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Apoptotic neuron death in rat substantia nigra induced by striatal excitotoxic injury is developmentally dependent

William J. Kelly, Robert E. Burke*

Department of Neurology, Columbia University, Black Building, 650 West 168th Street, New York, NY 10032, USA

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Abstract

We have previously observed that an axon-sparing lesion of the striatum during development is associated with an induction of apoptotic cell death in the substantia nigra (SN). We have postulated that the induced death is due to a loss of striatum-derived trophic support. In other paradigms of neural development, it is often found that a need for trophic support is primarily observed only during a critical development period. We have therefore examined the time course for early striatal lesion to induce cell death in substantia nigra. We find that induction of apoptotic cell death is largely restricted to the first 2 postnatal weeks. After that time, induction of death in SN pars compacta abates. In SN pars reticulata, apoptotic death also abates, but by postnatal day 28, a non-apoptotic morphology of death appears. Thus, induced apoptotic death in SN is restricted to a critical developmental period. Copyright © 1996 Elsevier Science Ireland Ltd.

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We have previously observed that an axon-sparing injury to the striatum made with the excitotoxin quinolinic acid (QA) early in development results in a diminished number of nigro-striatal dopaminergic neurons in maturity [2]. This decrease occurs in the absence of any direct injury to the substantia nigra (SN), and suggests that the nigro-striatal dopaminergic system may be dependent on its target, the striatum, for support during development. Such a possibility is also supported by many earlier in vitro studies demonstrating the ability of different striatal preparations to support the viability and differentiation of cultured embryonic mesencephalic dopaminergic neurons [15]. If the target striatum does support the developing nigro-striatal dopaminergic system, then it would be anticipated, on the basis of classic neurotrophic theory, that such support would be mediated, at least in part, by retrogradely transported factors derived from striatum which act to suppress a natural cell death event in SN. We have found that natural cell death does occur postnatally in the SN pars compacta (SNpc) [4], and that the magnitude of this death event is augmented by an excitotoxic injury to the striatum on postnatal day (PND) 7 [10].

For many developing neural systems which are dependent on their target, there is a critical period of time during which the support is necessary for viability [12]. For example, rat sensory neurons lose nerve growth factor (NGF) dependence over a 2 week period after birth [8]. To evaluate whether there may be a similar period limited to development when striatal injury results in induced death in the nigro-striatal system, we examined the effect of extensive excitotoxic striatal injury in adult animals on the SNpc [17]. Such a lesion did not result in the induction of cell death in SNpc, in keeping with the observation by Lundberg et al. [9] that there is no loss of nigro-striatal neurons. Although a striatal excitotoxic lesion in adults results in induced transneuronal neuron death in SN pars reticulata (SNpr) [16,17], the morphology is not apoptotic, so it may be unrelated to the apoptotic death observed during development.

In order to define the period of time during development when nigro-striatal neurons are dependent on striatal support, we have performed striatal lesions at successive developmental ages and have quantified the level of induced apoptotic cell death in SNpc and SNpr.

* Corresponding author. Room 308. Tel.: +1 212 3057374; fax: +1 212 3055450; e-mail: rb43@columbia.edu

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Timed pregnant female rats were obtained from Charles River Lab. (Wilmington, MA, USA). On PND 7, rat pups were anesthetized with metofane by inhalation and hypothermia. A burr hole was placed 3.0 mm lateral to bregma along the coronal suture, and a 28-gauge cannula was inserted into the brain to a depth of 4.0 mm below the skull. QA, at a concentration of 480 nmol/μl (in 0.1 M phosphate-buffered saline (PBS), pH 7.2) was infused by pump at a rate of 0.5 μl/min. At the end of the infusion, the cannula was slowly withdrawn after an interval of 2.0 min. Control animals received vehicle alone. A similar procedure was used for PND 14 pups. At PND 21 and 28, the cannula was inserted to a depth of 5.0 mm. PND 28 animals were anesthetized with pentobarbital 60 mg/kg. On postlesion day (PLD) 2 or 4, rats were sacrificed by induction of deep anesthesia and perfusion fixation. Rats were perfused transcardially with 0.9% cold saline for 1 min followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.1). Brains were then post-fixed for at least 1 week. After cryoprotection overnight in 20% sucrose in fixative, each brain was rapidly frozen in 2-methylbutane in dry ice. The entire SN was sectioned serially at 30 μm for silver staining, and representative striatal sections from Paxinos and Watson [13] 9.2–9.7 were collected for Nissl staining to confirm lesion placement, and to determine its size by image analysis.

Apoptotic profiles in SN were identified by the silver impregnation technique, as previously described [10].

Quantitative analysis of the number of silver-impregnated apoptotic profiles was performed by scanning the SN both ipsilateral and contralateral to the striatal lesion in QA and vehicle-injected controls. Each SN was scanned at 400 × over its entire dorsal-ventral and medial-lateral extent. Profiles were identified as apoptotic and counted only if they contained one or more deeply stained, rounded chromatin clumps, surrounded by a cellular profile, as previously described [4,10]. Bare extracellular chromatin clumps were not counted. In order to sample the complete rostro-caudal extent of the SN, two or more sections from each of Paxinos and Watson planes 4.2, 3.7 and 3.2 were examined. Within each plane, values for apoptotic profiles for all sections were averaged to give a mean value for the plane; the mean values from the planes were then added to provide a measure of the number of apoptotic profiles in the SN for each animal. In order to examine the specific induction of cell death by the lesion, the number of apoptotic profiles due to natural cell death on the non-lesioned side was subtracted for each animal.

In order to quantify the extent of the striatal lesion induced by QA, image analysis of the lesion was performed on Nissl-stained sections from planes 9.2–9.7; a mean of 3.6 sections per animal were examined. Each section was digitized on a Loats Associates inquiry image analysis system, and the maximum and minimum optical density values were measured. Values for preserved Nissl staining were defined as all optical density values ≥50% of the difference between maximum and minimum staining. The area of the striatum was measured. The percent lesion was defined as 100% − ((area of preserved Nissl stain/striatal area) × 100%). The percent lesion values for each section were averaged to provide a mean value for each animal.

On PND 7, QA lesion of the striatum led to a clear induction of apoptotic cell death in both SNpc and SNpr at 2 days postlesion, as we had previously observed [10] (Fig. 1A,B). In the present study, we find that striatal lesion at PND 14 results in a comparable level of induced apoptotic cell death in both SNpc and SNpr (Fig. 1A,B). However, on PND 21 and 28, while there was a tendency for a low level of induction to occur, this did not achieve significance in comparison to effects at PND 7 and 14, for

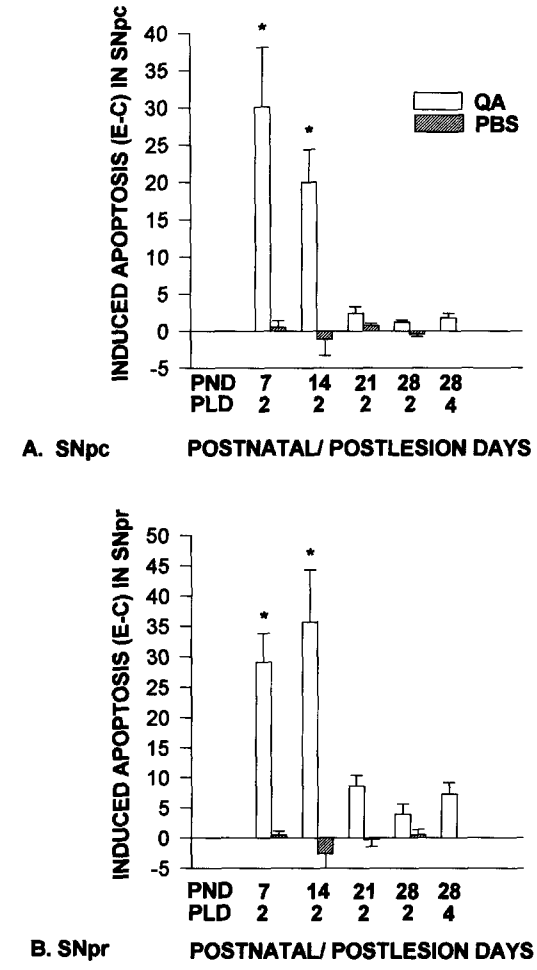

Fig. 1. Quantitative analysis of the induction of apoptotic cell death in SN by striatal QA lesion at varying postnatal and postlesion times. (A) SNpc. Striatal QA lesion led to an induction of apoptotic cell death at PND 7 and 14 ($P < 0.0001$, ANOVA). Post hoc analysis (Newman–Keuls) revealed a significant difference between the QA groups on PND 7 and 14 and all other conditions. During postnatal development, there are varying levels of natural cell death in SNpc [4]. Therefore, for ease of comparison at different ages, the number of induced apoptotic profiles is expressed as the difference between the experimental (E) and control (C) sides. (B) SNpr. Striatal lesion led to an induction of apoptotic cell death on PND 7 and 14 ($P < 0.0001$, ANOVA).

both SNpc and SNpr. We considered the possibility that maturation may lead to a change in the time course of induced death in SN. For this reason, we also examined PND 28 animals at PLD 4. However, even at this later postlesion time, there was no significant induction of death in comparison to PND 7 and 14.

We also considered the possibility that the apparent developmental dependence of induced apoptotic death could be due to a change in the extent of striatal injury induced by QA rather than a change in target dependence. It has previously been shown that PND 7 animals are more sensitive to QA than older animals [18]. We therefore analyzed the extent of striatal injury in PND 14 animals as compared to PND 28. PND 14 animals showed a 90.2 ± 2.9% lesion of striatum, as compared to 87.6 ± 2.9% lesion in PND 28 animals; this was not a significant difference. A comparison of typical striatal lesions at PND 14 and 28 is shown in Fig. 2A,B.

We have previously observed that extensive striatal excitotoxic injury in adult animals is not associated with induction of apoptotic cell death in either SNpc or SNpr, but is associated with a non-apoptotic appearance of cell death in SNpr [17]. In the present study, non-apoptotic profiles in SNpr first made their appearance in PND 21 animals in very small numbers. They became more abundant on PND 28 when there were a mean of 8.5 ± 3.8 non-apoptotic cells in the SNpr among the three planes assessed (Paxinos and Watson 4.2, 3.7, 3.2). The morphology of these cells on silver stain was identical to that which we described in adults: the entire nucleus was silver-impregnated (without the appearance of apoptotic chromatin clumps), and the cytoplasm contained diffuse, punctate silver deposits [17].

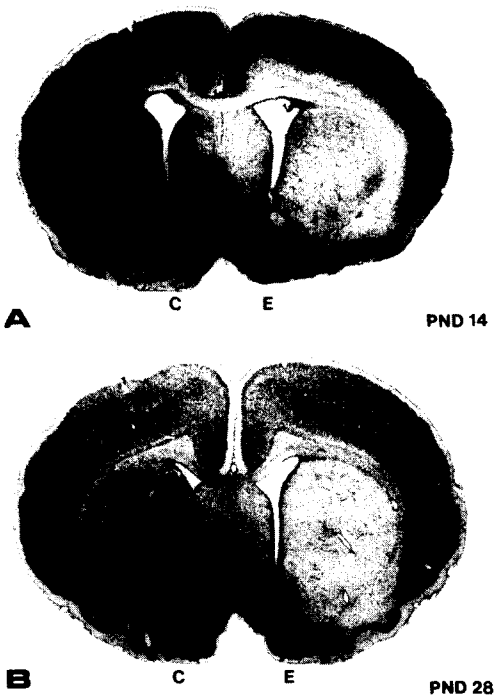

Fig. 2. Comparison of the extent of striatal lesion induced by QA in PND 14 and 28 animals. (A) PND 14. Nissl stain reveals pallor throughout the entire striatum, indicating an extensive loss of neurons. There is also evidence of neuron loss in overlying and ventral cortex. (B) PND 28. Like the lesion induced on PND 14, the lesion on PND 28 extends throughout the cross-section of the striatum. There is also evidence of neuron loss in adjacent ventro-lateral cortex.

These results show that the ability of an early axon-sparing injury of the striatal target to induce apoptotic cell death in SN [10] is developmentally dependent. The induction occurs with equal magnitude on PND 7 and 14, but is much diminished by PND 21 and 28. This developmental decrease in induced apoptotic death does not appear to be due to a shift in the time course, because the level of cell death at PND 28 was as low on PLD 4 as on PLD 2. Nor does the decrease in induced death appear to be due to a developmental decrease in the extent of the striatal injury induced by QA. We therefore conclude that the decrease in induced death in SN is probably due to a loss of dependence on striatum during the course of development.

This interpretation is compatible with many established precedents for developmental dependence on trophic support which wanes with maturity [12]. In relation to SNpc dopaminergic neurons in particular, the interpretation that they are developmentally dependent on their target, the striatum, is compatible with many prior observations in vitro demonstrating the ability of striatal preparations to support the viability of these neurons [15]. The concept that developing dopaminergic neurons are dependent on their target is also compatible with our recent observation that early destruction of dopaminergic terminals with the neurotoxin 6-hydroxydopamine, which spares intrinsic striatal neurons but eliminates target contact, also leads to induced apoptotic death [11]. Interestingly, in this model of induced apoptotic death in SNpc, there is a time course of developmental dependence which is identical to that which we have observed in the QA striatal injury model; i.e. a marked decrease in induced death after PND 14.

The consequences of striatal injury in mature animals are quite different for SNpr as compared to SNpc. While such injury does not result in any form of induced cell death in SNpc [9,17], in SNpr it results in a non-apoptotic form of cell death in its central region [17]. The present study shows that there is a transition from an apoptotic form of cell death at PND 7 and 14 to a non-apoptotic form by PND 28. The relationship between the early apoptotic form of death, and the later non-apoptotic form is unknown. It is possible that they share a similar mechanism, which is that loss of input from the striatum leads to the loss of afferent trophic support [7]. In both the early and later settings, this loss of support may lead to programmed cell death which is apoptotic in young animals, but non-apoptotic in older animals. There are precedents

for changes in the morphology of programmed cell death in the course of development [3]. Alternatively, it is possible that the early apoptotic death and the later non-apoptotic death in SNpr have different mechanisms. The death in mature animals has been postulated to be due to loss of inhibitory GABAergic input from the striatum [16], with a resulting imbalance in excitatory input, leading to neuron death. If such a mechanism were responsible, then it would be dependent on synaptic input. Our current observation, that such non-apoptotic death begins to appear on PND 21 is compatible with the possibility that it is mediated by a synaptic mechanism, because a major period of synapse formation in SN occurs from PND 15 to 30 [5].

While there is substantial evidence that dopaminergic neurons of the SNpc depend on striatal target-derived support during normal development, the factors mediating such support are unknown. It has been suggested that glial cell line-derived neurotrophic factor (GDNF) may play such a role, based on its ability to support dopamine neurons in embryonic culture [6], and its mRNA expression in striatum during development [14]. Interestingly, the highest levels of GDNF mRNA are expressed through the second postnatal week; after PND 14, they fall considerably [1]. This time course parallels the observations of the present study on the time course of target dependence of the SNpc.

In conclusion, the SN appears to be dependent on its anatomical relationships with the striatum through the second postnatal week, and this therefore appears to be the critical period during development when striatal factors may regulate natural cell death in SN.

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