In Vivo Observation of Active Neurons in the Mouse Habenular System Using Mn^{2+}-Enhanced MRI

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Introduction

Previous studies have suggested that the dysfunction of the habenula, the chief relay nucleus of the descending dorsal diencephalic system, is associated with cognitive impairment of schizophrenics [1]. Respective rodent models have been developed [2]. Mn^{2+}-enhanced MRI [3,4] may be useful for functional assessment of the habenular system [5]. The purpose of this work was to investigate the use of Mn^{2+} to characterize morphologic and functional properties of the habenular pathway in behaving mice.

Methods

T1-weighted 3D MRI data sets (rf-spoiled 3D FLASH, TR = 17 ms, TE = 7.6 ms, flip angle = 25°, 117 µm isotropic resolution, measuring time 84 min) [6] were acquired at 2.35 T (Bruker Biospin, Germany). Excitation and signal reception were accomplished with use of a Helmholtz coil (100 mm) and an elliptical surface coil (20 x 12 mm), respectively.

Intracerebroventricular Mn^{2+} administration: Three mice received a single injection of $MnCl_2$ (5 mM, 0.25 µl) in the left lateral ventricle as previously described [7]. MR images were acquired 6 hours after Mn^{2+} administration.

Systemic Mn^{2+} administration: Three mice received $MnCl_2$ (120 mM, 5 ml/kg body weight) subcutaneously. MR images were acquired before as well as 2, 6, and 24 h after Mn^{2+} administration.

Results and Discussion

Intracerebroventricular (i.c.v.) injection of $MnCl_2$ highlighted the entire habenulo-interpeduncular pathway. Figure 1 shows a sagittal as well as a coronal MRI section along the pathway. The tissue uptake of Mn^{2+} resulted in a pronounced MRI signal enhancement in the habenula (Hb). From the Hb, enhanced bundles (arrowheads) can be further traced more ventrally across the thalamus to the interpeduncular nucleus (IP) in the midbrain. In agreement with previous studies of the Hb using conventional intraneuronal tracers, this connection most likely represents the fasciculus retroflexus (FR). The results indicate the Mn^{2+} uptake and subsequent transport by habenular neurons. However, a SNR analysis of the Hb reveals an insufficient reproducibility (SNR 38.4 ± 12.8, n = 3), which hampers the i.c.v. approach as an administration technique for functional assessments of habenular neurons.

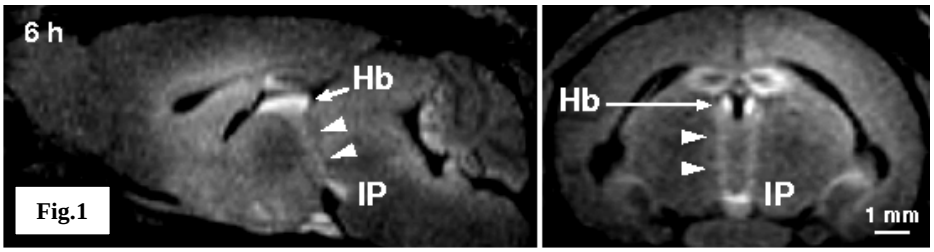

Fig.1

Subcutaneous (s.c.) injections of $MnCl_2$ depicted the Hb as demonstrated in a previous study using a low dose (20 mM, 5 ml/kg BW) [5]. However, a much better delineation of the FR was achieved by administering a high dose as shown in Figure 2. The coronal MRI section (middle) obtained 6 h after s.c. injection clearly depicts the whole Hb-FR-IP pathway. At 24 hours after injection the soft tissue contrast is faded out (right). In contrast to the i.c.v. approach, systemic administration achieved increases of the SNR with lower inter-individual variability (Table). A comparison of SNR values revealed conditions of unsaturated Mn^{2+} accumulation 2 hours and optimal SNR values 6 hours after injection. 24 hours SNR tends to decrease. These results suggest that MRI investigations of the dynamics of intra-axonal Mn^{2+} accumulation could be performed during the first 2 hours after injections.

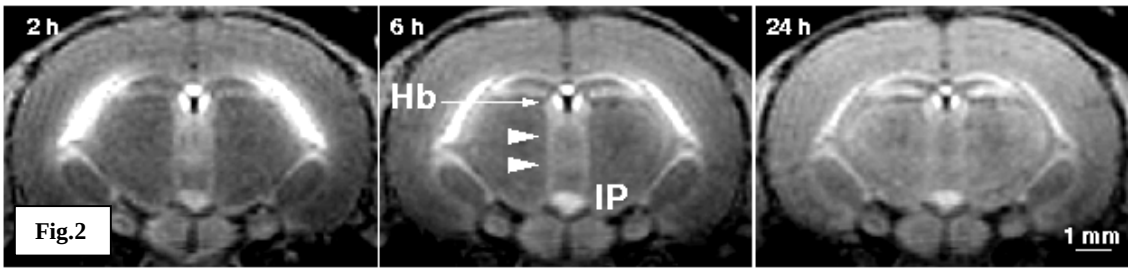

Fig.2

SNR after Systemic Mn^{2+} Administration (Mean ± SD, n = 3)				
Region	Basal	2 h	6 h	24 h
Hb	28.7 ± 1.8	68.2 ± 5.0	71.0 ± 2.4	60.4 ± 2.6
FR	25.7 ± 2.6	35.2 ± 4.0	41.4 ± 0.5	41.4 ± 3.7
IP	23.4 ± 2.9	43.2 ± 7.1	52.3 ± 1.6	47.1 ± 3.5

In summary, the results suggest that Mn^{2+}-enhanced MRI can be used for structural and functional screening of mouse models involving disturbances of the descending dorsal diencephalic system. Whereas the intracerebroventricular administration is advantageous for morphological assessments of the Hb-FR-IP pathway, systemic administration offers a reproducible quantitative evaluation of regional Mn^{2+} enhancement. This strategy is currently applied for an assessment of novel schizophrenic mouse models.

References

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