Increased GABA levels in postnatal development alter cortical inter-hemispheric circuits

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The corpus callosum (CC) is the largest fiber tract of the brain, connecting the two cerebral hemispheres and integrating sensory, motor and higher-level cognitive information. The excitatory-inhibitory (E/I) balance is crucial for sculpting cortical networks during the early postnatal period. Here, we developed an experimental setup that alters E/I balance in mice by injecting Diazepam –an agonist of the inhibitory neurotransmitter GABA– at specific postnatal windows. The number and location of callosal neurons were characterized via stereotaxic injections of the retrograde tracer cholera toxin subunit B (CTB) in the CC of the primary somatosensory and visual areas (S1 and V1). Diazepam injections result in a reprogramming of the interhemispheric adult circuit. Interestingly, injections during the first postnatal week preferentially altered S1 over V1, while later treatments produce greater changes in V1 compared to S1. Furthermore, immunostaining of GABAergic markers to evaluate the status of the inhibitory circuit revealed a decrease in the total number of somatostatin interneurons and an increase in the parvalbumin population. Overall, our data show that disrupting the activity during development leads to alterations in both the interhemispheric and interneuron networks. We show that Diazepam-dependent plasticity is restricted temporally depending on the sensory area, possibly related to each area's critical period of plasticity.

CTB injection in the corpus callosum (CC)

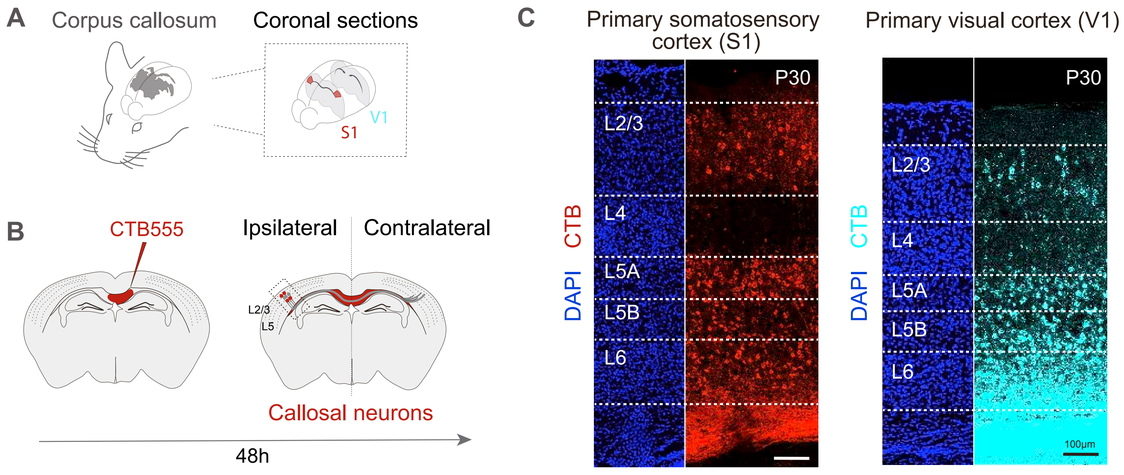


Figure 1. CTB injection in the mouse corpus callosum revealing S1 and V1 callosal neurons. (A) 3D view of the CC and representation of the coronal sections at the level of S1 and V1. (B) CTB injection in the CC and processing of the brain 48h later. (C) Cortical column of S1 and V1 in the adult mouse (P30).

GABA neurotransmission and Diazepam treatment

- $GABA_AR$s are the major inhibitory neurotransmitter receptors in the mammalian central nervous system.
- It is a chloride selective ion channel.
- Diazepam is a $GABA_AR$ allosteric activator.
- The most common isoform of the receptor is $\alpha_1\gamma_2\beta_2\alpha_1\beta_2$
- $GABA_AR$s that contain the α_1 subunit have high affinity for benzodiazepines.
- Diazepam injection disrupts the E/I balance.

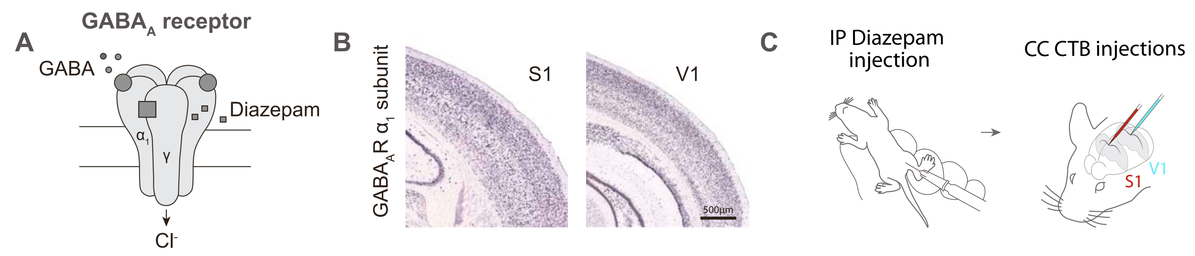


Figure 2. (A) Receptor GABA type A showing the two GABA binding sites and the allosteric binding site for benzodiazepines (like Diazepam). (B) *In situ* hibridization of the $GABA_AR$ subunit α_1 from the *Allen Brain Atlas*. (C) Intraperitoneral (IP) Diazepam injections were followed by CC CTB injections to evaluate the effect of a disbalance in neuronal activity in callosal development.

Early GABA-agonist treatment

Primary somatosensory cortex

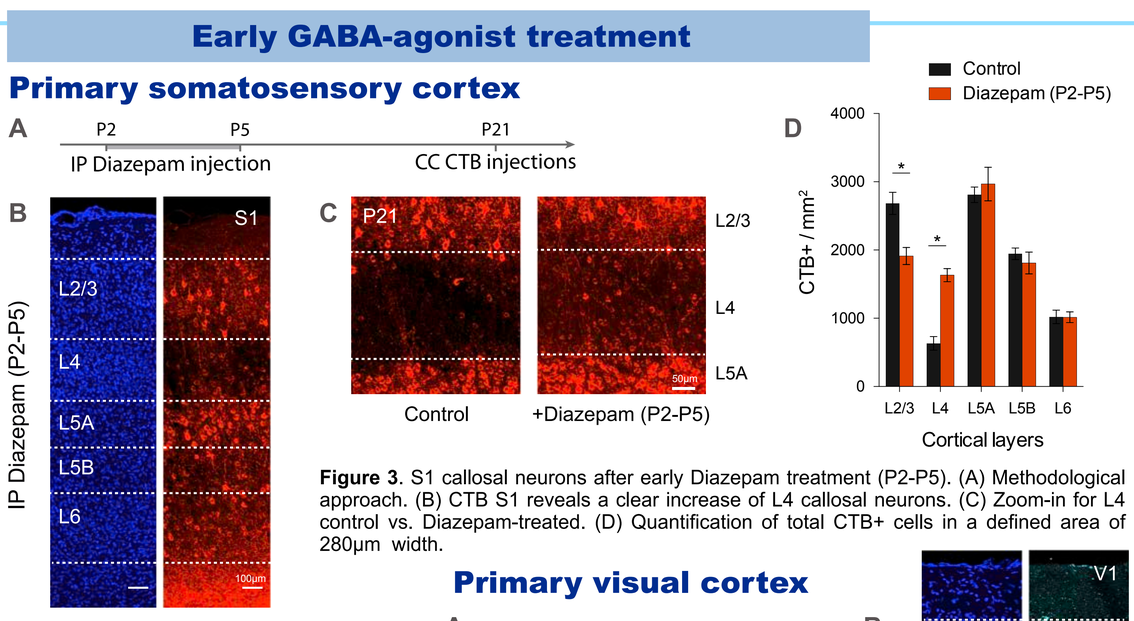


Figure 3. S1 callosal neurons after early Diazepam treatment (P2-P5). (A) Methodological approach. (B) CTB S1 reveals a clear increase of L4 callosal neurons. (C) Zoom-in for L4 control vs. Diazepam-treated. (D) Quantification of total CTB+ cells in a defined area of 280µm width.

Primary visual cortex

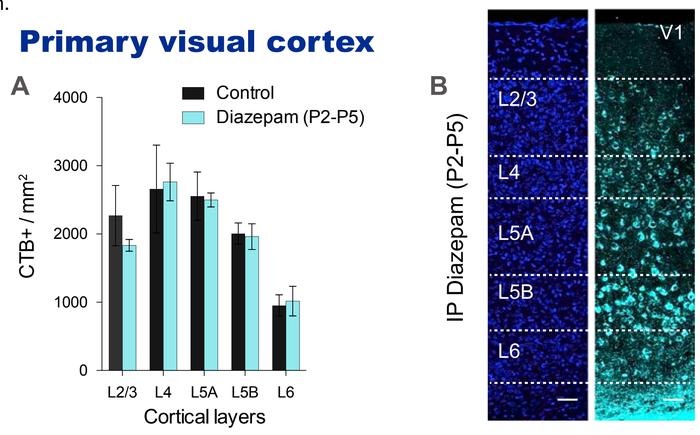


Figure 4. V1 callosal neurons after early Diazepam treatment (P2-P5). (A) Quantification of total CTB+ cells in a defined area of 280µm width (B) CTB V1 does not reveal a significant change in callosal neurons.

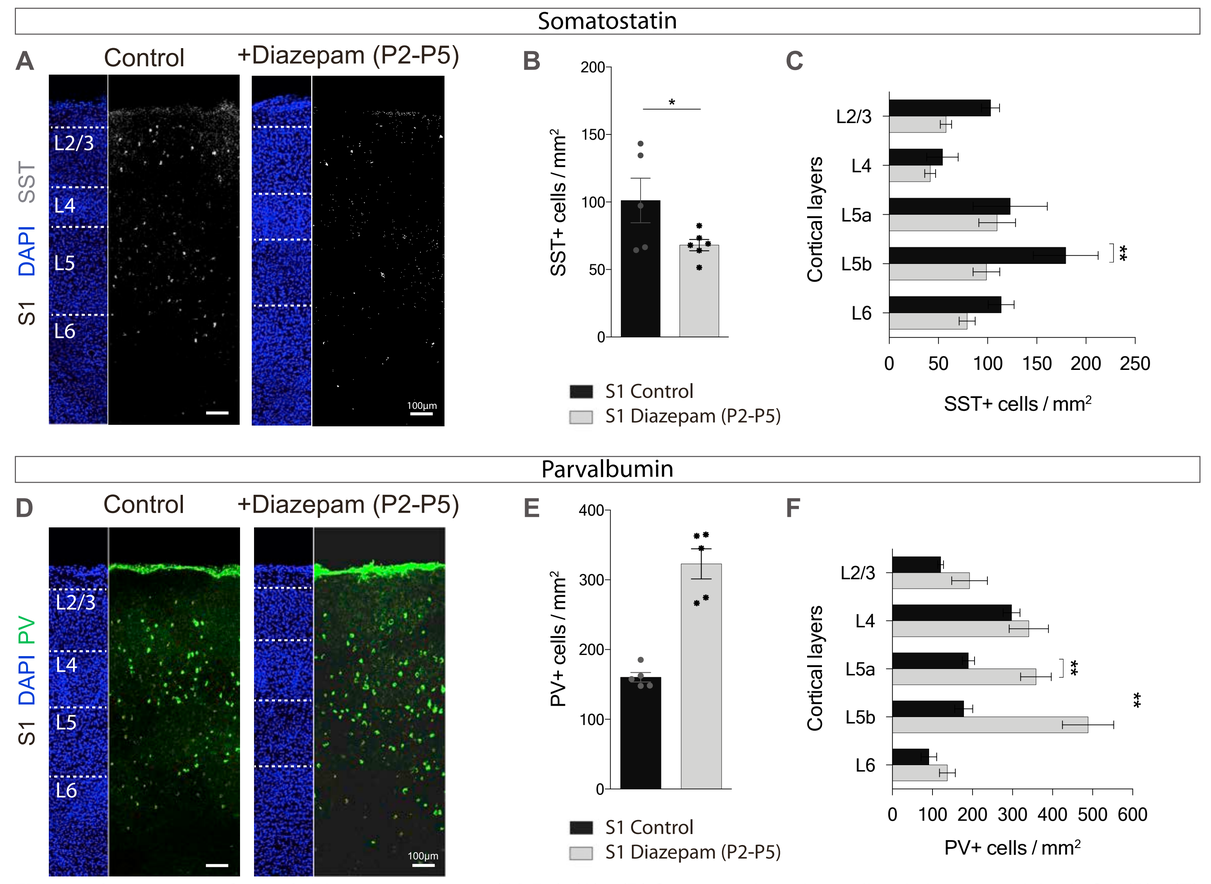
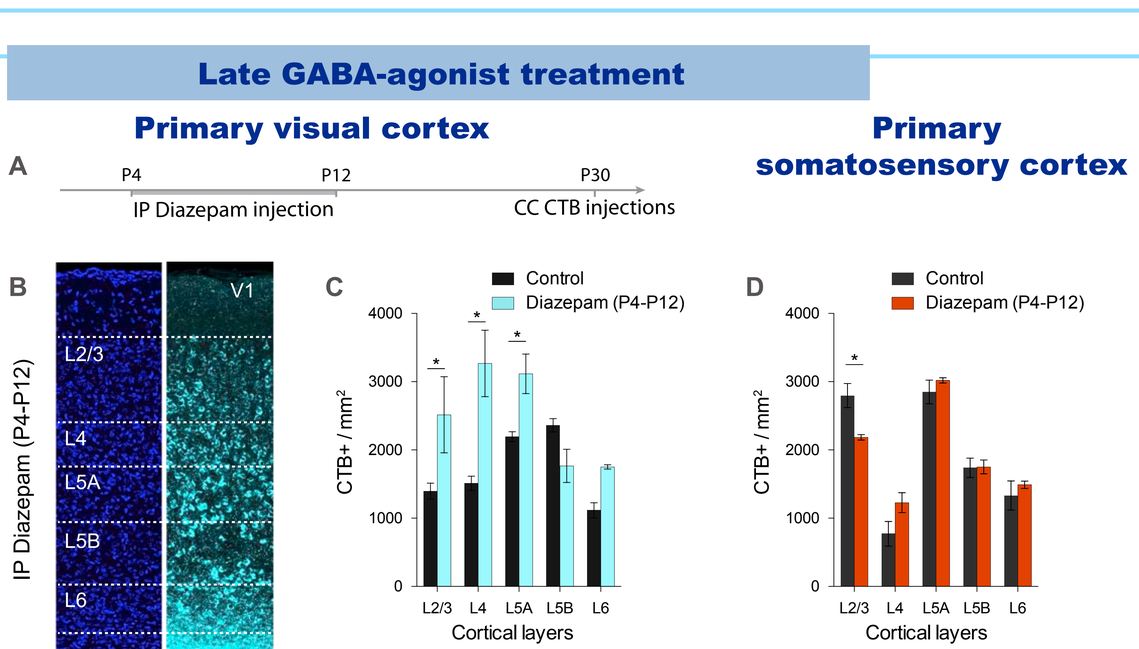


Figure 5. Interneurons after early Diazepam treatment (P2-P5). (A) y (D) Somatostatin and parvalbumin immunostaining in S1. (B) y (E) Mean of the SST+ and PV+ S1 cells. (C) y (F) SST+ and PV+ cells distribution per cortical layer. Quantifications were made in a defined area of 280Mm width.

Late GABA-agonist treatment

Primary visual cortex



Primary somatosensory cortex

Figure 6. (A) Methodological approach. (B) CTB V1 reveals an increase in the callosal neurons of the superficial layers (C) Quantification of total CTB+ cells in V1 (D) Quantification of total CTB+ cells in S1. Quantifications were made in a defined area of 280µm width.

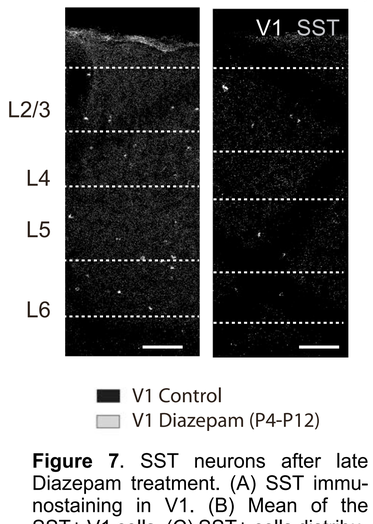


Figure 7. SST neurons after late Diazepam treatment. (A) SST immunostaining in V1. (B) Mean of the SST+ V1 cells. (C) SST+ cells distribution per cortical layer.

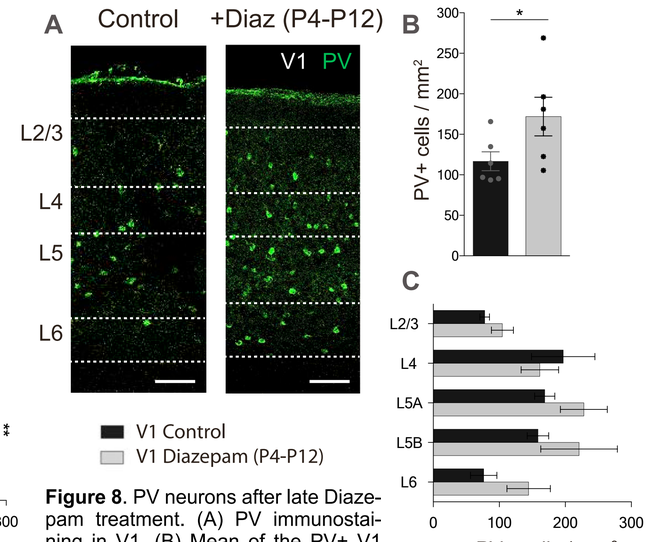


Figure 8. PV neurons after late Diazepam treatment. (A) PV immunostaining in V1. (B) Mean of the PV+ V1 cells. (C) PV+ cells distribution per cortical layer.

References:

Smith, T.A. (2001) Type A gamma-aminobutyric acid (GABA) receptor subunits and benzodiazepine binding: significance to clinical syndromes and their treatment. Br J Biomed Sci. 58(2):111-21.

Wogsamitkul, N., Maldifassi, M.C., Simeone, X., Baur, R., Ernst, M., and Sigel, E. (2017) α subunits in GABAA receptors are dispensable for GABA and Diazepam action. Scientific Reports 7, 15498.

Conclusions

Diazepam treatment change E/I balance and produces changes both in pyramidal neurons and in the interneurons populations.

- Early Diazepam treatment preferentially affects S1 over V1 callosal neurons.
- Late Diazepam treatment preferentially affects V1 over S1 callosal neurons.
- Both treatments produce alterations in somatostatin and parvalbumin interneuron populations.

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