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Research report

Maturation but not survival of dopaminergic nigrostriatal neurons is affected in developing and aging BDNF-deficient mice

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Abstract

Brain-derived neurotrophic factor (BDNF) promotes survival of injured dopaminergic nigrostriatal neurons of the adult rodent substantia nigra pars compacta, as well their development in vitro. BDNF deficiency may play a role in Parkinson's disease, as the surviving dopaminergic nigrostriatal neurons have reduced levels of BDNF, and a BDNF gene polymorphism is present in a subpopulation of patients. Here, we investigated whether a lack of BDNF in early postnatal BDNF−/− mice or a chronic 50% reduction in BDNF levels in aging BDNF+/− mice would affect the survival of the dopaminergic nigrostriatal neurons. In general terms, BDNF−/− and BDNF+/− mice had morphologically and quantitatively normal nigrostriatal neurons at any time between postnatal day 14 (P14) and 18 months, when compared to their wild-type littermates. BDNF−/− mice (P14 and P21 only) had fewer dopaminergic dendrites in the substantia nigra, suggesting that BDNF plays a role in phenotypic maturation, but not in neuronal birth or survival. BDNF−/− mice also had aberrant tyrosine hydroxylase (TH) positive cell bodies in the pars reticulata. During adulthood and aging, BDNF+/− mice performed equally well as their wild-type littermates in tests of motor coordination, and both showed aging-related decreases in the size of the dopaminergic neurons as well as in motor coordination. These results suggest that chronic deficits in BDNF alone do not affect survival or function of dopaminergic nigrostriatal neurons during aging or potentially even in Parkinson's disease.

Theme: Development and regeneration
Topic: Neurotrophic factors: biological effects

Keywords: Brain-derived neurotrophic factor; Substantia nigra; Knockout mice; Parkinson's disease; Tyrosine hydroxylase

1. Introduction

BDNF can promote neuronal survival, differentiation and functional activity in many regions of the mammalian brain [5,24,30,46,47,75,77]. This ubiquitous neurotrophic factor is synthesized by the dopaminergic nigrostriatal neurons and is both anterogradely transported to the neostriatum and retrogradely transported to the cell bodies [4,63]. The TrkB receptor is expressed by these neurons [54], suggesting that BDNF has an autocrine or paracrine action.

The trophic effects of BDNF in the substantia nigra have been studied extensively both in vitro and in vivo. Cultured fetal ventral mesencephalon neurons can be protected from developmental cell death by BDNF [30,31,36,69,78], whereas blockade of BDNF translation with antisense oligonucleotides decreases survival of dopaminergic cul-

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tures [40]. BDNF also protects against 6-OHDA, MPP+, glutamate and NO neurotoxicity in vitro [22,30,41,67,70] and can reduce the extent of nigral neuron death caused by neurotoxins or axotomy in adult rodents [3,22,25,35,41,65].

The role of endogenous BDNF in the adult nigrostriatal system is less clear. Mice that overexpress BDNF in noradrenergic neurons projecting to the nigra have 52% more dopaminergic nigrostriatal neurons [2]. BDNF−/− mice are not viable beyond 2–3 weeks of age, thus studies have focused on their early postnatal development. Several groups have found no qualitative difference in the dopaminergic neuron population in the BDNF−/− mice, although detailed quantification has not been performed [12,19,30,33,36].

In Parkinson's disease, progressively more dopaminergic nigrostriatal neurons degenerate over time. The surviving neurons have reduced levels of BDNF protein and mRNA [28,51,56], raising the possibility that chronically decreased BDNF contributes to neuronal loss. In addition, a single nucleotide polymorphism of the human BDNF gene, and subsequent BDNF deficiency, may be linked to idiopathic Parkinson's disease [48,52]. BDNF+/− mice have BDNF protein levels that are 50% of normal [4,38], and thus present an opportunity to study the potential harmful effects of a chronic deficit in trophic factor over time.

Here, we investigated in more quantitative detail the early postnatal fate of the dopaminergic nigrostriatal neurons in BDNF−/− mice and whether a chronic long-term reduction in BDNF would affect the survival and related motor function of nigrostriatal neurons in aging BDNF+/− mice.

2. Materials and methods

2.1. Animals

All animal procedures were in accordance with NIH guidelines, and all animals were euthanized by an overdose of sodium pentobarbital. Mice heterozygous for a null mutation in the BDNF gene locus (Stock $Bdnf^{tm1Jae}$, No. 002267, The Jackson Laboratory, Bar Harbor, ME) were bred to produce BDNF null mutant (−/−), heterozygote (+/−) and wild-type control (+/+) littermates. Pups were implanted subcutaneously with an identification microchip during the first postnatal week (Avid Canada, Calgary, AB) immediately before obtaining tail clippings for genotyping. Genotypes were determined by a published polymerase chain reaction (PCR) protocol [75]. As confirmation of the genotype, ear clippings from anesthetized animals were obtained just before euthanasia.

BDNF−/−, +/− and +/+ littermates were euthanized at P14 or P21 (n = 5, 8, 4 and 2, 5, 3, respectively). A second cohort of the same BDNF mutant line bred in a different facility (Queen's University, Canada) was used for analysis of the P14 and P21 mice (n = 5, 5, 3 and 0, 5, 4, respectively). Additional groups of both female and male heterozygote and wild-type littermates were housed together in groups of 4–5, fed ad libitum and lived for 3, 6, 12 or 18 months (n = 7, 6, 7, 11, and 6, 5, 6, 14 for BDNF+/− and wild-type, respectively). Older mice were not analyzed as the mortality rate of this strain increases sharply at greater ages. All data were collected in a manner so that the experimenter was blind to the animals' genotypes.

2.2. Behavioral testing

Groups of 6-, 12- and 18-month-old BDNF+/− and wild-type littermates were tested for motor-related behaviors in sequential tests during the 4 days before euthanasia. All testing was performed under low level red light conditions during the dark portion of the animals' 12 h:12 h light/dark cycle when the mice are naturally more active. The mice were not exposed to white light at any time during transport between the vivarium and the testing facility in an adjacent room.

During the first dark period, mice were videotaped from above while exploring an elevated plus maze for 3 min. A trained observer, blind to experimental conditions, completed the analysis of motor activity using event recording software which was able to provide the amount of time spent in open and closed arms, as well as the number of head dips, rearing and stretch-attend postures, and amount of grooming, in order to evaluate the level of anxiety for each mouse [44,60,64]. Anxiety can influence performance in movement-based behaviors [57].

During the second and third dark periods, mice were tested for motor coordination and balance on an Accu-Rotor Rotarod with a 3 cm diameter rod appropriate for mice (AccuScan Instruments, Columbus, OH). Animals were placed in the middle of a rotating rod in the direction opposite to rotation. The mice walked forward as the rod accelerated from 0.0 to 40.0 rpm over 300 s. When animals could not maintain pace with the rod, they fell a short distance into a holding container, triggering an infrared motion detector which recorded the latency to fall from the rod. The mean latency to fall over 10 trials (5 trials per dark period) was calculated. Shorter latencies indicate impaired motor coordination and balance, and the nigrostriatal system has a clear influence on rotarod performance [8,20,18,58,76].

Bradykinesia is one of the hallmarks of Parkinson's disease, and delayed initiation of movement in rodents may represent a behavioral correlate [11,42,71]. Therefore, during the fourth dark period, the mice were placed in the center of a 1.3 cm wide elevated beam with painted lines every 10 cm and allowed to voluntarily explore for 3 min while being videotaped from above. The time taken to initiate movement was measured and recorded to a maximum of 180 s, and the number of lines crossed was counted.