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The neurotoxic effect of monosodium glutamate (MSG) on the retinal ganglion cells of the albino rat

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Summary. Monosodium glutamate (MSG) administered postnatally to the albino rat causes extensive destruction of the retina. This MSG effect does not result in complete blindness. Ganglion cells surviving the MSG treatment are healthy and functional. Using retrogradally transported HRP and Nissl staining in whole mounted retinas, it was found that the ganglion cells left after MSG treatment are nut smaller than those in controls, that these cells do not belong to one cell size group, and that no cell size group is selectively missed. The results explain why photic entrainment of MSG treated animals is still possible.

Key words: Monosodium glutamate - Retinal ganglion cells - Rat

Introduction

Physiological and behavioural abnormalities have been demonstrated in rodents, following neonatal parenteral administration of MSG (Nemeroff et al., 1977; Olney, 1969). Precocious puberty, sterility and obesity have been reported in the literature (Nemeroff et al., 1977; Olney. 1969).

Considerable histological changes have been found in the hypothalamic arcuate nuclei (Marani et al., 1982; Schiethart et al., 1983) and in the optic system (Lucas et al., 1957; Cohen, 1967; Olney, 1969; Hansson, 1970; hlarani et al., 1984) after administration of MSG. The neurotoxicity of MSG causes massive degeneration of the inner layer of the retina, i.e. the bipolar cell layer and the ganglion cell layer, but leaves unharmed the outer layer of the retina: the receptor cells (Lucas et al., 1957; Cohen 1967; Olney, 1969; Hansson, 1970). This degeneration does not result in complete blindness (Groos, 1981).

However, indications that the visual system is not unaffected by MSG-induced retinal degeneration are: a) the absence of the visual placing response in such animals (Nemeroff et al., 1977), b) on the electroretinogram only the a-wave is found and the b-wave is lost (Potts et al., 1960), c) the MSG-treated animals demonstrate a reduction in the density of retinal terminals in the superior colliculi and in the dorsal lateral geniculate nuclei (Piekard, 1982).

On the other hand, the MSG-induced retinal damage does not lead to abnormalities in the photic entrainment of circadian rhythms (hlarani et al., 1985, Rietveld, in press), an indication that the retinal-suprachiasmatic projections are still functional. Groos (1981) found a normal proportion of dorsal lateral geniculate nucleus cells still responding to visual stimulations in MSG-treated rats. Moreover, MSG-treated animals have a normal direct pupil response (Cohen, 1967). These observations indicate that the ganglion cells left after MSG treatment are healthy and functional.

Reports concerning the ganglion cells of MSG-treated animals mention that the remaining ganglion cells are smaller than those of controls (Cohen, 1967; Hansson, 1970). Hansson (1970) thought this to be due to immaturity, while Cohen (1967) considered it likely to be an atrophy or an enhanced survival of a smaller ganglion cell variety. Olney (1969) reported that only a few ganglion cells appeared normal.

The aim of this study was to visualize the ganglion cells after MSG administration, in order to study their morphology. This has been achieved by retrogradely transported horseradish peroxidase (HRP) and by Nissl staining.

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Materials and methods

Wistar albino rats (W.A.G. strain) were treated neonatally with MSG, according to Marani (1982). Newborn albino rats were injected with MSG, 2.2 - 4.2 mg per gram birthweight, subcutaneously, during the first ten postnatal days.

Mature MSG-treated rats and mature control animals were kept under standard conditions (Marani et al., 1982). Mature rat weight varied from 300 g to 500 g for MSG-treated animals (n = 6) and from 250 g to 280 g for controls (n = 6).

Three MSG rats and three controls received 10 µl of 30% HRP (Sigma VI) in the nervus opticus bilaterally, under Hypnorm and Nembutal anaesthesia; survival time was 24 hours. The animals were perfused intracardially by lactated Ringer's solution with 5% sucrose, followed by Karnovsky's fixative (1.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M sodiumphosphate buffer, pH 7.6), again followed by lactated Ringer (Stone, 1981). The retinas were dissected from the eyecups and the cobalt intensification protocol of Leventhal (Stone, 1981) was followed to visualize the HRP. The retinas were mounted with the ganglion cells oriented upward. Three animals were used to label four retinas. The control retinas for endogenous peroxidase showed no labelled somata. The HRP was also visualized in transverse sections. The incubation was carried out on the retina in situ in the eyecup, followed by cutting the eyecups on a cryostate: (14 µm thick sections). One eye of a MSG rat and one eye of a control rat were used for this purpose.

Nissl coloured whole mounted retinas were obtained by placing the eyeballs of two MSG rats and two control rats in 4% formaldehyde for 4 days. The retinas were then dissected from the eyecups, mounted and stained with cresylviolet.

Moreover, transverse Nissl coloured sections were obtained from eyes embedded in nitrocellulose. Sections were 12 µm thick. One MSG rat and one control rat were used.

In order to ensure that the visual system was indeed impaired by MSG, the nervus opticus was examined and found to be reduced in diameter in all experimental animals (Marani et al., 1984).

The ganglion cells in the whole mounts were measured on photomicrographs, (magnification 700x). The mean of the longest and the shortest axes of the soma was taken as its diameter. No corrections for shrinkage of the retina nor for possible oedema of the ganglion cells were made, as the MSG retinas and the controls were treated in the same way. No attempt was made to quantify the number of measured ganglion cells left per retina (4).

Results

The cell density of the ganglion cell layer in MSG-treated rat retinas was much lower than the density in the control retinas, as can be clearly seen in the Nissl stained sections (Figs. 1,2,3,4). This agrees with earlier publications (Lucas, et al., 1957; Cohen, 1967; Olney, 1969; Hansson, 1970).

It is difficult to distinguish between ganglion cells and glia cells in Nissl stained retinas. Fukuda (1977) assumed that cell somata bigger than 6 µm in diameter containing irregularly stained Nissl granules belonged to the ganglion cell population. Applying Fukuda's criteria we found that not only glia cells were left after MSG treatment in the inner layer of the retina, but ganglion cells as well, since we measured cells with somata as big as 22 µm in diameter containing Nissl granules. Retinal ganglion cells can be defined in another way too, namely by the localization of the somata in the innermost neuronal layer and by the course of their axons through the nerve fibre layer entering the optic nerve (Bunt et al., 1974). HRP injected in the optic nerve is transported retrogradally to the cell somata in the retina. HRP loaded cells in the retina will therefore be ganglion cells. In this way, we were able to show that there were ganglion cells left after MSG administration and not only glia cells (Figs. 5,6,7,8).

Based upon the dimension of the cell diameter, ganglion cells have been classified into three groups: large (15 µm — 28 µm diameter), medium (10 µm — 14 µm diameter) and small (5 µm — 9 µm diameter) (Fukuda, 1977; Perry, 1979). In our experiments Nissl stained cell diameters varied from 8 µm to 22 µm for MSG-treated retinas and from 8 µm to 23 µm for controls.

Although only cells with Nissl granules were considered, the measured cells may still include glia cells as well (See Table 1).

In the HRP-labelled retinas, cell sizes varied from 11 µm to 24 µm in diameter in MSG-treated retinas and from 11 µm to 26 µm for controls (see Table 1).

Table 1. Ganglion cell diameter (µm) in normal and MSG-treated rat retina.

	NORMAL Retina			MSG treated retina		
	diameter (µm)			diameter (µm)		
	small	medium	large	small	medium	large
Data found in literature		5-28		small		
	5-9	10-14	15-28	no data present		
Nissl stained retinas	8-23 (N = 4)			8-22 (N = 4)		
	+	+	+	+	+	+
HRP labelled retinas	11-26 (N = 3)			11-24 (N = 3)		
	—	+	+	—	+	+

\+ Cells fitting in this group are found with the staining technique used.

— Cells fitting in this group are not found with the staining technique used.

N Number of retinas examined, in each retina more than twenty ganglion cells were counted.

Fig. 1. Normal retina. Photomicrograph of the ganglion cell layer in Nissl stained whole mounted retina. Bar represents 40 µm.

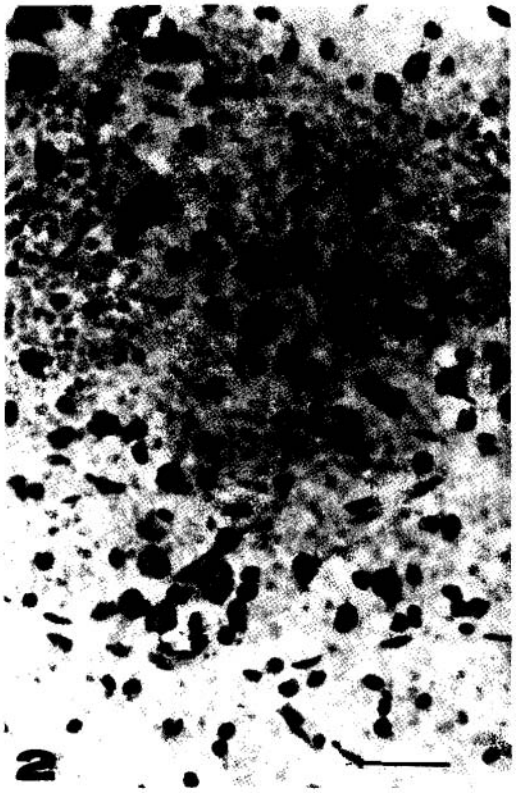

Fig. 2. MSG-treated retina. Photomicrograph of the ganglion cell layer in Nissl stained whole mounted retina. Bar represents 40 µm.

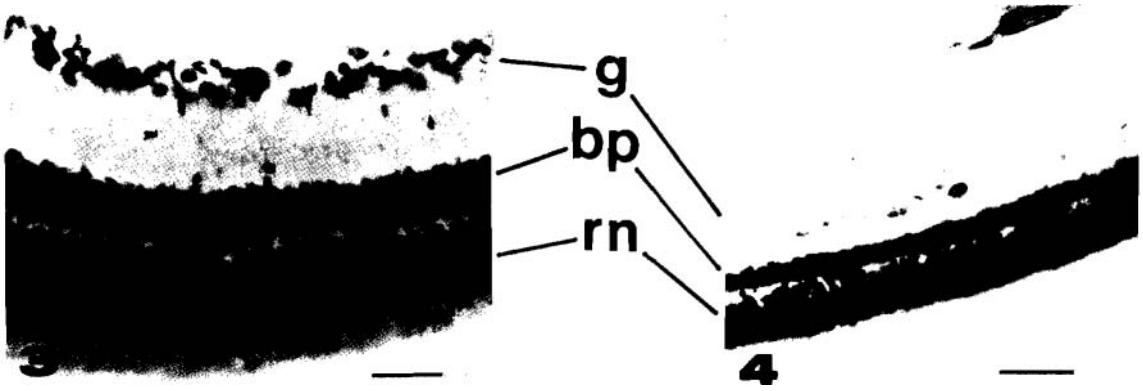

Fig. 3. Normal retina. Photomicrograph of Nissl stained transversal section of the retina: 12 µm thick. The levels indicated are those of the receptor cell nuclei (rn), the bipolar cell layer (bp) and the ganglion cell layer (g). Bar represents 40µm.

Fig. **4.** MSG-treated retina. Photomicrograph of Nissl stained transversal section of the retina: 12 µm thick. The levels indicated are those of the receptor cell nuclei (rn), the bipolar cell layer (bp) and the ganglion cell layer (g). Bar represents 40 µm.

Fig. 5. Normal retina. Photomicrograph of the ganglion cell layer in whole mounted retina. The rats were sacrificed 24 hours after injection of 10 µl 30% HRP in the nervus opticus. Bar represents 40 pm.

Fig. **6.** MSG-treated retina. Photom~crographof the ganglion cell layer in whole mounted retina. The rats were sacrificed 24 hours after injection of 10 µl 30% HRP in the nervus opticus. Bar represents 40 µm.

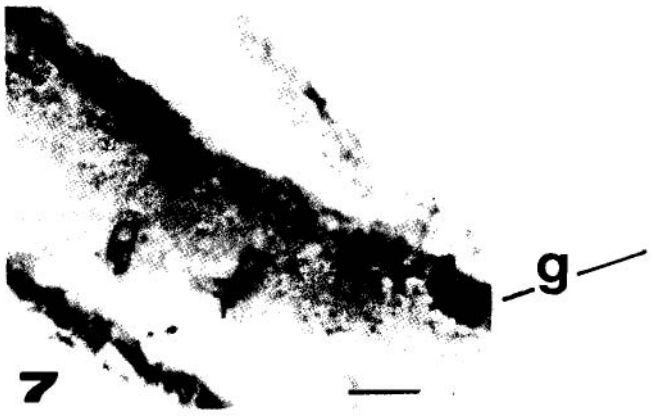

Fig. 7. Normal retina. Photomicrograph of transversal section of the retina, 14 µm thick. The rats were sacrificed 24 hours after injection of 10 µl 30% HRP In the nervus opticus. The level indicated (g) in the retina is the ganglion cell layer. Bar represents 40 pm.

Fig. **8.** MSG-treated retina. Photomicrographs of transversal section of the retina, 14 µm thick. The rats were sacrificed 24 hours after injection of 10 µl 30% HRP in the nervus opticus. The level indicated (g) in the retina is the ganglion cell layer. Bar represents 40 µm.

Discussion

The HRP results show that at least medium-sized and large ganglion cell can be found in the MSG-treated retina. From the Nissl stained retinas it can be concluded that, based upon Fukuda's criteria, small cells are present in the retina of MSG-treated rats as well.

It is remarkable that the small cells in the ganglion cell layer of the retinas examined (MSG as well as controls) remain free of HRP label. This was also found by Bunt et al., (1974), although further morphological studies are necessary to interprete this renewed finding.

Earlier reports suggest that the cells left in the ganglion cell layer of the retina after MSG administration are much smaller than those in controls, due to either immaturity (Hansson, 1970), atrophias or enhanced survival of a smaller ganglion cell variety (Cohen, 1967), as found in the arcuate nucleus (Schiethart, 1983) too.

Our results suggest that the ganglion cells left after MSG treatment are not smaller than those in controls and that, as far as the cell soma size is concerned, no cell groups are selectively missed.

Analysis of the axon caliber distribution in the optic nerve of MSG-treated animals by Marani et al., (1984) showed a decrease in the number of axons with a small diameter as well as a decrease in the number of axons with a large diameter, mainly leaving the axons with intermediate diameters unharmed. It has been assumed that the bigger the soma size of the ganglion cell, the bigger is the diameter of its extra-retinal axon (Fukuda, 1977). Analysis of the axon diameter distribution therefore suggests a decrease in the small and large ganglion cells. In order to verify this suggestion, quantification of the diameter of the ganglion cells in normal and MSG retinas is necessary. In the present study, however, no such quantification has been undertaken.

It can be concluded, therefore, that the cell density of the ganglion cell layer in MSG-treated rat retinas is much smaller than the density in the control retinas and that not only glia cells but also real ganglion cells can be found remaining in the ganglion cell layer.

The ganglion cells left in the retina after MSG administration are not smaller than those in controls, they do not belong to one cell size group, nor is one cell size group selectively missed. The results explain why photic entrainment of MSG-treated animals is still possible.

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