggered by the $Na_V1.7$ p.I739V mutation [29, 30] could reduce receptor susceptibility to agonist. By partially inhibiting $Na_V1.7$ p.I739V with guanfacine and restoring more negative membrane potentials, α_{2A}-adrenergic receptors may become more sensitive to agonists. As such, guanfacine could reduce neuronal hyperexcitability via direct $Na_V1.7$ inhibition and thereby increase its own affinity for the α_{2A}-adrenergic receptor to correct sympathetic outflow. This dual synergistic effect is but one possible explanation for the low dosage needed in patients (1 mg/day) to achieve symptom improvement.

While this limited study provides insights into the off-label efficacy of guanfacine, a few limitations are worth mentioning. First, although guanfacine inhibits $Na_V1.7$ p.I739V, we found that other Na_V channel subtypes are also susceptible to a varying degree. Therefore, a beneficial effect of guanfacine may not only be directly linked to $Na_V1.7$ but also may entail inhibition of multiple Na_V channel subtypes which cumulatively can reduce membrane excitability. Second, in our dysautonomia clinic we very occasionally have to stop prescribing guanfacine due to the onset of adverse patient complaints such as xerostomia, somnolence, and lightheadedness, symptoms that have also been associated with altered $Na_V1.7$ function [18]. However, we found that night-time administration coupled with a slow increase in the titration of the dose of guanfacine, preferably no faster than 1 mg per 2-3 weeks, assists in tolerability in the vast majority of patients. Third, our observations are based on only two nonrelated families with the $Na_V1.7$

p.I739V variant which do not fully represent the diverse population of patients with neuropsychiatric symptoms and Na_V channelopathies. Nonetheless, our data suggest that the non-selectivity of guanfacine towards Na_V channel subtypes could be a beneficial trait in patients with mutations in other Na_V channel subtypes. Fourth, our study is observational in nature lacking guanfacine data in a control group with similar symptoms but without a Na_V channelopathy. Since our observational data were not part of a randomized trial to assess the efficacy and safety of guanfacine in genetically stratified patients, future studies in a more controlled setting will be needed. Fifth, although the COMPASS-31 questionnaire is a widely used and validated tool for assessing multiple dysautonomia indications, its scoring algorithm may not fully capture the complexity of all symptoms. Some symptoms, such as hyperhidrosis - which improved in both of our patients -, may be underrepresented or not adequately weighted, leading to a potential bias in the extent of the overall score improvement with guanfacine treatment. Sixth, as exemplified by our third patient whose erythromelalgia was provoked by a mononucleosis infection, dysautonomia is a complex condition and other genetic as well as environmental factors that may contribute to both the observed symptoms and treatment responses.

Overall, the results of this study substantiate a possible pathological role for $Na_V1.7$ mutations in disorders related to an increased sympathetic tone and concomitant anxiety. Moreover, these data illustrate the potential benefits of low-dose short-acting guanfacine treatment, perhaps aided by an inhibitory effect on Na_V channels. Even though anxiety and dysautonomia symptoms in our studied families most likely involve a complex interplay between genetics and environmental cues, it is encouraging that inhibition of Na_V channels, including presumed gain-of-function genetic aberrations, can result in patients self-reporting substantial symptom improvement. In such a complex disease, a single drug given at a low dose being so effective, indicates a likelihood of guanfacine acting synergistically on multiple targets, including direct ion channel inhibition and a drug-induced augmented affinity to activate the α_{2A}-adrenergic receptor. Our observations suggesting that inhibition of Na_V channels by guanfacine may serve as an off-label target is clinically relevant since it promotes the possibility that guanfacine could one day be utilized in genetically stratified patients with neuropsychiatric symptoms resulting from, among other causes, anomalous Na_V channel behavior.

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Disclosures

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References

1. Abela AR, Chudasama Y (2014) Noradrenergic alpha2A-receptor stimulation in the ventral hippocampus reduces impulsive decision-making. Psychopharmacology (Berl) 231:521-531
2. Ahern CA, Payandeh J, Bosmans F, Chanda B (2016) The hitchhiker's guide to the voltage-gated sodium channel galaxy. J Gen Physiol 147:1-24
3. Al Dhaheri N, Wu N, Zhao S, Wu Z, Blank RD, Zhang J, Raggio C, Halanski M, Shen J, Noonan K, Qiu G, Nemeth B, Sund S, Dunwoodie SL, Chapman G, Glurich I, Steiner RD, Wohler E, Martin R, Sobreira NL, Giampietro PF (2020) KIAA1217: A novel candidate gene associated with isolated and syndromic vertebral malformations. Am J Med Genet A 182:1664-1672
4. Alamo C, Lopez-Munoz F, Sanchez-Garcia J (2016) Mechanism of action of guanfacine: a postsynaptic differential approach to the treatment of attention deficit hyperactivity disorder (adhd). Actas Esp Psiquiatr 44:107-112
5. Albadrani A (2017) Clonidine is effective for the treatment of primary idiopathic hyperhidrosis and hot flushes: a case report. J Med Case Rep 11:16
6. Alexandra Kredlow M, Fenster RJ, Laurent ES, Ressler KJ, Phelps EA (2022) Prefrontal cortex, amygdala, and threat processing: implications for PTSD. Neuropsychopharmacology 47:247-259
7. Arnsten AF (2009) The Emerging Neurobiology of Attention Deficit Hyperactivity Disorder: The Key Role of the Prefrontal Association Cortex. J Pediatr 154:I-S43
8. Arnsten AFT (2020) Guanfacine's mechanism of action in treating prefrontal cortical disorders: Successful translation across species. Neurobiol Learn Mem 176:107327
9. Brackx W, Collaco RC, Theys M, Vander Cruyssen J, Bosmans F (2023) Understanding the physiological role of Na(V)1.9: Challenges and opportunities for pain modulation. Pharmacol Therapeut 245:108416
10. Branco T, Tozer A, Magnus CJ, Sugino K, Tanaka S, Lee AK, Wood JN, Sternson SM (2016) Near-Perfect Synaptic Integration by Nav1.7 in Hypothalamic Neurons Regulates Body Weight. Cell 165:1749-1761
11. Cannon SC (2018) Sodium Channelopathies of Skeletal Muscle. Handb Exp Pharmacol 246:309-330
12. Caye A, Swanson JM, Coghill D, Rohde LA (2019) Treatment strategies for ADHD: an evidence-based guide to select optimal treatment. Mol Psychiatry 24:390-408
13. Chini M, Hanganu-Opatz IL (2021) Prefrontal Cortex Development in Health and Disease: Lessons from Rodents and Humans. Trends Neurosci 44:227-240

14. Connor DF, Grasso DJ, Slivinsky MD, Pearson GS, Banga A (2013) An open-label study of guanfacine extended release for traumatic stress related symptoms in children and adolescents. J Child Adolesc Psychopharmacol 23:244-251
15. Conrad F, Baumgartner H, Wiedermann C, Klein G (1983) Clonidine and hyperhidrosis. Ann Intern Med 99:570
16. Devigili G, Eleopra R, Pierro T, Lombardi R, Rinaldo S, Lettieri C, Faber CG, Merkies ISJ, Waxman SG, Lauria G (2014) Paroxysmal itch caused by gain-of-function Nav1.7 mutation. Pain 155:1702-1707
17. Dib-Hajj SD, Rush AM, Cummins TR, Hisama FM, Novella S, Tyrrell L, Marshall L, Waxman SG (2005) Gain-of-function mutation in Nav1.7 in familial erythromelalgia induces bursting of sensory neurons. Brain 128:1847-1854
18. Dormer A, Narayanan M, Schentag J, Achinko D, Norman E, Kerrigan J, Jay G, Heydorn W (2023) A Review of the Therapeutic Targeting of SCN9A and Nav1.7 for Pain Relief in Current Human Clinical Trials. J Pain Res 16:1487-1498
19. Eijkelkamp N, Linley JE, Baker MD, Minett MS, Cregg R, Werdehausen R, Rugiero F, Wood JN (2012) Neurological perspectives on voltage-gated sodium channels. Brain 135:2585-2612
20. Estacion M, Dib-Hajj SD, Benke PJ, Te Morsche RH, Eastman EM, Macala LJ, Drenth JP, Waxman SG (2008) NaV1.7 gain-of-function mutations as a continuum: A1632E displays physiological changes associated with erythromelalgia and paroxysmal extreme pain disorder mutations and produces symptoms of both disorders. J Neurosci 28:11079-11088
21. Fahrenholz II JM, Shibao CA (2023) Mast cell activation and autonomic disorders. In: Primer on the Autonomic Nervous System. Elsevier, p 627-629
22. Fesharaki-Zadeh A, Lowe N, Arnsten AF (2023) Clinical experience with the α2A-adrenoceptor agonist, guanfacine, and N-acetylcysteine for the treatment of cognitive deficits in “Long-COVID19”. Neuroimmunology Reports 3:100154
23. Fox H, Sinha R (2014) The role of guanfacine as a therapeutic agent to address stress-related pathophysiology in cocaine-dependent individuals. Adv Pharmacol 69:217-265
24. Fox HC, Seo D, Tuit K, Hansen J, Kimmerling A, Morgan PT, Sinha R (2012) Guanfacine effects on stress, drug craving and prefrontal activation in cocaine dependent individuals: preliminary findings. J Psychopharmacol 26:958-972
25. Freeman R, Wieling W, Axelrod FB, Benditt DG, Benarroch E, Biaggioni I, Cheshire WP, Chelimsky T, Cortelli P, Gibbons CH, Goldstein DS, Hainsworth R, Hilz MJ, Jacob G, Kaufmann H, Jordan J, Lipsitz LA, Levine BD, Low PA, Mathias C, Raj SR, Robertson D, Sandroni P, Schatz I, Schondorff R, Stewart

JM, van Dijk JG (2011) Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. Clin Auton Res 21:69-72

26. Gilchrist J, Dutton S, Diaz-Bustamante M, McPherson A, Olivares N, Kalia J, Escayg A, Bosmans F (2014) Nav1.1 modulation by a novel triazole compound attenuates epileptic seizures in rodents. ACS Chem Biol 9:1204-1212
27. Goldstein DS (2020) The extended autonomic system, dyshomeostasis, and COVID-19. Clin Auton Res 30:299-315
28. Goodwin G, McMahon SB (2021) The physiological function of different voltage-gated sodium channels in pain. Nat Rev Neurosci 22:263-274
29. Han C, Hoeijmakers JG, Ahn HS, Zhao P, Shah P, Lauria G, Gerrits MM, te Morsche RH, Dib-Hajj SD, Drenth JP, Faber CG, Merkies IS, Waxman SG (2012) Nav1.7-related small fiber neuropathy: impaired slow-inactivation and DRG neuron hyperexcitability. Neurology 78:1635-1643
30. Han C, Hoeijmakers JG, Liu S, Gerrits MM, te Morsche RH, Lauria G, Dib-Hajj SD, Drenth JP, Faber CG, Merkies IS, Waxman SG (2012) Functional profiles of SCN9A variants in dorsal root ganglion neurons and superior cervical ganglion neurons correlate with autonomic symptoms in small fibre neuropathy. Brain 135:2613-2628
31. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, Duda SN, Consortium RE (2019) The REDCap consortium: Building an international community of software platform partners. J Biomed Inform 95:103208
32. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG (2009) Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 42:377-381
33. Im ST, Jo YY, Han G, Jo HJ, Kim YH, Park CK (2018) Dexmedetomidine Inhibits Voltage-Gated Sodium Channels via alpha2-Adrenoceptors in Trigeminal Ganglion Neurons. Mediators Inflamm 2018:1782719
34. Iwanami A, Saito K, Fujiwara M, Okutsu D, Ichikawa H (2020) Safety and efficacy of guanfacine extended-release in adults with attention-deficit/hyperactivity disorder: an open-label, long-term, phase 3 extension study. Bmc Psychiatry 20:485
35. Johnson JP, Focken T, Khakh K, Tari PK, Dube C, Goodchild SJ, Andrez JC, Bankar G, Bogucki D, Burford K, Chang E, Chowdhury S, Dean R, de Boer G, Decker S, Dehnhardt C, Feng M, Gong W, Grimwood M, Hasan A, Hussainkhel A, Jia Q, Lee S, Li J, Lin S, Lindgren A, Lofstrand V, Mezeyova J, Namdari R, Nelkenbrecher K, Shuart NG, Sojo L, Sun S, Taron M, Waldbrook M, Weeratunge D, Wesolowski S,

Williams A, Wilson M, Xie Z, Yoo R, Young C, Zenova A, Zhang W, Cutts AJ, Sherrington RP, Pimstone SN, Winquist R, Cohen CJ, Empfield JR (2022) NBI-921352, a first-in-class, Na(V)1.6 selective, sodium channel inhibitor that prevents seizures in Scn8a gain-of-function mice, and wild-type mice and rats. Elife 11

36. Jones AR, Overly CC, Sunkin SM (2009) The Allen Brain Atlas: 5 years and beyond. Nat Rev Neurosci 10:821-828
37. Kanellopoulos AH, Koenig J, Huang H, Pyrski M, Millet Q, Lolignier S, Morohashi T, Gossage SJ, Jay M, Linley JE, Baskozos G, Kessler BM, Cox JJ, Dolphin AC, Zufall F, Wood JN, Zhao J (2018) Mapping protein interactions of sodium channel Na(V)1.7 using epitope-tagged gene-targeted mice. EMBO J 37:427-445
38. Kenwood MM, Kalin NH, Barbas H (2022) The prefrontal cortex, pathological anxiety, and anxiety disorders. Neuropsychopharmacology 47:260-275
39. Kuritzky A, Hering R, Goldhammer G, Bechar M (1984) Clonidine treatment in paroxysmal localized hyperhidrosis. Arch Neurol 41:1210-1211
40. Lacerda AE, Kramer J, Shen KZ, Thomas D, Brown AM (2001) Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs. Eur Heart J Suppl 3:K23-K30
41. Mantegazza M, Cestele S, Catterall WA (2021) Sodium channelopathies of skeletal muscle and brain. Physiol Rev 101:1633-1689
42. McKee SA, Potenza MN, Kober H, Sofuoglu M, Arnsten AF, Picciotto MR, Weinberger AH, Ashare R, Sinha R (2015) A translational investigation targeting stress-reactivity and prefrontal cognitive control with guanfacine for smoking cessation. J Psychopharmacol 29:300-311
43. Mechler K, Banaschewski T, Hohmann S, Hage A (2022) Evidence-based pharmacological treatment options for ADHD in children and adolescents. Pharmacol Therapeut 230:107940
44. Minett MS, Nassar MA, Clark AK, Passmore G, Dickenson AH, Wang F, Malcangio M, Wood JN (2012) Distinct Nav1.7-dependent pain sensations require different sets of sensory and sympathetic neurons. Nat Commun 3:791
45. Mulcahy JV, Pajouhesh H, Beckley JT, Delwig A, Du Bois J, Hunter JC (2019) Challenges and Opportunities for Therapeutics Targeting the Voltage-Gated Sodium Channel Isoform Na(V)1.7. J Med Chem 62:8695-8710
46. Nickel K, Tebartz van Elst L, Domschke K, Glaser B, Stock F, Endres D, Maier S, Riedel A (2018) Heterozygous deletion of SCN2A and SCN3A in a patient with autism spectrum disorder and Tourette syndrome: a case report. Bmc Psychiatry 18:248

47. Ota T, Yamamuro K, Okazaki K, Kishimoto T (2021) Evaluating Guanfacine Hydrochloride in the Treatment of Attention Deficit Hyperactivity Disorder (ADHD) in Adult Patients: Design, Development and Place in Therapy. Drug Des Devel Ther 15:1965-1969
48. Pasierski M, Kolba W, Szulczyk B (2023) Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons. Pharmacol Rep 75:331-341
49. Payandeh J, Hackos DH (2018) Selective Ligands and Drug Discovery Targeting the Voltage-Gated Sodium Channel Nav1.7. Handb Exp Pharmacol 246:271-306
50. Peddareddygari LR, Grewal RP (2021) Intrafamilial Phenotypic Variability Associated with the I1739V Mutation in the SCN9A Gene. Case Rep Neurol 13:135-139
51. Posey JE, Martinez R, Lankford JE, Lupski JR, Numan MT, Butler IJ (2017) Dominant Transmission Observed in Adolescents and Families With Orthostatic Intolerance. Pediatr Neurol 66:53-58 e55
52. Rees E, Carrera N, Morgan J, Hambridge K, Escott-Price V, Pocklington AJ, Richards AL, Pardinas AF, Investigators G, McDonald C, Donohoe G, Morris DW, Kenny E, Kelleher E, Gill M, Corvin A, Kirov G, Walters JTR, Holmans P, Owen MJ, O'Donovan MC (2019) Targeted Sequencing of 10,198 Samples Confirms Abnormalities in Neuronal Activity and Implicates Voltage-Gated Sodium Channels in Schizophrenia Pathogenesis. Biol Psychiatry 85:554-562
53. Remme CA (2023) SCN5A channelopathy: arrhythmia, cardiomyopathy, epilepsy and beyond. Philos Trans R Soc Lond B Biol Sci 378:20220164
54. Rinne A, Birk A, Bunemann M (2013) Voltage regulates adrenergic receptor function. Proc Natl Acad Sci U S A 110:1536-1541
55. Sagvolden T (2006) The alpha-2A adrenoceptor agonist guanfacine improves sustained attention and reduces overactivity and impulsiveness in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). Behav Brain Funct 2:41
56. Salzer I, Ray S, Schicker K, Boehm S (2019) Nociceptor Signalling through ion Channel Regulation via GPCRs. Int J Mol Sci 20
57. Savchenko VL, Boughter JD, Jr. (2011) Regulation of neuronal activation by Alpha2A adrenergic receptor agonist. Neurotox Res 20:226-239
58. Sivilotti L, Okuse K, Akopian AN, Moss S, Wood JN (1997) A single serine residue confers tetrodotoxin insensitivity on the rat sensory-neuron-specific sodium channel SNS. FEBS Lett 409:49-52
59. Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W (2012) COMPASS 31: a refined and abbreviated Composite Autonomic Symptom Score. Mayo Clin Proc 87:1196-1201

60. Sobreira N, Schiettecatte F, Boehm C, Valle D, Hamosh A (2015) New tools for Mendelian disease gene identification: PhenoDB variant analysis module; and GeneMatcher, a web-based tool for linking investigators with an interest in the same gene. Hum Mutat 36:425-431
61. Solish N, Bertucci V, Dansereau A, Hong HC, Lynde C, Lupin M, Smith KC, Storwick G, Canadian Hyperhidrosis Advisory C (2007) A comprehensive approach to the recognition, diagnosis, and severity-based treatment of focal hyperhidrosis: recommendations of the Canadian Hyperhidrosis Advisory Committee. Dermatol Surg 33:908-923
62. Soriano A, Gutgsell TL, Davis MP (2014) Diencephalic storms from leptomeningeal metastases and leukoencephalopathy: a rare and clinically important complication. Am J Hosp Palliat Care 31:98-100
63. Srour H, Pandya K, Flannery A, Hatton K (2018) Enteral Guanfacine to Treat Severe Anxiety and Agitation Complicating Critical Care After Cardiac Surgery. Semin Cardiothorac Vasc Anesth 22:403-406
64. Stoetzer C, Martell C, de la Roche J, Leffler A (2017) Inhibition of Voltage-Gated Na+ Channels by Bupivacaine Is Enhanced by the Adjuvants Buprenorphine, Ketamine, and Clonidine. Region Anesth Pain M 42:462-468
65. Strawn JR, Compton SN, Robertson B, Albano AM, Hamdani M, Rynn MA (2017) Extended Release Guanfacine in Pediatric Anxiety Disorders: A Pilot, Randomized, Placebo-Controlled Trial. J Child Adolesc Psychopharmacol 27:29-37
66. Tanaka BS, Nguyen PT, Zhou EY, Yang Y, Yarov-Yarovoy V, Dib-Hajj SD, Waxman SG (2017) Gain-of-function mutation of a voltage-gated sodium channel Na(V)1.7 associated with peripheral pain and impaired limb development. J Biol Chem 292:9262-9272
67. Taylor FB, Russo J (2001) Comparing guanfacine and dextroamphetamine for the treatment of adult attention-deficit/hyperactivity disorder. J Clin Psychopharmacol 21:223-228
68. Torch EM (2000) Remission of facial and scalp hyperhidrosis with clonidine hydrochloride and topical aluminum chloride. South Med J 93:68-69
69. Tovote P, Fadok JP, Luthi A (2015) Neuronal circuits for fear and anxiety. Nat Rev Neurosci 16:317-331
70. van Coevorden MH, Schoofs MW, Venhovens J (2021) Paroxysmal localized Hyperhidrosis, a case-report: When excessive sweating occurs in combination with severe headaches. Cephalalgia 41:1124-1127

71. van de Poll Y, Cras Y, Ellender TJ (2023) The neurophysiological basis of stress and anxiety - comparing neuronal diversity in the bed nucleus of the stria terminalis (BNST) across species. Front Cell Neurosci 17:1225758
72. Wadhawan S, Pant S, Golhar R, Kirov S, Thompson J, Jacobsen L, Qureshi I, Ajroud-Driss S, Freeman R, Simpson DM, Smith AG, Hoke A, Bristow LJ (2017) Na(V) channel variants in patients with painful and nonpainful peripheral neuropathy. Neurol Genet 3:e207
73. Wang M, Ramos BP, Paspalas CD, Shu Y, Simen A, Duque A, Vijayraghavan S, Brennan A, Dudley A, Nou E, Mazer JA, McCormick DA, Arnsten AF (2007) Alpha2A-adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. Cell 129:397-410
74. Weaver J (2016) Clinical Characterization of Inherited Erythromelalgia Due to Sodium Channel Mutations. Neurology Alert 35
75. Weiss LA, Escayg A, Kearney JA, Trudeau M, MacDonald BT, Mori M, Reichert J, Buxbaum JD, Meisler MH (2003) Sodium channels SCN1A, SCN2A and SCN3A in familial autism. Mol Psychiatry 8:186-194
76. Wilde AAM, Amin AS (2018) Clinical Spectrum of SCN5A Mutations: Long QT Syndrome, Brugada Syndrome, and Cardiomyopathy. JACC Clin Electrophysiol 4:569-579
77. Wu S, Morgenstern A, Rice T, Coffey B (2022) Psychopharmacological Strategies Employing Guanfacine in an Adolescent Girl with Postural Orthostatic Tachycardia Syndrome and Severe Posttraumatic Stress Disorder. J Child Adolesc Psychopharmacol 32:244-248
78. Xenakis MN, Kapetis D, Yang Y, Gerrits MM, Heijman J, Waxman SG, Lauria G, Faber CG, Westra RL, Lindsey PJ, Smeets HJ (2021) Hydropathicity-based prediction of pain-causing NaV1.7 variants. BMC Bioinformatics 22:212
79. Xu J, Cao S, Hubner H, Weikert D, Chen G, Lu Q, Yuan D, Gmeiner P, Liu Z, Du Y (2022) Structural insights into ligand recognition, activation, and signaling of the alpha(2A) adrenergic receptor. Sci Adv 8:eabj5347
80. Xu P, Chen A, Li Y, Xing X, Lu H (2019) Medial prefrontal cortex in neurological diseases. Physiol Genomics 51:432-442
81. Yang Y, Mis MA, Estacion M, Dib-Hajj SD, Waxman SG (2018) Na(V)1.7 as a Pharmacogenomic Target for Pain: Moving Toward Precision Medicine. Trends Pharmacol Sci 39:258-275
82. Yu S, Shen S, Tao M (2023) Guanfacine for the Treatment of Attention-Deficit Hyperactivity Disorder: An Updated Systematic Review and Meta-Analysis. J Child Adolesc Psychopharmacol 33:40-50
83. Zeng SL, Sudlow LC, Berezin MY (2020) Using Xenopus oocytes in neurological disease drug discovery. Expert Opin Drug Discov 15:39-52

84. Zhang Z, Schmelz M, Segerdahl M, Quiding H, Centerholt C, Jureus A, Carr TH, Whiteley J, Salter H, Kvernebo MS, Orstavik K, Helas T, Kleggetveit IP, Lunden LK, Jorum E (2014) Exonic mutations in SCN9A (NaV1.7) are found in a minority of patients with erythromelalgia. Scand J Pain 5:217-225

Figure legends

Figure 1: A 20-year-old male (proband, black arrow), harboring the $Na_V1.7$ p.I739V variant, presented with hyperhidrosis and concurrent symptoms of generalized anxiety, cardiac palpitations, cognitive deficits, chronic fatigue, insomnia, and an unusually high pain tolerance. Family consisted of three generations with pre-existing dysautonomia as shown. Symptoms are indicated with scores mentioned in pedigree. CS = COMPASS-31 Score with pre- and post-treatment score for the proband, HDSS = Hyperhidrosis Disease Severity Scale. Color/symbol legends are provided at the top of the figure.

Figure 2: The matriarch (proband, black arrow) of a large family with an inherited $Na_V1.7$ p.I739V substitution and three generations of pre-existing dysautonomia presented in our clinic in 2022 with brain fog and increased anxiety. This pedigree was drawn when the family was initially seen in our hyperhidrosis clinic pre-pandemic in 2017 with a chief complaint of hyperhidrosis, generalized anxiety disorder, and concomitant multiple symptoms of dysautonomia as shown. The maternal aunt of the proband presented with similar symptoms and her COMPASS-31 score decreased from 53.01 to 34.77 (post) after guanfacine treatment. Symptoms are indicated with scores mentioned in pedigree. CS = COMPASS-31 Score, HDSS = Hyperhidrosis Disease Severity Scale. Color/symbol legends are provided at the top of the figure.

Figure 3: The inhibitory effect of guanfacine on $Na_V1.7$ is illustrated here. A: Normalized G-V and channel availability relationships before and after guanfacine administration including current trace upon depolarization to 0 mV, the peak voltage of the G-V relationship (right) before (black) and after (red) guanfacine perfusion. Figure represents mean values ± SEM with n = 5-6. B: Normalized G-V and channel availability relationships before and after guanfacine administration. Data in grey (control) and green (guanfacine addition) represent inhibition of the p.I739V mutation. Figure represents mean values ± SEM with n = 5-8. C: $Na_V1.7$ recovery from inactivation before (black) and after (red) guanfacine addition obtained using a double-pulse protocol as described in the text. Y-axis represents fractional current recovery in function of the variable recovery interval in ms (X-axis). Figure represents mean values ± SEM with n = 5. D: state-dependent inhibition of $Na_V1.7$ by guanfacine. State dependence was determined using the test protocol described in the text. Representative current trace shown was obtained upon depolarization to 0 mV before (black) and after (red) guanfacine incubation.

Figure 4: The inhibitory effect of guanfacine on $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$ is illustrated here. Left: current trace upon depolarization to the peak voltage of the conductance (G) – voltage (V) relationship

(right) before (black) and after (red) guanfacine perfusion. Arrow indicates current inhibition after drug application. Right: Normalized G-V and channel availability relationships before and after guanfacine administration. Figure represents mean values ± Standard Error of the Mean (SEM) with n = 5-6.

Figure 5: The inhibitory effect of guanfacine on $Na_V1.4$, $Na_V1.5$, and $Na_V1.8$ is illustrated here. Left: current trace upon depolarization to the peak voltage of the G-V relationship (right) before (black) and after (red) guanfacine perfusion. Arrow indicates current inhibition after drug application. Right: Normalized G-V and channel availability relationships before and after guanfacine administration. Figure represents mean values ± SEM with n = 5-8.

Table legend

Table 1: Gating parameters of all tested Na_V channel subtypes in control conditions and after application of 86 µM guanfacine. Parameters shown include the $V_{1/2}$ and slope factor of the conductance (G) – voltage (V) relationship and steady-state inactivation (SSI; channel availability). Average current inhibition (%) with SEM at peak voltage (G-V) is also indicated.

Figure 1

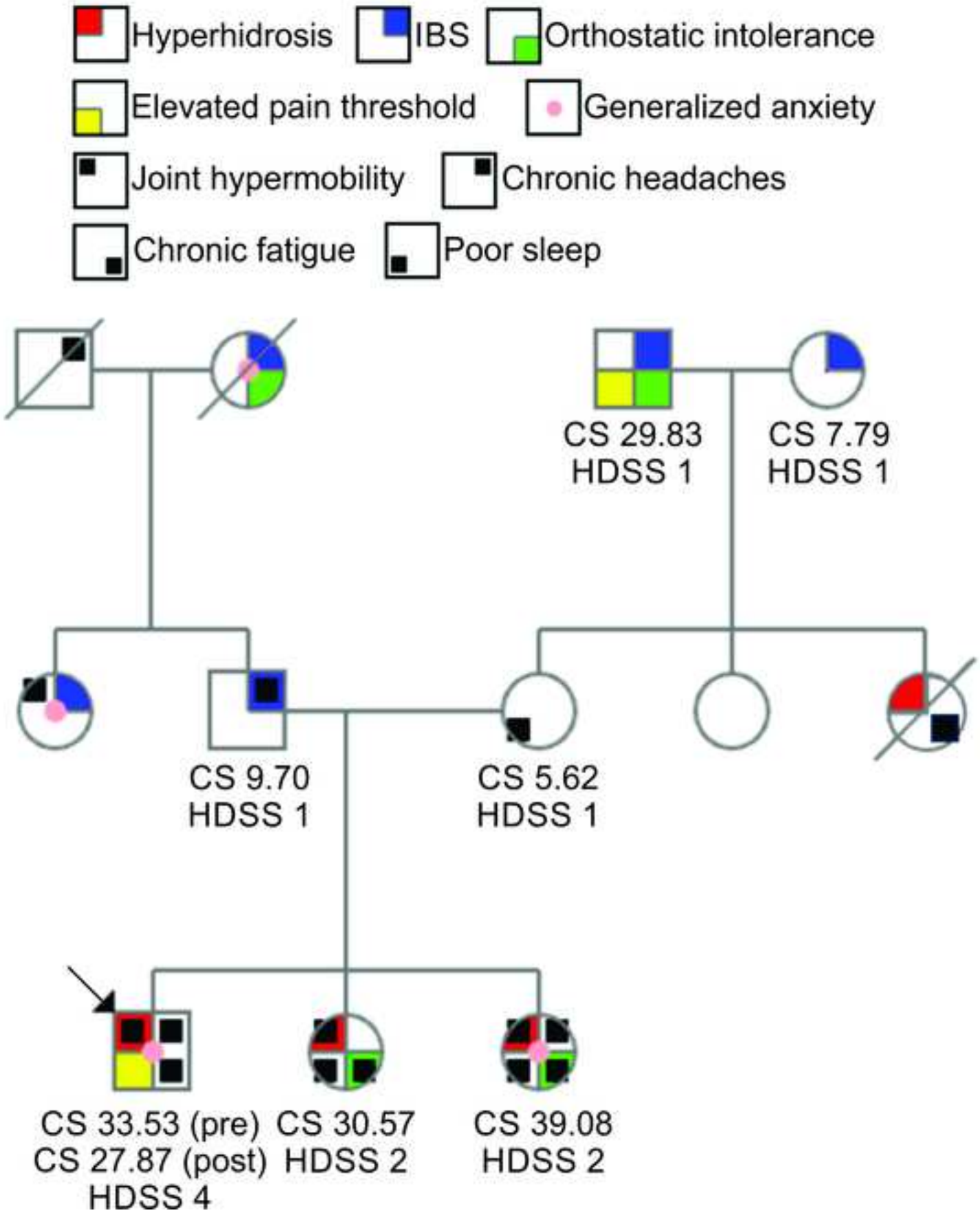
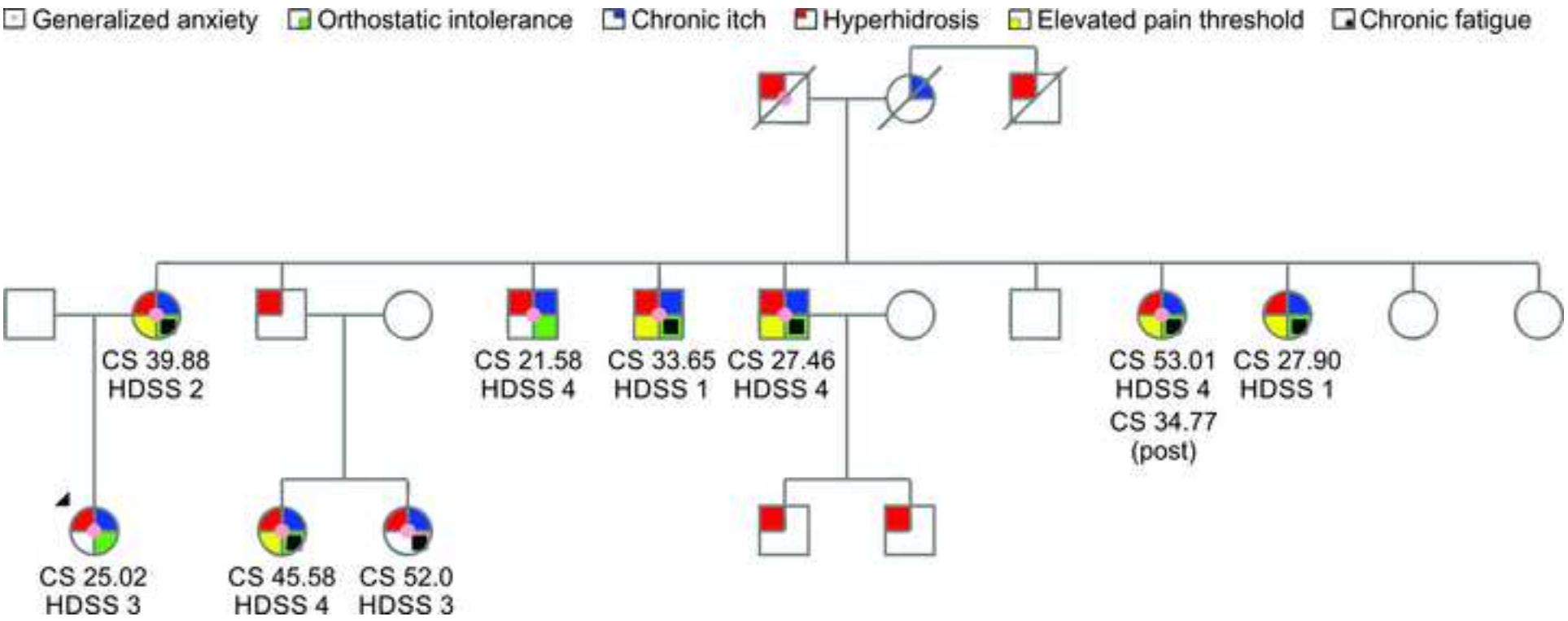
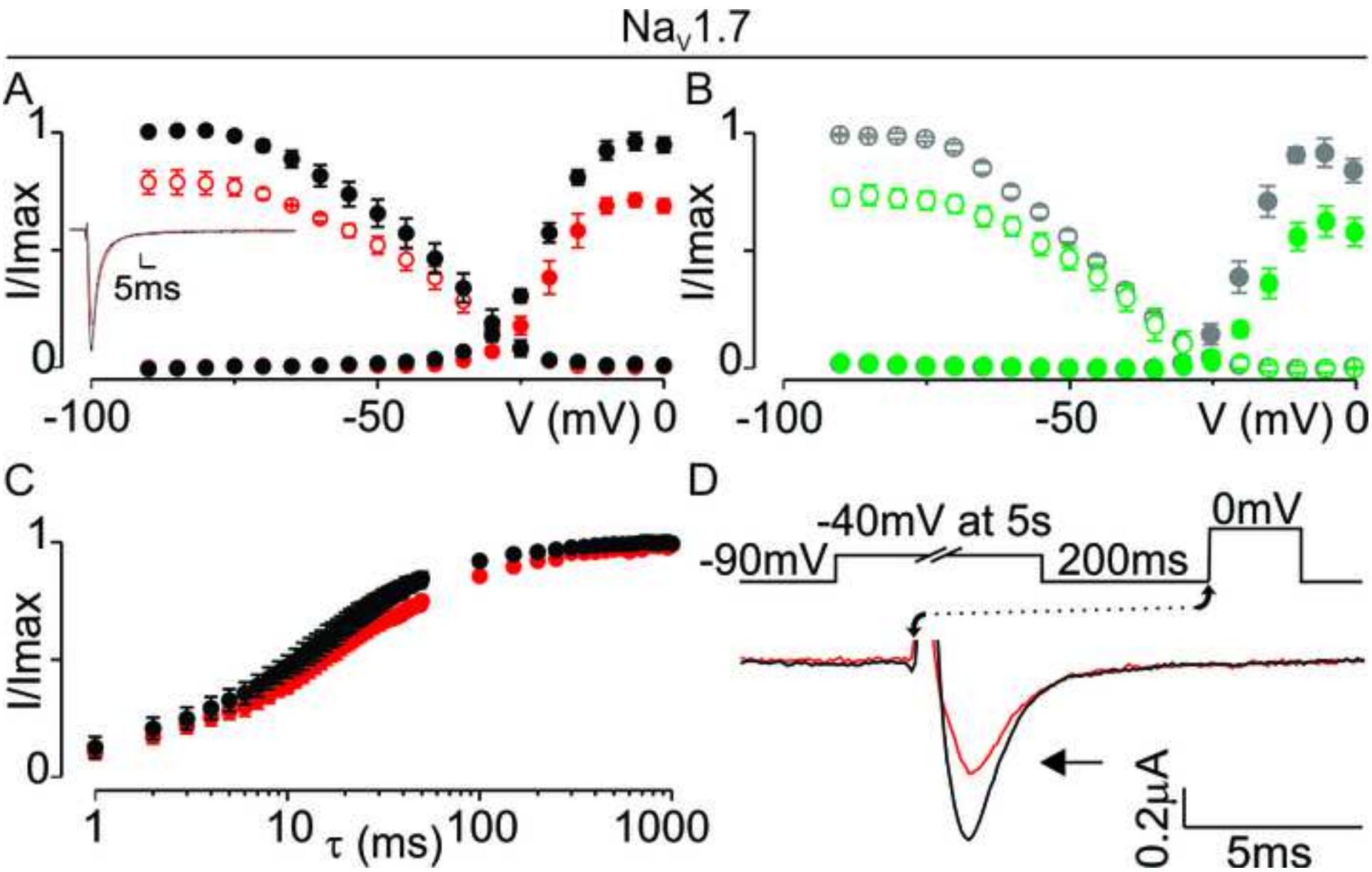


Figure 2





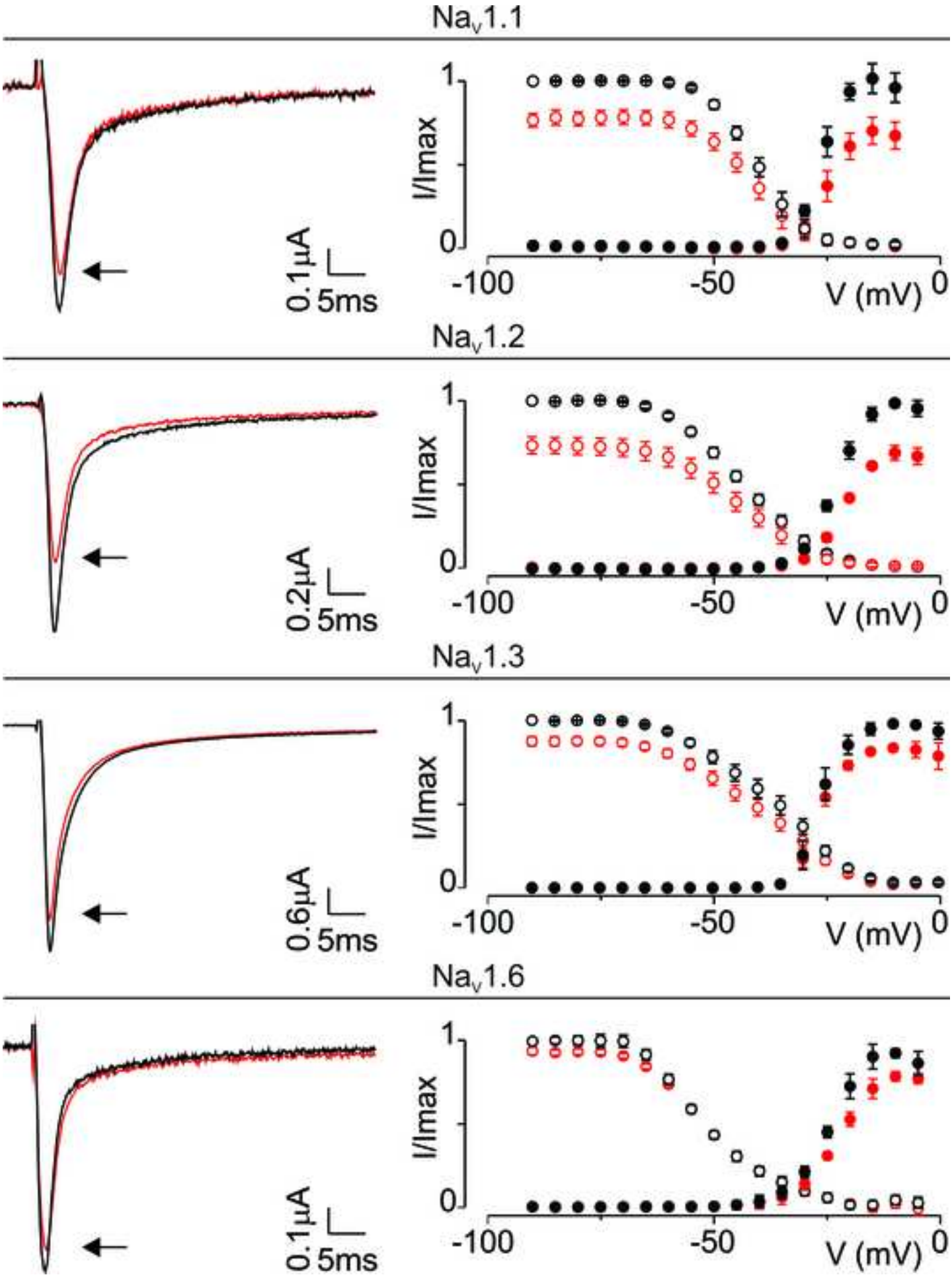


Figure 5

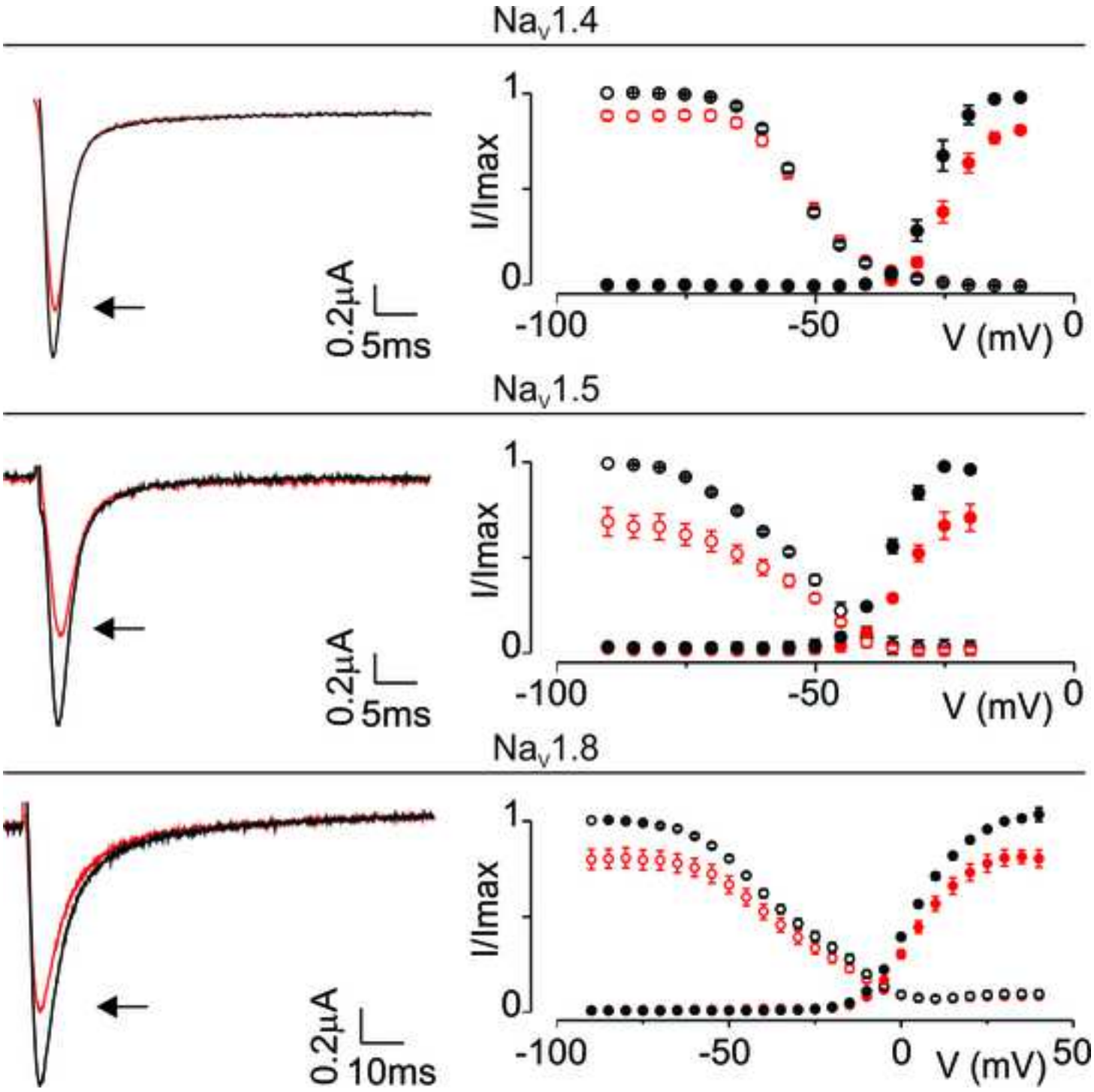


Table 1

Na_V channel	G-V - control		G-V + guanfacine		SSI - control		SSI + guanfacine		% block
	$V_{1/2}$ (mV)	slope	$V_{1/2}$ (mV)	slope	$V_{1/2}$ (mV)	slope	$V_{1/2}$ (mV)	slope	
$Na_V1.1$	-26.5±0.2	2.5±0.1	-25.5±0.2	2.8±0.2	-40.8±0.1	5.1±0.1	-41.4±0.2	5.6±0.2	23±4
$Na_V1.2$	-23.3±0.1	3.4±0.1	-21.6±0.1	3.3±0.2	-43.3±0.2	8.1±0.2	-43.5±0.2	7.8±0.2	27±5
$Na_V1.3$	-26.4±0.1	2.7±0.1	-26.7±0.1	2.6±0.1	-36.4±0.6	10±0.6	-37.9±0.6	10±0.6	12±3
$Na_V1.4$	-27.3±0.1	3.0±0.1	-24.4±0.1	3.3±0.1	-52.6±0.2	5.4±0.2	-51.2±0.3	5.5±0.2	12±2
$Na_V1.5$	-35.8±0.1	3.3±0.1	-33.3±0.1	3.5±0.1	-54.9±0.7	8.3±0.7	-53.6±0.8	8.3±0.8	31±7
$Na_V1.6$	-25.1±0.3	3.8±0.3	-23.0±0.2	4.3±0.2	-52.3±0.6	7.3±0.5	-50.9±0.5	8.0±0.4	6±2
$Na_V1.7$	-21.6±0.2	4.4±0.2	-20.6±0.2	3.8±0.2	-42.7±0.8	11±0.8	-41.6±0.9	11±0.9	22±5
p.I739V	-14.2±0.3	4.2±0.2	-10.8±0.3	2.8±0.2	-42.5±0.5	10±0.5	-39.2±0.5	10±0.5	25±4
$Na_V1.8$	-4.0±0.2	7.2±0.2	-4.3±0.2	6.7±0.2	-34.6±0.7	13±0.7	-33.3±0.6	12±0.5	20±5