Manganese enhanced MR neuronal tract tracing: a passive stochastic process.

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INTRODUCTION

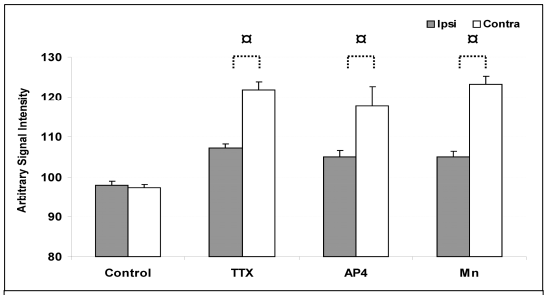
Manganese (Mn) enhanced magnetic resonance imaging (MEMRI) is a technique with considerable potential applications. MEMRI anatomical tract tracing relies on the intra-cellular uptake of Mn, its transportation along axons and subsequent accumulation at terminal fields. A number of studies suggest the uptake and/or transport of Mn may be activity dependent [1,2], while a recent study suggested that neuronal activity is actually perturbed and transport apparently unaffected by concentrations of Mn required for imaging [3]. While such measurements of transport were made at the optic chiasm at 2.5 hours post injection and transportation rates reported as consistent with fast anterograde transport, the contribution of passive diffusion in the distal portion of the optic nerve was not considered. A recent *in-vivo* murine DTI study [4] suggests that such apparent transport could be achieved purely by passive diffusion (apparent diffusion coefficient of $1.6x10\text{-}3mm^2s^{-1}$ in the direction parallel to the optic nerve). Therefore, in this study we have examined the effects of partial or complete pharmacological blockade of retinal ganglion activity on the relative degree of terminal field enhancement at 24 hours post-injection. The specificity of determining terminal field enhancement negates contributions from free diffusion and is perhaps more pertinent to the technique of interest.

METHODS

Conducted under licence from the UK Home Office. **Intra-ocular injections:** Approximately 24 hours prior to MR imaging, adult male C57/BL6 mice (31±2g) were anaesthetised and a pulled glass capillary inserted into the intra-ocular space at the temporal retinal margin. Manganese chloride (25mM – [5]), dissolved in physiological saline, was injected to a volume of 0.5µL (n=5); or co-localised with with either 14mM APB, an mGluR6 glutamate agonist (DL-2-amino-4-phosphonobutyric acid) (n=5), or 0.2mM tetrodotoxin (TTX) (n=4). Naïve subjects were used as imaging controls (n=6). **3D MR Imaging:** Under isoflurane anaesthesia (2% in 60:40 nitrous oxide and oxygen), animals were 3D spoiled gradient echo imaged: acquisition matrix 720x160x160; TR=35ms; TE=4ms; nex=4; α=21°; scan time=60minutes. **Post-processing:** Zero-filling providing an effective image resolution of 78x78x78µm. Images were B1 corrected [6] and normalised to an in-house template, being interpolated down to a resolution of 156x156x156µm. **Analysis:** The superior colliculi (SC), being the largest primary terminal fields of retinal ganglion cells, were manually segmented on the in-house template dataset. The SC ROIs were used to determine the mean signal intensity, which was ratio-scaled to the signal intensity of a small region of forepaw primary somatosensory cortex (FP_S1), ipsi-lateral to the injected eye.

RESULTS

Agents co-localised in intra-ocular injections of manganese chloride that should have produced partial (APB) or complete (TTX) blockade of retinal ganglion cell activity, failed to reduce the positive contrast changes within the contra-lateral superior colliculus (SC) (figure). Univariate analysis of variance for signal intensities within the SC, normalised to an area of the forepaw somatosensory cortex demonstrated main effects of Mn ($F(1,33)=70.8$, $p<0.001$), side ($F(1,33)=34.3$, $p<0.001$) but not blockade ($F(2,33)=0.79$, $p=0.46$). Interactions between Mn and side ($F(1,33)=23.1$, $p<0.001$) reached statistical significance, while blockade and side failed to reach significance ($F(1,33)=0.96$, $p=0.33$). Equal variances were demonstrated between groups ($F(6,33)=1.83$, $p=0.124$). Post-hoc tests between sides (within group) are illustrated in the figure.



Arbitrary Signal Intensity Within The Contoured SC. Post-hoc independent t-tests: (¤) statistically significant differences between ipsi-lateral and contra-lateral normalised signal intensities within each group ($p \leq 0.029$).

DISCUSSION AND CONCLUSION

Magnetic resonance tract tracing of retinal ganglion cells provided a favourable assay with which to examine the effect of activity on axonal transport of experimentally applied Mn. The apparent lack of difference between the relative signal intensity within the SC in animals co-administered APB and Mn versus the Mn only group (figure) suggests that the axonal transport of Mn is not dependent on retinal ganglion cell activity. This was further confirmed by the complete blockade of retinal ganglion cell activity with the sodium channel blocker TTX. The administered dose of APB in this study was equivalent to a vitreal concentration of approximately 1200µM, which compares to 700µM used by Chapman and Gödecke [7] and should therefore have been sufficient to block most visually-driven activity in ON-centre retinal ganglion cells, approximately 50% of the population. The effective vitreal concentration of TTX applied was 17µM, which is substantially larger than that previously demonstrated to block voltage sensitive sodium channels and therein abolish all retinal ganglion cell activity in mice (3µM [8]).

The expected blockade of activity and apparent activity independent axonal transport of Mn however poses a conceptual problem: activity blockade should have also blocked the uptake of Mn through activity dependent voltage gated calcium channels. This apparent paradox may have been unique to the choice of intra-ocular loading for the activity study – a vitreal concentration of 2mM. In rat pre-synaptic nerve endings this concentration of Mn has been shown to evoke a maximal influx of Mn, and competitive blockade of calcium, into synaptosomes following potassium stimulation influx [9]. Further the intra-cellular uptake of Mn cations is approximately 50 fold greater than normal calcium influx in the absence of Mn (incubated in 0.02mM calcium and potassium stimulated). Given the stochastic nature of voltage gated channels in the retina [10], such competitive mass influx of Mn at our applied concentration may have been adequate to achieve sufficiently high intra-cellular concentrations for tract tracing in the absence of activity per se. Thus, any additional activity and resultant greater intracellular influx would not be apparent in the rate of transported Mn to the terminal fields. Interestingly, the relative influx of calcium and Mn into synaptosomes [9] only becomes comparable when the applied Mn concentration equals the applied extra-celluar concentration of calcium (0.02mM), which is low compared to normal in-vivo extra-cellular calcium levels of 1.2mM. While it is difficult to compare synaptosome preparations directly with the in-vivo situation, it is perhaps worth noting that a vitreal concentration of 0.02mM failed to exhibit any MR visible axonal transport in the optic nerve of the rat [11]. Thus at the concentrations of Mn required to cause a change in MR contrast in the visual system, the influx and subsequent axonal transport of Mn may be dominated by passive stochastic processes.

REFERENCES

[1] Lin YJ, Koretsky AP. (1997) Magn. Reson. Med. 38(3):378-88. **[2]** Pautler RG, Silva AC, Koretsky AP. (1998). Magn. Reson. Med. 40(5):740-8. **[3]** Bearer EL, Falzone TL, Zhang XW, Biris O, Rasin A, Jacobs RE. (2007). Neuroimage. 37 Suppl 1:S37-46. **[4]** Wu Q, Butzkueven H, Gresle M, Kirchhoff F, Friedhuber A, Yang Q, Wang H, Fang K, Lei H, Egan GF, Kilpatrick TJ. (2007). MR diffusion changes correlate with ultra-structurally defined axonal degeneration in murine optic nerve. NeuroImage. 37(4):1138-47. **[5]** Lowe AS, Thompson ID and Sibson NR (2006) ISMRM Ab 225. **[6]** Wicks DAG, Barker G and Tofts, PS. (1993) Magn Reson Imag 11:183-196. **[7]** Chapman B, Godecke I. (2000). J. Neurosci. 2000;20(5):1922-30. **[8]** Robson JG, Maeda H, Saszik SM, Frishman LJ. (2004). Vision Res. 44(28):3253-68. **[9]** Drapeau P, Nachshen DA. (1994). J Physiol. 1994;348:493-510. **[10]** van Rossum MC, O'Brien BJ, Smith RG. (2003). J. Neurophysiol. 89(5):2406-19. **[11]** Thuen M, Singstad TE, Pedersen TB, Haraldseth O, Berry M, Sandvig A, Brekken C. (2005). J. Magn. Reson. Imaging. 22(4):492-500.