Experimental Neurology 249 (2013) 178–186

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

# Neurotrophic factors rescue basal forebrain cholinergic neurons and improve performance on a spatial learning test

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ARTICLE INFO

*Article history:*
Received 15 May 2013
Revised 5 August 2013
Accepted 22 August 2013
Available online 7 September 2013

*Keywords:*
Basal forebrain
ChAT
Choline acetyltransferase
Cholinergic
Neurotrophins
NGF
NT3
Target selection
Neuronal plasticity

ABSTRACT

This study investigated whether animals sustaining experimental damage to the basal forebrain cholinergic system would benefit from treatment with exogenous neurotrophic factors. Specifically, we set out to determine whether neurotrophic factors would rescue damaged cholinergic neurons and improve behavioral performance on a spatial learning and memory task. Adult rats received bilateral injections of either saline (controls) or 192 IgG-saporin to damage basal forebrain cholinergic neurons (BFCNs). Two weeks later, animals received implants of an Alzet mini-pump connected to cannulae implanted bilaterally in the lateral ventricles. Animals received infusions of nerve growth factor (NGF), neurotrophin 3 (NT3), a combination of NGF and NT3, or a saline control over a 4-week period. Compared to saline-treated controls, animals sustaining saporin-induced damage to BFCNs took significantly more trials to learn a delayed match to position task and also performed more poorly on subsequent tests, with increasing delays between test runs. In contrast, animals infused with neurotrophins after saporin treatment performed significantly better than animals receiving saline infusions; no differences were detected for performance scores among animals infused with NGF, NT3, or a combination of NGF and NT3. Studies of ChAT immunnocytochemical labeling of BFCNs revealed a reduction in the numbers of ChAT-positive neurons in septum, nucleus of diagonal band, and nucleus basalis in animals treated with saporin followed by saline infusions, whereas animals treated with infusions of NGF, NT3 or a combination of NGF and NT3 showed only modest reductions in ChAT-positive neurons. Together, these data support the notion that administration of neurotrophic factors can rescue basal forebrain cholinergic neurons and improve learning and memory performance in rats.



## Introduction

Basal forebrain cholinergic neurons (BFCNs) comprise a population of neurons that are distributed through the medial septum, diagonal band, substantia innominata, and medial globus pallidus (Butcher and Woolf, 2004; Mesulam et al., 1983). Cholinergic neurons in the substantia innominata and medial globus pallidus are sometimes considered to form the nucleus basalis, a term more appropriate for the human brain (nucleus basalis of Meynert) but also sometimes applied to rodents (nucleus basalis magnocellularis, nBM). The axons of this system provide much of the cholinergic innervation of the cerebral cortex (Baratta et al., 2001; Bruel-Jungerman et al., 2011; Butcher and Woolf, 2004; Mesulam et al., 1983). Experimental damage to BFCNs in laboratory animals has been shown to lead to deficits in learning and memory tasks, particularly those related to spatial memory (Deiana et al., 2011; Johnson et al., 2002; Marques Pereira et al., 2005; Ricceri et al., 2004). In addition, specific cholinergic lesions of the nBM can lead to the long-lasting reduction of acetylcholinesterase (AChE) positive fibers in the cerebral cortex (Szigeti et al., 2013). These results from laboratory animals have supported the idea that loss of cholinergic function in humans is associated with diminished cognitive abilities and may contribute to the development of Alzheimer's disease (Auld et al., 2002; Bartus et al., 1982; Craig et al., 2011; Mufson et al., 1999; Perry et al., 1992; Tuszynski et al., 2005).

The idea that cholinergic loss may contribute to Alzheimer's disease has led to the suggestion that treatment regimens that target the cholinergic system, primarily neurotrophic factors, may offer a viable therapy against the development of Alzheimer's disease (Tuszynski et al., 2005). Several studies have demonstrated that BFCNs are influenced by several neurotrophic factors, including nerve growth factor (NGF) and neurotrophic factor 3 (NT3) (Alderson et al., 1990; Dreyfus, 1989; Gähwiler et al., 1987; Ha et al., 1996, 1999; Li et al., 1995; Morse et al., 1993; Nonomura et al., 1996). These neurotrophic factors are produced by cells in the region of BFCN cell bodies, as well as in the cortical target regions of BFCN axonal projections, and they contribute to the

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0014-4886/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2013.08.012

development and maintenance of these cholinergic neurons and their projections (Dreyfus, 1989; Gähwiler et al., 1987; Ha et al., 1999; Lauterborn et al., 1994; Robertson et al., 2006; van der Zee et al., 1992).

The present study used an experimental model to determine whether exogenous neurotrophic factors might reduce the deleterious consequences of BFCN damage. Following treatment of rats with 192 IgG-saporin to partially damage the basal forebrain cholinergic system, animals were tested to determine if administration of NGF, NT3, or a combination of NGF and NT3 would improve performance on a delayed match to position (DMP) test and whether these treatment regimens would reduce atrophy of BFCNs.

## Experimental procedures

### *Animals*

Experiments were performed with adult Sprague–Dawley rats, purchased from Charles River (Hollister, CA) and housed in the UCI School of Medicine vivarium. At the beginning of these studies, rats were approximately 8 weeks of age and weighed approximately 180 g. Animals were placed on a restricted food diet to maintain body weight at 85% of normal (normal weights reported on the Charles River website) as described below.

All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of California, Irvine, Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

### *Materials*

Reagents were purchased from Sigma Chemical Co. (St Louis, MO) unless otherwise noted.

### *Surgical procedures*

Animals were deeply anesthetized with sodium pentobarbital (40 mg/kg; IP) and placed in a stereotaxic apparatus. Injections of 192 IgG-saporin (Chemicon, Temecula, CA) in sodium phosphate-buffered saline (PBS) or PBS alone were made using a Hamilton microsyringe and an attached glass micropipette. Four injections were made in each animal; bilaterally in the medial septum (relative to the bregma skull suture, AP +0.2 mm, ML +/−0.4 mm, DV −7.5 mm) and bilaterally in the basal nucleus (AP −0.4 mm, ML +/−1.8 mm, DV −8.0 mm) (Paxinos and Franklin, 2004). Injections (per site) were 0.2 μl of either saline (0.1 M sodium-phosphate buffered 0.9% saline) or 200 ng/μl 192 IgG-saporin, injected slowly over a period of 5 min, and the pipette tip left in place for an additional 5 min to facilitate diffusion into the tissue. Animals were placed in a temperature-controlled incubator until they recovered normal activity and then returned to the home cage. These injection parameters were selected in order to produce partial damage of BFCNs. An incomplete lesion was important to allow a remaining component of BFCNs as a target for treatment with neurotrophic factors.

Two weeks after the saporin injections, animals were again deeply anesthetized with pentobarbital (40 mg/kg; IP) and placed in a stereotaxic apparatus. Two 28G cannulae were implanted bilaterally in the lateral ventricles (stereotaxic targets AP: −0.9 mm; ML: +/−2.0 mm; DV: −3.5 mm below the brain surface). Cannulae were connected through a 'Y' adapter to a polyethylene tube leading to an Alzet minipump (Alzet, Cupertino, CA) that was implanted under the skin at the nape of the neck. The Alzet pumps used were model 2004 pumps, which deliver 0.25 μl/h, or a total of 200 μl over 4 weeks.

The Alzet pumps contained either NGF (Life Technologies, Invitrogen; Carlsbad, CA) or NT3 (BioSource, Invitrogen; Carlsbad, CA), or a combination of both, dissolved in PBS (pH 7.4; 0.1 M) at a concentration of 66 ng/μl, for a delivery of 400 ng/day. This treatment dosage is a relatively low dose (Johnson et al., 2002; Marques Pereira et al., 2005; Pappas and Sherren, 2003; Robertson et al., 1998). Six different sets of animals were used, with 5 conditions in each set. Condition 1 (Sap + NGF group) received saporin treatment followed by infusions of NGF alone, over 4 weeks. Condition 2 (Sap + NT3 group) received saporin treatment followed by infusions of NT3 alone. Condition 3 (Sap + NGF, NT3 Combo group) received saporin treatment followed by a combination of NGF and NT3, each at 200 ng/day so the total dose of neurotrophins would be similar to the single neurotrophin groups. Condition 4 (Sap + Sal Control) received saporin followed by vehicle control infusions of sodium-phosphate buffered saline. Condition 5 (Sal + Sal Control) received control intracranial injections of saline followed by infusions of saline. Each set of animals (with 5 conditions in each set) received surgeries, implants and behavioral testing together and was euthanized and tissues processed together for immunocytochemistry.

### *Behavioral tests*

The behavioral task employed was the delayed match to position (DMP) T-maze; performance on this test has been demonstrated to be impaired by lesions of the forebrain cholinergic system (Johnson et al., 2002). Further, this test offsets the rats' natural tendency to alternate between arms of the maze (Rabinovitch and Rosvold, 1951; Still, 1966). The experimenters in all cases were blinded to the treatment condition.

All animals were placed on a restricted food diet to maintain body weight at 85% of normal (normal weights as described on the Charles River website). Animals were housed 1 per cage and were provided 3 g of rat chow per 100 g body weight per day. Each day, after completion of the behavioral tests, animals were weighed and returned to their home cage, at which time they receive 3 g of standard rat chow per 100 g body weight. This procedure resulted in animals being food motivated when they underwent training the following day.

Behavioral testing began 2 weeks after the onset of the neurotrophin treatments. A standard T maze was used for the DMP test. Maze components were 5 in. wide and 5 in. high. The runway was 14 in. long and each arm was 12 in. long. During the first week, animals were adapted to the T maze by placing them in the maze with 2–3 reward pellets (45 mg sucrose tab/peanut butter pellets; Test Diet, Richmond, IN) for 10 min each day for 5 days. On the second week, animals were trained to run to the end of the maze arms by a series of forced choices over another 5 days. The DMP training consisted of 10 trial pairs/day; each trial consisted of a forced choice to one side or the other, followed immediately by an open choice. A two pellet reward was given when the subject correctly returned to the same arm; an incorrect run to the opposite arm resulted in no pellet reward and brief (10 s) confinement to that arm. Thus, the task was for the animal to remember which side of the maze was entered in the last trial and to return to that side. The rewarded side was randomized and balanced (right or left). Between trials within a session, animals were returned to their cage for approximately 5 min. Animals received 10 trial pairs/day until the criterion of at least 8 out of 10 correct choices was met for two consecutive sessions.

An additional 6 days of testing followed the training sessions; these tests incorporated increasing delays between the forced and open choices of the trial pairs. Delays were 60, 90, and 120 s, with 2 days of tests per delay period.

The data from the animals were averaged for each of the groups and groups were compared for the acquisition phase and delay tests. Analysis was performed on days to criterion, percent correct choices over acquisition, and post-criterion performance compared with the five groups (GraphPad InStat; La Jolla, CA). In addition, groups were compared by days to criterion using a non-parametric Kruskal–Wallis