COMBINED LONG-TERM NERVOUS SYSTEM EFFECTS OF *FUSARIUM* MYCOTOXINS EXAMINED ON RAT BRAIN SLICES

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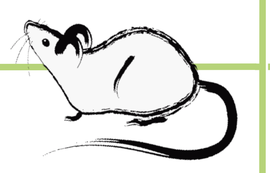
Background

Mycotoxins are toxic secondary metabolites produced by microscopic fungi; the most common *Fusarium* toxins in Europe – **fumonisin B1 (FB1), deoxynivalenol (DON)** and **zearalenone (ZEA)** – may contaminate the food chain.

FB1 is known for inhibiting de novo sphingolipid biosynthesis, DON decreases protein synthesis, while ZEA can interact with estrogen receptors. There is increasing evidence that these substances may affect nervous system functions as well.

Moreover, crops often contain these toxins in combinations and interactions between them are also possible.



Aims of the study

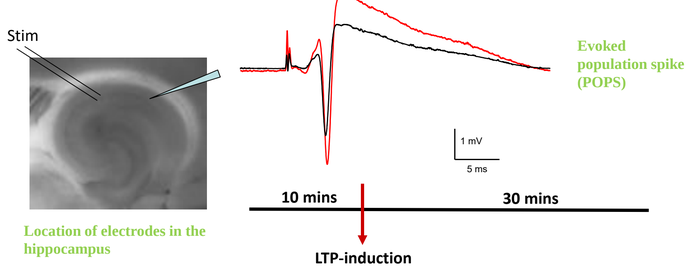
- examine the combined, long-term neuronal effects of the three mycotoxins
- analyze neuronal network functions in neocortex and hippocampus with microelectrophysiological methods

Materials & Methods

♀, ♂ rats treated for 28 days *via* gavage with
- FB1 + DON (50 and 20 µg/kg/day)
- FB1 + ZEA (50 and 20 µg/kg/day)
- DON + ZEA (20 and 20 µg/kg/day)

400 µm thick brain slices - electrophysiology

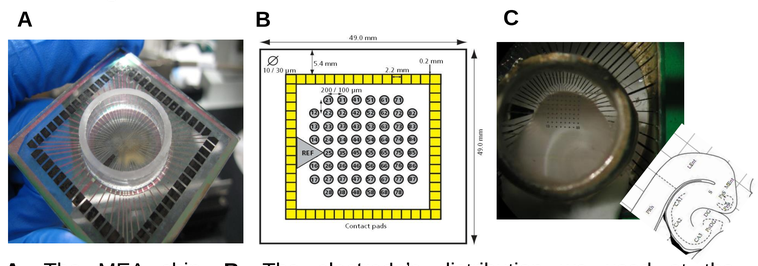
LTP in hippocampus



- long-term potentiation in CA1 (induced with a theta-burst stimulus train - TBS)

Seizure susceptibility – MEA recordings

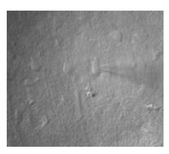
- epileptiform activity in the lateral entorhinal cortex induced with magnesium-free ACSF – MFR



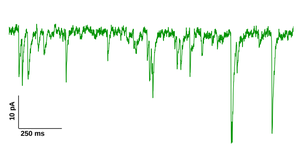
A, The MEA chip. **B,** The electrode's distribution we used at the experiments (8x8). The electrodes' diameter were 30 µm, and they are 200 µm apart from each other (200/30 MEA chip). **C,** An adult horizontal brain slice emplacement on the chip (the black dots represents the electrodes' exact position).

Whole-cell patch clamp

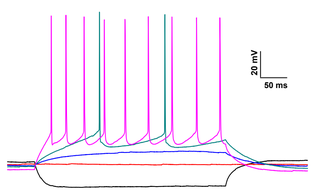
- pyramidal cells in lateral entorhinal cortex (♀, ♂ pooled)
- cell characteristics: current step protocol (-200 – 200 pA)
- synaptic inputs: EPSC recording in voltage clamp (-70 mV)



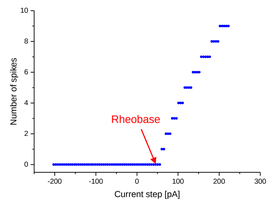
Patched pyramidal cell in layer 2/3



Spontaneous EPSC-s



Sample curves from current step protocol

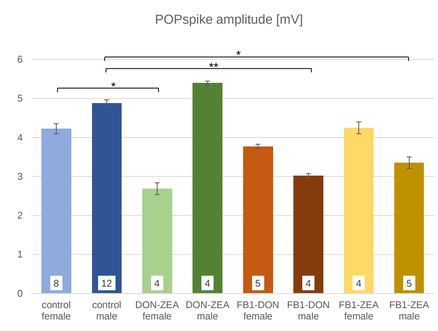


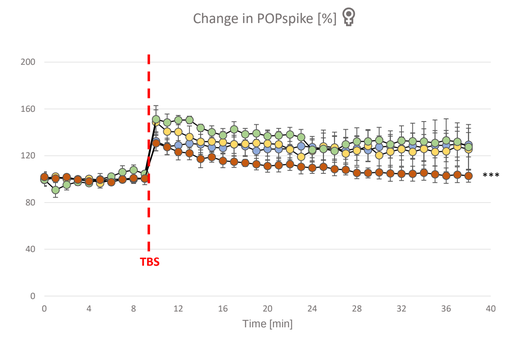
Number of spikes vs. injected current

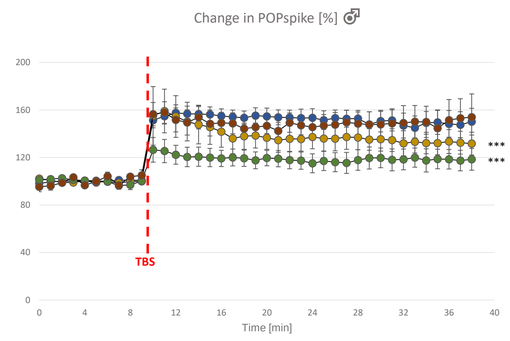
Statistical analyses: Student's t-test or repeated-measures ANOVA (* : p<0.05)

Results

LTP in hippocampus



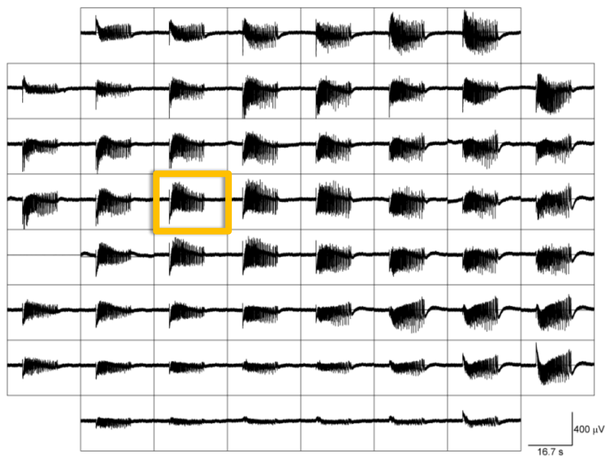


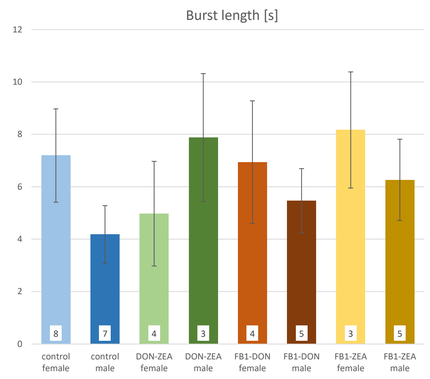


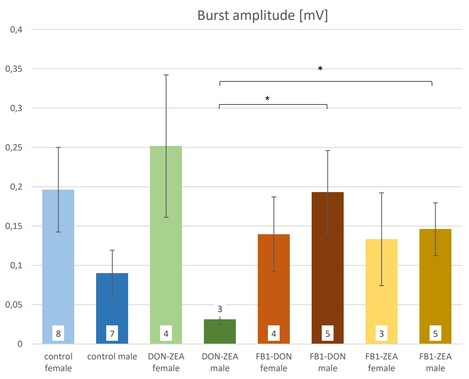
Basic excitability of hippocampal slices was altered differently depending on sex. DON-ZEA treatment in females and FB1-DON and FB1-ZEA treatment in males inhibited evoked population spikes.

On the other hand, the efficacy of LTP was not affected by DON-ZEA treatment in females nor by FB1-DON treatment in males. The treatments causing inhibition of LTP were FB1-DON in females and DON-ZEA and FB1-ZEA in males.

Seizure susceptibility – MEA recordings



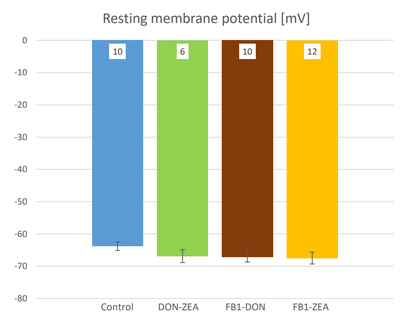


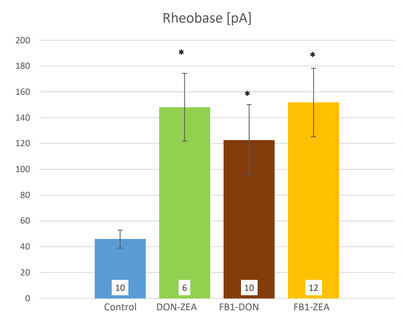


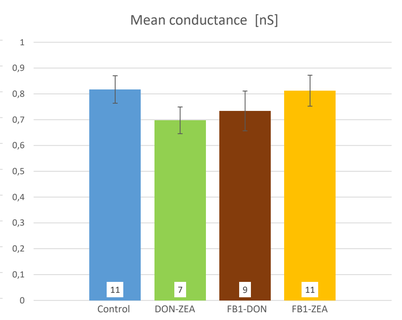
The MEA chip allows us to take measurements from the whole region of entorhinal cortex. During analysis of field potentials, the most active channel was picked to determine the burst length and the amplitude of MFR-induced epileptiform activities.

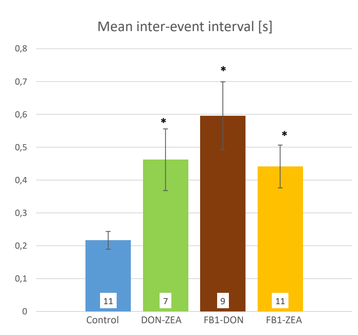
The mycotoxins did not modify the burst length of the MFR-induced epileptiform activities. However, their amplitude showed differences between DON-ZEA and FB1-DON and DON-ZEA and FB1-ZEA treated groups. The activities from the group of DON-ZEA had significantly lower amplitude in males than in the other groups.

Whole-cell patch clamp









Current clamp: Treatment with all 3 mycotoxin combinations did not change the resting membrane potential of the patched entorhinal pyramidal cells, but significantly decreased their excitability as shown by the increase in rheobase current value. Meanwhile, the input resistance of the neurons decreased (data not shown).

Voltage clamp: the mean conductance mediated by glutamatergic EPSCs arriving on the cells was not influenced by either treatment. However, the mean inter-event interval was significantly increased by all 3 mycotoxin combinations, suggesting a marked decrease in the synaptic input.

Discussion

In previous experiments, the above-mentioned mycotoxins were tested individually with the same experimental protocol as in this study, applying the current doses and a higher dose. FB1 and DON alone had an overall excitatory effect on hippocampal and cortical circuits, while ZEA mostly altered the pattern of induced epileptiform activity. The intensity of effects strongly depended on the sex of the rats, also. It seems that male rats are more sensitive to ZEA while females are more affected by DON.

However, given in pairwise combinations, FB1-DON, FB1-ZEA and DON-ZEA all inhibited neuronal network functions to a different extent. Synaptic plasticity was also inhibited by the toxin combinations. Here also, similar gender differences could be seen as with individual toxins. Patch clamp results indicate that individual neuronal characteristics as well as synaptic activity are altered by the mycotoxins.

To summarize, the three *Fusarium* toxins exert markedly different effects on the nervous system when applied in combination as compared individual effects; this suggests the presence of toxin interactions on the level of the brain.

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