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Apoptosis in the mechanisms of neuronal plasticity in the developing visual system

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ABSTRACT. *Visual experience during early postnatal life is essential for normal development of synaptic connections in the visual system. In fact, altered visual experiences such as monocular deprivation (MD) or abnormal visual stimulation (e.g. strabismus, anisometropia) during this period disrupt the physiologic organization of the visual pathway, leading to loss of visual responses in cortical neurons and reduction in visual acuity of the affected eye, so that it becomes amblyopic. The authors review the main functional and morphologic changes induced by altered visual experiences in the developing visual system and focus on the recent discovery that MD induces apoptotic cell death in the lateral geniculate nucleus of newborn rats. Particular attention is given to the authors' studies documenting that, during development, MD leads retinal terminals to release excessive glutamate in the lateral geniculate nucleus where it elevates nitric oxide and causes DNA fragmentation. The latter event is known to activate poly-(ADP-ribose) polymerase, which in turn may trigger apoptosis. Better understanding of the mechanisms underlying the morphologic changes induced by altered visual experiences during development may open new venues for studying novel neuroprotective strategies for amblyopia and, more generally, for the treatment of ophthalmic diseases associated with neuronal apoptosis.* Eur J Ophthalmol 2003; 13: (Suppl3): S36-S43

KEY WORDS. *Amblyopia, Apoptosis, Lateral geniculate nucleus, Monocular deprivation*

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INTRODUCTION

The mammalian visual pathway is a model system for studying activity-dependent mechanisms that regulate the development and refinement of connections in the central nervous system. The foundation for this statement was laid down three decades ago by the experiments of Wiesel and Hubel (1). They first established that in kittens, at about 1 month of age, most cortical neurons respond to stimulation of either eye with varying degrees of ocular dominance. If the animal is allowed to develop in a normal visual environment, these binocular connections are maintained; however, they also observed that during a critical period of postnatal life, deprivation of vision in one eye (monocular deprivation [MD]) causes dramatic changes in ocular dominance, leading the large majority of cortical neurons to lose responsiveness to the deprived eye. This selective depression of synaptic transmission is associated with severely reduced spatial sensitivity, so that the deprived eye becomes blind, although it is anatomically intact at reopening (2). Similar forms of deprivation-induced synaptic depression have been observed in many species, including monkeys (3), rats (4), and mice (5). In humans, it is well known that altered visual experience during postnatal life, such as monocular or binocular visual deprivation (i.e., congenital cataract, ptosis) or abnormal visual stimulation

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(strabismus, anisometropia), induces a progressive decline of the visual performance of the affected eye. This neuro-ophthalmologic disease, termed amblyopia, affects approximately 4% of the world population (6), accounting for more than all other forms of blindness considered together. During the last 35 years, many studies have been devoted to the understanding of the mechanisms underlying this dramatic change in the physiology of the visual system, but new insights have been gained only recently. In this article, we briefly review some of the functional and morphologic changes induced by MD in the visual system. Particular emphasis is given to the recent discovery of the mechanism underlying neuronal cell death caused by MD in the lateral geniculate nucleus (LGN) because the latter may open a new venue for studying novel therapeutic strategies for the treatment of amblyopia.

Functional characteristics of MD-induced plasticity

The sensitivity of the visual system to MD is limited to a time period, also referred to as the critical period, during early postnatal life (7). At the peak of this time window, brief episodes of monocular vision, as short as 4 hours, are sufficient to reduce binocularity and to shift the ocular performance of visual cortical neurons toward the nondeprived eye (8). At the end of the critical period, ocular preference of cortical neurons is stabilized and no longer will be influenced by manipulation of the visual environment.

If the eye that was originally deprived is reopened and the previously open eye is closed (reverse lid suture) the effects of MD are reversible (9). This demonstrates another degree in the plasticity of the geniculocortical pathway. However, the rate and the extent of the physiologic changes produced by reverse closure are conditioned by the age of the animal and the length of the original deprivation (10). Electrophysiologic recovery is usually accompanied by an equally rapid improvement of the visual performance of the originally closed eye as determined behaviorally. However, the recovery is often temporary, and frequently the animals are left with severe bilateral amblyopia after consecutive periods of reverse occlusion (11). These findings suggest that the impaired morphology of both eyes' pathways after reverse suture may have behavioral consequences that could not be predicted from the currently documented physiologic changes (12).

MD induces greater synaptic depression than does binocular deprivation (10) and, as reported, the effects of MD are more severe than the effects of binocular deprivation (10). These data support the concept that the ocular dominance of cortical neurons is established and retained by a mechanism of activity-mediated competition between the pathways carrying information from the two eyes. It should be noted, however, that binocular deprivation produces significant depression of visual responsiveness with a rapid time course (10), thus indicating that noncompetitive mechanisms are also involved in the processes of MD-induced plasticity. Finally, it has been observed that synaptic depression in the visual cortex may also be induced by other experimental procedures, such as applying a contact lens that alters the visual image projected to the retina in one eye or deviating the eye (10).

MD-induces reversible morphologic changes

The LGN is the relay visual station of the visual pathway that is most affected by MD. Wiesel and Hubel (1) first observed in kittens that, in the laminae of the LGN receiving afferents from the deprived eye, the diameter of the neuronal cells is about two-thirds of that of neurons in the experienced layers. Further studies have confirmed the occurrence of neuronal cell shrinkage (13) and, more importantly, the latter has also been reported postmortem in the LGN of patients with amblyopia (14). Interestingly, of the two main classes of relay cells in the LGN, Y cells seem particularly sensitive to physiologic, metabolic, and morphologic effects elicited by visual deprivation (13, 15). For instance, it has been shown that deprived Y cells are 58% as large as nondeprived Y cells and that lid suture is associated with a slight reduction in the proportion of Y cells in deprived laminae (a ratio of Y cells to X cells of 0.8 versus 1.0 in nondeprived laminae) (16). In addition, several reports have documented a decrease in the proportion of physiologically recordable Y cells in the deprived layers of the LGN (13).

MD causes apoptotic cell death in the LGN

The electrophysiologic findings described above may be in part the result of sampling bias arising from the reduced cell size; it has been proposed that MD may

render Y cells inactive or may convert them into elements with electrophysiologic properties similar to those of X cells. Another possibility is that, besides the anatomic and physiologic changes occurring in some of the Y cell populations, there may be a loss of neuronal cells induced by MD. Indeed, we recently reported that MD causes neuronal cell death in the LGN cells of newborn rats (17, 18) (Fig. 1). In particular, high magnification light microscopy analysis of hematoxylin and eosin (H&E)–stained tissue sections from the brain of MD rats documented the occurrence of marginalization and condensation of the nuclear matrix (19, 20) (Fig. 1C). The onset of nuclear DNA fragmentation has also been revealed by the observation in adjacent tissue sections, obtained from rats monocularly deprived for 2, 7, and 14 days, of LGN cells positive to the terminal transferase-based TUNEL staining technique (21) (Fig. 1B). Finally, cell death by MD was accompanied by the appearance in the LGN of cells that were immunopositive for the tumor suppressor protein p53 (22), a gene product often associated with apoptosis, and most of these cells presented pyknotic nuclei and apoptotic bodies (Fig. 1D). Collectively, these three criteria support the hypothesis that cell death caused by MD in the LGN of newborn rat may be of the apoptotic type (see 19 and 23). The latter deduction is also supported by the lack of activation/proliferation of neuroinflammatory cells (e.g., astrocytes or microglia) in the LGN as shown by immunohistochemical experiments using the glial fibrillary acid protein (GFAP) (specific marker for astroglia) (20) and OX-42, a specific marker for resting and amoeboid microglia (24). As a consequence of cell death, we observed that the number of viable cells in the deprived LGN progressively decreases as the interval of deprivation is prolonged. In fact, in H&E-stained sections the number of cells per mm^3 in the LGN contralateral to the deprived eye was reduced by approximately 10%, 27%, and 44% after 2, 7, or 14 days of deprivation, respectively.

These results have been obtained by estimating the total number of neurons per volume unit using the optical dissector (18). This method is based on the ability to optically section histologic planes by using microscope objectives with high numerical apertures that produce images with relatively shallow depths of focus. The focal plane (optical section) can be moved through the thickness of the tissue section, producing a continuous series of superimposed planes within which cell counting can be carried out with the dissector counting rules. In practice, this consists of counting the number of new objects that come into view as one focuses through a known tissue volume. The

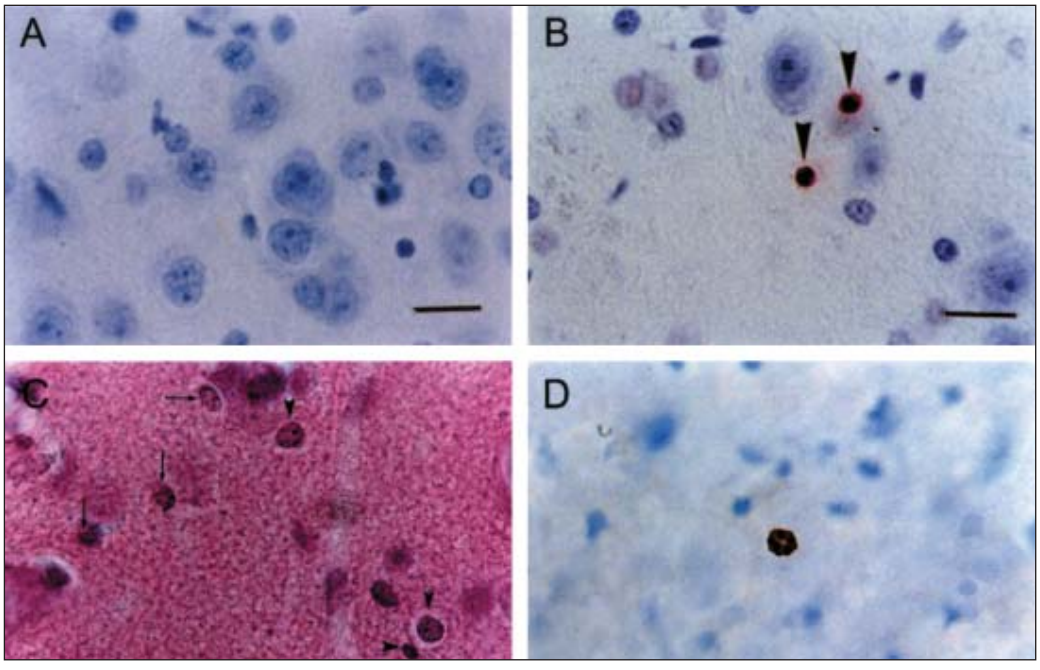

Fig. 1 - *Evidence that monocular deprivation (MD) triggers apoptosis in the lateral geniculate nucleus (LGN) of newborn rats.* In situ *DNA fragmentation (TUNEL-positive cells) is shown in the LGN of a brain tissue section obtained from a monocularly deprived rat (***B***). No TUNEL-positive cells were observed in the LGN of control rats (***A***). (***C***) Nuclei with condensed (arrowheads) and marginalized (arrows) chromatin are apparent in a haematoxylin and eosin–stained section from a newborn rat deprived in one eye for 7 days. (***D***) A cell immunopositive for the tumor suppressor protein p53 in a brain tissue section corresponding to the LGN of a monocularly deprived rat.*

dissector method permits the researcher to follow cells or synapses throughout their thickness and this avoids overestimation of profiles, which often results from splitting of cells or synapses during the process of tissue sectioning. Accordingly, it has been demonstrated that traditional counting techniques based on counting cell profiles or synapses in a reference area of two-dimensional probes may give rise to biases by as much as 40% when compared to an unbiased counting method such as the dissector (25).

Thus, our results indicate that MD is associated with neuronal cell death that is responsible for a progressive decrease in the total number of viable cells in the LGN. Incidentally, MD has been shown to reduce the number of LGN cells immunopositive for Cat-301, a chondroitin sulphate proteoglycan expressed at the surface of Y cells in the cat LGN (26); the magnitude of the reduction increased as the period of deprivation was extended, and these data have been interpreted by the authors as representing a genuine decrease in the number of Y cells in the LGN. More recently, Bickford et al (27) reported a significant reduction of SMI-32-positive neurons in the laminae of the LGN that received inputs from the deprived eye. The SMI-31 antibody stains the nonphosphorylated form of the high molecular weight neurofilament protein that in the LGN is expressed uniquely by Y cells. Similar results were obtained in the substantia nigra from the brain of patients with Parkinson's disease (28) and have been interpreted as reflecting an initial stage of neuronal degeneration in which neurofilaments are one of the first proteinaceous targets to be degraded. The occurrence of apoptotic cell death in the LGN of MD rats is in apparent contrast with the evidence that during the critical period of postnatal life the electrophysiologic and functional changes induced by monocular light deprivation are mostly reversible if the previously occluded eye is reopened and the originally opened eye is closed for a proportional period of time (10). However, it can be assumed that functional recovery may reflect the capacity of surviving neurons to compensate for the lost cells. This resource may also be conditioned by a reduction in the total number of viable neurons in the contralateral LGN that probably occurs during reverse lid suture. Thus, it can be hypothesized that the normal binocular vision after reverse lid suture results from a new equilibrium in the total number of viable neurons in the LGN of either side of the brain. This equilibrium is likely to be reached when MD is maintained for a limited period of time during the critical period, whereas prolonged visual deprivation leads to very few residual viable cells in the LGN and irreversible damage to vision.

Excitotoxicity is involved in MD-evoked apoptosis

Studies in kittens have demonstrated that residual activity in the deprived retina is implicated in the functional changes induced by MD in the visual cortex (29, 30). In fact, it has been observed that elimination of retinal activity by intraocular injection of tetrodotoxin prevents the synaptic depression induced by MD in the visual cortex (30). Presynaptic activity is an important element in maintaining synaptic effectiveness. It has been proposed (31) that activation of an excitatory input will lead to an increase or decrease in synaptic effectiveness depending on whether the coincident activity of the postsynaptic neuron falls above or below a critical value. The condition required for long-term synaptic potentiation (LTP) is the pairing of presynaptic activity with strong postsynaptic depolarization, whereas the suggested condition for long-term synaptic depression (LTD) is presynaptic activity that consistently fails to evoke or correlate with a postsynaptic response large enough to trigger LTP (32). The occurrence of LTD has been documented in the visual cortex of MD kitten (33) and because during MD the depression occurs only at the synapses that receive the presynaptic stimulation, the phenomenon has been referred to as homosynaptic LTD (30). In accordance with these data, we have observed that presynaptic retinal activity is also necessary for the induction of neuronal death in the LGN of MD rats. In fact, we have shown that optic nerve transection prevents cell death in the LGN (18). The importance of retinal activity has been further confirmed by experiments in which we injected the tetanus toxin (TeNT), a neurotoxin that blocks the synaptic release of neurotransmitter (see 34), into the eye. Forty-eight hours after injections—the approximate time required for the toxin to reach the LGN via the optic nerve—the right eyelids of newborn rats were sutured for 48 hours. Interestingly, we observed that intraocular injections of TeNT 48 hours prior to MD significantly reduced the number of apop-

totic cells in the LGN compared to those in uninjected monocularly deprived animals (35). The involvement of retinal activity in the induction of LTD and cell death during MD suggests that these two events could be related. It can be hypothesized that apoptosis represents a morphologic change consequent to the induction of LTD, and that the neurochemical modifications responsible for the induction of LTD in the visual cortex may retrogradely be involved in the mechanisms of MD-evoked apoptosis in the LGN.

The most likely candidate to mediate these events is glutamate, an excitatory neurotransmitter involved in the mechanisms of synaptic plasticity that is continuously released in the LGN by optic nerve terminals. Several studies have documented that N-methyl-D-aspartate (NMDA) and non-NMDA subtypes of glutamate receptors, besides their physiologic function as mediators of visual transmission, are also involved in the mechanisms of activity-dependent refinement of visual topography; for example, during segregation of kitten geniculocortical afferent into ocular dominance columns (36) or in the maintenance of retinotectal topography of frogs during development (37). A role for the NMDA subtype of glutamate receptor has also been documented in the mechanisms of synaptic plasticity in the visual cortex. In particular, it has been observed that infusion of the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (APV) into the visual cortex reduces the ocular dominance shift and prevents the neuronal shrinkage induced by MD (38). In agreement with these data, we documented that the glutamatergic pathway is also involved in the mechanisms of MD-evoked cell death in the LGN of newborn rats. In fact, systemic administration of selective antagonists of the NMDA (e.g., MK801 and CGP040116) or non-NMDA (GYKI52466) glutamate receptor subtypes protected against cell death in the LGN evoked by MD for 7 days. In particular, administration of MK801 or CGP040116 reduced the number of TUNEL-positive cells by 73% and 79%, respectively, whereas administration of GYKI52466 yielded a 91% reduction (18). In addition, we observed that both NMDA and non-NMDA glutamate receptor antagonists protected rats from MD-induced cell loss in the LGN. Thus, excessive glutamatergic stimulation seems to be involved in the mechanisms of cell death evoked by MD (18).

Transient glutamate stimulation can lead to sustained opening of NMDA and non-NMDA receptor-gated ionic channels and the subsequent rise in cytosolic Ca^{2+} can stimulate nitric oxide (NO) production (see 39), a highly reactive radical species that plays important physiologic roles in the CNS (40) and has also been involved as a downstream mediator in various neurodegenerative processes (41). Interestingly, it has been reported that NO is necessary for the transmission of visual input under normal visual stimulation and it is directly involved in visual information processing at the level of the LGN (42); in the latter nucleus, NOS immunoreactive perikarya are observed from birth and increase until the end of the third postnatal week, when they achieve the staining observed in adulthood (43). Interestingly, evidence indicates that NO acts together with NMDA receptors in the activity-dependent refinement of the visual connections during development. In particular, in ferret, the formation of ON/OFF sublaminae in the retinogeniculate connection is disrupted in vivo by treating the animals with NMDA receptor antagonists (44) or with inhibitors of NO synthesis (45). In the chick, transient retinotectal projections are removed in an activity- and NMDA receptor–dependent manner (46) whereas NOS inhibitors prevent their removal (47). NO also has been involved in the process of neuronal plasticity induced by MD because monocular lid suture in kitten increases the expression of NADPH-diaphorase in Y cells of the LGN (48).

To investigate NOS activity in the LGN, we measured the content of citrulline, a coproduct of NO synthesis, at different times after MD by high performance liquid chromatography. We observed that citrulline levels increase significantly in the LGN of newborn rats after 1 and 2 days of MD. This increase was transient; its levels were again similar to those of age-matched nondeprived controls after 7 days of MD (49).

Interestingly, the increase in citrulline levels observed after 1 and 2 days of MD was abolished by treatment with either the NMDA receptor antagonist MK801 or the non-NMDA receptor antagonist GYKI (50). In addition, the prevention of augmented citrulline production by the NOS inhibitor, L-NAME, confirmed that NOS becomes active after MD. As control, we used D-NAME (18), which is structurally similar to L-NAME, but inhibits NOS very poorly. Accordingly, D-NAME was ineffective.

To test whether the observed activation of NOS during MD was involved in the induction of neuronal apop-

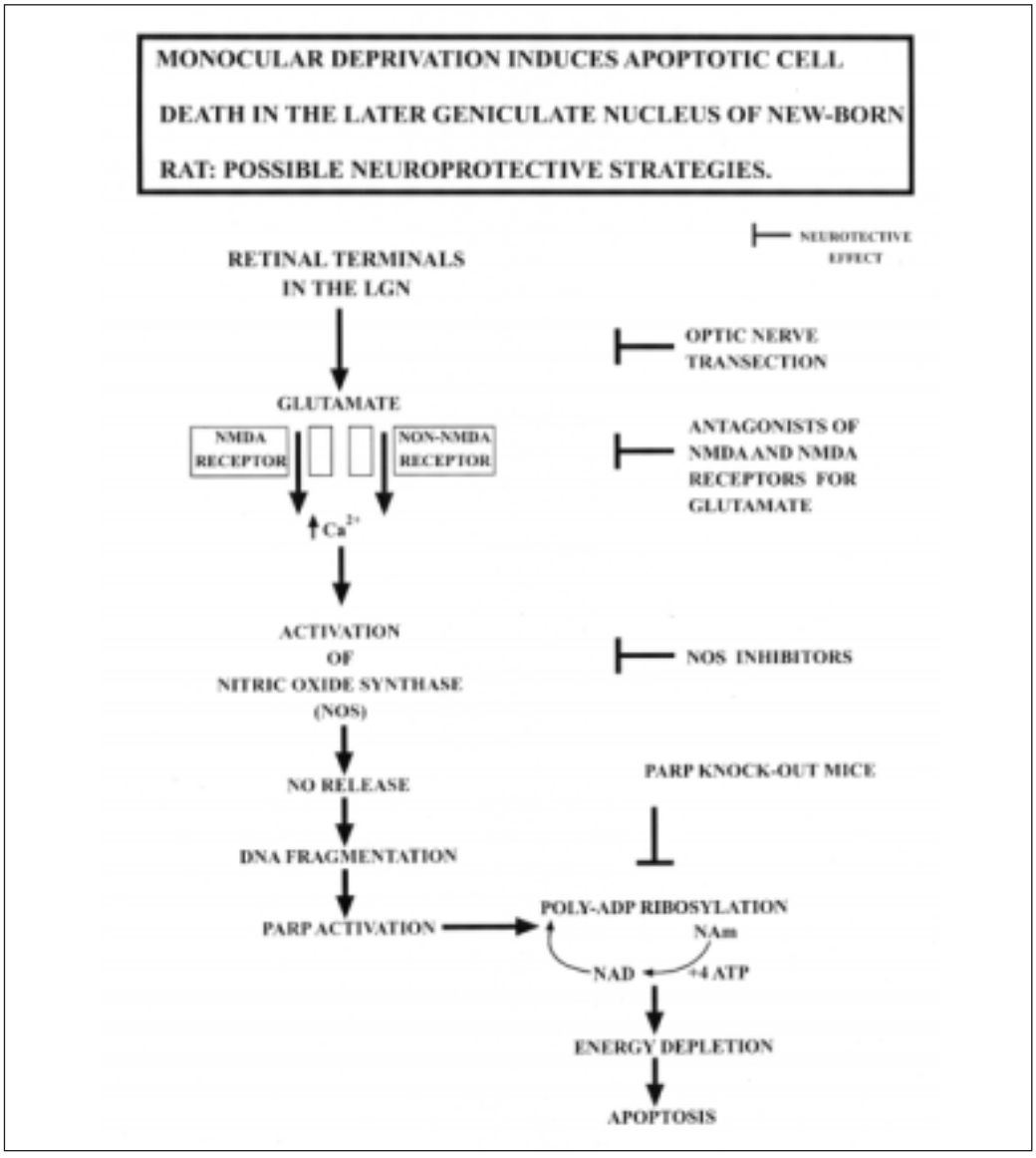

Fig. 2 - *Monocular deprivation (MD) during early postnatal life induces excitotoxic, nitric oxide–mediated cell death in the lateral geniculate nucleus (LGN) that appears of the apoptotic type and requires poly(ADP)ribose polymerase (PARP) activation. Blocking retinal signalling to the LGN by optic nerve transection or systemic treatments with either glutamate receptor antagonists or inhibitors of nitric oxide synthase prevented MD-induced cell death (as indicated by the T bars). Also, absence of the PARP gene seems to confer significant neuroprotective properties in the LGN.*

tosis, we treated the animals with L-NAME or D-NAME during 7 days of MD and we observed that administration of L-NAME but not D-NAME prevented apoptosis in the LGN of deprived rats.

Collectively, these data suggest that during the early period of postnatal life, excessive stimulation of NMDA and non-NMDA glutamate receptors may elevate NO synthesis and this may be involved in the mechanism of apoptosis induced by MD.

Notably, it has been proposed that the activation of poly(ADP)ribose polymerase (PARP) is a key mechanism for NO toxicity and glutamate toxicity, which requires NOS activation (51). PARP is a nuclear enzyme that is activated by DNA strand breaks to participate in DNA repair. However, excessive activation of PARP can deplete tissue stores of nicotinamide adenine dinucleotide, with the resultant depletion of energy and cell death.

In particular, a role for PARP has been demonstrated in neuronal death in stroke, in excitotoxin-exposed cortical neurons, and in substantia nigra of MPP-exposed animals. To investigate PARP in MD-triggered

apoptosis, we used mice lacking the PARP gene, which were donated by Z.Q. Wang (IARC, Lyons, France) (52). Initial experiments showed that MD in mice caused neuronal apoptosis in the LGN with features virtually indistinguishable from those observed in rats. Then, both wild-type and -/- mice were treated as described for rats, and apoptosis in the LGN was scored. Interestingly, we observed that apoptosis was reduced in a statistically significant fashion in PARP -/- mice as compared to wild-type mice (53).

Based on these findings, the most likely sequence of events triggered by MD during development involves glutamatergic signals, which lead to NOS activation with increased NO production, activation of PARP, and apoptosis (Fig. 2).

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