## Title
aGABRacadabra: A surprising new role for GABAA receptors in cortical development



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been identified and associated with treatment response,[9,10] the results of this study further highlight the importance of small non-coding RNAs in brain disorders. Namkung et al. identified a neurobiological pathway associated with the AMPAR-dependent regulation of behavioral dimensions relevant to the psychopathology of both SZ and BD.[4] If confirmed, this finding may lead to the discovery of new pharmacological interventions beyond the mechanisms of action of current antipsychotic drugs.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## REFERENCES

1. Whiteford, H.A., Degenhardt, L., Rehm, J., Baxter, A.J., Ferrari, A.J., Erskine, H.E., Charlson, F.J., Norman, R.E., Flaxman, A.D., Johns, N., et al. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet *382*, 1575–1586. https://doi.org/10.1016/s0140-6736(13)61611-6.

2. The International Schizophrenia Consortium, et al. (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature *460*, 748–752. https://doi.org/10.1038/nature08185.

3. Ruderfer, D.M., Fanous, A.H., Ripke, S., McQuillin, A., Amdur, R.L., Gejman, P.V., O'Donovan, M.C., Andreassen, O.A., Djurovic, S., Hultman, C.M., et al.; Schizophrenia Working Group of the Psychiatric Genomics Consortium; Bipolar Disorder Working Group of the Psychiatric Genomics Consortium; Cross-Disorder Working Group of the Psychiatric Genomics Consortium (2014). Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. Mol Psychiatry *19*, 1017–1024. https://doi.org/10.1038/mp.2013.138.

4. Namkung, H., Yukitake, H., Fukudome, D., Lee, B.J., Tian, M., Ursini, G., Saito, A., Lam, S., Kannan, S., Srivastava, R., et al. (2022). The miR-124-AMPAR pathway connects polygenic risks with behavioral changes shared between schizophrenia and bipolar disorder. Neuron *111*, 220–235. https://doi.org/10.1016/j.neuron.2022.10.031.

5. Feron, F., Perry, C., McGrath, J.J., and Mackay-Sim, A. (1998). New techniques for biopsy and culture of human olfactory epithelial neurons. Arch. Otolaryngol. Head Neck Surg. *124*, 861–866. https://doi.org/10.1001/archotol.124.8.861.

6. Peng, J., Liu, J., Liu, H., Huang, Y., and Ren, Y. (2022). Prognostic value of microRNAs in patients with small cell lung cancer: a meta-analysis. World J. Surg. Oncol. *20*, 381. https://doi.org/10.1186/s12957-022-02851-4.

7. Akhlaghipour, I., Taghehchian, N., Zangouei, A.S., Maharati, A., Mahmoudian, R.A., Saburi, E., and Moghbeli, M. (2022). MicroRNA-377: A therapeutic and diagnostic tumor marker. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2022.11.236.

8. O'Brien, K., Breyne, K., Ughetto, S., Laurent, L.C., and Breakefield, X.O. (2020). RNA delivery by extracellular vesicles in mammalian cells and its applications. Nat. Rev. Mol. Cell Biol. *21*, 585–606. https://doi.org/10.1038/s41580-020-0251-y.

9. Lopez, J.P., Lim, R., Cruceanu, C., Crapper, L., Fasano, C., Labonte, B., Maussion, G., Yang, J.P., Yerko, V., Vigneault, E., et al. (2014). miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. Nat Med *20*, 764–768. https://doi.org/10.1038/nm.3582.

10. Lopez, J.P., Fiori, L.M., Cruceanu, C., Lin, R., Labonte, B., Cates, H.M., Heller, E.A., Vialou, V., Ku, S.M., Gerald, C., et al. (2017). MicroRNAs 146a/b-5 and 425-3p and 24-3p are markers of antidepressant response and regulate MAPK/Wnt-system genes. Nat. Commun. *8*, 15497. https://doi.org/10.1038/ncomms15497.

# aGABRacadabra: A surprising new role for $GABA_A$ receptors in cortical development

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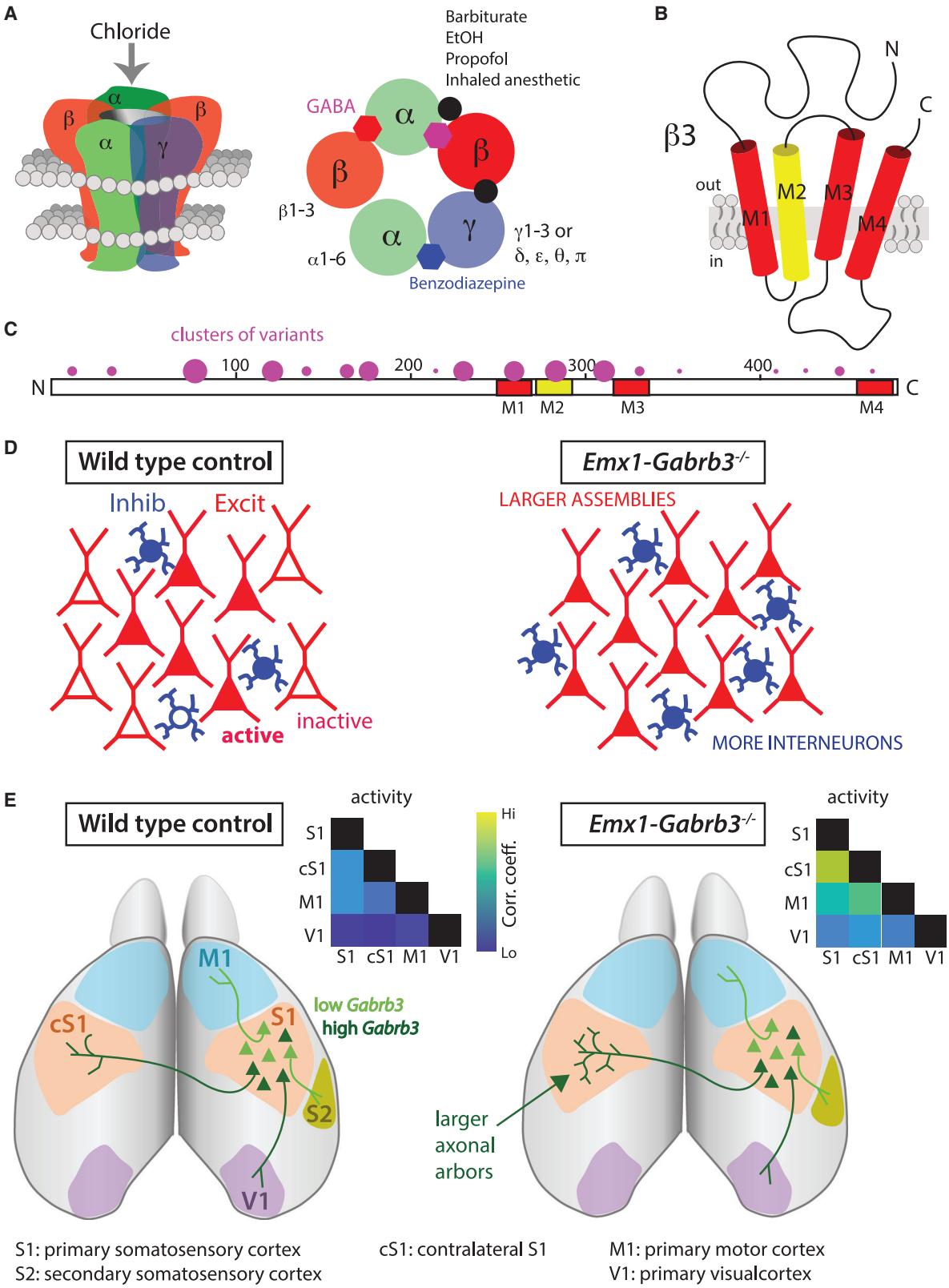
**In this issue of *Neuron*, Babij, Ferrer, and colleagues provide new evidence that β3 subunit of $GABA_A$ receptors is critical for the maturation of functional networks in the neonatal somatosensory cortex.**

Imagine for a moment that you are an excitatory pyramidal neuron in a 5-day-old mouse. You were born a week ago in the subventricular zone, and you finally migrated to your permanent home in layer (L) 2/3, where you will live for the next two years. You are busy growing dendrites, frantically extending and retracting dendritic protrusions in search of suitable axon boutons to synapse with. You will grow an axon too, but that's a lot trickier because you must decide whether to project its branches within the same hemisphere or go contralaterally across the corpus callosum. As this is all happening, you are also learning how to fire action potentials, both in response to sensory stimuli that reach the cortex and as a function of spontaneous network depolarizations that seem to pop up periodically around you. You quickly realize that for now, you are part of a collective that discourages individuality: when a wave of activity courses across the neonatal cortex, you must fire with your neighbors, even if they are GABAergic inhibitory neurons. Whether or not you participate in these early synchronous patterns of network activity is a matter of life and death. If you fail to make enough synapses and remain active, you will succumb to programmed cell death.[1]

These structural and functional aspects of cortical development are orchestrated by precise genetic programs. These processes are stochastic, but the brain can




S1: primary somatosensory cortex
S2: secondary somatosensory cortex
cS1: contralateral S1
M1: primary motor cortex
V1: primary visualcortex

*(legend on next page)*

accommodate some genetic variability and still achieve developmental goals to ensure survival of the animal. Still, certain mutations in key genes can steer the trajectory of cortical development far enough away from the intended plan that they will cause seizures, intellectual disability, or autism symptoms. Further research into these developmental processes is likely to yield a much clearer understanding of the causes of various neurodevelopmental conditions and how to treat any troublesome symptoms.

Babij, Ferrer, et al.[2] investigate the role of $GABA_A$ receptors, which are ligand-gated chloride channels that mediate fast inhibition in the central nervous system in early cortical development. $GABA_A$ receptors are heteropentameric structures composed of two $\alpha$ subunits, two $\beta$ subunits, and a $\gamma$ subunit, with each of these subunits having 4 transmembrane domains (Figures 1A and 1B).[3] The authors characterize the impact of loss of function of the β3 subunit using genetically modified mice in which the gene *Gabrb3* has been deleted from all excitatory neurons. To do so, they cross a conditional mouse line with the floxed allele for this gene (*Gabrb3*$^{fl/fl}$) to *Emx1-Cre* mice, which restricts knockdown of *Gabrb3* to neurons of the dorsal telencephalon starting around embryonic day 9. These experiments have high clinical significance because mutations in *GABRB3* are strongly associated with several neurodevelopmental conditions in humans (Figure 1C).

The authors first document that *Emx1-Gabrb3* null mice lack any gross anatomical differences in brain size, cortical lamination, or dendritic complexity, even though they are smaller than wild-type (WT) controls during the first month. Next, they record network activity using *in vivo* 2-photon calcium imaging in primary somatosensory cortex (S1) of unanesthetized *Emx1-Gabrb3* mice at postnatal day (P) 7 and P14. They observe that early network activity in L2/3 at P7 is dominated by infrequent events where local neuronal assemblies fire synchronously, but subsequently decorrelate by P14, as previously described.[4,5] Notably, *Emx1-Gabrb3* null mice show a higher degree of synchrony than WT controls at both ages as assessed by pairwise correlation coefficients (Figure 1D). A greater recruitment of L2/3 neurons in S1 is also observed after contralateral whisker stimulation in the mutants. Using slice electrophysiology, the authors report that loss of *Gabrb3* leads to a lower density of inhibitory synapses onto L2/3 pyramidal neurons at P7 compared to controls and a significantly lower frequency of inhibitory post-synaptic currents (IPSCs) and elevated excitation-to-inhibition ratio at P14. While not unanticipated, these results suggest that β3 subunits and GABAergic inhibition are important for restricting network synchrony in the developing neocortex. Much more surprising is their observation that *Emx1-Gabrb3* mutant mice have a much higher density of parvalbumin and somatostatin interneurons in S1. The authors suggest this could be due to improved survival from programmed cell death because of the greater activity of excitatory neurons.[1] That may be the case, but it is noteworthy because a lower (not higher) density of parvalbumin neurons has been reported in many mouse models of neurodevelopmental conditions, including some with similar network hypersynchrony.[6]

Things get more interesting when they analyze axonal projections of L2/3 neurons at P14 and find an increase in arborizations within the homotopic S1 region of the contralateral hemisphere (cS1). In contrast, axonal projections from S1 L2/3 neurons to other brain regions are unaffected in mutants, including those to ipsilateral S1, motor cortex, or prefrontal cortex (Figure 1E). The increase in S1-cS1 projections was confirmed using retrograde virus tracing. To further investigate this functionally, the authors use widefield calcium imaging at P14 and discover that some *Emx1-Gabrb3* mutant mice show global network events that spread in both hemispheres. In the remaining mutants, which exhibit only slightly higher amplitude of calcium signals than WT controls, they note higher correlation coefficients for activity between S1 and cS1 than for activity between ipsilateral regions. Future studies will be needed to determine whether the greater axonal coverage of cS1 in *Emx1-Gabrb3* mice is due to impaired pruning or initial overgrowth and whether whisker stimulation also elicits greater responses in the ipsilateral S1 compared to controls. Finally, in a translational effort, the authors analyze publicly available MRI and transcriptomic data from humans diagnosed with autism spectrum disorder (ASD) to show that atypical circuit connectivity in ASD correlates with expression of high-risk autism genes, including *GABRB3*, but not with expression of genes that have not been linked to ASD.

We are accustomed to elegant studies from the De Marco-García lab, and this is no exception. They utilize the latest techniques in modern neuroscience, meticulously confirm their results using multiple mouse lines, and perform important controls. And for the non-experts, let's just say that doing calcium imaging in 1-week-old mice is not trivial. The authors ultimately conclude that *Gabrb3* is critical for proper functional network connectivity between S1 and its homotopic cS1, but not for ipsilateral connections, and much of the data supports this.

Still, the widefield calcium imaging shows higher correlation coefficients in mutant mice not just for S1-cS1 but also for ipsilateral S1-V1, and there are similar trends for S1-cM1, S1-TeA, and V1-cV1 (the sample size may have been too small to reach significance). Thus, loss of *Gabrb3* might just lead to widespread network hypersynchrony with subtle regional differences. The axon phenotype may also be widespread and reflect delayed pruning due to excessive network synchrony. And it's not just *Gabrb3*. The authors find similar phenotypes of elevated network synchrony[7] and exuberant axonal

**Figure 1. β3 subunit of $GABA_A$ receptors and main circuit phenotypes of *Emx1-Gabrb3* null mice**
(A) Structure of $GABA_A$ receptor.
(B) Structure of β3 subunit.
(C) Approximate locations of >40 pathogenic missense variants in *Gabrb3*.
(D) *Emx1-Gabrb3* mice exhibit larger synchronous assemblies than WT controls.
(E) *Emx1-Gabrb3* mice show larger arborizations of axons projecting from S1 and cS1 than to S2, M1, or V1. Activity is also more correlated across ipsi- and contralateral cortical areas in mutants.

innervation of cS1 in *Emx1-Gabrg2* mice,[2] which lack the γ2 subunit of $GABA_A$ receptors. This goes to show that functional $GABA_A$ receptors are critical to limit the spread of synchronous network events in early postnatal mice. However, though *Gabrg2* and *Gabrb3* mutants have much higher network synchrony, they both undergo significant decorrelation between P7 and P14, suggesting these subunits may not be necessary for desynchronization. Whether subtle differences exist between *Gabrb3* and *Gabrg2* mutants remains unclear.

To put these results in context, it is important to mention the global *Gabrb3* knockout mice (*Gabrb3*$^{-/-}$), most of which die soon after birth with severe palate deformities.[8] The few that survive to adulthood display obvious behavioral phenotypes, including seizures of various semiologies, and notably, EEG recordings show subclinical seizures and interictal epileptiform discharges. In contrast, *Camk2-Gabrb3* mice engineered to delete the β3 subunit from forebrain excitatory neurons after the third postnatal week do not have seizures or obvious behavioral phenotypes.[9] The *Emx1-Gabrb3* mutants likely represent an intermediate of β3 expression and clearly manifest neonatal network hypersynchrony that would predispose them to seizures. Even though the authors do not observe clinical seizures, one wonders what the EEG might have shown at P14 since overt clinical seizures may be harder to notice in neonatal mice.

The potential clinical relevance of this study cannot be understated given how dozens of mutations in the *GABRB3* gene in humans are associated with developmental delay, autism, intellectual disability, and severe treatment-resistant epileptic encephalopathies.[10] Angelman syndrome, which is characterized by intellectual disability and seizures, is also associated with *GABRB3*, as the gene is located within a 15q11-q13 region that includes the *UBE3A* gene and those encoding other GABA receptor subunits. *Gabrb3* mutant mice are therefore potential models of human neurodevelopmental conditions from epilepsy to autism. Future studies should carefully examine *Gabrb3* and *Gabrg2* mutant mice for behavioral deficits both at 2 weeks and as adults.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## REFERENCES

1. Wong, F.K., Bercsenyi, K., Sreenivasan, V., Portales, A., Fernandez-Otero, M., and Marin, O. (2018). Pyramidal cell regulation of interneuron survival sculpts cortical networks. Nature *557*, 668–673. https://doi.org/10.1038/s41586-018-0139-6.

2. Babij, R., Ferrer, C., Donatelle, A., Wacks, S., Buch, A.M., Niemeyer, J.E., Ma, H., Duan, Z.R.S., Fetcho, R.N., Che, A., et al. (2022). Gabrb3 is required for the functional integration of pyramidal neuron subtypes in the somatosensory cortex. Neuron *111*, 256–274. https://doi.org/10.1016/j.neuron.2022.10.037.

3. Ghit, A., Assal, D., Al-Shami, A.S., and Hussein, D.E.E. (2021). GABA(A) receptors: structure, function, pharmacology, and related disorders. J. Genet. Eng. Biotechnol. *19*, 123. https://doi.org/10.1186/s43141-021-00224-0.

4. Golshani, P., Goncalves, J.T., Khoshkhoo, S., Mostany, R., Smirnakis, S., and Portera-Cailliau, C. (2009). Internally mediated developmental desynchronization of neocortical network activity. J. Neurosci. *29*, 10890–10899. https://doi.org/10.1523/JNEUROSCI.2012-09.2009.

5. Rochefort, N.L., Garaschuk, O., Milos, R.I., Narushima, M., Marandi, N., Pichler, B., Kovalchuk, Y., and Konnerth, A. (2009). Sparsification of neuronal activity in the visual cortex at eye-opening. Proc. Natl. Acad. Sci. USA *106*, 15049–15054. https://doi.org/10.1073/pnas.0907660106.

6. Contractor, A., Ethell, I.M., and Portera-Cailliau, C. (2021). Cortical interneurons in autism. Nat. Neurosci. *24*, 1648–1659. https://doi.org/10.1038/s41593-021-00967-6.

7. Duan, Z.R.S., Che, A., Chu, P., Modol, L., Bollmann, Y., Babij, R., Fetcho, R.N., Otsuka, T., Fuccillo, M.V., Liston, C., et al. (2020). GABAergic Restriction of Network Dynamics Regulates Interneuron Survival in the Developing Cortex. Neuron *105*, 75–92.e5. https://doi.org/10.1016/j.neuron.2019.10.008.

8. Homanics, G.E., DeLorey, T.M., Firestone, L.L., Quinlan, J.J., Handforth, A., Harrison, N.L., Krasowski, M.D., Rick, C.E.M., Korpi, E.R., Makela, R., et al. (1997). Mice devoid of γ-aminobutyrate type A receptor β3 subunit have epilepsy, cleft palate, and hypersensitive behavior. Proc. Natl. Acad. Sci. USA *94*, 4143–4148. https://doi.org/10.1073/pnas.94.8.4143.

9. Ferguson, C., Hardy, S.L., Werner, D.F., Hileman, S.M., DeLorey, T.M., and Homanics, G.E. (2007). New insight into the role of the β3 subunit of the GABAA-R in development, behavior, body weight regulation, and anesthesia revealed by conditional gene knockout. BMC Neurosci. *8*, 85. https://doi.org/10.1186/1471-2202-8-85.

10. Absalom, N.L., Liao, V.W.Y., Johannesen, K.M.H., Gardella, E., Jacobs, J., Lesca, G., Gokce-Samar, Z., Arzimanoglou, A., Zeidler, S., Striano, P., et al. (2022). Gain-of-function and loss-of-function GABRB3 variants lead to distinct clinical phenotypes in patients with developmental and epileptic encephalopathies. Nat. Commun. *13*, 1822. https://doi.org/10.1038/s41467-022-29280-x.