Study shows distinct types of cerebellar neurons control motor and social behaviors

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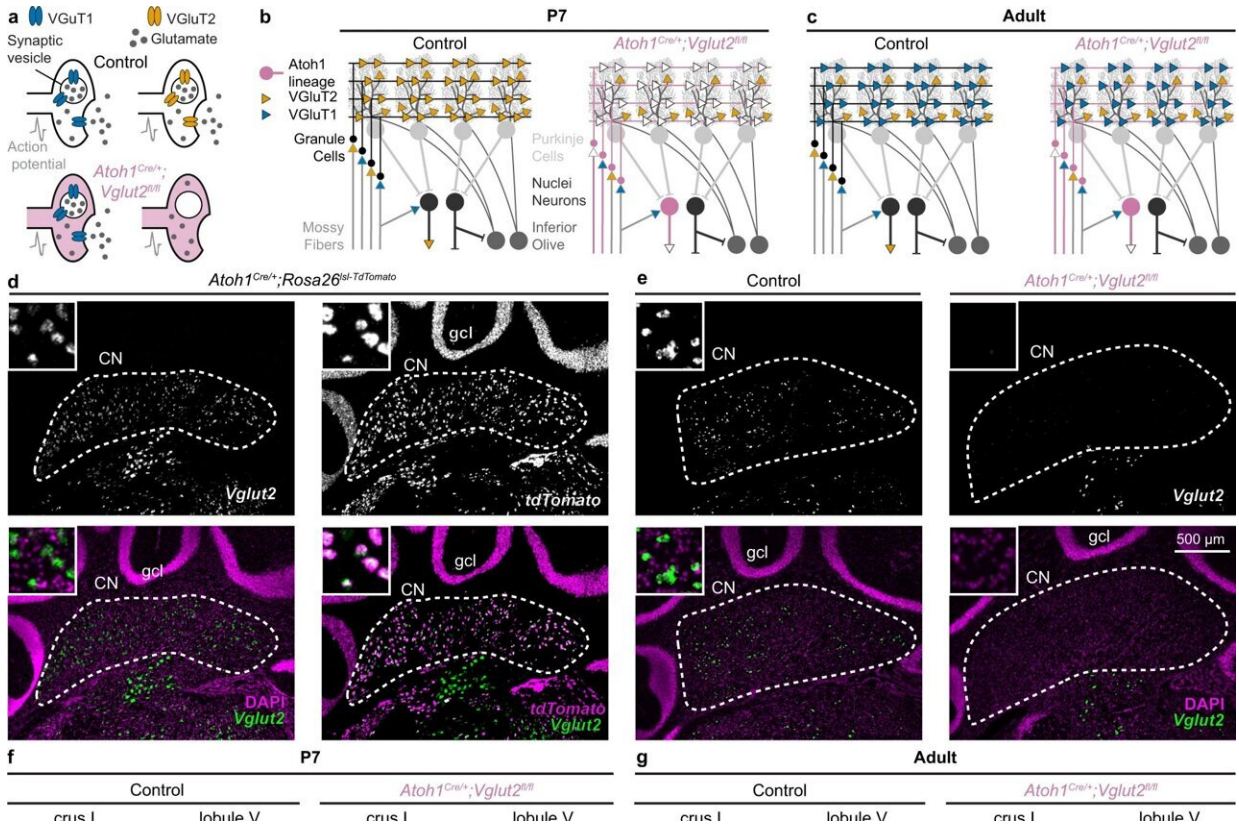

Conditional *Vglut2* deletion from *Atoh1* lineage neurons. **a** Schematic showing how conditional deletion of VGluT2 only affects fast neurotransmission in VGluT2-expressing neurons. **b** Schematic of cerebellar connectivity and vesicular transporter expression for the glutamate subtypes (VGluT1 and VGluT2) in the cerebellar circuit in P7 control mice and $Atoh1^{Cre/+};Vglut2^{fl/fl}$ conditional knockout mice. **c** Same as **b**, but in adult mice. For **a–c** VGluT1 (blue); VGluT2 (orange); Control mice (black); $Atoh1^{Cre/+};Vglut2^{fl/fl}$ mice (reddish purple). **d** Expression of *Vglut2* (green) with DAPI (purple, left) or

tdTomato (purple, right) in the cerebellar nuclei (CN) and granule cell layer (gcl) of adult *Atoh1*$^{Cre/+}$;*Rosa26*$^{lsl\text{-}tdTomato}$ mice. **e** Expression of *Vglut2* (green) and DAPI (purple) in the CN and gcl of adult control mice and *Atoh1*$^{Cre/+}$;*Vglut2*$^{fl/fl}$ mice. **d**, **e** insets are 125 by 125 µm high magnification images. **f** VGluT2^{+} synapses (green) and DAPI (purple) in the molecular layer (ml) and gcl of P7 control and *Atoh1*$^{Cre/+}$;*Vglut2*$^{fl/fl}$ mice, e-gcl = external granule cell layer containing actively proliferating granule cell precursor cells. **g** VGluT2^{+} synapses (green) and DAPI (purple) in the ml and gcl of adult control mice and *Atoh1*$^{Cre/+}$;*Vglut2*$^{fl/fl}$ mice. **d–e** are shown at the same scale. **f**, **g** are shown at the same scale. Images are representative of $N = 3$ mice. Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-38475-9

The cerebellum, a major part of the hindbrain in all vertebrates, is important for motor coordination, language acquisition, and regulating social and emotional behaviors. A study led by Dr. Roy Sillitoe, professor of Pathology and Neuroscience at Baylor College of Medicine and investigator at the Jan and Dan Duncan Neurological Research Institute (Duncan NRI) at Texas Children's Hospital, shows two distinct types of cerebellar neurons differentially regulate motor and non-motor behaviors during development and in adulthood.

The study, published in *Nature Communications*, provides the first in vivo evidence supporting the critical role of a specific subset of excitatory glutamatergic neurons in acquiring motor and sensory/emotional behaviors. Further, it shows that neurons present in different regions of the cerebellum contribute differently to motor versus non-motor behaviors during development and in adulthood.

Cerebellar circuits are established by two major types of neurons

The cerebellar nuclei are present in the deepest layer of the cerebellum. These nuclei are encased by an outer highly convoluted sheet of tissue called the cerebellar cortex, which contains most of the other types of neurons in the cerebellum. The cerebellar cortex receives information from most parts of the body and other brain regions. These inputs are integrated by many types of cerebellar neurons and the deep-set cerebellar nuclei—the sole output structures in the cerebellum—then send those signals to the other parts of the brain.

During development, cerebellar injury in preterm infants is often associated with movement disorders, language impairments, and social deficits. However, growing evidence in patients and animal models suggests that the site of injury and its relative severity determines the type and extent of the resulting symptoms.

Unraveling the function of two types of cerebellar neurons

"Our goal in undertaking this study was to determine if excitatory neurons in the cerebellar cortex and cerebellar nuclei act differentially to establish and maintain motor and social behaviors during developmental stages and in adulthood," lead author, Dr. Meike van der Heijden, a postdoctoral fellow in the Sillitoe lab at the time of the study, said.

"Several recent studies have hinted at discrete roles for various cerebellar neuronal types and these findings inspired us to conduct a deeper examination of how relatively few neuron types in the cerebellum contribute to a wide range of motor and non-motor functions. When we embarked on this study, very little was known about how circuit cerebellar-associated behaviors originate, and whether the same neuronal subtypes contribute equally to the acquisition of these diverse behaviors."

Dr. van der Heijden and graduate student in the Sillitoe lab, Alejandro G. Rey Hipolito, focused on the excitatory glutamatergic neuronal lineages in the cerebellum because it is commonly believed that these neuronal lineages drive the majority of cerebellar behaviors.

Dissecting the functional contributions of these two neuronal lineages in the acquisition of different cerebellar-dependent behaviors requires the use of non-invasive and cell-type- specific manipulations during circuit development. They employed a combination of intersectional genetics and behavioral paradigms which allowed them to address this question with unparalleled precision and specificity in mice models of various developmental ages.

Neurons of the cerebellar cortex control social behaviors whereas cerebellar nuclei neurons regulate motor function

The team found silencing the excitatory lineages in the cerebellar cortex and cerebellar nuclei in early postnatal stages by genetically removing the Vglut2 gene from Atoh1-expressing neurons caused severe impairments in both motor and social vocalization behavior in early prenatal stages.

However, by the time these Atoh1mutant mice reach adulthood, natural molecular transitions result in the normalization of the cerebellar cortex function, which to their surprise coincided with the restoration of social behaviors and only mild motor deficits in these mice. This finding indicated that early social deficits and delayed acquisition of normal social behaviors in these mice were likely due to the progressive normalization of the function of cerebellar cortex neurons.

To test if this hypothesis was true, they eliminated neurotransmission

from a subset of glutamatergic nuclei neurons using *Ntsr1-cre* driver. Upon repeating the same behavioral paradigms, they did not observe any social deficits but observed severe motor deficits in early postnatal mice that were fully resolved with age.

"Together, several major novel findings emerged from our experiments," co-first author, Alejandro Rey Hipolito, said. "First, we were surprised to find that silencing the excitatory neurons did not impair all the cerebellar functions. Second, we observed that glutamatergic neurotransmission from cerebellar cortical versus cerebellar nuclei neurons regulates the acquisition of motor and social behaviors differentially—the cerebellar cortex neurons control the acquisition of social skills whereas the cerebellar nuclei affect the establishment of motor behaviors. Finally, it appears that the brain is able to compensate for some, but not all, perturbations that occur in the developing cerebellum."

"This study has not only led to several important discoveries about the roles of different cerebellar neurons but has opened several interesting questions about the role of inhibitory GABAergic nuclei neurons in compensating for the loss of excitatory glutamatergic neurons and restoring the function, which we intend to explore in the future," Dr. Sillitoe, added. "Moreover, these findings offer several exciting and new possibilities to regulate specific cerebellar lineages to restore motor and non-motor functions after brain injury and disease."

More information: Meike E. van der Heijden et al, Glutamatergic cerebellar neurons differentially contribute to the acquisition of motor and social behaviors, *Nature Communications* (2023). DOI: 10.1038/s41467-023-38475-9

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