Maternal administration of histamine H_1 receptor (H_1R) antagonist chlorpheniramine affect rat offspring cerebral cortex development

Rocío Valle-Bautista, Berenice Márquez-Valadez, Gabriel Herrera-López, Emilio J. Galván, Ernesto Griego-Melo, Néstor F. Díaz, José Antonio Arias-Montaño and Anayansi Molina-Hernández

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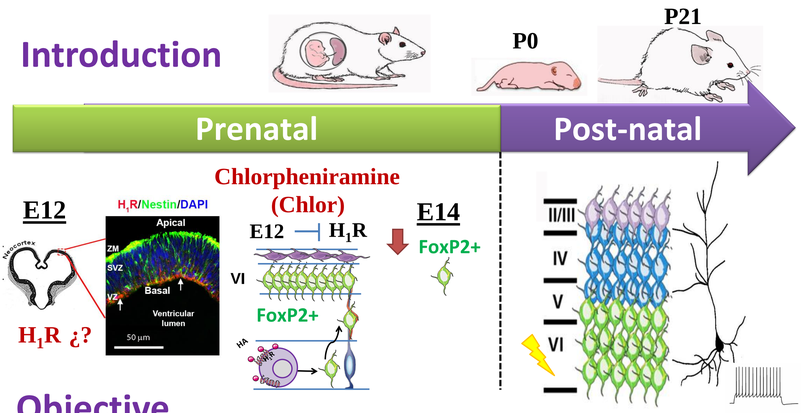
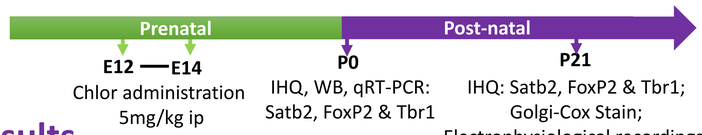
Introduction



Objective

The aim of this study was to evaluate the effect of maternal administration of chlorpheniramine on post-natal cortical development.

Methods



Results

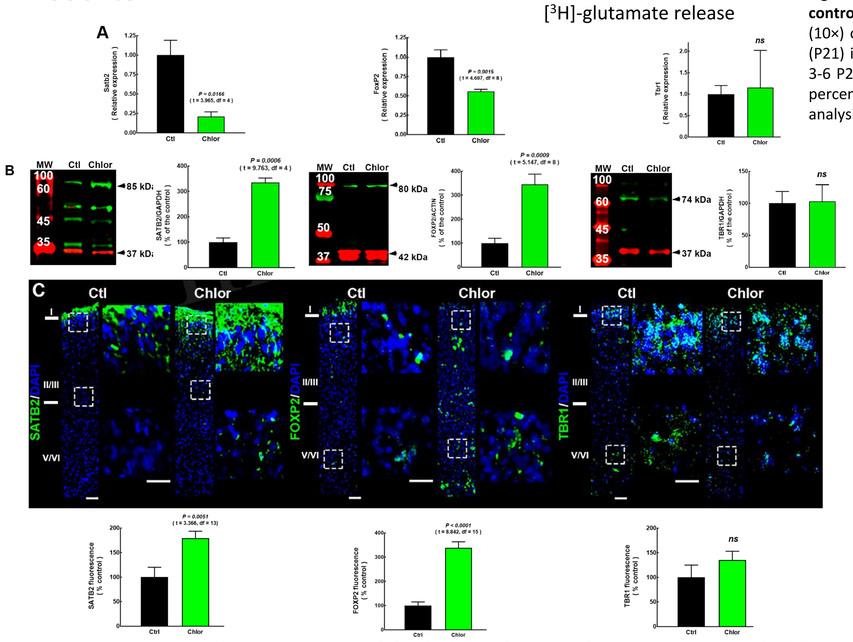


Figure 1. SATB2, FOXP2 and TBR1 expression and distribution in the neonatal primary motor cortex (M1) from control and chlorpheniramine treated dams. A, quantitative analysis of the qRT-PCR of Satb2, FoxP2 and Tbr1. Values were normalized to the relative expression of the control using the $2^{-\Delta\Delta CT}$ method, and are means ± SEM (n = 3-5; P0). **B**, left, representative Western blots for SATB2 (green, ~85 kDa), FOXP2 (green, ~80 kDa), TBR1 (green, ~74 kDa), and the internal controls GAPDH (red, ~37 kDa) or β-actin (red, ~42 kDa) bands on the left lane are the molecular weight ladders (MW). Right, quantitative fluorometry analysis for SATB2, FOXP2 and TBR1. Values are expressed as percentage of the fluorescence ratio of controls, and are means ± SEM (n = 3-5; P0). **C**, upper panel, representative M1 reconstructions from five micrographs (10×) of SATB2, FOXP2, and TBR1 (green) immunodetection and DAPI-stained nuclei (blue) at P0 from control (Ctl) and chlorpheniramine (Chlor) groups. Amplifications (400%) of the dotted squares of the superficial and deep neocortex are shown on the right. Scale bars correspond to 50 and 25 µm for 10× and zoom, respectively. Lower panel, quantitative analysis of the immunofluorescence of SATB2, FOXP2 and TBR1. Values are expressed as percentage of the fluorescence of controls and are means ± SEM (n = 3-6; P0). The statistical analyses in A, B, and C were performed with unpaired Student´s *t*-test. *ns, non significant*.

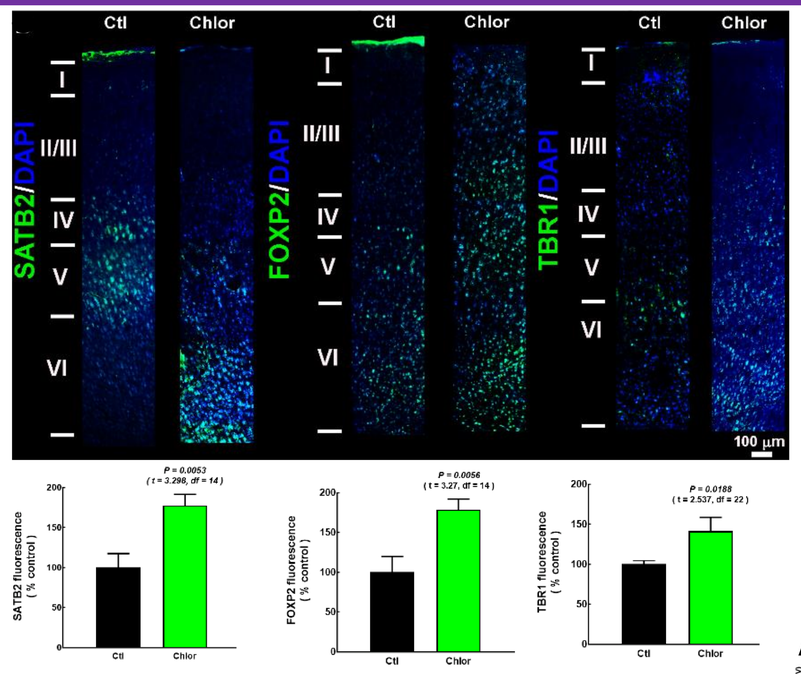


Figure 2. SATB2, FOXP2 and TBR1 distribution in the primary motor cortex (M1) of 21 day old offspring from control and chlorpheniramine-treated dams. Upper panel, representative reconstruction from seven micrographs (10×) of SATB2, FOXP2, and TBR1 (green) immunodetection and DAPI-stained nuclei (blue), at post-natal day 21 (P21) in the M1 from control (Ctl) and chlorpheniramine-treated (Chlor) groups. Images are representative from 3-6 P21 animals. Scale bar = 100 µm. Lower panel, quantitative fluorometric analysis. Values are expressed as a percentage of the fluorescence obtained in the controls and are means ± SEM from 3-6 P21 animals. The statistical analysis was performed with unpaired Student´s *t*-test.

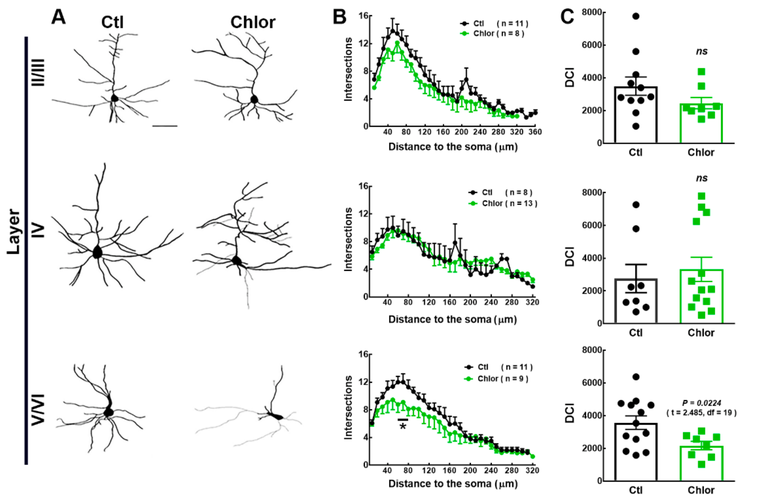


Figure 3. Analysis of the dendritic arbor in P21-day-old offspring from control and chlorpheniramine-treated rats. A, representative reconstruction of Golgi-Cox stained pyramidal projection neurons in cortical layers (II-VI). **B**, neuron Sholl analysis per layer (II-VI). Values are means ± SEM. *$P < 0.05$ versus control values; multiple *t*-test. **C**, graphs of the dendritic complexity index (DCI) of neurons obtained from the offspring of control (Ctl; black bars) and chlorpheniramine (Chlor; green bars) groups (n = 3-4), values are the mean ± SEM (n = 8-13 neurons per pair of layers). The statistical analyses were performed with unpaired Student´s *t*-test. *ns, non significant*.

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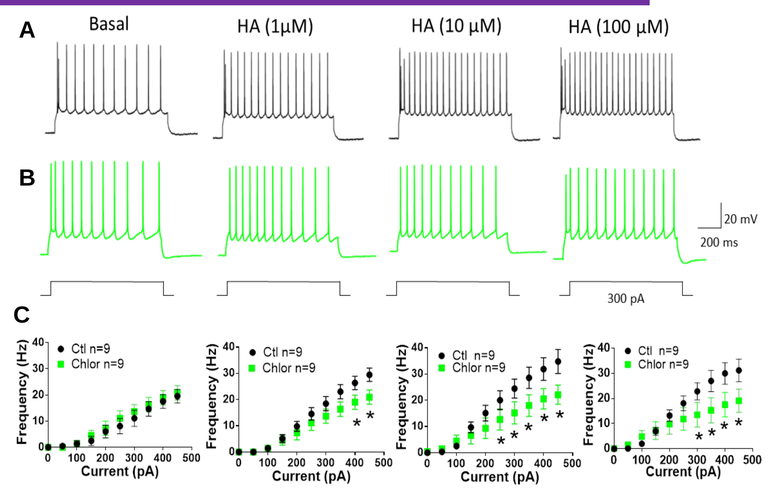


Figure 4. Analysis of action potential frequency in pyramidal neurons from V-VI cortical layers of the primary motor cortex at P21. **A** and **B**, representative traces of action potentials produced by 300 pA in offspring of control (Ctl; black) and chlorpheniramine-treated (Chlor; green) groups, under basal or stimulated 1-100 µM of histamine (HA) conditions. **C**, graphs of the analysis of action potentials frequency at different stimulation current intensities in basal and HA conditions per group, values are means ± SEM from 9 neurons for each condition from 6, P21 animals from three different litters per group. Differences between groups were analyzed with Repeated Measures ANOVA followed by Sidak´s test. *$P < 0.05$ *vs* control.

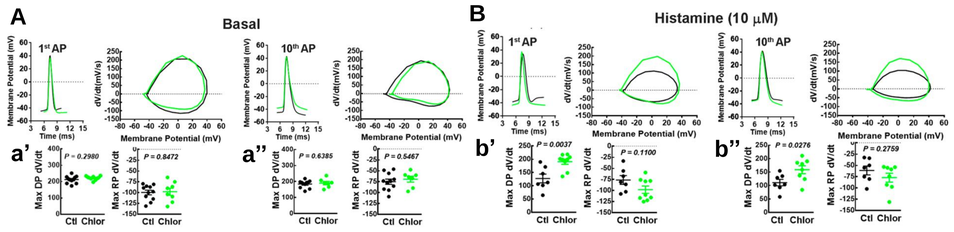
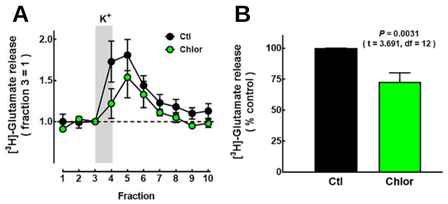


Figure 5. Phase plot analysis of the action potentials. A and **B**, representative action potentials (left) from the first (1st AP) and tenth (10th AP) spikes and the corresponding phase plots (right) from the deep layers cortical neurons from the offspring of control (Ctl; black) and chlorpheniramine-treated (Chlor; green) groups in the absence (A, basal) or presence of 10 µM of histamine (B, Histamine). Action potentials were induced by the injection of depolarizing current (300 pA) for 1 s. Analysis was performed on the maximum rate of the depolarization (DP) and repolarization (RP) phases of the 1st and 10th AP under basal (**a'** and **a''**) or histamine stimulation (**b'** and **b''**) for Ctl and Chlor groups. Values are means ± SEM from 7 to 11 neurons for each condition of 6, P21 animals from three different litters per group. The statistical analysis was performed with unpaired Student's *t*-test. *ns*, *non-significant*.

Figure 6. Effect of chlorpheniramine administration to pregnant rats on [3H]-glutamate release in striatal slices from 21-day-old offspring. A, representative experiment for depolarization evoked [3H]-glutamate release from striatal slices of P21 offspring from control (Ctl; black) and chlorpheniramine (Chlor; green). Values are means ± SEM from striatal pools of four P21 animals per group. **B**, Analysis of the area under the curve after subtraction of basal release. Values are expressed as percentage of the release from slices of Ctl animals in the same experiment and are means ± SEM from striatal pools of four P21 animals per litter from seven different dams per condition. The statistical analysis was performed with unpaired Student´s *t*-test.



Conclusion

Through H_1R activation, histamine regulates the development of primary motor cortex and pharmacological receptor blockade with chlorpheniramine during corticogenesis will altered post-natal cortical deep-layer neurons distribution and function.