Observation of Neural Activity in Insect Brain *In Vivo* Using Mn^{2+}-Enhanced 3D MRI

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Introduction

Mn^{2+}-enhanced MRI [1,2] is widely used for the observation of active brain tissue in rodents. The distribution of Mn^{2+} in neural tissue, under physiologic and pathologic conditions, still remains to be elucidated [3]. The relatively simple but fundamental organization of insect brain may serve for a better understanding of the central nervous system in mammals. For example, the antennal lobe of the sphinx moth (*Manduca sexta*) serves as a model for the olfactory system of mammals and its development. Extending a recent MRI study of *M. sexta* at different pupal stages [4], the purpose of this *in vivo* study was (i) to establish an application method of $MnCl_2$ suitable for the pupae, and (ii) to investigate Mn^{2+} accumulation within the antennal lobes after transecting one antenna.

Methods

Animals. Transection of either left or right antenna at its base was performed in nine male pupae of *M. sexta* (P12—P17). After 0—24 hours eight pupae (#1—#8) received an injection of $MnCl_2$ (20 µl, 20 mM) into the hemolymph near the proboscis. The damages of the cuticle due to the transection and injection were sealed with melted wax.

MRI. T1-weighted 3D MRI data sets at 100 µm isotropic resolution (rf-spoiled 3D FLASH, TR/TE = 20/7.8 ms, α 25°, FOV 12.8×25.6×25.6 mm^3, matrix 128×256×256, 2 averages, 44 min measuring time) were acquired before (#8 and #9) and at various times (0.5—44 hours) after (#1—#8) Mn^{2+} administration at 2.35 T (Bruker Biospin, Germany). Excitation and signal reception were accomplished with use of a Helmholtz coil (∅100 mm) and a circular surface coil (∅10 mm), respectively. In some cases, T1-weighted 3D MRI data sets were obtained at 60 µm isotropic resolution (rf-spoiled 3D FLASH, TR/TE = 20/8.1 ms, α 25°, FOV 7.68×15.36×15.36 mm^3, matrix 128×256×256, 16 averages, 6 h measuring time). Regional SNR analysis followed a strategy previously described [3].

Results and Discussion

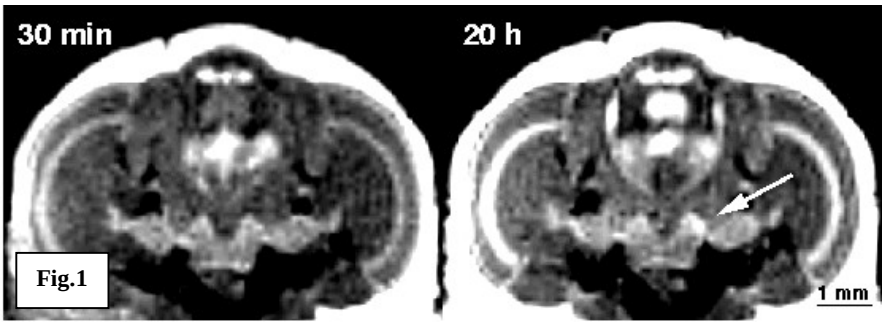

Fig.1

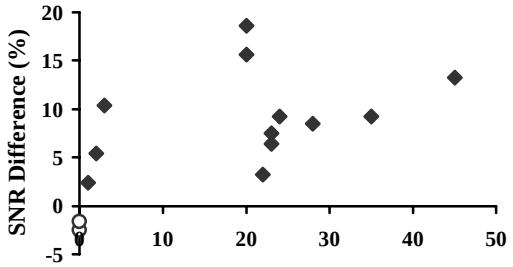

Fig.2

In comparison to a previous study without Mn^{2+} enhancement [4], the use of a smaller surface coil which was adapted to the shape of the pupal head, allowed for 3D MRI with similar SNR but at considerably reduced measuring time (by factor of 4).

In a technical sense, injections of $MnCl_2$ into the hemolymph near the proboscis of the pupa turned out to be feasible with sufficient reproducibility. Two animals had to be excluded from the analysis because of a lack of an enhancement. Due to the systemic circulation of the hemolymph, Mn^{2+} is supplied to the brain. Figure 1 shows horizontal MRI sections (100 µm isotropic resolution) at the level of the antennal lobes (AL) 30 min (left) as well as 20 h (right) after $MnCl_2$ injection (applied 24 hours after the transection of the right antenna at stage P13). The accumulation of Mn^{2+} resulted in a pronounced MRI signal enhancement in the intact left AL (arrow), indicating preserved neural activity. The axotomy-induced degeneration of the respective afferent fibers caused a lack of local Mn^{2+} accumulation in the right AL.

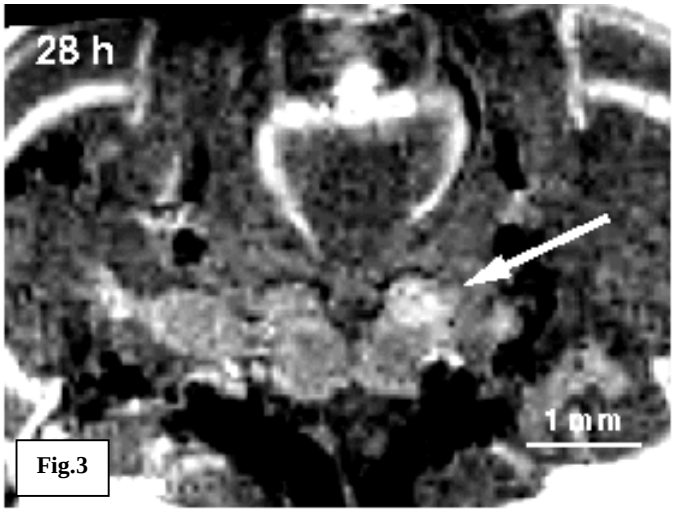

Fig.3

Figure 2 shows the SNR difference between the intact AL and the antenna-transected AL from all animals as a function of time after Mn^{2+} administration. Except for the data obtained before (#8 and #9, open circles) Mn^{2+} administration, SNR increases of 2—20% were observed in the intact AL.

Figure 3 shows a zoomed horizontal MRI section of a pupa (around P15) with an isotropic resolution of 60 µm (28 hours after Mn^{2+} administration, 34 hours after transection). Again, a pronounced signal enhancement was observed in the AL with the intact antenna (arrow). The synaptic transmission from receptor axons to the AL neurons is known to be already present in the glomeruli at P12. Therefore, at this high spatial resolution one may speculate if the bright ring within the enhanced AL represents the assembly of glomeruli (known as functional units for odor processing).

In summary, this study demonstrated the feasibility of Mn^{2+}-enhanced MRI in pupae of *M. sexta in vivo*. The observed Mn^{2+} enhancement in the AL indicates neural activity. Mn^{2+}-enhanced MRI of insect brain has the potential to unravel patterns of neural activity during the development of specifically targeted brain systems.

References

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