Impaired cerebellar plasticity hypersensitizes sensory reflexes in SCN2A-associated ASD

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ABSTRACT

Children diagnosed with autism spectrum disorder (ASD) commonly present with sensory hypersensitivity, or abnormally strong reactions to sensory stimuli. Such hypersensitivity can be overwhelming, causing high levels of distress that contribute markedly to the negative aspects of the disorder. Here, we identify the mechanisms that underlie hypersensitivity in a sensorimotor reflex found to be altered in humans and in mice with loss-of-function in the ASD risk-factor gene SCN2A. The cerebellum-dependent vestibulo-ocular reflex (VOR), which helps maintain one's gaze during movement, was hypersensitized due to deficits in cerebellar synaptic plasticity. Heterozygous loss of SCN2A-encoded Na+1.2 sodium channels in granule cells impaired high-frequency transmission to Purkinje cells and long-term potentiation, a form of synaptic plasticity important for modulating VOR gain. VOR plasticity could be rescued in adolescent mice via a CRISPR-activator approach that increases Scn2a expression, highlighting how evaluation of simple reflexes can be used as quantitative readout of therapeutic interventions.

INTRODUCTION

Altered sensitivity to sensory input is a hallmark of autism spectrum disorder (ASD); over 90% of children with ASD experience heightened sensitivity to sensory stimuli, spanning sensory modalities (1–4). Although the neuronal mechanisms that contribute to this hypersensitivity have been examined extensively in forebrain circuits (5–8), emerging evidence suggests that changes in sensory sensitivity can occur even at much earlier stages of sensory processing, including those that support sensory reflexes (9–12). Thus, changes in reflexive behavior can provide a window into cellular and circuit dysfunction that underlie altered sensory experience in ASD.

The vestibulo-ocular reflex (VOR) is one such reflex that is affected in ASD. This reflex transforms vestibular sensory information generated by head movements in one direction into eye movements in the opposite direction, thereby helping stabilize images on the retina. In neurotypical subjects, this reflex is engaged well by fast head movements, but not by slower head movements. By contrast, VOR assessed in children with ASD appears to be sensitive to both fast and slow movements, reflecting a hypersensitivity not seen in neurotypical children (10, 12). The mechanisms for this heightened sensitivity are unknown.

Here, we show that VOR gain is hypersensitized in children and mouse models of SCN2A loss-of-function (LoF), a condition that carries the highest risk for ASD of any gene identified via clinical exome sequencing (13, 14). SCN2A encodes the neuronal sodium channel Na+1.2 (15). This channel supports action potential (AP) initiation and propagation in multiple cell classes (16–19), including cerebellar granule cells whose activity is key for modulating VOR gain (20). SCN2A LoF impaired plasticity between granule cells and Purkinje cells, in turn hypersensitizing VOR by preventing synaptic plasticity that typically readjusts VOR amplitude (21–24). Remarkably, VOR plasticity could be restored in adolescent mice by upregulating Scn2a expression levels via a CRISPR-activator (CRISPRa) based approach. Overall, these data demonstrate how innate reflexes provide a window into cerebellar dysfunction in ASD that is both well-conserved across species and sensitive to therapeutic intervention.

RESULTS

SCN2A loss-of-function hypersensitizes VOR gain in humans and mice

Children with SCN2A LoF variants typically have limited to no verbal repertoire and have difficulty following instructions, but often are very comfortable with caregivers (15). With these behavioral aspects in mind, we developed a lightweight, helmet-mounted infrared eye-tracking system paired with an inertial measurement unit (IMU) to assess both eye and head movement, respectively (Fig. 1A, S1). Children aged 3-10 years were seated either alone or on a caregiver’s lap, and VOR gain was assessed with ±5° sinusoidal head oscillations in the dark (0.4 Hz, peak angular velocity: 12.5°/s). Consistent with previous work, VOR gain was well below unity in neurotypical children (10). By contrast, VOR gain was near unity in children with SCN2A LoF variants (Fig. 1B, E).

Protein truncation and resultant nonsense-mediated decay accounts for the vast majority of SCN2A LoF cases (13, 15). As such, mice heterozygous for Scn2a are an ideal model system for studying LoF-related physiology. We therefore assessed VOR in Scn2atm mice head-fixed on a rotational platform using identical test conditions as above (±5° rotation at 0.4 Hz
VOR gain is elevated in Scn2a haploinsufficiency conditions

A: Schematic of purpose-built eye tracking apparatus for VOR assessment in children. Head movements are measured from a device located at the center of the head while the right eye is imaged under infrared illumination (940 nm LED). Participants are seated in a swivel chair and oscillated ±5° at ~0.4 Hz to assess VOR gain.

B: Head angle (purple) and contraversive eye angle for neurotypical (Nt, black) and Scn2A LoF conditions (cyan). Lines represent the average of a single cycle from all participants; shaded area is SEM. Dashed red lines indicate ±5° range.

C: VOR was assessed in mice head-fixed at the center of a rotating table, imaged under IR illumination.

D: VOR at 0.4 Hz rotation frequency, displayed as in panel B, for Scn2a+/− (left, black) and Scn2a−/− mice (right, cyan).

E: Baseline VOR gain in human and mouse. Circles are individuals; box plots are medians, quartiles and 90% tails. n: 11 Nt, 5 LoF humans; 13 +/+, 12 +/- mice. Mann Whitney test p-values shown.

F: VOR gain across rotation frequencies in mouse. Lines connect repeated tests in single mice. Asterisks: p < 0.0001, Friedman test on overall distribution; Mann Whitney test on individual frequencies, Holm Sidak correction.

in the dark). Like children with SCN2A LoF, Scn2a−/− mice showed a VOR gain near unity, significantly higher than wild type (WT) littermates. This elevated gain persisted over a wide range of rotation frequencies (Fig. 1F). This suggests that aberrantly high VOR gain may serve as a readout for Scn2a+/− mice both in mouse and human.

VOR hypersensitivity is associated with an inability to reduce VOR gain

VOR gain is plastic and is adaptively increased or decreased throughout life to maintain image stability on the retina. The high gain in humans and mice suggests that VOR plasticity has been compromised, and that SCN2A LoF impairs one’s ability to reduce VOR gain. To test this, we implemented a standard protocol that decreases VOR gain, termed “VOR gain-down”. Here, instead of rotating the animal in the dark, the animal was seated in a swivel chair and oscillated ±5° at ~0.4 Hz to assess VOR gain.

Within the cerebellum, NaV1.2 expression appears limited to granule cells (Fig. 3A-C) (29–31), with highest density in their parallel fiber axons that make excitatory synapses onto Purkinje cells. Gain-down plasticity is correlated with long-term potentiation (LTP) of parallel fiber-to-Purkinje cell synapses (32–34). Thus, deficits in granule cell function in Scn2a−/− mice may affect synaptic plasticity that mediates VOR plasticity. To test this, we made acute slices of cerebellum and stimulated parallel fibers with a high-frequency burst-based LTP protocol and monitored EPSC amplitude and paired-pulse ratio (PPR) in Purkinje cells. This protocol evoked robust potentiation of EPSCs in WT mice (Fig. 3D), and was correlated with a reduction in PPR, suggesting that this induction protocol acts, at least in part, to increase release probability (35, 36). By contrast, no LTP was observed in Scn2a−/− mice, and PPR was unchanged (Fig. 3D). Thus, a lack of VOR gain-down plasticity may be due to difficulty in potentiating synapses between granule and Purkinje cells in Scn2a−/− mice.

To determine the underlying cellular excitability deficits that impair plasticity, we examined intrinsic and synaptic excitability features of the granule cell-Purkinje cell circuit. Consistent with immunostaining localization of NaV1.2 (37), intrinsic excitability was impaired in granule cells in Scn2a−/− mice. Granule cell APs were slower to rise, and fewer APs were evoked per given somatic current stimulus (Fig. 3E). By contrast, APs in Purkinje cells, which lack NaV1.2 channels (29, 37), were unaffected preferring clockwise head rotations over counterclockwise rotations (Fig. S4). In addition, Purkinje cell firing rates were increased following gain-down induction in WT mice (Fig. 2E–F). Firing rate changes occurred within 10 minutes of induction onset (Fig. 2G) and were consistent across all animals studied (Fig. 2H). But in Scn2a−/− mice, Purkinje cell activity did not increase following gain-down induction. Instead, firing rate decreased modestly, indicating that normal plasticity mechanisms were impaired in Scn2a−/− mice (Fig. 2E–H).

Scn2a heterozygosity alters granule-to-Purkinje cell synaptic transmission and plasticity

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by Scn2a heterozygosity (Fig. 3B, C, E). This suggests that changes to Purkinje cell firing rate plasticity in vivo are due to alterations in presynaptic granule cell input.

Granule cells often fire bursts in vivo, with average instantaneous frequencies of 160-170 Hz (38, 39). These high-frequency bursts are critical for gain-down plasticity, as they are a major signal that evokes LTP that is impaired in Scn2a+/− mice (40–43). A high axonal Na⁺ density is required to sustain a burst of APs at high-frequency (44), suggesting that lower Na⁺,1.2 density in parallel fiber axons may impair transmission. To test this, we assayed AP conduction fidelity in parallel fibers. At a lower frequency (50 Hz), parallel fiber volleys were sustained at normal levels in Scn2a+/− mice; however, fiber volley amplitude was attenuated markedly at the higher frequency used for LTP induction (166 Hz) (Fig. 3G). Since the amplitude of axonal APs affects transmitter release probability (45), these smaller fiber volleys would be expected to evoke less transmitter at granule-Purkinje synapses. To test this, we again stimulated parallel fibers at high-frequency and now recorded resultant EPSCs in Purkinje cells. Similar to fiber volley measurements, we found that EPSC charge transfer was impaired in Scn2a+/− mice, with more pronounced deficits occurring later in trains when volley waveform was more markedly reduced (Fig. 3H).

Together, these data suggest that deficits in VOR plasticity in Scn2a+/− mice are strongly associated with deficits in transmission required for LTP at parallel fiber to Purkinje cell synapses.
This approach will only upregulate gene activity throughout life. Furthermore, previous research has shown that this approach only upregulates genes in tissues where the targeted regulatory element is active (46). To control for this, and to further confirm that floccular complex granule cell function is critical for VOR gain-down plasticity, we injected Cre bilaterally into the floccular complex of Scn2a+/− mice. Injections were performed at postnatal day 30 to determine which components of VOR gain and its plasticity require normal Na+,1,2 levels at this later state of development. Since granule cells are the only cell class at the injection site that express Scn2a, this approach will result in selective Scn2a heterozygosity in floccular granule cells alone. As observed in alpha6-Cre: Scn2a+/− mice, VOR gain-down plasticity was blocked. Interestingly, it did not alter baseline VOR gain from WT-like levels (Fig. S5), even 3 months after injection (Fig. S6). This suggests that baseline gain is maintained despite loss of gain-down plasticity, perhaps because there is no pressure to change baseline values once established in early development, as there is likely limited error in visual scene stabilization that would drive such changes. Consistent with this, VOR measured while presenting a static visual stimulus was near unity for both WT and Scn2a−/− mice, confirming that Scn2a−/− mice can respond to visual stimuli (Fig. S2).

VOR gain-down plasticity can be rescued by upregulating Scn2a expression

These observations add to a growing body of literature showing that Scn2a is a key gene for synaptic and behavioral plasticity (47–49). In neocortical circuits, we found recently that plasticity could be reinvigorated if Scn2a expression was restored in Scn2a−/− mice (50). This was achieved using a CRISPRa-based therapeutic approach that targets a transcriptional activator to the residual, functional allele present in Scn2a heterozygotes. Since neurons are non-dividing cells, a single CRISPRa administration can effectively upregulate gene activity throughout life. Furthermore, previous research has shown that this approach only upregulates genes in tissues where the targeted regulatory element is active (51). Therefore, Scn2a upregulation would only occur in cells that express Scn2a endogenously, such as cerebellar granule cells. To test whether cerebellar plasticity could be similarly rescued with this approach, we injected a pair of blood-brain-barrier penetrant adeno-associated viruses containing all necessary CRISPRa components and the fluorescent protein mCherry via the retro-orbital sinus at P30 to upregulate Scn2a across the brain. To assess viral infection levels across cerebellum, we performed quantitative PCR.
Feedforward inhibition to PCs, interneuron activity is not necessary for granule cells also synapse onto molecular layer interneurons that provide apparent due to alterations at parallel fiber-to-Purkinje cell synapses. Though result, for both human and mouse models of cell-mediated inhibition of VOR circuit through the vestibular nuclei. The net transmission. This, in turn, altered mechanisms for regulation of Purkinje patient and family outcomes. Furthermore, we show that plasticity in VOR (52), detectable within the first months of life in humans (53, non-syndromic ASD, where heightened VOR gain or dysfunction in other vertebrates. We observed that its dysfunction is also conserved in mouse neurodevelopmental disorders as well. VOR function is conserved across shed light on dysfunction in cellular, circuit, and behavioral plasticity in neurological disorders. Here, we highlight how examination of VOR can

**Figure 4: Scn2a heterozygosity in granule cells alone impairs VOR gain-down plasticity**

A: Experimental design for VOR gain-down induction as in Fig. 2, but for Scn2a+/− mice crossed to the alpha6-Cre driver line (α6-Cre), which restricts Cre expression largely to cerebellar granule cells. B: Head angle (purple) and contraversive eye angle before (darker shade) and after (lighter shade) gain-down induction for α6-Cre not crossed to Scn2a+/− animals (black, WT-equivalent), or α6-Cre::Scn2a+/− mice (green). C: VOR gain before and after gain-down induction. Data color coded as in B. Bars connect data from individual mice. *: p < 0.001, Wilcoxon signed rank test, #: p < 0.0001, Mann Whitney test. D: Putative Purkinje cell unit recordings before and after gain-down induction. Traces aligned to table rotation onset. E: Average Purkinje cell simple spike firing frequency during sinusoidal head rotation, before and after gain-down induction, displayed as in C. α6-Cre WT (gray, open circles, n = 24 units, 5 mice) and α6-Cre::Scn2a+/− mice (green, n = 28 units, 5 mice). *: p < 0.005, all conditions, Mixed-effects modeling, #: p = 0.01, Mann Whitney test. F: Average Purkinje cell simple spike firing frequency during induction protocol, normalized to firing rate in first minute per cell. Circles and bars are mean ± SEM, binned per minute. G: Normalized change in VOR gain vs. normalized change in simple spike rate (normalized per unit and averaged across units per animal). Circles are color-coded as in C and represent individual animals. Data from Scn2a+/− (gray) and Scn2a+/− (light cyan) are in background for comparison.

Discrimination

Oculo-motor reflexes are commonly assessed for diagnosis of neurological disorders. Here, we highlight how examination of VOR can shed light on dysfunction in cellular, circuit, and behavioral plasticity in neurodevelopmental disorders as well. VOR function is conserved across (qPCR) for Scn2a mRNA in cerebellar lysates from all CRISPRa injected animals. The qPCR results showed elevation in normalized Scn2a mRNA expression levels in 5 of the 6 injected animals (Fig. 5A-C). Remarkably, VOR gain-down plasticity was restored in those 5 animals, but not in the last animal (Fig. 5D). Plasticity restoration coincided with increases in Purkinje cell firing rate during and after VOR gain-down induction (Fig. 5E-G), suggesting that both synaptic and behavioral plasticity could be rescued even when restored in later stages of development.

**DISCUSSION**

neurodevelopmental disorders as well. VOR function is conserved across vertebrates. We observed that its dysfunction is also conserved in mouse and human SCN2A haploinsufficiency. This parallels observations in non-syndromic ASD, where heightened VOR gain or dysfunction in other aspects of vestibular sensation were also observed (10, 12). VOR is detectable within the first months of life in humans (52, 53), well before ASD is typically diagnosed (54). Given the importance of early diagnosis (55), examination of VOR and related innate behaviors may help improve patient and family outcomes. Furthermore, we show that plasticity in VOR gain is sensitive to therapeutic intervention in mouse models. Here, deficits in VOR were due specifically to deficits in granule cell transmission. This, in turn, altered mechanisms for regulation of Purkinje cell-mediated inhibition of VOR circuit through the vestibular nuclei. The net result, for both human and mouse models of SCN2A LoF, was a VOR gain saturated near unity, even at slow head angular velocities. These effects appear due to alterations at parallel fiber-to-Purkinje cell synapses. Though granule cells also synapse onto molecular layer interneurons that provide feedforward inhibition to PCs, interneuron activity is not necessary for gain-down plasticity (33). Furthermore, our understanding of interactions between parallel fiber firing rates and plasticity at synapses onto Purkinje cells is consistent with impaired LTP in Scn2a+/− conditions. At parallel fiber-to-Purkinje cell synapses, high-frequency bursts of APs are required for LTP related to VOR gain-down plasticity (34). By contrast, long-term depression can be supported by shorter bursts of granule cell activity paired with Purkinje cell depolarization, and likely remains intact even in Scn2a+/− conditions (Fig. 3F) (42, 56–58). Thus, loss of LTP may bias VOR gain towards unity and render VOR inflexible to gain-down protocols.

The simplicity of cerebellar anatomy — composed of relatively few cell classes with stereotyped synapses — belies its functional complexity. Indeed, the cerebellum contributes to a range of processes, including social and cognitive processing that are affected in ASD (59–64). Here, we build upon decades of work that established a role for parallel fiber-Purkinje cells synaptic plasticity and VOR adaptation to understand links between cellular dysfunction and alterations in reflexive behavior in SCN2A haploinsufficiency. While this behavior requires the floccular complex alone, Na, 1.2 is expressed ubiquitously in granule cells across the cerebellum, as is high-frequency, burst-based LTP to Purkinje cells (40, 41, 43, 65). Thus, vestibular plasticity deficits observed here may hint toward broader cerebellar plasticity deficits, affecting a range of phenotypes in children with SCN2A LoF (15). Furthermore, this work parallels studies of cerebellar plasticity in mouse models of other ASD-associated genes (66–70). Thus, cerebellum-dependent reflexes may prove to be generalizable and translatable biomarkers for ASD and potential therapeutics, allowing for quantitative assessment of behavior throughout development.

Limitations of the study

This work represents the first study, to our knowledge, in which VOR gain was assessed in children with profound developmental delay. Children with SCN2A LoF, including those in this study, are often non-verbal, unlikely to follow complex instruction, and, from consultation with caregivers, very

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variants that either dampen or eliminate channel function, but do not lead to a majority of LoF cases in the human population, it does not model missense mutations that underlie VOR is unknown. Furthermore, mouse models with Scn2a+/- typically avoided (71). How such medications interact with the physiology of the brain in the setting of autism spectrum disorder is currently not understood. In this work, we tested whether CRISPR activation can be used to rescue VOR gain plasticity in Scn2a+/- mice with and without autism.

## Methods and Materials

### A. Retro-orbital injection at P30

**AAV-PrP-eb-U6-sgRNA-CMV-mCherry + AAV-PrP-eb-CMV-sadCas9-VP64**

**Systemic CRISPRa delivery**

Retro-orbital injection at P30

**mCherry mRNAs**

500 µm

**B.** Cerebellar coronal section from injected animal. Note broad, but incomplete, infection across cerebellum, as signaled by mCherry fluorescence. A region of cerebellum containing the left floccular complex was dissected immediately after harvesting brain for quantitative PCR and is missing from section.

**C.** Quantitative PCR result showing Scn2a mRNA detection in mouse cerebellum of “empty vector” controls, which contain all viral elements except the guide RNA. In 5/6 animals, marked upregulation was noted (closed circles). In 1 animal, no upregulation was witnessed (open circle).

**D.** VOR gain before and after gain-down induction in Scn2a+/- mice with Scn2a CRISPRa (red, 6 mice) and empty vector (blue, 4 mice), depicted as in Fig 2D. *: p < 0.05 Wilcoxon-signed rank test; all data included.

**E.** Average Purkinje cell simple spike firing frequency during gain-down induction for all CRISPRa (6 mice) and empty vector (4 mice) injected animals. Circles and bars are mean ± SEM, binned per minute.

**F.** Normalized change in VOR gain vs. normalized change in simple spike rate (normalized per unit and averaged across units per animal). Circles are color-coded as in D and represent individual units. *: p < 0.005, Mixed-effects modeling.

**G.** Normalized change in VOR gain vs. normalized change in simple spike rate (normalized per unit and averaged across units per animal). Circles are color-coded as in D and represent individual units. Data from Scn2a+/- (gray) and Scn2a+/- (light cyan) are in background for comparison. Note overlap of CRISPRa population with Scn2a+/- population and different slopes of the plots.

## Discussion

We are grateful to the children, parents, and caregivers associated with the FamilieSCN2A Foundation for participating in this study. We thank Drs. B. Barbour, M. Casado, J. Christie, F. Dunn, A. Nelson, P. Reeson, M. Scanzi, and M. Schonewille for feedback on this work, and to Dr. J. Christie for discussions that motivated this study. This work was supported by an Action Potential Award from the FamilieSCN2A Foundation (CW), a research grant from BioMarin Pharmaceutical Inc. (KJB), the Well Neurohub Investigator Program (KJB, NA), SFARI 629287 (KJB, NA), Howard Hughes Medical Institute funds awarded to M. Scanzi (GB), and National Institutes of Health grants F32 MH125536 (ADN) and R01 MH125978 (KJB).

## Author contributions

Conceptualization—CW, GB, KJB

Methodology—CW, KDD, EH, XZ, ADN, HK, NA, GB, KJB

Software—CW, KDD, HK, GB

Formal analysis—CW

Investigation—CW, KDD, EH, XZ, ADN, HK, NA, GB, KJB

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Supervision—NA, GB, KJB

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## Disclosures

NA is the cofounder and on the scientific advisory board, Regal Tx. KJB is on the scientific advisory board of Regel Tx. NA and KJB receive funding from BioMarin Pharmaceutical Incorporated.

## Supplementary materials

Supplementary materials contain Methods and Supplemental Figures 1-6.

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