Assessment of cholinergic synaptic transmission modulation in the mouse brain using resting-state functional Magnetic Resonance Imaging (rsfMRI)

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Introduction: Resting state functional magnetic resonance imaging (rsfMRI) is an upcoming technique used to evaluate functional connectivity (FC) in the brain. This technique has been applied in neurodegenerative disorders (ND) such as Alzheimer's disease (AD). Synaptic dysfunction is thougth to be a key event in AD and occurs at a relatively early stage [1], leading to alterations in neuronal transmission. We hypothesize that these synaptic transmission deficits could be reflected as altered FC in the brain. In the current study we investigate whether synaptic transmission defects, induced by the muscarinic acetylcholine receptor (mAChR) antagonist scopolamine, can be detected as altered FC using rsfMRI in mice. Furthermore, we investigate whether scopolamine induced FC deficits can be reversed with milameline (an AChR agonist).

Material and methods: All MR imaging procedures were performed on a 9.4T Biospec MRI system (Bruker BioSpec, Germany). RsfMRI measurements were acquired using a single shot gradient echo EPI sequence (TE 15ms, TR 2s, FOV 20mm, matrix 128x64, 16 axial slices). C57BL/6 mice (N=15/group) were subcutaneously injected with saline (10ml/kg), methylscopolamine (3mg/kg) or scopolamine (0.5;1;3mg/kg), after which they were subjected to rsfMRI. For an additional group of mice (N=8), rsfMRI scans of the same animal were acquired first at baseline, then after the administration of scopolamine (1mg/kg) and finally after the additonal injection of milameline (1mg/kg). All mice were anesthetized using medetomidine (0.3mg/kg) [2]. The rsfMRI data were analyzed by means of a whole brain region-of-interest (ROI) analysis and a seed-based analysis.

Results: For the highest dose of scopolamine, the whole brain ROI-analysis showed significant differences in FC between the hippocampus (HC) and the thalamus (T) (two sample T-test,$p<0.05$) (**Figure 1**). We focussed more on this functional connection using a seed-based analysis with the left hippocampus as seed: on the FC-maps decreased FC was observed for the hippocampus-thalamus connection as well as between the left and right hippocampus (two sample T-test, Bonferroni correction, $p<0.05$). These functional connections were also assessed at two lower doses of scopolamine, demonstrating a dose dependent response of scopolamine on FC between these regions (one way anova, Bonferroni correction, $p<0.05$). Moreover, these FC deficits induced by scopolamine were reversed by the administration of milameline (**Figure 2**) (one way anova within-subject, Bonferroni correction, $p<0.05$). Methylscopolamine did not show a significant effect on brain FC.

Discussion: Scopolamine showed a significant decrease of FC between the hippocampus and the thalamus and the hippocampus bilaterally, connections which are important for learning and memory. This impaired hippocampus-thalamus FC induced by scopolamine can be reversed with milameline, which has proven to enhance cognitive functions in animal models of cholinergic dysfunction[3].

Conclusions: Our study shows that rsfMRI can detect pharmacologically induced synaptic dysfunctions as altered FC in the mouse brain as well as restoration of these FC deficits by treatment. These results have implications for studies in animal models of neurodegenerative disorders, where rsfMRI can be used as a non-invasive tool to detect the occurrence and modulation of synaptic dysfunctions that occur at a presymptomatic stage.

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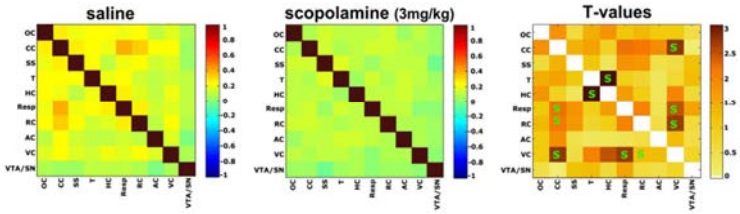

Figure 1: The effect of scopolamine on brain FC. This figure shows the FC matrices of the saline (left) and scopolamine (3mg/kg) (middle) groups. The color scale represents the strength of FC. The T-values demonstrating the difference in FC between the two groups are represented on a color map (right), warmer colors representing higher T-values. The functional connection between the hippocampus (HC) and the thalamus (T) showed the biggest difference between the two groups i.e. the highest T-value. Abbreviations: S= significant differences ($p<0.05$), OC= orbitofrontal cortex, CC= cingulate cortex, SS= somatosensory cortex, T= thalamus, HC= hippocampus, Resp= retrosplenial cortex, RC= rhinal cortex, AC= auditory cortex, VC= visual cortex, VTA/SN= ventral tegmental area/substantia nigra.

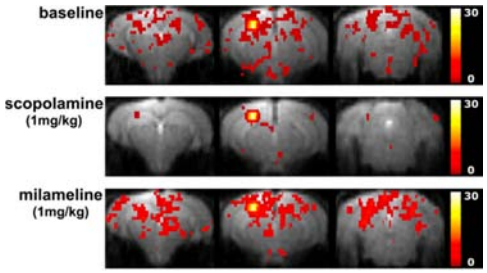

Figure 2: Scopolamine-induced FC deficits can be reversed by milameline. This figure shows 3 slices from the FC maps (corrected for multiple comparisons,$p<0.05$) resulting from the seed-based analysis with the left hippocampus as seed. At baseline conditions there is significant FC between the hippocampus-thalamus and the hippocampus bilaterally, which disappears after the administration of scopolamine. The administration of milameline recovers this scopolamine-induced decrease in FC back to baseline conditions.

References

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