# The Effect of Peripheral Administration of Monosodium Glutamate on Ionotropic Glutamate Receptor Signalling in the Mouse Brain *in vivo* Shown through Manganese Enhanced MRI

**M. K. Hankir[1,2], J. R. Parkinson[1], S. R. Bloom[2], and J. D. Bell[1]**
[1]Metabolic and Molecular Imaging Group, Imperial College, London, United Kingdom, [2]Investigative Medicine, Imperial College, London, United Kingdom

## Introduction

Peripheral administration of monosodium glutamate (MSG) to adult animals increases feeding (Reddy et al., 1985). This is possibly due to the direct activation of ionotropic glutamate receptors (iGluRs) which comprise either NMDA or AMPA/Kainate receptors and are both expressed in the hypothalamus. We used Manganese Enhanced MRI (MEMRI) to determine whether intraperitoneal (i.p.) administration of MSG increases the $Mn^{2+}$ influx into the arcuate nucleus (ARC) of the hypothalamus as a surrogate for neuronal excitability and activation of which specific ionotropic glutamate receptor subtype mediates this effect.

## Methods

Adult male C57BL/6 mice were anaesthetised and scanned in a 9.4T horizontal-bore MR scanner. Intravenous (i.v.) infusion of $MnCl_2$ via the tail vein commenced after 5 baseline MRI image acquisitions. Each mouse received 5µl/g body weight of 100mM $MnCl_2$ at a rate of 8µl/g body weight/h. Spin-echo multi-slice T1-weighted sequence imaging was carried out with the following scanning parameters; repetition time (TR) = 1800 msec, echo time (TE) = 5.2 msec, matrix = 256 x 256, field of view (FOV) = 25 mm x 25 mm. Image processing software was used to define and calculate the relative signal intensity (SI) within specific regions of interest (ROI) from T1-weighted scan data. ROI corresponding to the arcuate nucleus (ARC) was generated with reference to a standard mouse brain atlas (Franklin and Paxinos, 1997). This ROI was selected on the basis that it is in close proximity to the median eminence where glutamate gains access to the brain (Hawkins, 2009) and also because iGluRs are expressed in this region of the mouse brain (Kia et al., 2002). MEMRI scans were performed on mice given bolous i.p. injections of MSG (100mg and 300mg) or saline (n=5/group) at the commencement of baseline acquisitions. MEMRI scans were then performed on mice first given either bolous i.p. injections of the NMDA receptor antagonist MK-801 (1mg) or the AMPA receptor antagonist NBQX twenty minutes prior to baseline acquisitions, and then followed by MSG (100mg) at the commencement of baseline acquisitions. All data are presented as mean ± sem. Differences in SI profile between the ROI in all experimental groups were analysed using generalized estimating equations (GEE) and the Mann-Whitney-U test. For assessment of disruption of the blood brain barrier (BBB), 30mg/kg Evans Blue dye was injected intravenously 10 minutes after i.p. injection of 300mg MSG under identical conditions to $MnCl_2$ infusion. Brains were removed frozen in liquid nitrogen and coronal slices were fixed on microscope slides for analysis.

## Results

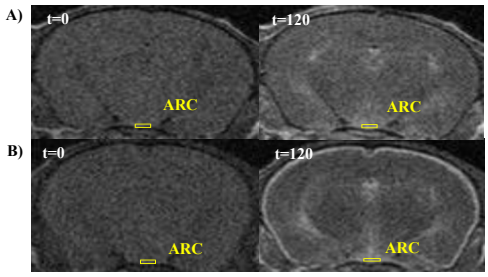


**Figure 1. T1-weighted image of a coronal slice of mouse brain 0 and 120 mins after $MnCl_2$ infusion in A) saline treated mice and B) 300mg treated mice MSG treated mice.**

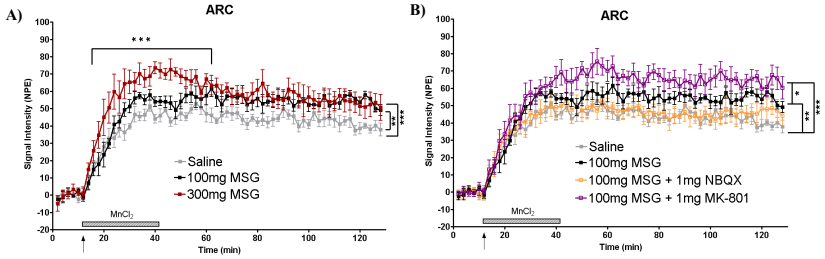


**Figure 2. Normalised percent enhancement (NPE) of MEMRI images of the hypothalamic arcuate nucleus (ARC) of mice treated with either A) saline, 100mg MSG or 300mg MSG or B) saline, 100mg MSG, 100mg MSG+1mg NBQX, or 100mg MSG + 1mg MK-801.**

Administration of MSG i.p. produced a significant dose-dependent increase in signal intensity (SI) in the ARC of the hypothalamus compared to saline treated controls ($p<0.01$ 100mg MSG vs saline; $p<0.001$ 300mg MSG vs Saline) (Figure 2A). This was entirely suppressed by coadministration of 1mg of the AMPA receptor antagonist NBQX, but not by 1mg of the NMDA receptor antagonist MK-801, which actually produced a significant increase in SI in the ARC compared to 100mg MSG alone ($p<0.05$) (Figure 2B). Higher doses of MSG also caused an increased SI in the region corresponding to the entire cerebral cortex (Figure1) which may be due to disruption of the blood brain barrier (BBB) rather than neuronal activation. Intravenous infusion of Evans Blue dye did not reveal any changes in BBB integrity however after treatment with 300mg MSG.

## Discussion

These results show that i.p. administration of 100mg MSG leads to the stimulation of hypothalamic neurons which is detectable with MEMRI *in vivo*. They further suggest that i.p. administration of 300mg of MSG causes a widespread cortical stimulation rather than BBB disruption, and also that activation of specifically the AMPA receptor is mainly responsible for the changes in neuronal excitability in the ARC following i.p. administration of 100mg MSG. They may also provide indirect evidence for the existence of presynaptic NMDA receptors in the mouse ARC.

## References

Franklin,K.B.J. and Paxinos,G. (1997). The Mouse Brain *in Stereotaxic Coordinates*. Academic Press. Hawkins,R.A. (2009). The blood-brain barrier and glutamate. Am. J. Clin. Nutr. Kia,H.K., Yen,G., Krebs,C.J., and Pfaff,D.W. (2002). Colocalization of estrogen receptor alpha and NMDA-2D mRNAs in amygdaloid and hypothalamic nuclei of the mouse brain. Brain Res. Mol. Brain Res. *104*, 47-54. Reddy,V.M., Meharg,S.S., and Ritter,S. (1986). Dose-related stimulation of feeding by systemic injections of monosodium glutamate. Physiol Behav. *38*, 465-469.