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Evaluation of $Na_v1.8$ as a therapeutic target for Pitt Hopkins Syndrome

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Pitt Hopkins Syndrome (PTHS) is a rare syndromic form of autism spectrum disorder (ASD) caused by autosomal dominant mutations in the Transcription Factor 4 (*TCF4*) gene. *TCF4* is a basic helix-loop-helix transcription factor that is critical for neurodevelopment and brain function through its binding to cis-regulatory elements of target genes. One potential therapeutic strategy for PTHS is to identify dysregulated target genes and normalize their dysfunction. Here, we propose that *SCN10A* is an important target gene of *TCF4* that is an applicable therapeutic approach for PTHS. *Scn10a* encodes the voltage-gated sodium channel $Na_v1.8$ and is consistently shown to be upregulated in PTHS mouse models. In this perspective, we review prior literature and present novel data that suggests inhibiting $Na_v1.8$ in PTHS mouse models is effective at normalizing neuron function, brain circuit activity and behavioral abnormalities and posit this therapeutic approach as a treatment for PTHS.

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INTRODUCTION

Pitt Hopkins Syndrome (PTHS) is a rare neurodevelopmental disorder resulting from autosomal dominant mutations on chromosome 18 at the *TCF4* (also known as ITF2, SEF2, E2-2, not T-cell factor 4 which is encoded by *TCF7L2* gene) locus. Disease-causing mutations are primarily de novo with rare instances of parental mosaicism [1, 2] and result in TCF4 haploinsufficiency or dominant negative mechanisms [3–7]. PTHS patients display features of ASD and are more generally characterized by intellectual disability, developmental delay, breathing abnormalities, absent or limited speech, motor delay, seizure, constipation, and facial features including wide mouth and a broad nasal base with high bridge [8–11]. The exact disease-causing mechanisms downstream of *TCF4* remains an open question, and to date, no therapeutic interventions have been tested in a clinical trial. However, several studies using PTHS animal models have identified a variety of phenotypes that provide important biological insights into this disorder. These phenotypes are observed across the lifespan, beginning with alterations in cortical development, cell fate specification, neuron development and eventually lead to altered neuronal excitability, synaptic plasticity, and behavioral deficits in adult mice [12–14]. Here, we highlight evidence that suggests mutations in *Tcf4* lead to ectopic expression of *Scn10a*/$Na_v1.8$ which partially underlies neuronal excitability, network synchronicity and behavioral deficits observed in PTHS mouse models. Moreover, we discuss evidence that inhibition of $Na_v1.8$ is effective at acutely rescuing these phenotypes and discuss the potential of $Na_v1.8$ as a therapeutic target for the treatment of PTHS (Fig. 1).

IDENTIFICATION OF SCN10A/$NA_V1.8$ IN PTHS

Scn10a/$Na_v1.8$ was first identified as a downstream dysregulated gene of *Tcf4* in a rat model of PTHS [6]. In this model system, shRNA and CRISPR/Cas9 constructs specific to *Tcf4* were delivered by in utero electroporation leading to cellular transgenesis of layer 2/3 pyramidal neurons and knockdown of *Tcf4*. This knockdown resulted in a significant reduction in the intrinsic excitability of transfected neurons. Molecular profiling of transfected neurons via translating ribosome affinity purification (iTRAP) led to the identification of two upregulated ion channel genes, *Scn10a* and *Kcnq1*. Rescue experiments with antagonists to these two channels and phenocopy experiments via overexpression of *Scn10a* in wildtype neurons validated the causal role of *Scn10a* and *Kcnq1* in these intrinsic excitability deficits. Further confirmation of the TCF4-dependent excitability deficits was obtained in two different PTHS mouse models. One contained a puromycin cassette replacing the bHLH domain which led to expression of a truncated TCF4 protein ($Tcf4^{+/tr}$), and the other mouse model contained a missense mutation in the bHLH domain (R579W) which models the R580W mutation commonly found in patients [6, 8, 15]. The germline mutations in both of these mouse models are predicted to produce dominant-negative TCF4 protein [5, 16]. In the $Tcf4^{+/tr}$ mouse model, it was shown that SCN10a expression was upregulated, and consistent with the rat model, pharmacological blockade of $Na_v1.8$ normalized intrinsic excitability deficits [6]. Regulation of *Scn10a* by *Tcf4* appears to be direct, as TCF4 ChIP-seq analysis in rat neuroprogenitor cell cultures indicated that *Tcf4* binds directly to regions of the *Scn10a* genetic locus and therefore is predicted to act as a repressor of *Scn10a* gene expression in the central nervous system (CNS) [6]. Together, these

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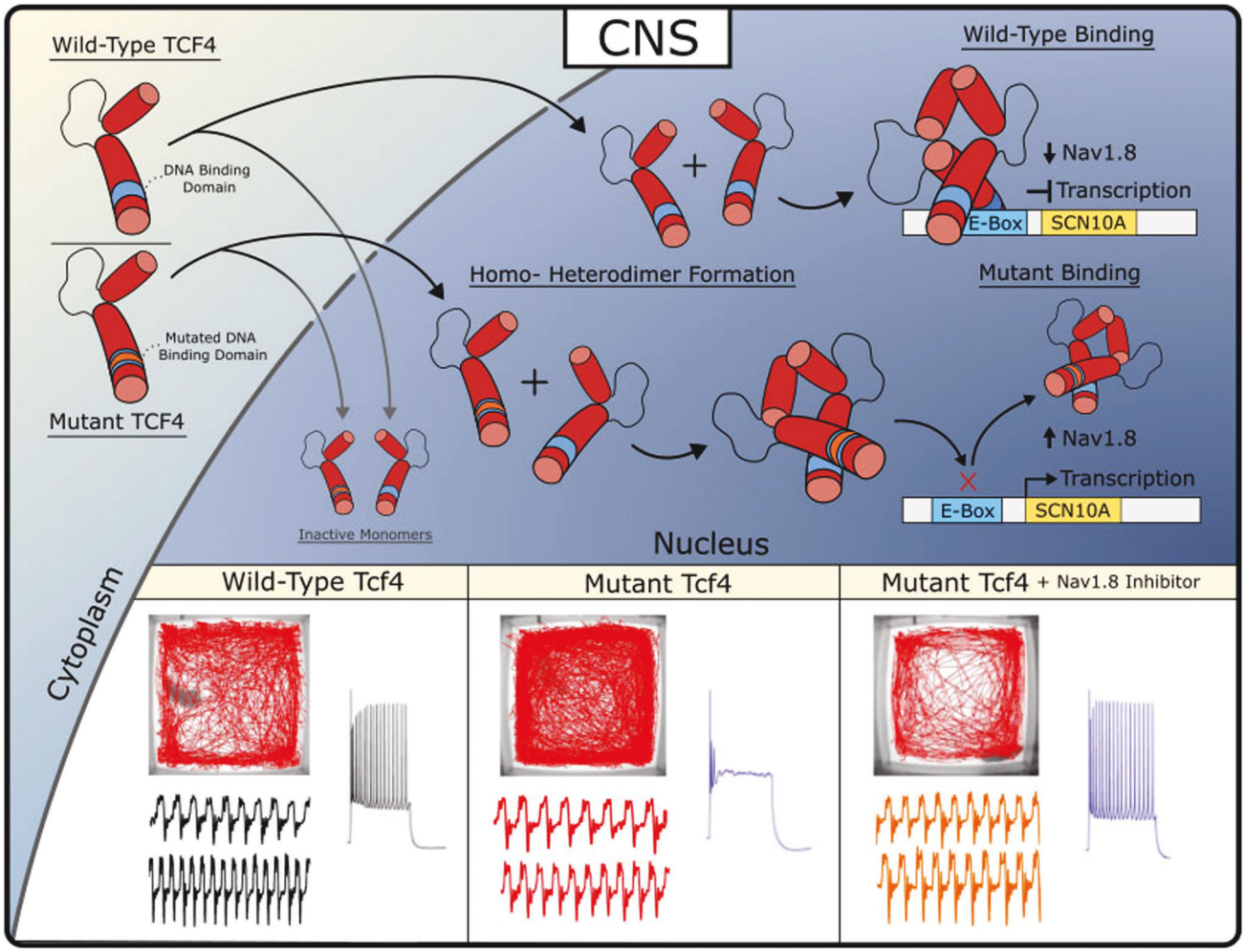


Fig. 1 Disease-causing mutations in *Tcf4* dysregulates *Scn10a*/$Na_v1.8$ expression in the CNS and results in abnormal physiology and behavior. TCF4 is imported into the nucleus and forms homo- or heterodimers with itself or other bHLH-domain containing transcription factors. Dimers containing wild-type TCF4 directly bind the genomic locus of *Scn10a* and repress *Scn10a* expression. Dimers containing mutant TCF4 are incapable of binding DNA and result in de-repression of *Scn10a* expression. Resulting ectopic *Scn10a*/$Na_v1.8$ expression leads to hyperlocomotion, breathing abnormalities and intrinsic excitability deficits which are all rescued by inhibition of $Na_v1.8$.

initial findings indicated $Na_v1.8$ was dysregulated in PTHS rodent models and that its ectopic expression was a key molecular mechanism underlying TCF4-dependent intrinsic excitability deficits (Fig. 1). Fortunately, the unique properties of $Na_v1.8$ make it a suitable drug target.

SCN10A/$NA_V1.8$ FUNCTION AND PHARMACOLOGY

SCN10a/$Na_v1.8$ is primarily a peripherally expressed, TTX resistant, voltage-gated sodium channel [17]. However, its expression and function in the central nervous system is reported [6, 18, 19] and SCN10a variants are associated with epileptic disorders [20]. In the peripheral nervous system, $Na_v1.8$ is thought to play an important role in nociception [21–24] and in dorsal root ganglion cells (DRGs) $Na_v1.8$ is responsible for a substantial proportion of the inward current needed to generate an action potential [25]. In addition, $Na_v1.8$ also appears to regulate the frequency of action potential firing and spike-frequency adaptation due to its unique kinetic properties [26, 27]. $Na_v1.8$ channels display prominent slow inactivation [17] and DRGs show a pronounced adaptation of action potential firing in response to stimulation [27]. Selective inhibitors of $Na_v1.8$ have been developed and have shown promise in rodent pain models as well as in early phase human trials. The selective $Na_v1.8$ inhibitor A-803467 has shown significant effects on the maximal amplitude and kinetic properties of the TTX-resistant sodium current in rats [18]. A-803467, exhibited high affinity and selectivity for blocking human $Na_v1.8$ channels and effectively inhibited spontaneous and evoked DRG neuronal action potentials in an in vivo rat preparation. A-803467 also dose-dependently reduced nociception in neuropathic and inflammatory pain models [22]. However, A-803467 in preclinical models has limited oral bioavailability [22]. PF-04531083 ($Na_v1.8$ IC50 = 700 nM) and PF-06305591 ($Na_v1.8$ IC50 = 15 nM) were developed as potent and highly selective $Na_v1.8$ inhibitors with acceptable oral bioavailability and showed effectiveness in preclinical pain models [28, 29]. PF-04531083 was tested in a clinical trial for treatment of post-surgical dental pain and was found to have no serious adverse side effects [30]. Moreover, PF-04531083 can pass the blood brain barrier (data not shown), and was shown to rescue CNS phenotypes in a PTHS mouse model, whereas PF-06305591 is non brain penetrant (Fig. 1) [19, 28, 29]. More recently, VX-548 an oral selective $Na_v1.8$ inhibitor has shown success in two phase 2 clinical trials for acute pain in patients who had recently undergone abdominoplasty or bunionectomy [31, 32], however the ability of VX-548 to penetrate the blood brain barrier is not reported.

NORMALIZATION OF BREATHING AND BEHAVIORAL ABNORMALITIES

A common symptom observed in PTHS patients is disordered breathing characterized by hyperventilation and intermittent apnea or breath holding [33, 34]. These breathing abnormalities severely impact the patient's quality of life and often contribute to aspiration-induced pneumonia, which is the leading cause of death in PTHS [35, 36]. Remarkably, similar breathing abnormalities were observed in a PTHS mouse model [19]. *Tcf4*$^{+/tr}$ mice display frequent episodes of hyperventilation, reduced sigh activity, increased post-sigh apnea, and fail to increase inspiratory and expiratory output in response to CO_2 (Fig. 1). Cleary and colleagues deduced that these breathing abnormalities may result from abnormal function of the retrotrapezoid nucleus (RTN) because similar breathing abnormalities are found in Rett Syndrome and are known to involve chemoreception. In addition, acetazolamide, a carbonic anhydrase inhibitor, used to induce metabolic acidosis and hyperventilation, improved breathing in PTHS patients [37–42]. They showed that *TCF4* mutation resulted in selective loss of parafacial Phox2b+ neurons, altered connectivity between Phox2b+ neurons and the pre-BotC complex, and suppressed excitability of chemosensitive RTN neurons. All these phenotypes were consistent with previously observed phenotypes in various brain regions of PTHS mouse models [6, 15, 43, 44]. They demonstrated that *Scn10a* expression is not normally detected in the RTN of *Tcf4*$^{+/+}$ mice, however *Scn10a* expression was observed in *Tcf4*$^{+/tr}$ mice. Remarkably, pharmacological block of $Na_v1.8$ with i.p. injection of PF-04531083 was effective at rescuing breathing abnormalities in *Tcf4*$^{+/tr}$ mice. Moreover, they showed that acute $Na_v1.8$ block was also effective at rescuing hyperlocomotion and anxiety (Fig. 1). Importantly, they demonstrated that rescue by PF-04531083 was specific to inhibition of $Na_v1.8$ in the CNS, because i.p. injection of PF-06305591, which does not penetrate the blood brain barrier, was ineffective at normalizing behavior.

Together, Cleary and colleagues provided direct in vivo evidence showing that central inhibition of $Na_v1.8$ was effective at normalizing breathing and behavioral abnormalities in a PTHS mouse model, further supporting the idea of $Na_v1.8$ as a therapeutic target. In another set of studies, Ekins and colleagues performed a high throughput screen to identify FDA approved drugs for inhibition on recombinant $Na_v1.8$ expressed in HEK cells [45]. Their screen identified a number of dihydropyridine calcium channel antagonists that were effective at blocking $Na_v1.8$ channels, with nicardipine being the most potent having a sub micromolar IC50 (0.6 μM). They went on to show that administration of nicardipine improved several behavioral deficits in a PTHS mouse model, including social recognition, nesting, self-grooming, fear conditioning, and hyperlocomotion [45]. However, the exact mechanism of rescue by nicardipine is not entirely clear, as it is likely inhibiting both sodium and calcium channels. Overall, these studies provide evidence that inhibition of $Na_v1.8$ is effective at rescuing breathing and behavioral abnormalities in PTHS mouse models and therefore support therapeutic targeting of $Na_v1.8$.

NORMALIZATION OF AUDITORY EVOKED POTENTIALS

Event-related potentials (ERPs) are stereotyped patterns of voltage fluctuation measured in response to sensory stimuli, which consist of temporal components that reflect physiological response. Levels of spectral power and phase coherence during ERP components are thought to reflect strength and connectivity in cortical circuits that mediate sensory information processing [46]. Following our previously published methods [47], we recorded auditory ERPs in 12 week old C57Bl6/J male wild-type (*Tcf4*$^{+/+}$) and *Tcf4*$^{+/tr}$ mice approximately 35 min after vehicle treatment (baseline) or acute administration of the Nav1.8 antagonist PF-04531083 (i.p., 10 mg/kg). We used component and time-frequency analysis of the ERP to identify changes in patterns of synchronized oscillatory activity during the ERP in this PTHS mouse model at baseline and following $Na_v1.8$ antagonism. Component analysis of the ERP showed that there is a significant effect of genotype in reducing the N40 amplitude peak (Fig. 2B–D). In addition, the event-related spectral perturbation (ERSP) power showed changes relative to tone onset in *Tcf4*$^{+/tr}$ mice and alterations in phase locking as measured by intertrial phase coherence (ITC, Fig. 3). Specifically, we observed no difference in ERSP at baseline between genotypes (Fig. 3A and data not shown) but observed significant delay in the latency of low (theta) frequency activity (Fig. 3B, E), and increased level of coherence in the high (gamma) frequency ITC at baseline (Fig. 3B, D). The delayed oscillatory activity and increased gamma synchrony in response to auditory stimuli suggests impairments in the neural correlates of sensory information processing in this PTHS mouse model. The N40 in rodents is thought to be functionally analogous to the P50 in humans. A reduction in P50 amplitude has been strongly associated with schizophrenia and validated in both genetic and pharmacological rodent models of schizophrenia [48–50]. Gamma synchrony is often associated with the function of fast-spiking parvalbumin-positive interneurons [51, 52] and therefore the observed alterations in this frequency band could indicate abnormal functioning of these gabaergic interneurons in the PTHS mouse model. Given prior evidence that Nav1.8 is upregulated in this mouse model and that Nav1.8 antagonists were effective at normalizing both intrinsic excitability and behavior, we quantified the acute effect of PF-04531083 on ERPs.

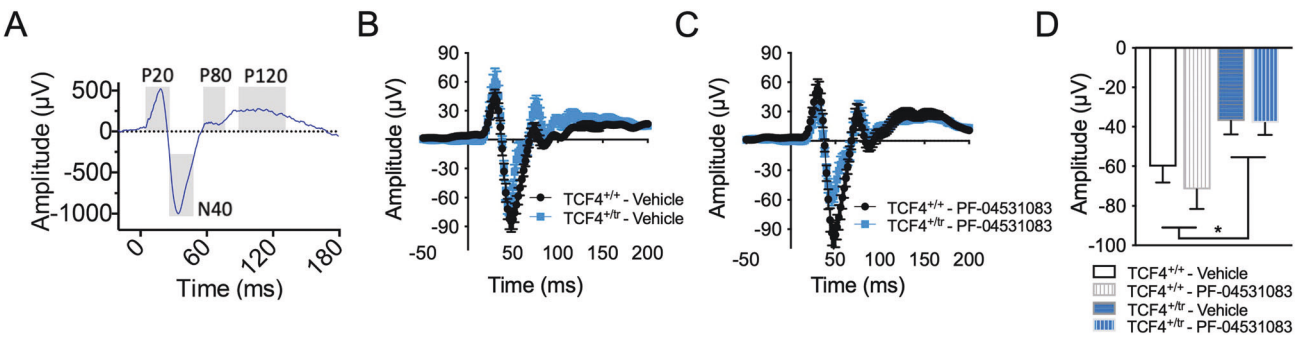
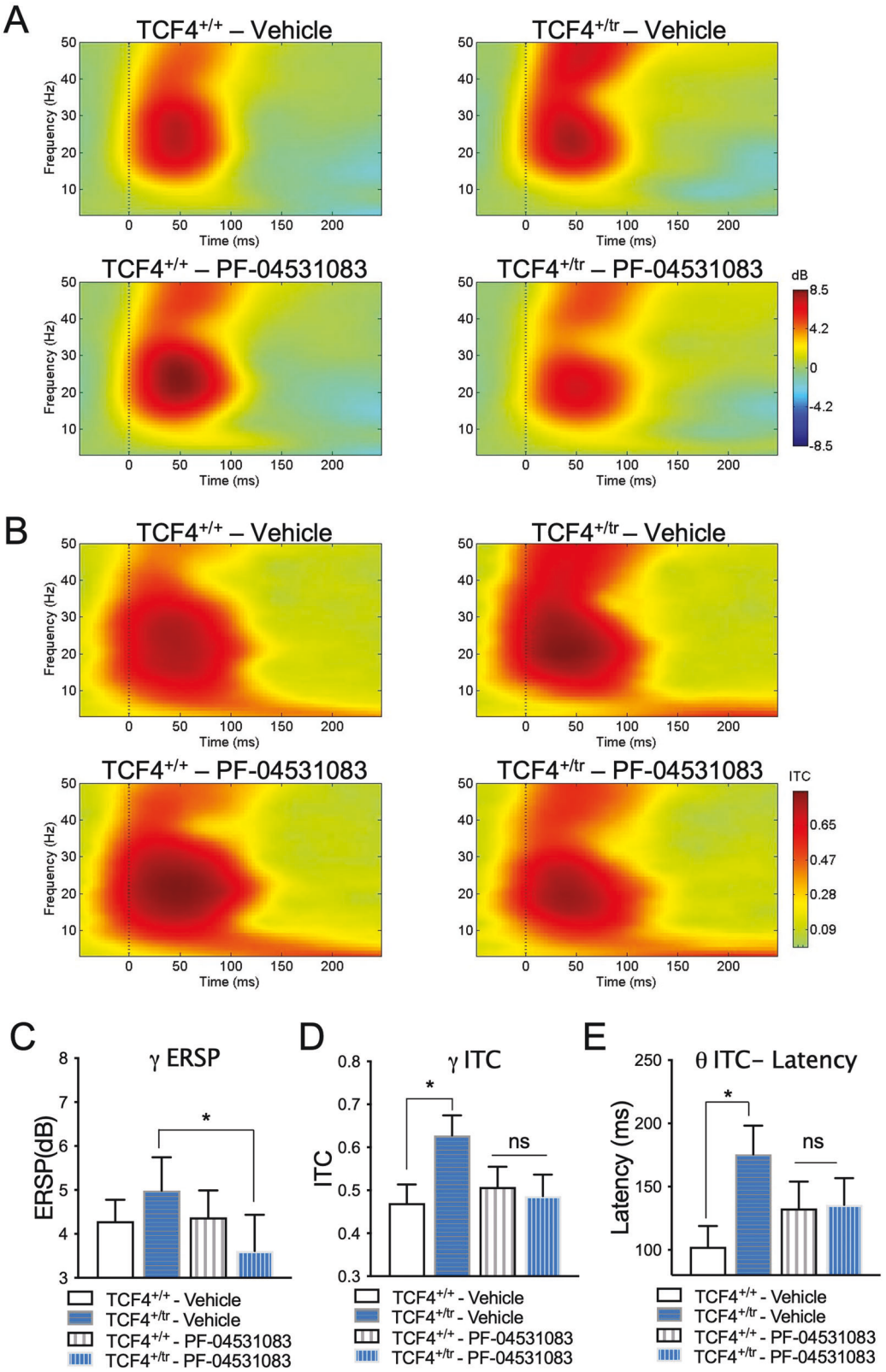


Fig. 2 Sensory information processing deficits in the PTHS mouse model. A Example event-related potential (ERP) grand averages from individual temporal components (P20, N40, P80 and P120) where time 0 = auditory stimulus onset. **B** Grand average ERPs in *Tcf4*$^{+/tr}$ ($n = 10$) compared to *Tcf4*$^{+/+}$ ($n = 12$) animals at baseline and (**C**) following PF-04531083 administration. **D** Summary component analysis showing significantly reduced amplitudes in N40 peaks in *Tcf4*$^{+/tr}$ mice compared to *Tcf4*$^{+/+}$ animals, which are not altered by PF-04531083 administration (2-way RM ANOVA, $^*p = 0.0163$, main effect of genotype; ns $p = 0.1317$, main effect of treatment). All descriptive statistics and p values for the data presented in this figure are provided in Supplementary Table 1.



PF-04531083 had no effect on N40 peak amplitude in *Tcf4*$^{+/+}$ or *Tcf4*$^{+/tr}$ mice (Fig. 2B–D), suggesting this particular aspect of the AEP is unrelated to $Na_v1.8$ and results from additional mechanisms downstream of TCF4. However, PF-04531083 did significantly reduce gamma ERSP in *Tcf4*$^{+/tr}$ mice, but not in *Tcf4*$^{+/+}$ mice (Fig. 3A, C). In addition, acute $Na_v1.8$ blockade also normalized the latency of theta ITC and gamma ITC (Fig. 3B, D, E). These results suggest acute $Na_v1.8$ antagonism is effective at normalizing abnormal synchronous activity in the PTHS mouse model and provides further support that $Na_v1.8$ may have utility for treating

Fig. 3 Sensory information processing deficits are normalized by $Na_v1.8$ inhibition. A Heat maps of event-related spectral perturbation (ERSP) in *Tcf4*$^{+/+}$ (left, $n = 12$) and *Tcf4*$^{+/tr}$ (right, n = 10) animals depicting ERP-related changes due to genotype (vehicle) and rescue with PF-04531083 (SCN10a). **B** Heat maps of intertrial coherence (ITC) in *Tcf4*$^{+/+}$ (left, $n = 12$) and *Tcf4*$^{+/tr}$ (right, n = 10) animals depicting ERP-related changes due to genotype (vehicle) and rescue with PF-04531083 (SCN10a). **C** Reduction of gamma ERSP following SCN10a antagonism in *Tcf4*$^{+/tr}$, but not in *Tcf4*$^{+/+}$ animals (2-way RM ANOVA, $p = 0.0453$ interaction of genotype X treatment; Bonferroni post hoc, $^*p = 0.0269$ vehicle-treated *Tcf4*$^{+/tr}$ versus PF-04531083-treated *Tcf4*$^{+/tr}$; ns $p > 0.05$ vehicle-treated *Tcf4*$^{+/+}$ versus PF-04531083-treated *Tcf4*$^{+/+}$). **D** High frequency disturbances in *Tcf4*$^{+/tr}$ mice are corrected by SCN10a antagonist. There is significantly higher gamma ITC in vehicle-treated *Tcf4*$^{+/tr}$ compared to *Tcf4*$^{+/+}$ vehicle-treated mice in the first 75 ms post-tone. Following SCN10a treatment, there is no effect of genotype (2-way RM ANOVA, $p = 0.0027$ interaction of genotype X treatment; Bonferroni post hoc, $^*p = 0.0446$ vehicle-treated *Tcf4*$^{+/+}$ versus *Tcf4*$^{+/tr}$; ns $p > 0.05$ PF-04531083-treated *Tcf4*$^{+/+}$ versus *Tcf4*$^{+/tr}$). **E** Low frequency disturbances in *Tcf4*$^{+/tr}$ mice are corrected by PF-04531083. Latency to peak theta (3–8 Hz) ITC is significantly increased in vehicle-treated *Tcf4*$^{+/tr}$ compared to vehicle-treated *Tcf4*$^{+/+}$ mice. No significant effect of genotype is detected following treatment with the SCN10a antagonist (2-way RM ANOVA, $p = 0.0123$ interaction of genotype X treatment; Bonferroni post hoc, $^*p = 0.0303$ vehicle-treated *Tcf4*$^{+/+}$ versus *Tcf4*$^{+/tr}$; ns $p > 0.05$ PF-04531083-treated *Tcf4*$^{+/+}$ versus *Tcf4*$^{+/tr}$).

symptoms in PTHS. Moreover, these data represent a potential electrophysiological biomarker that could be utilized for screening $Na_v1.8$ antagonists for therapeutic efficacy. Patterns of oscillatory activity are well-conserved across species, and if similarly altered ERP responses were detected by scalp EEG recordings in PTHS patients, the translational worth of this biomarker would be invaluable.

SCN10A/$NA_V1.8$ AND MYELINATION

Another mechanistic link that supports the use of $Na_v1.8$ antagonists for the treatment of PTHS could be through its relation to demyelinating disorders. Transcriptional profiling of several different PTHS mouse models showed that differentially expressed genes were enriched in neurons and oligodendrocytes (OLs), and analysis of OLs and myelination in the *Tcf4*$^{+/tr}$ mouse showed a significant reduction in OL density, myelination and function [43]. These results suggest that re-myelination could be a potential therapeutic avenue for PTHS but may also provide another link to therapeutic targeting of *Scn10a*/$Na_v1.8$. Several groups have shown that a variety of diseases associated with demyelination result in maladaptive ectopic expression of *Scn10a*/$Na_v1.8$. For instance, hereditary demyelinating neuropathy leads to an upregulation of *Scn10a*/$Na_v1.8$ and abnormal axonal excitability [53], and ectopic *Scn10a*/$Na_v1.8$ is observed in the cerebellum of the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis (MS) and in MS patients [54]. Motor deficits are common to both MS and PTHS patients and it has been observed that paroxysmal dysarthria and ataxia in MS patients responds to treatment with sodium channel blocker such as carbamazepine [55, 56]. These results have led to the notion that $Na_v1.8$ antagonists may be a beneficial treatment for demyelinating diseases and neuropathies [24]. It was subsequently shown that an administration of an orally bioavailable $Na_v1.8$ antagonist (PF-01247324) improved cerebellar-dependent motor coordination in a transgenic mouse model overexpressing *Scn10a* as well as the EAE mouse model of MS [57, 58]. The link between demyelination and *Scn10a* expression is intriguing, and a similar maladaptive mechanism could be at play in PTHS in response to the TCF4-dependent reduction in myelination. Overall, these results suggest inhibition of $Na_v1.8$ in PTHS patients may provide a dual benefit by normalizing neuronal excitability and improving myelin related excitability deficits.

CONCLUSION

Currently there are no approved medications for the core symptoms of ASD or even subsets of ASD like PTHS. Here, we discuss the results of a variety of rodent studies on PTHS that all converge on $Na_v1.8$ as being a plausible therapeutic target. Rodent models of PTHS have routinely shown that disruption of *Tcf4* function leads to upregulation of *Scn10a*/$Na_v1.8$ and pharmacological blockade of $Na_v1.8$ is effective at normalizing both physiological and behavioral phenotypes (Fig. 1). Potent and selective $Na_v1.8$ antagonists are developed and their safety in humans is demonstrated in clinical trials [59, 60]. Given all these factors, we recommend testing antagonists of $Na_v1.8$ as a therapeutic approach for PTHS.

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AUTHOR CONTRIBUTIONS

KM and BJM designed the experiments. YM performed and analyzed the experiments. KM analyzed the results. DD, SRS and BJM wrote the manuscript. RFK generated the illustration. KM and BJM reviewed and edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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