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Unspooling the Thread: VIP Interneurons Linked With Autism Spectrum Disorder Behaviors but Not Seizures in Dravet Syndrome

VIP Interneuron Impairment Promotes In Vivo Circuit Dysfunction and Autism-Related Behaviors in Dravet Syndrome

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Dravet syndrome (DS) is a severe neurodevelopmental disorder caused by loss-of-function variants in SCN1A, which encodes the voltage-gated sodium channel subunit Nav1.1. We recently showed that neocortical vasoactive intestinal peptide interneurons (VIP-INs) express Nav1.1 and are hypoexcitable in DS ($Scn1a^{+/-}$) mice. Here, we investigate VIP-IN function at the circuit and behavioral level by performing in vivo 2-photon calcium imaging in awake wild-type (WT) and $Scn1a^{+/-}$ mice. VIP-IN and pyramidal neuron activation during behavioral transition from quiet wakefulness to active running is diminished in $Scn1a^{+/-}$ mice, and optogenetic activation of VIP-INs restores pyramidal neuron activity to WT levels during locomotion. VIP-IN selective Scn1a deletion reproduces core autism-spectrum-disorder-related behaviors in addition to cellular- and circuit-level deficits in VIP-IN function, but without epilepsy, sudden death, or avoidance behaviors seen in the global model. Hence, VIP-INs are impaired in vivo, which may underlie non-seizure cognitive and behavioral comorbidities in DS.

Commentary

Inhibitory interneurons have been long studied in the epilepsy field, as the balance of excitation and inhibition in the brain is critical to seizure initiation. These interneurons, however, are incredibly diverse, with one major subtype expressing vasoactive intestinal peptide (VIP).[1] These VIP interneurons preferentially target other inhibitory interneurons, leading to the activation of excitatory cells and acting as a source of disinhibition in the cortex. Vasoactive intestinal peptide interneurons have been previously implicated in Dravet syndrome,[2] one of the most common genetic epilepsy syndromes resulting from mutations in the sodium channel gene *SCN1A*. These mutations in *SCN1A* are primarily loss-of-function and result in haploinsufficiency, leading to a 50% reduction of functional $Na_v1.1$, the sodium channel encoded by the *SCN1A* gene. Dravet syndrome is characterized by drug-resistant seizures, cognitive dysfunction, and developmental delay, and is often associated with autism-like behaviors. Despite the fact that these cognitive and behavioral comorbidities significantly affect the quality of life of Dravet syndrome patients and families, they have been relatively less studied than the seizure phenotype of this devastating epileptic encephalopathy. However, a recent study by Goff and colleagues demonstrate that dysfunction of VIP interneurons may underlie the autism-like behavior and cognitive impairment observed in Dravet syndrome.[3]

Vasoactive intestinal peptide interneurons play a prominent role in sensory processing and behavior-state transitions such as the onset of locomotion.[4] In these transitions, they are excited by cholinergic modulation and inhibit other inhibitory interneurons, notably those expressing somatostatin (SST).[5] This enhances the response of local excitatory neurons and results in overall network disinhibition. Goff and colleagues used the $Scn1a^{+/-}$ model, a frequently used Dravet syndrome mouse model with heterozygous loss of *Scn1a*, to show that this cholinergic modulation and subsequently, VIP function in a behavior-state transition is impaired in Dravet syndrome. Using patch-clamp electrophysiology, Goff and colleagues showed that cholinergic modulation, which typically excites VIP interneurons, actually suppresses VIP interneuron activity in $Scn1a^{+/-}$ mice via a decrease in sodium channel current. To examine the circuit *in vivo*, cranial windows were implanted to allow for calcium imaging in awake mice, and behavior-state transitions were assessed using measurements of pupil diameter along with GCaMP7 fluorescence as a reporter for the recruitment of VIP and non-VIP neurons. While baseline brain activity levels remained similar between wild-type and $Scn1a^{+/-}$ mice, the study showed that $Scn1a^{+/-}$ VIP interneurons and pyramidal cells were less responsive to the onset of locomotion and that their activity was less correlated with pupil diameter. Furthermore, the authors demonstrated that

optogenetic activation of VIP interneurons during locomotion restored non-VIP cell activity to normal levels, indicating that VIP interneuron function is critical in this behavioral transition deficit.

The importance of VIP interneurons to the non-seizure phenotype of Dravet syndrome was further illustrated when researchers conditionally deleted one copy of the *Scn1a* gene selectively from VIP interneurons, generating VIP-Cre.*Scn1a*$^{+/fl}$ mice. This led to impairment of VIP interneurons physiologically, yet *in vivo* circuit activity during locomotion did not indicate any deficit in behavior-state transitions. However, the authors saw similar behavior-state transition deficits to the global *Scn1a*$^{+/-}$ mouse model in a state of increased cholinergic modulation. Pilocarpine, which is often used as a convulsant in models of temporal lobe epilepsy,[6] was used at a low dose to act as a muscarinic agonist and increase cholinergic activity. In wild-type mice, this significantly increased VIP interneuron activity both at baseline and during locomotion, but this effect was significantly reduced in VIP-Cre.*Scn1a*$^{+/fl}$ mice. Generally, cholinergic modulation is known to heavily impact VIP interneuron function and their response to locomotion, as VIP interneurons receive cholinergic input from the basal forebrain.[5] This aligns with the cholinergic modulation-dependent deficit in VIP interneuron activity at behavior-state transitions. Additionally, activity of wild-type non-VIP cells was similarly increased during locomotion, yet non-VIP cells in VIP-Cre.*Scn1a*$^{+/fl}$ mice also exhibited a reduced response. The demonstrated deficits in behavior-state transitions in both *Scn1a*$^{+/-}$ and VIP-Cre.*Scn1a*$^{+/fl}$ mice are incredibly important to the overall cognitive phenotype: these impairments in sensory and motor processing have been associated heavily with the other core symptoms of autism, particularly sociability and social development.[7]

In their final set of experiments for this study, Goff and colleagues assessed the behavioral consequences of conditional *Scn1a* deletion in VIP interneurons. Previous work has shown that global heterozygous deletion of *Scn1a* in *Scn1a*$^{+/-}$ mice leads to seizures along with autism-like behavior such as hyperactivity, spatial memory impairments, and decreased sociability.[8] VIP-Cre.*Scn1a*$^{+/fl}$ mice showed significant impairments in spatial memory tasks along with decreased social interaction when compared to wild-type mice. Notably, VIP-Cre.*Scn1a*$^{+/fl}$ mice were not hyperactive and did not exhibit spontaneous or temperature-induced seizures.

Although VIP-Cre.*Scn1a*$^{+/fl}$ mice provide an interesting and novel model to examine the loss of *Scn1a* in VIP interneurons specifically, the idea that VIP impairment *in vivo* could only be observed in the presence of cholinergic modulation may suggest some limitations to the VIP-Cre.*Scn1a*$^{+/fl}$ model. It is possible that normal excitability in other parts of the cortical network might mask VIP interneuron-related deficits or that specific heterozygous deletion in VIP interneurons may lead to unintended compensatory mechanisms, particularly related to their output onto other inhibitory interneurons.

Nevertheless, these findings begin to disentangle the roles of various interneurons in the phenotype of Dravet syndrome, as many previous studies have elucidated the role of other inhibitory interneurons. $Na_v1.1$ is primarily expressed in inhibitory interneurons, and conditional heterozygous deletion in all inhibitory interneurons leads to a similar phenotype to the global model, exhibiting seizures as well as premature death.[9] Haploinsufficiency in either parvalbumin (PV) or SST inhibitory interneurons leads to seizures without premature death.[10] Interestingly, *Scn1a* haploinsufficiency in SST interneurons leads to hyperactivity without other behavioral hallmarks of autism, somewhat opposite to VIP-Cre.*Scn1a*$^{+/fl}$ mice, whereas social interaction deficits are present with conditional deletion of *Scn1a* in PV interneurons. Although other interneurons may play some role in Dravet syndrome's non-seizure symptoms, these findings distinctly separate VIP interneurons from the other major interneuron subtypes as a more prominent contributor to the behavioral comorbidities of Dravet syndrome rather than the seizure phenotype. As therapies move toward personalized medicine, it is imperative that we better understand the differential consequences of reduced $Na_v1.1$ function in various cell types if we wish to create more individualized treatments for Dravet syndrome.

Inhibitory interneurons in general are grouped heterogeneously—VIP interneurons are no exception. Within the VIP interneuron grouping, there are multiple different morphologies, firing patterns, and synaptic outputs,[1] making it difficult to pinpoint an exact target for the autism-like features of Dravet syndrome. However, the study by Goff and colleagues permits an important distinction between cell types in the pathology of Dravet syndrome, potentially allowing future research and therapeutic applications to address cognitive impairment in Dravet separately from the seizure phenotype.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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