Neurophysiological Assessment of Peripheral Nerve, Somatosensory, and Brainstem Auditory Function After Perinatal Exposure to Emamectin in Long Evans Rats

Garyn L. Jung[1], Emily M. Pitzer[1], Tracey E. Beasley[1], Kathy L. McDaniel[1], Robert C. Sills[2], Ronald Herbert[2], Charan K. Ganta[2,3], Renee Fisher[2], Heather Jensen[2], G. Jean Harry[4], and David W. Herr[1]

[1]CPHEA/PHITD/NETB, [2]NIEHS/DTT/CMPB. [3]Inotiv, [4]NIEHS/MTB

U.S. Environmental Protection Agency, Office of Research and Development

Garyn Jung | Jung.garyn@epa.gov | 919-541-2217

Introduction

Emamectin is a positive allosteric modulator of receptor-gated chloride channels (GABA, glycine). We have previously described an altered locomotor activity ontogeny, uncoordinated hindlimb gait, and decreased startle response in adult animals following perinatal treatment with emamectin benzoate. To examine possible neuroanatomical sites related to the behavioral changes, we measured neurophysiological endpoints in peripheral nerves, as well as somatosensory and brainstem auditory function.

Methods

Treatment & Surgery: Pregnant Long Evans rats were gavaged with deionized water (vehicle), 2.29, or 3.78 mg/kg EB (5 mL/kg dosage volume) from gestational day 6 to postnatal day (PND) 21. On approximately PND76 the male offspring (n=18-21/dose) were surgically implanted with epidural screw electrodes over the somatosensory cortex and cerebellum, along with ground and reference electrodes.

Auditory Function: Brainstem auditory evoked responses (BAER) were recorded using rarefaction clicks or tone pips of 4, 16 or 64 kHz. Three sound pressure levels were presented for each stimulus.

Peripheral Nerve Function: Compound nerve action potentials (CNAP) and nerve conduction velocity (NCV) were measured in the ventral caudal tail nerve. Three levels of stimulus current were used.

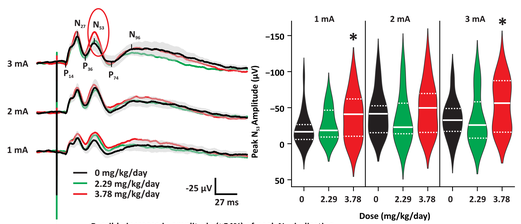
Somatosensory Function: Simultaneously with the CNAPs, somatosensory evoked potentials (SEPs) were recorded from electrodes located either over the somatosensory cortex or cerebellum.

Histology: A subset of the animals (n=8/dose) was perfused with buffered saline and paraformaldehyde, fixed *in situ*, and stained with haematoxylin and eosin (H&E) or immunochemical staining for parvalbumin.

Data Analysis: Peak amplitudes, latencies, area, and NCV were analyzed using generalized linear mixed models with appropriate distributions and covariance structures (SAS 9.4). Group average waveforms represent the neurophysiological responses, with circles around peaks that appear altered. Violin plots illustrate the non-parametric kernal density estimate of the probability of data occurring at a certain amplitude or latency, and were constructed using SRPlot at https://www.bioinformatics.com.cn/. These plots were made for peaks that appear altered in the average waveform response (indicated in red circles and on y-axis). Lines represent the 25%, 50% (median), and 75% quartiles. Difference in the distributions compared to controls was assessed using a 2-sample Kolmogorov-Smirnov Statistic.

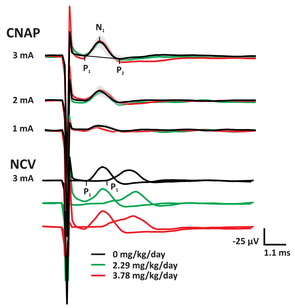
Results

Somatosensory Cortex

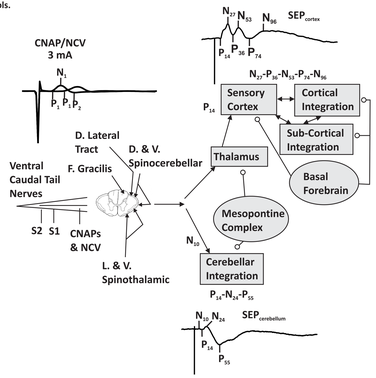


Possible increase in amplitude (~54%) of peak N_{21}, indicating changes in intra-cortical processing. Shaded area is 95% confidence interval around control response. * = Distribution differs from controls.

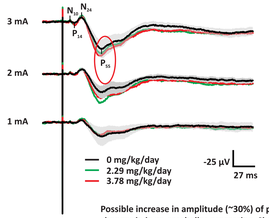
Peripheral Nerve

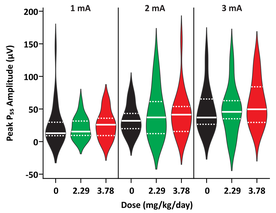


No changes were indicated in tail peripheral nerve action potentials or nerve conduction velocity. Shaded area is 95% confidence interval around control response.

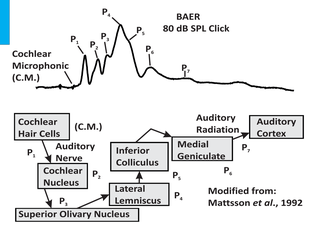


Cerebellum



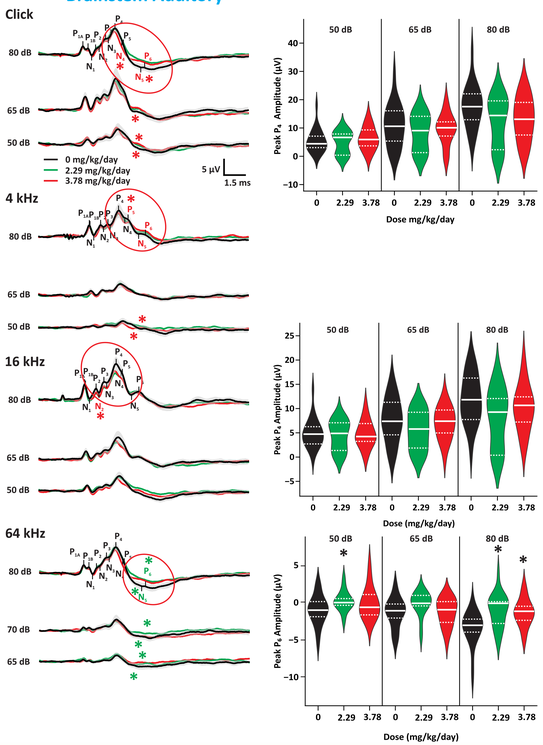


Possible increase in amplitude (~30%) of peak P_{55}, indicating changes in intra-cerebellar processing. Shaded area is 95% confidence interval around control response.

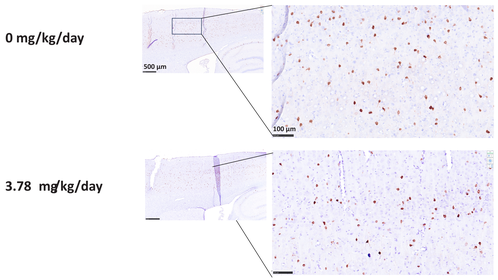


Changes in BAERs differed between stimuli and intensities. Possible decrease in amplitude (~24-15%) of peak P_x, for click or 16 kHz stimulus. Alterations in later parts of BAER waveforms in regions of peaks N_x-P_x. These changes in BAERs suggest altered neurotransmission in the brain regions between the lateral lemniscus -> medial geniculate nucleus. Shaded area is 95% confidence interval around control response. * = 2.3 mg/kg/day amplitude or latency differs from controls. * = 3.8 mg/kg/day amplitude or latency differs from controls. * = Distribution differs from controls.

Brainstem Auditory



Parvalbumen Staining



H&E staining showed no evidence of altered cortical layers or eosin positive cells in the somatosensory cortex in adult animals (not shown). Parvalbumen immunoreactivity in GABAergic neurons showed a similar distribution of staining patterns, and no evidence of cell loss, between control and treated animals. However, localized changes in connectivity cannot be ruled out. Stained cells showed features of interneurons, with small cell bodies and either bipolar or branching thin and short dendrites.

Conclusions

Results

- The evoked responses differed between the different stimuli, showing that they were under stimulus control.
- Perinatal treatment with emamectin did not alter neurophysiological measures of peripheral tail nerve function.
- Treatment with emamectin resulted in subtle alterations in evoked responses in the somatosensory and auditory systems.
- The changes in neurophysiological responses localize any effects to more apical regions of the sensory pathways: cerebellum, somatosensory cortex, and brainstem-midbrain auditory areas.
- H&E and parvalbumin staining did not reveal evidence of neurodegeneration or an overt loss of GABAergic interneurons in the somatosensory cortex.
- Altered excitatory/inhibitory (E/I) balance may be involved in producing the changes in evoked responses.
- See posters [#]P293 and P303 for related research in this project.

Future Directions

- Assessment of neurophysiological responses in these brain regions will be required to determine if changes in E/I balance are detectable.
- Examination of GABAergic neuron morphology/connections in these brain regions could assist with visualization of altered connectivity.