Evidence for coupling between Cerebellum and Basal-ganglia loops – An MEMRI study

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Introduction The cerebellum and the basal ganglia (BG) are known to play an important role in the integration of sensory-motor information, motor coordination, motor learning, and cognitive functions such as attention and language. Due to their common functions, the question whether sensory-motor information is processed in separate loops or that the loops associated with the BG and the cerebellum integrates, particularly in pathological conditions, is of major basic and clinical significance. Using various staining procedures, previous studies have demonstrated the existence of direct and indirect projections from the BG to (i) the cerebellar dentate nucleus and (ii) to the red nucleus (RN) (main target for cerebellar projection), suggesting these sites as possible locations for mutual influence between BG and cerebellum loops. To test this, we dynamically followed the anterograde connectivity from the deep cerebellar nuclei (DCN), the dentate; to the RN *in vivo* using manganese enhance MRI (MEMRI) method. Particularly, we have tested if the information transferred from the dentate is being affected by BG abnormality such as in Parkinson's disease (PD). Anterograde connectivity in control and in 6-OHDA injected rats, a unilateral Parkinson's disease model, was compared. A unique analysis, integrating MEMRI with principal component analysis (PCA) that enables the observation of distinct spatial-temporal connectivity patterns was sued.

Method Male Sprague-Dawley rats (250-350g) were stereotaxically injected into the right SNc with either 4.5 µl of 10mM 6-hydroxydopamine (6-OHDA group, N=6) or with saline solution (sham group, N=6). Lesion severity was tested by TH immunoflorescent staining. Two weeks after 6-OHDA/saline injection, rats were intracranially injected with $MnCl_2$, (0.4µL; 0.12M) into the right lateral cerebellar nucleus. A 4.7 T Bruker BioSpec scanner was used to measure six MEMRI sessions before and at 3h, 24h, 48h, 72h and 96h post injection. 2D T1-weighted GE coronal images were obtained. Analysis was performed with IDL self-written software. Data were (i) realigned, (ii) arranged into a 2D array with spatial, the dependent variable, and temporal the independent variable and (iii) standardized PCA applied for each animal separately. Significant weights were overlaid in color on the anatomical images to generate PC-maps. Overlaying all PC-maps from each group generate incident maps. Pixels with different degree of overlap are shown in different colors.

Results Signal intensity in the red nucleus slowly increased with maximum at 24/48 h post injection with all time points (except at 3h) significantly higher then baseline. No significant differences in signal enhancements between control and 6-OHDA groups were observed. PCA analysis identified two common temporal patterns: (i) A moderate ascending slope followed by a steady or slow descent (PC1, variance 49±6% and 33±8% for the control and 6-OHDA groups). (ii) A moderate increase to a maximum at 24h followed by a sharp decrease (PC2, variance 21±5% and 33±8%). Figure 1 shows incident PC1-maps demonstrating better overlap of high weighted pixels in control compared with 6-OHDA rats. Similar results were obtained for PC2. 'Effective-ROIs', the area with >50% overlap of the control group for each PC, show significant lower PC1 weighting for the 6-OHDA group with no difference for PC2 weightings.

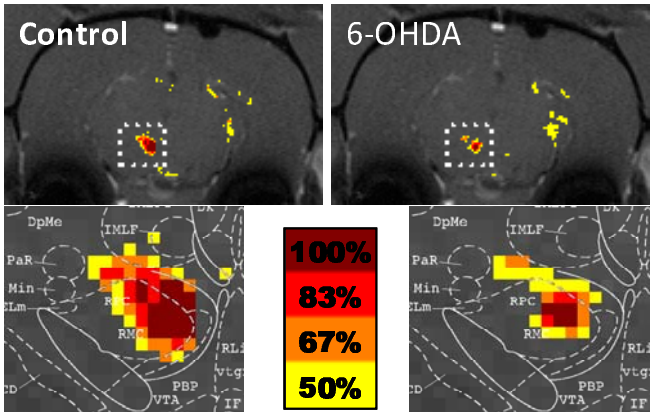

Figure 1 PC1 incident maps in a slice containing the red nucleus. PCA was performed on each animal separately, PC1-maps generated and all PC1-maps from the same group overlaid together to generate the incident maps. Color indicates the degree of overlap between rats. **Left** Incident PC1 map for control rats and **Right** for 6-OHDA rats. Bottom images show the area around the RN in both maps with rat brain atlas overlaid to help in anatomical identification.

Discussion Assuming that PC temporal patterns in the RN, correspond to signal enhancements resulting from intracellular manganese propagation and that PC weighting is proportional to the effective connectivity strength, our findings suggest that the effective connectivity between the DCN and the RN can be decomposed into two independent temporal processes of which the efficiency of only one is reduced by BG abnormality. In contrast to PC2 that represents the expected mono-synaptic DCN→RN process, PC1 describes a complex di-synaptic process which can be: (i) A cerebellar nucleo-cortical projection followed by cortinuclear projection. (ii) Reciprocal projections of the RN. (iii) Intra-dentate projection or (iv) Intra RN projection. Our results favor the last scenario. The finding that reduction in PC1 weighting of the 6-OHDA rats corresponds to a di-synaptic DRN→ RN projection is in line with the fact that in parkinsonian state, GPi increases its inhibitory output and since it was shown to directly project to the RN, RN activity is expected to be reduced. This finding coincides with clinical observations of decreased arm-swinging and akinesia in PD patients.

In summary, we have shown the coexistence of two distinct temporal patterns of signal enhancement in the RN following the intra-cranial injection of manganese ions into the dentate. We suggest that one pattern corresponds to a mono-synaptic pathway while the other corresponds to a di-synaptic pathway. The most likely concretization of the di-synaptic pathway is an additional intra RN projection. The weighting of the di-synaptic pattern was significantly reduced in the 6-OHDA group, demonstrating active coupling between the cerebellum-RN loop and the BG, while the weighting of the other, mono-synaptic, pattern did not change. These findings suggest that interaction between the BG and the cerebellum takes place in the RN since in parkinsonian state RN activity is reduced due to enhanced inhibition from the GPi. Consequently, processes which involve manganese transfer by an intra-RN projection are expected to be less efficient while processes that involve manganese transfer through dentate-RN projection are not expected to be affected, these expectations fit our observations.