PNAS Nexus, 2024, 3, pgae132
https://doi.org/10.1093/pnasnexus/pgae132
Advance access publication 30 March 2024
Brief Report

Dysfunction of striatal parvalbumin interneurons drives motor stereotypies in Cntnap2−/− mouse model of autism spectrum disorders

Mathieu Thabault[a], Cloé Fernandes-Gomes[a], Anne-Lise Huot[b], Maureen Francheteau[a], Anaïs Balbous-Gautier[a,c], Pierre-Olivier Fernagut[a,1] and Laurie Galvan[a,*1]

[a]Laboratoire de Neurosciences Expérimentales et Cliniques, Inserm U1084, Université de Poitiers, 86073, Poitiers, France
[b]Prébios Animal Facility, Université de Poitiers, 86073, Poitiers, France
[c]Centre Hospitalier Universitaire, 86021, Poitiers, France
*To whom correspondence should be addressed: Email: lgalvan@unimes.fr
[1]P.-O.F. and L.G. contributed equally to this work.
Edited By: Eric Klann

Abstract

The involvement of parvalbumin (PV) interneurons in autism spectrum disorders (ASD) pathophysiology has been widely described without clearly elucidating how their dysfunctions could lead to ASD symptoms. The Cntnap2−/− mice, an ASD mouse model deficient for a major ASD susceptibility gene, display core ASD symptoms including motor stereotypies, which are directly linked to striatal dysfunction. This study reveals that striatal PV interneurons display hyperexcitability and hyperactivity in Cntnap2−/− mice, along with a reduced response in medium spiny neurons. We also provide evidence for a crucial role of striatal PV interneurons in motor stereotypies by demonstrating that their selective inhibition rescued a wild type-like phenotype. Our study identifies how PV interneuron dysfunctions disrupt striatal circuitry and drive the motor stereotypies in ASD.

Keywords: parvalbumin interneurons, motor stereotypies, autism spectrum disorders, Cntnap2

Introduction

Despite clear evidence for an involvement of parvalbumin (PV) interneurons in the pathophysiology of autism spectrum disorders (ASD) (1) and proofs of striatal dysfunctions in both patients and animal models (2), the role of striatal PV interneurons in repetitive behaviors, a main symptom of ASD, is poorly known. Partial depletion of these cells in the dorsal striatum elicited motor stereotypies in mice (3) but how PV interneurons contribute to this symptom remains to be elucidated. Using the Cntnap2−/− mouse model of ASD (4), we investigated the role of striatal PV interneurons on motor stereotypies, given the well-established link between the striatum and this phenotype (5). We first examined the functional properties of these interneurons, then tested their communication with striatal projection neurons (SPNs) that are their main targets. We then used chemogenetic modulation to probe their contribution to the expression of motor stereotypies.

Methods

The PV-Cre × Cntnap2 mice were housed at 20°C with 12/12 h light–dark cycle with ad libitum access to food and water. Mice were injected with either AAV-DiO-EYFP (Fig. 1A) or AAV-DiO-ChR2-EYFP (Fig. 1E) in the dorsolateral striatum for electrophysiological and optogenetic experiments. For chemogenetic modulation, mice were injected with AAV-DiO-hM4di-mCherry (Fig. 2A) and treated with clozapine-N-oxide (CNO 0.5 mg/kg/day, i.p.) for 28 days (Fig. 2B and C). This CNO dose does not induce changes in grooming phenotype (Fig. 2B). All procedures were approved by the local ethical committee and followed the guidelines of the European Union directive 2010/63/EU. See extended methodology in the supplementary material.

Results

We first recorded the spontaneous activity of PV interneurons. In brain slices, striatal Cntnap2+/+ PV interneurons were silent as previously reported (6), while Cntnap2−/− PV interneurons exhibited a complex pattern of alterations in their firing properties, with a majority of PV interneurons (87%) displaying hyperactivity, characterized by spontaneous firing, as determined via cell-attached recordings (Fig. 1B), with 28% of them displaying bursting activity as determined by Poisson surprise method (7). In addition, PV neurons that were initially silent (13%) in cell-attached recordings exhibited increased excitability, as indicated by an enhanced input–output curve obtained through whole cell recordings (Fig. 1C). These findings highlight the

Competing Interest: The authors declare no competing interest.
Received: May 31, 2023. **Accepted:** March 18, 2024

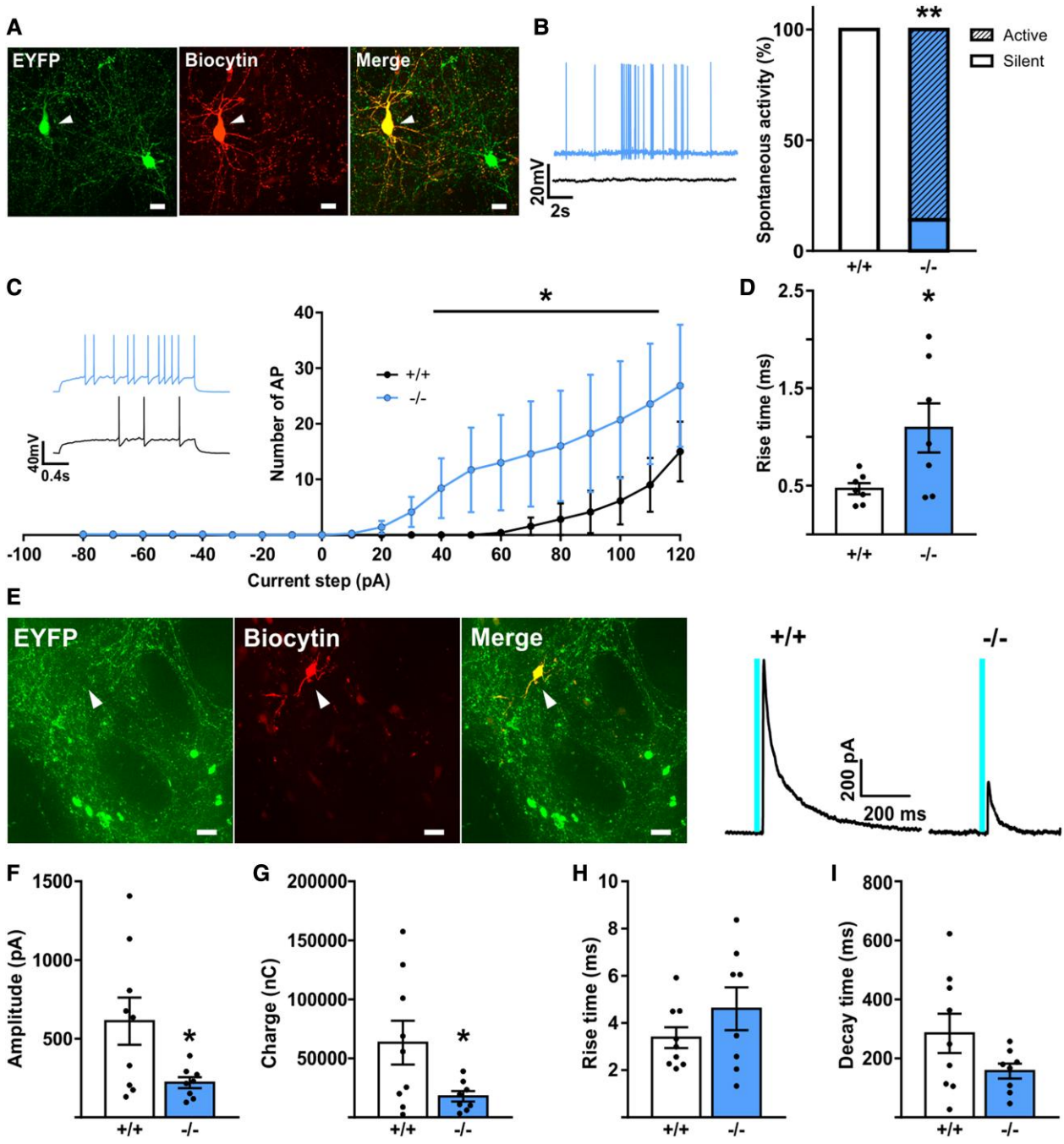

Fig. 1. Striatal Cntnap2−/− PV interneurons display hyperexcitability and hyperactivity, associated with decreased SPN response. A) EYPF- and biocytin-stained striatal PV interneurons. B) Spontaneous activity of PV interneurons: left, representative trace, right quantification (Fisher's exact test, $P = 0.0047$). C) Input–output curves (two-way ANOVA, $F(20, 252) = 3.654$, $P < 0.0001$, Fischer's posthoc). D) Action potential rise time (unpaired t test, $t = 2.411$, $df = 12$, $P = 0.0329$). Number of mice/number of neurons: $N/n = 6/7$ +/+ $N/n = 6/7$ −/−. E) EYFP-positive PV interneurons surrounding a biocytin-stained SPN and representative traces of response current following PV interneurons optical activation (top line). F) Response amplitude (unpaired t test, $t = 2.397$, $df = 15$, $P = 0.0300$). G) Current charge (unpaired t test, $t = 2.253$, $df = 15$, $P = 0.0396$). H and I) Current rise time and decay time. Number of mice/number of neurons: $N/n = 6/9$ +/+ $N/n = 7/8$ −/−.

heterogeneity of dysfunctions of these cells in Cntnap2−/− mice, despite no significant changes in their striatal density (Cntnap2 +/+: 10.70 ± 0.76/mm², Cntnap2−/−: 10.44 ± 0.70/mm²). We next investigated the PV interneuron–SPN communication by recording SPNs evoked responses to optogenetically activation of striatal PV interneurons. Both the response amplitude (Fig. 1F) and charge (Fig. 1G) were significantly decreased in Cntnap2−/− SPNs, with no differences of current kinetics (Fig. 1H–I). Intrinsic electrophysiological properties of SPNs and inhibitory postsynaptic currents were not altered in Cntnap2−/− mice (see supplementary material). This indicates that PV interneuron-mediated inhibition of SPN is reduced and not due to SPN intrinsic functional alteration. Given the well-established link between striatal dysfunctions and motor stereotypies (5), we hypothesized that this specific alteration of striatal microcircuit could be responsible for this phenotype. To test this hypothesis, we measured the effect

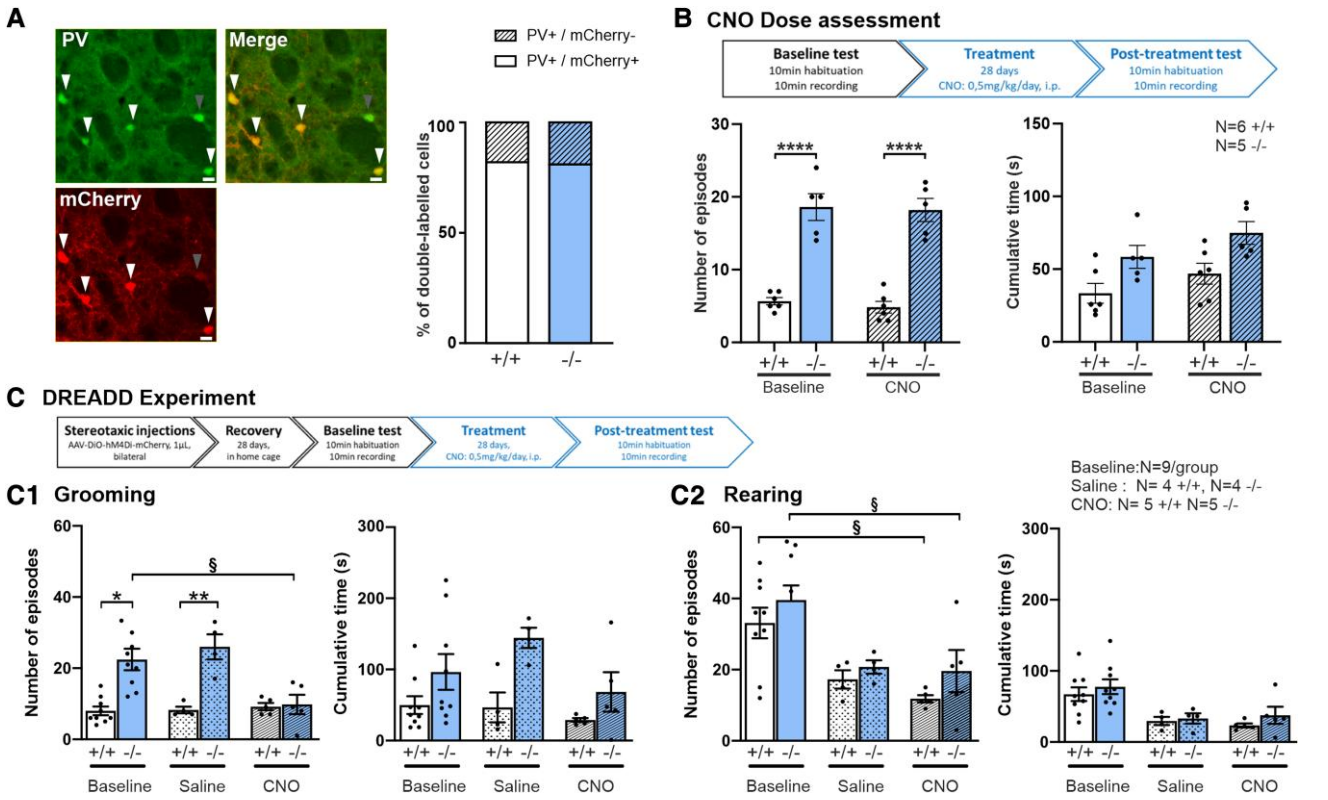

Fig. 2. Chemogenetic chronic inhibition of striatal PV interneurons restores WT-like stereotypy phenotype in Cntnap2−/− mice. A) Anti-PV and anti-mCherry staining showing striatal PV interneurons and an overlay. Left, the proportion of double-labeled cells was similar among groups (+/+: 81.67%, 24 sections, $N = 3$; −/−: 81.33%, 17 sections, $N = 3$;, Fisher's exact test, $P > 0.99$). B) Chronic injections of CNO (0.5 mg/kg/day) do not modify grooming behavior of Cntnap2+/+ and −/− mice. Top, Experimental timeline. Left, number of grooming episodes (rmANOVA, Genotype effect $F(1,9) = 68.93$ $P < 0.0001$. **** Sidak test for genotype differences, $P < 0.0001$). Right, cumulative time spent grooming (rmANOVA, not significant) following chronic injections of CNO in nonvirus-injected mice ($N = 6$+/+, $N = 5$−/−). C) DREADD experiment design. C1) Number of grooming episodes (left, rmANOVA, genotype × treatment effect $F(3,12) = 4.209$, $P = 0.0299$, Sidak posthoc: § −/− Baseline vs. −/− CNO $P = 0.0200$, * +/+ vs. −/− Baseline $P = 0.0391$, ** +/+ vs. −/− Saline $P = 0.0013$) and cumulative time spent grooming (right) following DREADD inhibition of striatal PV interneurons (rmANOVA, not significant). C2) Number of rearing episodes (left, rmANOVA, treatment effect $F(3,12) = 10.82$, $P = 0.0010$. Sidak test for treatment differences, § +/+ Baseline vs. CNO $P = 0.0351$, § −/− Baseline vs. CNO $P = 0.0265$ and cumulative time spent rearing (right, rmANOVA, not significant) following DREADD inhibition of striatal PV interneurons. Baseline: $N = 9$/group, Saline: $N = 4$/group, CNO: $N = 5$/group.

of selective chemogenetic inhibition of striatal PV interneurons on repetitive behaviors. The Cntnap2−/− mice displayed motor stereotypies, i.e. excessive number of grooming episodes (Fig. 2C), as previously described (4). Following chronic inhibition of striatal PV interneurons, we show that the motor stereotypies phenotype of Cntnap2−/− mice was rescued (Fig. 2.C1). Cntnap2+/+ and Cntnap2−/− mice exhibit a similar rearing behavior, measured as an index of global activity in the cylinder (Fig. 2.C2). Following chronic inhibition of striatal PV interneurons, we show that rearing behavior, a typical exploratory behavior, decreased similarly in Cntnap2+/+ and Cntnap2−/− mice (Fig. 2.C2). This could be explained by the combination of the preservation of spontaneous motor activity following CNO injections and habituation to the cylinder since mice were exposed multiple times to the test setup. The observed phenotypic restoration post-PV interneuron silencing primarily targets the abnormal grooming behavior, affirming the specificity of this modulation on the aberrant phenotype rather than nonspecific response (rearing).

Discussion and conclusion

Converging evidence highlighted that PV interneurons are dysfunctional in ASD (1) including in Cntnap2−/− mice (4, 8) on the molecular level but the functional consequences have remained elusive. Here, we demonstrate that striatal PV interneurons display hyperactivity and hyperexcitability which are linked to motor stereotypy outcomes. When these interneurons are optogenetically activated, the evoked response in SPNs is attenuated in Cntnap2−/− mice, while the SPNs do not exhibit the modifications of intrinsic properties. This discrepancy suggests a potential compensatory mechanism within the striatal network in response to hyperactive PV interneurons. Our optogenetic data support the idea that the network adjusts PV interneuron-mediated inhibition onto SPNs to counteract hyperexcitability. While the increase in PV interneuron activity is linked to motor stereotypy outcomes, the network's attempt to balance this excessive activity by reducing the inhibitory drive onto SPNs might, in turn, contribute to the development of these repetitive behaviors. Such a dynamic interplay between hyperactive PV interneurons and compensatory mechanisms within the network warrants further investigation to determine their respective contributions to core ASD symptoms such as motor stereotypies. Prior investigations demonstrated that a reduction in striatal PV interneurons density in adult wild-type (WT) mice led to repetitive movements and anxiety which are hallmarks of several psychiatric disorders (9) including ASD. Our study reveals a distinct mechanism in Cntnap2−/− mice exhibiting neurodevelopmental dysfunctions, wherein excessive activity of striatal PV interneurons leads to

the development of motor stereotypies independently of any changes in their density as previously reported (10). While our research focused on repetitive behavior, emerging evidence suggests the involvement of PV interneurons in other processes disrupted in ASD. Indeed, optically silencing fast-spiking PV interneurons in the medial prefrontal cortex results in anomalies in attentional processing necessary for goal-directed action (11). Furthermore, manipulating the excitation/inhibition balance of PV interneurons in mPFC rescued social behaviors in Cntnap2 −/− mice (12). Accordingly, both cortical and striatal PV interneurons could be involved in ASD symptoms. These results highlight the contribution of the striatal network and the importance of striatal PV interneuron in ASD. These observations suggest that preserving an optimal range of striatal PV interneuron activity could be crucial for maintaining proper striatal function and motor behavior. Therefore, strategies aiming at modulating PV interneuron activity may be beneficial for the management of ASD symptoms.

Acknowledgments

The Université de Poitiers and the Institut National de la Santé et de la Recherche Médicale provided infrastructural support. The authors thank Valentine Turpin, Éric Balado, and Catherine Le Goff for their help.

Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

Funding

This work was supported by Fondation Fyssen (grant #RAK20005GGA), Région Nouvelle-Aquitaine CPER 2015–2020/FEDER 2014–2020 programs. This study has benefited from PREBIOS and ImageUP core facilities. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

M.T.—performed experiments, collected and analyzed data, and contributed to the writing of the manuscript. C.F.-G.—performed experiments, collected and analyzed data. M.F.—performed some histological experiments and image acquisitions. A.L.H. and A.B.-G.—generation and management of transgenic lines. P.-O.F.—contributed to project management and writing of the manuscript. L.G.—designed, managed the project, analyzed data and contributed to the writing of the manuscript. All authors reviewed and approved the final version of the manuscript.

Data Availability

The data have been deposited in Harvard Dataverse at https://doi.org/10.7910/DVN/8YOMDC.

References

1 Filice F, Janickova L, Henzi T, Bilella A, Schwaller B. 2020. The parvalbumin hypothesis of autism spectrum disorder. *Front Cell Neurosci*. 14:577525.
2 Thabault M, *et al.* 2022. Cerebellar and striatal implications in autism spectrum disorders: from clinical observations to animal models. *Int J Mol Sci*. 23:2294.
3 Rapanelli M, *et al.* 2017. Targeted interneuron depletion in the dorsal striatum produces autism-like behavioral abnormalities in male but not female mice. *Biol Psychiatry*. 82:194–203.
4 Peñagarikano O, *et al.* 2011. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell*. 147:235–246.
5 Hollander E, *et al.* 2005. Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. *Biol Psychiatry*. 58:226–232.
6 Tepper JM, Tecuapetla F, Koós T, Ibáñez-Sandoval O. 2010. Heterogeneity and diversity of striatal GABAergic interneurons. *Front Neuroanat*. 12:91.
7 Legendy CR, Salcman M. 1985. Bursts and recurrences of bursts in the spike trains of spontaneously active striate cortex neurons. *J Neurophy*. 53:926–939.
8 Lauber E, Filice F, Schwaller B. 2018. Dysregulation of parvalbumin expression in the Cntnap2−/− mouse model of autism spectrum disorder. *Front Mol Neurosci*. 11:262.
9 Xu M, Li L, Pittenger C. 2016. Ablation of fast-spiking interneurons in the dorsal striatum, recapitulating abnormalities seen post-mortem in Tourette syndrome, produces anxiety and elevated grooming. *Neuroscience*. 324:321–329.
10 Briones BA, *et al.* 2022. Perineuronal nets in the dorsomedial striatum contribute to behavioral dysfunction in mouse models of excessive repetitive behavior. *Biol Psychiatry Glob Open Sci*. 2:460–469.
11 Kim H, Ährlund-Richter S, Wang X, Deisseroth K, Carlén M. 2016. Prefrontal parvalbumin neurons in control of attention. *Cell*. 164:208–218.
12 Selimbeyoglu A, *et al.* 2017. Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. *Sci Transl Med*. 9:eaah6733.