DELAYED MATURATIONAL CHANGES OF INHIBITORY NEUROTRANSMISSION IN THE PREFRONTAL CORTEX COMPARED TO SOMATOSENSORY CORTEX

Katerina Kalemaki[1,2], Kyriaki Sidiropoulou[2,3*] and Domna Karagogeos[1, 2*]

[1]Department of Basic Science, Faculty of Medicine, University of Crete, Heraklion, Greece

[2]Institute of Molecular Biology and Biotechnology (IMBB), FORTH, Heraklion, Greece

[3]Department of Biology, University of Crete, Heraklion, Greece

* Equal senior contribution

FORTH INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

UNIVERSITY OF CRETE

Figure 1. Differential development of cell density in the PFC vs BC.

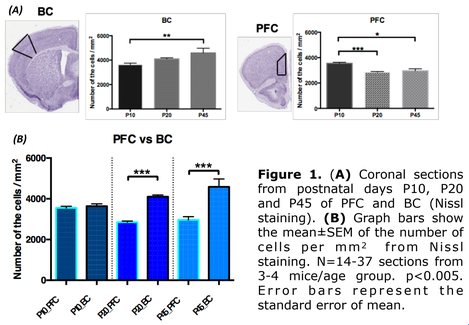


Figure 1. (A) Coronal sections from postnatal days P10, P20 and P45 of PFC and BC (Nissl staining). **(B)** Graph bars show the mean±SEM of the number of cells per mm² from Nissl staining. N=14-37 sections from 3-4 mice/age group. p<0.005. Error bars represent the standard error of mean.

Figure 2. Lhx6⁺ cell density is reduced in the PFC vs BC at early ages.

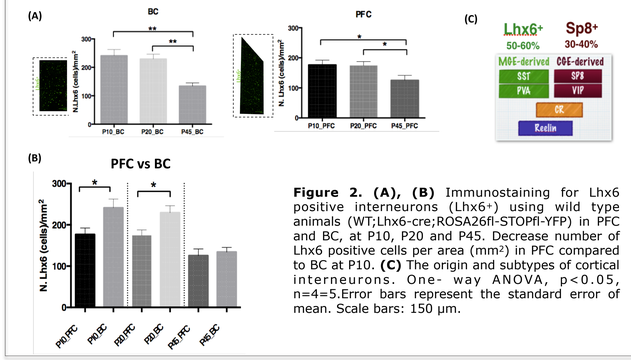


Figure 2. (A), (B) Immunostaining for Lhx6 positive interneurons (Lhx6⁺) using wild type animals (WT;Lhx6-cre;ROSA26fl-STOPfl-YFP) in PFC and BC, at P10, P20 and P45. Decrease number of Lhx6 positive cells per area (mm²) in PFC compared to BC at P10. **(C)** The origin and subtypes of cortical interneurons. One- way ANOVA, p<0.05, n=4=5.Error bars represent the standard error of mean. Scale bars: 150 μm.

Figure 3. The same numbers of Somatostatin (SST) expressing cells between the PFC and BC.

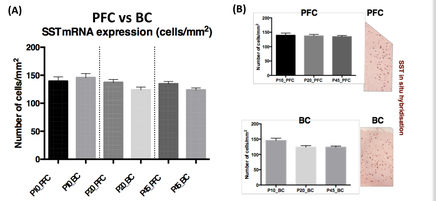


Figure 3. (A), (B) *In situ* hybridization for somatostatin positive cells (SST⁺) using wild type animals (WT;Lhx6-cre;ROSA26fl-STOPfl-YFP) in PFC and BC at P10, P20 and P45. One- way ANOVA, p<0.05, n=4=5.Error bars represent the standard error of mean. Scale bars: 150 μm.

Figure 4. Parvalbumin(PVA) is not expressed at P10 in the PFC.

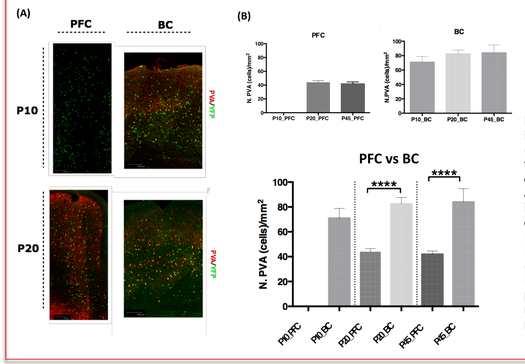


Figure 4. (A) Double immunostaining for PVA/YFP (PVA:parvalbumin) using wild type animals (WT;Lhx6-cre;ROSA26fl-STOPfl-YFP) in PFC and BC at P10, P20 and P45.No immunoreactivity of PVA positive cells in PFC compared to BC at P10. **(B)** Decrease number of PVA positive cells per area (mm²) in PFC compared to BC at P20 an P45. One- way ANOVA, p<0.05, n=4-5.Error bars represent the standard error of mean. Scale bars: 150 μm.

Figure 5. No Fast- Spiking profile at P10 in PFC.

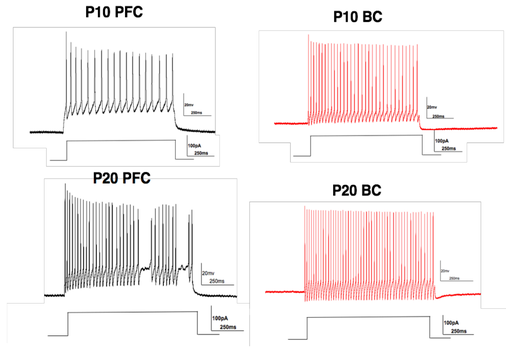
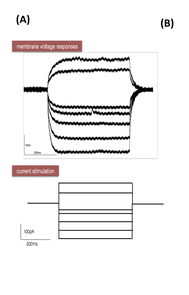


Figure 5. Patch-clamp recordings in the current-clamp mode were performed from layer II fluorescent Lhx6-expressing interneurons in the PFC at P10 and P20. The representative traces shown are in response to 500ms step-pulse protocol.

Figure 6. No Differences of Passive properties of pyramidal neurons in the PFC and BC.



(B)	PFC			BC	
Passive properties	P10_Pyr	P20_Pyr	P45_Pyr	P10_Pyr	P20_Pyr
Resting membrane potential , RMP (mv)	-65.55 ± 0.7033, n=9	-65.63 ± 0.4752, n=10	-61.6 ± 0.3882, n=10	-65.14 ± 0.8471, n=9	-65.45 ± 0.8172, n=8
Input Resistance (MΩ)	202.4 ± 9.124, n=9	179.8 ± 17.35, n=10	139.1 ± 12.63, n=12	182.3 ± 21.74, n=7	145.6 ± 13.90, n=7

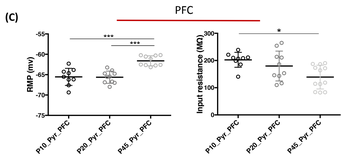


Figure 6. Patch-clamp recordings in the current-clamp mode were performed from layer II pyramidal neurons in the PFC and BC, at P10 and P20 from wild type mice. **(A)** Examples of subthreshold membrane potential during 500ms step-pulse protocol. The input resistance was measured in response to 500ms step-pulse protocol. **(B), (C)** Table and graphs (mean SD) summarise the data of resting membrane potential (RMP) and input resistance. One- way ANOVA, p<0.01, n=6-15. Error bars represent the standard error of mean.

During early development of PFC vs BC:

- ✓ Differential development of cell density.
- ✓ Decreased cell density of Lhx6⁺ interneurons in PFC at P10 and P20.
- ✓ The reduced number of Lhx6⁺ cell density is dependent on decreased PVA⁺ cell density and not SST⁺ expressing cells.
- ✓ The maturation of sodium channels is still ongoing in pyramidal neurons of PFC at P10.
- ✓ No Fast-spiking (PVA) interneurons recorded in PFC at P10.
- ✓ The synaptic activity of neuronal circuits of PFC are increased at P10.
- ✓ The effect of GABA may not be inhibitory at P10 in PFC vs BC (diazepam experiment).
- ✓ Decreased protein level of K-Cl co-transporter KCC2 at P10 in PFC.

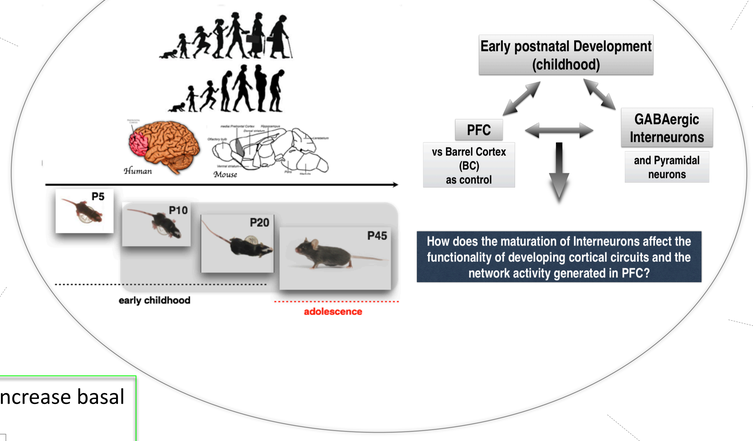


Figure 10. Decreased protein level of K-Cl co-transporter KCC2 at P10 in PFC.

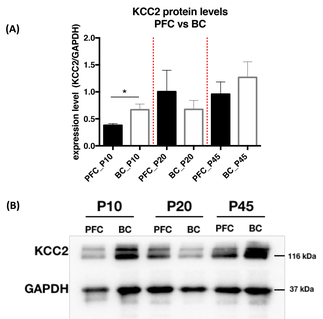


Figure 10. (A) Graph showing that K-Cl co-transporter KCC2 is decreased in PFC at P10 but not in BC at the same age. (n=3-4, one-way ANOVW, p=0,01). **(B)** Representative blots showing the changes of that K-Cl co-transporter KCC2, relative to GAPDH, during development of PFC and BC.

Acknowledgments: *Manasaki scholarship and* ΕΔΒΜ34/ ΚΑ10040, ΕΣΠΑ.

Επιχειρησιακό Πρόγραμμα Ανάπτυξη Ανθρώπινου Δυναμικού, Εκπαίδευση και Διά Βίου Μάθηση Ειδική Υπηρεσία Διαχείρισης Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

Ευρωπαϊκή Ένωση Ευρωπαϊκό Κοινωνικό Ταμείο

ΕΣΠΑ 2014-2020 ΕΠΑΝΕΚ · ΕΡΑΝΑΚ · ΕΠΑΝΕΔ

Figure 9. Increase of GABAR action leads to increase basal synaptic transmission of PFC at P10.

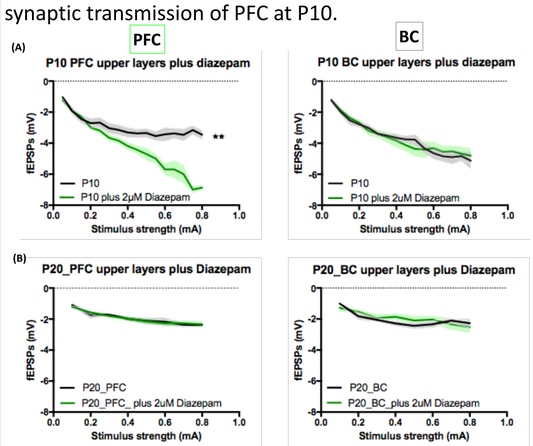


Figure 9. Field excitatory postsynaptic potential (fEPSP) responses were recorded in layer II of the PFC and BC in response to stimulation of layer II, before and after application of 2μM Diazepam at P10 and P20. At P10 , bath application of Diazepam (2μM) increases the fEPSPs in PFC. However, in the BC, diazepam does not have any effect in the fEPSP amplitude. 2-way repeated measures ANOVA, p<0.05, n=4-6. Error bars represent the standard error of mean.

Figure 8. The basal synaptic transmission is decreased, from P10 to P20, in PFC and BC.

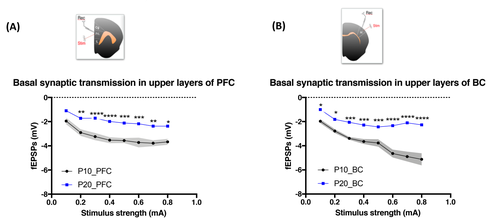
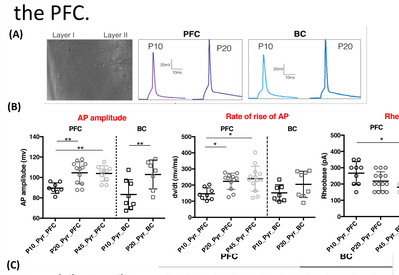


Figure 8. Differential changes in basal synaptic transmission during the early postnatal period (P10 and P20) in the PFC and BC of B6 male mice. Field excitatory postsynaptic potential (fEPSP) responses were recorded in layer II of the PFC and BC in response to stimulation of layer II, respectively. The basal synaptic transmission is decreased within layer II of PFC **(A)** and BC **(B)** . 2-way repeated measures ANOVA, p<0.05, n=6-7. Error bars represent the standard error of mean.

Figure 7. The maturation of sodium channels is still ongoing in Pyramidal neurons of the PFC.



(C)

Active properties	P10_Pyr	P20_Pyr	P45_Pyr	P10_Pyr	P20_Pyr
1. AP amplitude (mv)	**89.6 ± 1.823, n=8**	104.5 ± 3.130, n=12	103.9 ± 2.373, n=10	**83.27 ± 5.122, n=8**	**102.9 ± 5.354, n=7**
2. Rate of rise of AP: dV/dt (mv/ms)	**146.5 ± 12.98, n=8**	223.6 ± 14.43, n=12	239.8 ± 21.43, n=13	**151.8 ± 19.28, n=7**	**204.6 ± 28.84, n=8**
3. Duration of AP: Half-width (ms)	1.883 ± 0.1247, n=9	1.769 ± 0.08661, n=14	1.576 ± 0.09165, n=13	1.873 ± 0.2163, n=6	1.771 ± 0.1360, n=8
4. Rheobase (pA)	**266.7 ± 23.57, n=9**	**217.9 ± 15.38, n=14**	180.8 ± 12.06, n=13	**218.8 ± 28.25, n=8**	**237.5 ± 33.74, n=8**
5. Threshold (mv)	**-64.89 ± 0.8948, n=9**	**-65.96 ± 1.094, n=13**	-55.73 ± 2.14, n=13	**-63.43 ± 1.524, n=7**	**-63.36 ± 1.582, n=8**

Figure 7. Patch-clamp recordings in the current-clamp mode were performed from layer II pyramidal neurons in the PFC and BC, at P10 and P20 from wild type mice. **(A)** The active properties were measures in response to 5ms step-pulse protocol. **(B), (C)** Graphs (mean SD) and table summarise the data of active properties Significant differences are found in the action potential amplitude, between P10 and P20 only in the PFC, and rate of rise between P10 and P20 in both the PFC and BC. One- way ANOVA, p<0.01, n=6-15. Error bars represent the standard error of mean. (**Bold lettering,** on the table, indicates statistically significant).