

Folic Acid Supplementation Enhances Repair of the Adult Central Nervous System

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Folic acid supplementation has proved to be extremely effective in reducing the occurrence of neural tube defects (NTDs) and other congenital abnormalities in humans, suggesting that folic acid can modulate key mechanisms for growth and differentiation in the central nervous system (CNS). To prevent NTDs, however, supplemental folate must be provided early in gestation. This suggests that the ability of folic acid to activate growth and differentiation mechanisms may be confined to the early embryonic period. Here, we show that folic acid can enhance growth and repair mechanisms even in the adult CNS. Using lesion models of CNS injury, we found that intraperitoneal treatment of adult rats with folic acid significantly improves the regrowth of sensory spinal axons into a grafted segment of peripheral nerve in vivo. Regrowth of retinal ganglion cell (RGC) axons into a similar graft also was enhanced, although to a smaller extent than spinal axons. Furthermore, folic acid supplementation enhances neurological recovery from a spinal cord contusion injury, showing its potential clinical impact. The results show that the effects of folic acid supplementation on CNS growth processes are not restricted to the embryonic period, but can also be effective for enhancing growth, repair, and recovery in the injured adult CNS.

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Congenital or acquired defects in folate metabolism have been linked to a variety of disorders of central nervous system (CNS) development, such as neural tube defects (NTDs), developmental delay, dementia, myelopathy, and seizures.^{1,2} Supplementing the diet with folic acid can prevent some of these problems, provided the supplementation occurs sufficiently early in embryonic development. This became especially evident when the use of periconceptional folic acid in pregnant women was shown to significantly decrease the occurrence and recurrence of NTDs.^{3,4} Even though the direct mechanism of action has not been shown, the implication is that folate has an important role in growth and differentiation during brain and spinal cord development.⁵

In the adult nervous system, neurons formed during embryonic development can be damaged by spinal cord injury, stroke, neurodegenerative diseases, and other insults. The regeneration of injured CNS neurons may involve some of the same cellular and molecular mechanisms used during development. These may include a neuronal gene expression program that gets downregulated at birth, and which can be potentially

reactivated experimentally to promote neuronal regeneration.^{6,7} Or they may include glial processes that inhibit axonal growth⁸ and that can be overcome by experimental environmental manipulations such as replacing the CNS tissue with peripheral nerve grafts,^{9–11} exposing the neurons to neurotrophins,¹² or exposing the glia to antibodies against the inhibitory molecules.¹³ The folate metabolic pathway might well be an important constituent of such developmental processes. Because folic acid has been implicated in CNS growth, we have investigated whether folic acid supplementation also can enhance neuronal regeneration and recovery after experimental injury to the spinal cord and optic pathway.

Materials and Methods

Spinal Cord Regeneration Model

SURGERY. Sixty four adult male Sprague-Dawley rats (200–300gm) were subjected to surgery under ketamine/xylazine anesthesia, as previously described (Fig 1A).¹⁴ The cervical cord was exposed through a C3 laminectomy and dural opening. Using a pair of sharp jeweler's forceps, we made a well-defined 1mm-deep injury in both posterior columns. A sciatic nerve

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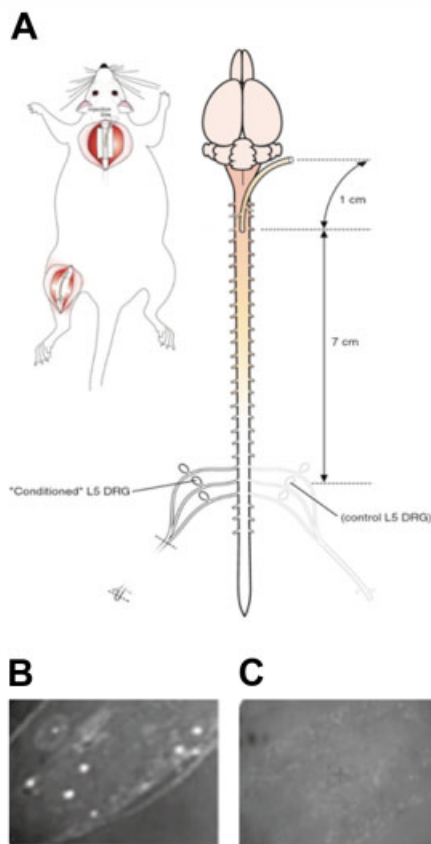


Fig 1. Model used to evaluate spinal sensory axon regeneration into a peripheral nerve graft *in vivo*. (A) Rat dorsal root ganglia (DRG) system after bilateral lesion of the dorsal columns of the spinal cord at a cervical level (C3) and introduction of a sciatic nerve graft. The nerve graft provided a supportive environment for axon growth. Removal of the nerve segment injured the peripheral axons of DRG neurons on one side (a so-called “conditioning” lesion), whereas neurons on the opposite side were unaffected by the peripheral injury (“unconditioned”). At 2 weeks, a fluorescent tracer was injected 1cm into the graft and was transported to the cell bodies of DRG neurons that were able to extend axons into the graft. The lower panel depicts DRG sections with (B) and without (C) fluorescence. The fluorescent tracer was taken up in the regenerated neurons only.

segment harvested from the left hindlimb then was implanted at the injury site using 10-0 nylon sutures in the pia. The other end of the graft was left to lie freely under the skin.

Two weeks later, 5 μ l of the retrograde tracer Fluorogold was placed into the nerve graft at a distance of 1cm from the spinal cord. It has been well established in previous studies that the fluorescent tracer does not diffuse down the graft and into the spinal cord. Instead, it is picked up solely by axons that have grown into the graft and transported retrogradely in these axons.¹⁴

PERFUSION AND TISSUE PREPARATION. Twenty four hours later, the animal was deeply anesthetized and perfused through the heart with 4% paraformaldehyde. The L5 dorsal

root ganglia (DRG) were removed bilaterally, postfixed, incubated in 30% sucrose overnight, and quick frozen in Histo Prep (TM). Sections were cut at 10 μ m with a cryostat, floated on pretreated glass slides, and stored at -80°C .

FOLIC ACID TREATMENT. Animals were treated with intraperitoneal doses of folic acid ranging from 10 to 800 μ g/kg, starting 3 days before the injury and given daily for 2 weeks.

ANALYSIS. Sections were examined under a fluorescence microscope and the number of fluorescently labeled cells was counted (see Fig 1B). The same sections then were counterstained with cresyl violet to reveal the cell somas of all neurons in the DRG. Fluorescently labeled cells and total cells were counted in treated and control animals by an observer unaware of the treatment conditions. The percentage of labeled DRG cells in each animal was calculated. To determine neuronal numbers accurately, we used the assumption-based method of Abercrombie, in which only cells with visible nucleoli are counted.^{15,16}

STATISTICS. The Wilcoxon rank-sum test and box-and-whiskers plots were used to compare the different groups.

IN VITRO ASSAYS. Three groups of adult male Sprague-Dawley rats (200–300gm) were used for the *in vitro* assays: Group I, or uninjured animals ($n = 8$); Group II, or animals with preceding spinal cord injury ($n = 8$), in which a dorsal column injury is made as described above, but without placement of a sciatic nerve graft; Group III, or animals with preceding sciatic nerve crush injury ($n = 16$). Half of the animals in each group received intraperitoneal injections of 80 μ g/kg of folic acid daily for 3 days before injury. The control animals were euthanized 3 days after treatment with folic acid was started. Groups II and III animals were euthanized 48 hours after the injury (or 5 days after starting the folic acid injections).

STANDARD DORSAL ROOT GANGLIA CULTURES. Immediately after euthanasia, the L4 and L5 ganglia were removed from the adult rats, dissociated, and centrifuged through a cushion of 10% Ficoll in F14 culture medium to remove myelinated axons, cellular debris, and nonneuronal cells as previously described.^{7,17} Neurons were resuspended in serum-free F14 medium containing N1 supplements and plated on polylysine/laminin coated glass coverslips. Cells from 12 to 14 ganglia were plated in a 24-well plate, and, after 18–24 hours, they were fixed in 4% paraformaldehyde for 30 minutes at room temperature.^{7,17}

VISUAL ANALYSIS OF NEURITE GROWTH. Quantitative analysis of neurite length and number of branch points per neurite was performed on fixed cultures displayed on a video monitor. Dilute cultures allowed unambiguous measurements of neurites. The longest processes from each cell were chosen for measurement, and all counts were made by an observer unaware of the treatment conditions. The percentage of DRG neurons that extended processes longer than 300 μ m was calculated in all groups of animals at various time intervals within a 48-hour period.

Functional Recovery Model

Adult male Sprague Dawley rats (200–300gm) were used with approval from the University of Wisconsin Research Animal Resources and Care Committee and in accordance with published NIH guidelines. Rats were anesthetized with halothane and underwent a T9 laminectomy under aseptic conditions. A 12.5gm/cm injury was created using the NYU impactor.¹⁸ Rats received folic acid (80µg/kg) in saline, or an equal volume of saline alone via an intraperitoneal injection every day for 2 weeks, beginning 3 days before the injury. The animals were videotaped for 4 minutes while ambulating in an open-field environment on the day after surgery, and weekly thereafter for a period of 6 weeks. Ambulatory function was scored blindly using the BBB rating scale,¹⁹ which assigns points for the frequency of occurrence of specific features of normal posture and locomotion.^{19,20} The scoring differences between folate and control groups were displayed in a graph illustrating the mean \pm standard error of the mean (SEM) at each time point. In addition, the primary end points of 6 weeks were analyzed using the Wilcoxon rank-sum test.

Optic Nerve Regeneration Model

SURGERY. Surgery was performed on 31 adult male Sprague-Dawley rats. There were 23 control animals that did not receive folic acid and 8 animals in which daily folic acid injections were started 3 days preoperatively and continued for 2 weeks postoperatively. The optic nerve was exposed through a lateral orbital approach and cut within 2mm of the globe. One end of an autologous sciatic nerve graft was attached to the optic stump, while the distal end was left to lie freely under the skin. Two months later, 5µl of the retrograde tracer Fluorogold was placed into the graft at a distance of 1.5cm from the globe. Twenty four hours later, the animal was deeply anesthetized and perfused intracardially

with 4% paraformaldehyde. The globe was removed and postfixed with 4% paraformaldehyde. The retina was dissected from the eye cup and examined under a fluorescence microscope. An observer unaware of the treatment conditions recorded the number of retinal ganglion cells (RGCs) labeled with Fluorogold. The Wilcoxon rank-sum test was used to compare the two groups.

Results

Spinal Cord Regeneration

UNCONDITIONED DORSAL ROOT GANGLIA. Without folic acid treatment, unconditioned DRG neurons (contralateral to the peripheral injury) show no detectable fluorescent labeling, that is, no regeneration of their spinal axons into the graft (Fig 2), which is consistent with previous reports.¹⁴ In contrast, animals treated with an optimal dose of folic acid (80µg/kg) show a mean of 54 labeled neurons per ganglion (1.69% \pm 0.40% SEM, $n = 15$), in the absence of peripheral nerve injury (see Fig 2). As shown in Figure 3, this is similar to the number of regenerating axons evoked in untreated animals on the side ipsilateral to the peripheral nerve injury (48 cells per ganglion or 1.37% \pm 0.25% SEM, $n = 11$). We found that all doses of folic acid from 10µg/kg to 400µg/kg resulted in statistically significant increases in the percentage of unconditioned neurons regenerating relative to untreated control animals ($p \leq 0.007$; Wilcoxon test); similarly, the 80µg/kg dose resulted in a statistically significant increase in the percentage of unconditioned neurons regenerating relative to the 0 to 40µg/kg doses and 180 to 800µg/kg doses ($p \leq 0.012$; Wilcoxon test).

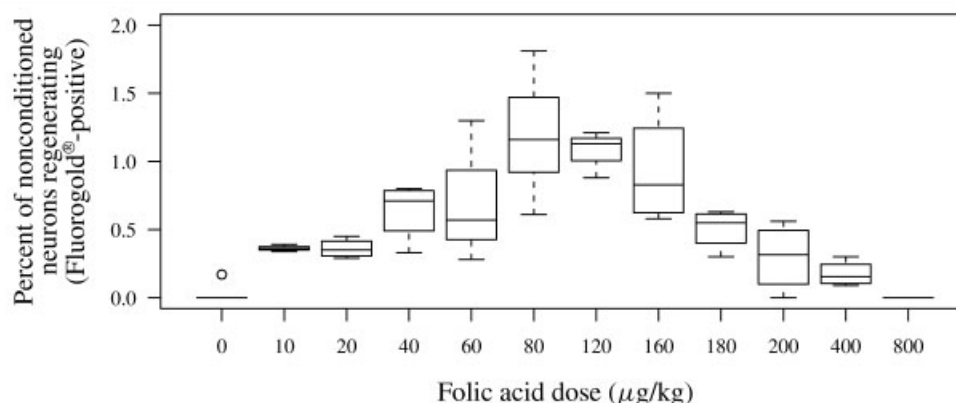


Fig 2. Folic acid enhances regeneration of spinal axons into a peripheral nerve graft. Dorsal root ganglia (DRG) axons in the dorsal columns of the spinal cord were lesioned in adult rats treated with the indicated daily doses of folic acid and a segment of peripheral nerve implanted into the lesion site as in Figure 1. After 2 weeks, axons regenerating through the graft were labeled with a retrograde tracer, and labeled DRG cell bodies were counted on the side opposite the peripheral nerve resection (right L4-6 DRGs in Fig 1). Eleven animals were used as no folate treatment controls; the others were treated with intraperitoneal injections of folic acid (10, 20, 40, 60, 80, 120, 160, 180, 200, 400, 800µg/kg in 4, 4, 4, 3, 15, 3, 4, 4, 4, 4, and 4 animals, respectively) starting 3 days before the surgery and given daily until 2 weeks postoperatively. Measurable regeneration occurred only in the folate-treated animals. This dose-related response occurred in all treated animals except for the group receiving the 800µg/kg dose. The boxes extend from the 25th to 75th percentiles and the whiskers from the minimum to the maximum except where there are outliers indicated by a circle.

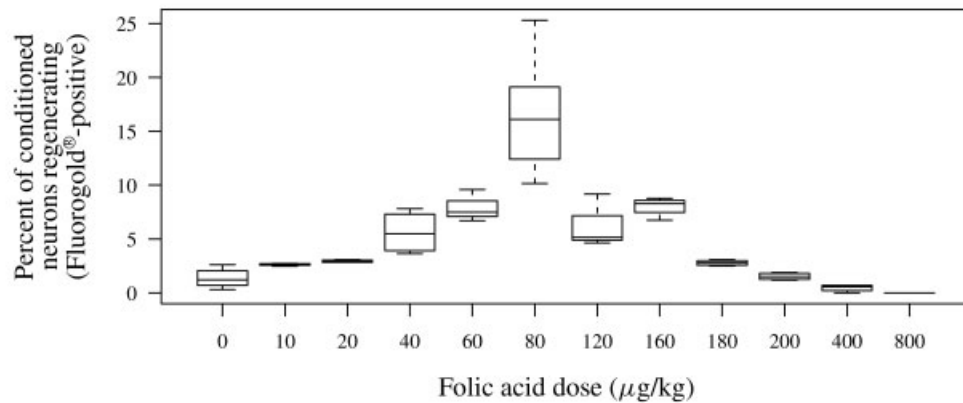


Fig 3. Effects of folic acid on spinal axon regrowth are synergistic with the effects of a conditioning peripheral nerve injury. Adult rats were treated daily with the indicated doses of folic acid, and regeneration of spinal sensory axons into a peripheral nerve graft was measured as described in legend to Figure 1. Axon regeneration by dorsal root ganglia (DRG) neurons subjected to a peripheral nerve lesion (left L4-6 DRGs in Fig 1) are shown here. The conditioning lesion promoted regeneration of the spinal axons even without folic acid supplementation (note the difference in scale for this graph compared to Fig 2). Furthermore, the addition of folic acid was synergistic with the peripheral nerve injury at enhancing the growth of the injured spinal axons ($n = 11, 4, 4, 4, 3, 15, 3, 4, 4, 4, 4, 4$ for folic acid doses 0, 10, 20, 40, 60, 80, 120, 160, 180, 200, 400, 800 µg/kg, respectively). The only exception was the highest dose tested of 800 µg/kg, which resulted in decreased regeneration compared with the control animals. The boxes extend from the 25th to 75th percentiles and the whiskers from the minimum to the maximum.

CONDITIONED DORSAL ROOT GANGLIA. Daily injections of folic acid (80 µg/kg), starting 3 days before the injury and continuing for 2 weeks after the injury, produced a large increase in the percentage of conditioned DRG neurons that were fluorescently labeled ($16.26\% \pm 1.12\%$ [SEM, $n = 15$] in folate-treated animals, vs $1.37\% \pm 0.25\%$ [SEM, $n = 11$] in untreated controls). Furthermore, higher or lower doses of folic acid appeared to be somewhat less effective, although regeneration was still much greater than in control animals (see Fig 3). All doses of folic acid from 10 to 180 µg/kg resulted in statistically significant increases in the percentage of conditioned neurons regenerating relative to the untreated control group ($p \leq 0.01$; Wilcoxon test). The regeneration of injured spinal axons into a graft seems to respond less to higher doses of folic acid (200–800 µg/kg), and, in fact, the 800 µg/kg dose resulted in a statistically significant decrease in the percentage of conditioned neurons regenerating relative to control ($p = 0.037$; Wilcoxon test). Finally, the optimal dose of 80 µg/kg of folic acid resulted in a statistically significant increase in the percentage of conditioned neurons regenerating relative to all other doses ($p < 0.008$; Wilcoxon test).

Although either folic acid treatment (optimal dose of 80 µg/kg, see Fig 2) or peripheral nerve injury alone (no treatment group, see Fig 3) permits regeneration of approximately 50 axons per ganglion under the conditions used here, folate treatment combined with peripheral injury promotes regeneration by a mean of 565 neurons per ganglion ($16.26\% \pm 1.12\%$ SEM, $n = 15$, see Fig 3), a synergistic effect not previously

observed with any experimental intervention. The responses did not change over the period of experimentation (15 months). In all but five animals, the conditioned response was greater than the unconditioned response ($p < 10^{-10}$; Wilcoxon test), and four of these five animals composed the 800 µg/kg group in which there was no regeneration in either the conditioned or unconditioned neurons. No neurological, behavioral, or hematological toxicity related to folic acid was detected in any of the groups tested at any dose; however, at the highest dose tested (800 µg/kg), there was no evidence of spinal regeneration in the nonconditioned group, similar to the untreated control animals, and even decreased regeneration in the conditioned animals compared with controls.

Notably, because the effect of folic acid seems to occur independently of the neuronal cell body responses evoked by peripheral nerve injury, we examined the effect of *in vivo* injections of folic acid on the neuronal cell bodies. We treated animals *in vivo* for 3 days with daily injections of folic acid and then used a short-term cell culture assay to monitor the ability of DRG cell bodies to support axon elongation.^{7,17} The number of branches was constant between the different groups as expected from regenerating (nonsprouting) neurons.^{7,17} At a dose that produced optimal regrowth of DRG axons *in vivo* (80 µg/kg), folate supplementation had no effect on the ability of isolated DRG neurons to support axon extension *in vitro*. A crush injury to the sciatic nerve 48 hours before removing the DRG has been shown to promote axonal elongation in the dissociated cells.^{7,17} However, even in animals whose sciatic nerve

or dorsal columns were preinjured, *in vivo* supplementation of folic acid caused no enhancement of axonal elongation in the dissociated cells. Furthermore, cresyl violet staining demonstrated that there was rare nuclear clumping in the DRG sections, and the average total number of DRG neurons was identical, approximating 3,500, regardless of the treatment conditions. Therefore, the differences between the various groups was unrelated to cell death.

Functional Recovery after Spinal Cord Injury

To assess the effects of folic acid on functional recovery from CNS damage in adult rats, we used the Basso, Beattie, and Bresnahan (BBB) scoring system^{19,21} to monitor neurological recovery from a standardized weight-drop contusion injury to the spinal cord. Animals received daily intraperitoneal injections of either folic acid (80 μ g/kg) or vehicle (saline), beginning 3 days before the injury and continuing for 2 weeks after injury. The animals then were videotaped once a week for 6 weeks and scored by an observer unaware of the treatment conditions.^{19,21} With the contusion injury used in our study, control animals recover a degree of voluntary locomotion, reaching a plateau at 2 to 3 weeks after spinal cord injury (Fig 4) with a median BBB score of 12. Animals at this level support weight on the plantar surfaces of their hindlimbs 50 to 90% of the time but drag their toes when stepping and fail to coordinate hindlimb with forelimb movements.

In contrast, folic acid treatment produced a significant improvement in the BBB score beginning 7 days after

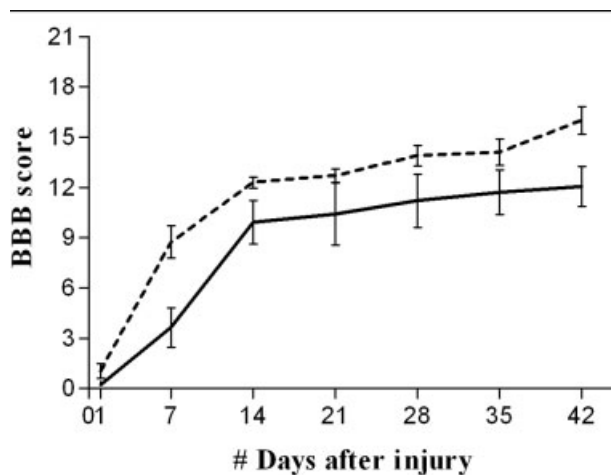


Fig 4. Functional recovery after spinal cord injury: Basso, Beattie, and Bresnahan (BBB) locomotor score in the folate-treated animals (dotted line, $n = 10$) compared with a control group (solid line, $n = 7$). The animals were videotaped once a week and scored by an observer unaware of the treatment conditions. This graph illustrates the mean \pm SEM at each time point, with the Wilcoxon test showing a six-point difference in the median outcome at 42 days ($p < 0.01$).

injury. This improvement was maintained throughout the study period ($p < 0.01$; Wilcoxon test), leading to an improvement in the mean of four points (median of six points) on the BBB scale 6 weeks after injury.^{7,19} Animals that scored at the median in the folate-treated group consistently bear weight on the plantar surfaces of their hindlimbs, lift their toes clear of the walking surface when stepping forward, and coordinate hindlimb and forelimb movements. The results indicate that folate supplementation in adult animals can substantially improve functional recovery from spinal cord injury (see Fig 4). In this animal model, a peripheral nerve graft is not used, thus showing that the effect of folic acid is independent of the presence of a graft.

Optic Nerve Regeneration

Enhanced regeneration of sensory axons (nearly 10-fold) after spinal cord injury suggests that folic acid treatment can improve regeneration of sensory axons in the spinal cord. To determine whether folic acid treatment has a similar effect on other CNS systems, we administered folic acid to adult rats with an optic nerve injury and peripheral graft. In control animals, the number of fluorescent RGCs per retina was 913.4 ± 11.83 (SEM, $n = 23$). In the folate-treated animals, the number of fluorescent RGCs was $1,373 \pm 73.42$ (SEM, $n = 8$), a 50% increase ($p < 0.0001$; Wilcoxon test; Fig 5).

Discussion

Recognition of the critical influence of folic acid on growth and differentiation in the embryonic CNS led to widespread dietary supplementation and a drastic reduction in NTDs and other developmental disorders. Our results now show that the effects of folic acid supplementation are not confined to the embryonic period but also can influence repair mechanisms in the adult CNS. In our studies, this is reflected in a significant increase in the regeneration of axons into peripheral nerve grafts after damage to the spinal cord or optic nerves, and a substantial improvement in functional recovery from spinal cord injury.

This nerve graft regeneration paradigm is designed to monitor the intrinsic capacity of injured neurons to support axon regeneration, by providing the damaged axons with a supportive environment for regrowth. It has been shown previously that whereas a small number of injured axons grow into the graft, spared (ie, uninjured) axons do not.¹⁴ In addition, injuring the peripheral axon of the DRG enhances the potential for growth of both the peripheral (sciatic) and central (dorsal column) components of the DRG.¹⁴ We were surprised to find that the effect of folic acid on spinal axon regrowth was not equivalent to the effect of a peripheral injury. Rather, the influence of folic acid on axon regrowth appears to be additive or synergistic

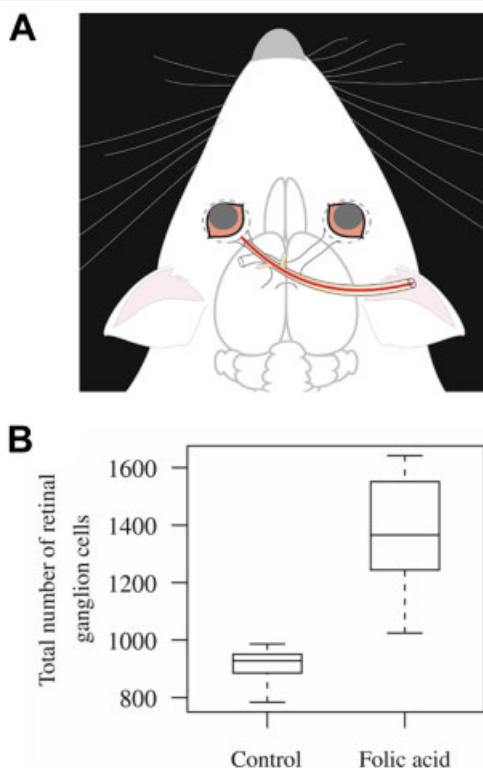


Fig 5. Optic nerve regeneration in vivo. (A) A sciatic nerve graft was attached to the axotomized left optic nerve. The axons that regenerated reached the end of the graft, where they were backfilled with the fluorescent tracer, Fluorogold. The tracer traveled to the cell body in the retina, where it was visualized by light microscopy. (B) This graph demonstrates that folic acid-pretreated animals showed a significant increase in the number of regenerated retinal ganglion cells. The boxes extend from the 25th to 75th percentiles and the whiskers from the minimum to the maximum.

with the neuronal cell body responses produced by peripheral nerve injury, which increase the intrinsic capacity of neurons for regeneration.¹⁷ In contrast, previous studies have suggested that the effects of peripheral nerve injury can be mimicked but were not augmented by the targeted expression of specific growth-associated genes,^{7,22,23} by infusion of appropriate neurotrophins,²⁴ or by a transient elevation of cyclic AMP.^{25,26} Our findings suggest that folic acid supplementation enhances the regeneration of spinal sensory axons in vivo by an independent mechanism that can act synergistically with conditions known to promote axon regeneration through an effect on the neuronal cell body and therefore potentially could be combined with these or other treatments to further improve axon repair.

If folic acid does not act at the level of the injured neuron's cell body, where does it act to promote axon regeneration into a peripheral nerve graft? One potential site is the glial scar that forms at lesion sites within

the adult CNS. Chondroitin sulfate proteoglycans and other molecules expressed by reactive glial cells in these scars can be potent inhibitors of axon regeneration and pose a substantial impediment to the growth of axons beyond the lesion site.^{27–29} One possibility therefore is that folic acid supplementation reduces or delays formation of the glial scar, or the expression of specific growth-inhibiting molecules within the glial scar, and thus increases the ability of damaged CNS axons to gain access to the peripheral nerve grafts. An alternative possibility is that folic acid could enhance regrowth by promoting changes within the nerve grafts themselves. Isolation of a peripheral nerve segment leads to a series of responses by Schwann cells and other elements within the nerve, resulting in an optimal environment for axon extension. Folic acid potentially could act to enhance these growth-supporting properties. Although the precise cellular targets for folic acid remain to be determined, our results indicate that a sustained increase in folic acid can improve the environment for axonal growth and repair in the adult brain and spinal cord by reducing inhibitory influences and/or increasing growth-promoting factors within the CNS environment. This suggests that folic acid supplementation could contribute not only to the regeneration of transected axons, but also to other forms of axonal remodeling and repair in the adult CNS.

It has been shown previously that folic acid deficiency and elevated homocysteine levels cause DNA damage and neuronal degeneration.^{30,31} To our knowledge, this is the first time that folic acid supplementation is shown to *enhance* repair processes in the adult CNS. Folic acid is widely used as a dietary supplement in humans, and its effectiveness in preventing developmental disorders of the CNS and cardiovascular system is well established.^{32,33} In this study, the folic acid dose (80 μg/kg) with the greatest effect on regeneration was equivalent to the daily dose of folic acid recommended by the Center for Disease Control in women with a previous NTD pregnancy (4mg). Although there has been no reported toxicity to folic acid, whether in our study or in the literature, it is reassuring that the pro-regenerative effect of folic acid does not require supra-maximal doses.

As in the case of folic acid supplementation during fetal development, the mechanisms through which folic acid influences growth and repair processes in the adult CNS remain to be established. The folate pathway is the main source of methyl groups for a variety of intracellular methylation reactions in humans.¹ Among other essential functions, methylation is required for DNA synthesis and transcriptional regulation, as well as the formation of myelin and neurotransmitters.¹ Folate therefore might serve to regulate growth-related gene expression in neurons or glial cells or act as a cofactor for proteins involved in axon growth and other repair pro-

cesses. Even before the mechanisms of action are resolved, however, the simplicity and established safety of folic acid supplementation, together with the magnitude of the effect demonstrated in this study, makes this vitamin an attractive candidate for intervention in cases of spinal cord and other CNS trauma, and perhaps in other neurodegenerative conditions. An important question is whether folic acid can also enhance CNS repair when given after the injury, rather than only as a preventative measure. This is currently under investigation at our institution. In any case, as with NTDs, the daily use of preventative doses of folic acid may have many unforeseen benefits in both health and disease.

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References

- Rosenblatt DS, Fenton WA. Inherited disorders of folate and cobalamine transport and metabolism. In: Scriver CR, Sly WS, Childs B, eds. *The metabolic and molecular bases of inherited disease*. 8 ed. New York: McGraw-Hill, 2000:3897–3927.
- Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends Neurosci* 2003;26:137–146.
- Berry RJ, Li Z, Erickson JD, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* 1999;341:1485–1490.
- Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832–1835.
- Martinasevic MK, Rios GR, Miller MW, Tephly TR. Folate and folate-dependent enzymes associated with rat CNS development. *Dev Neurosci* 1999;21:29–35.
- Neumann S, Woolf CJ. Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. *Neuron* 1999;23:83–91.
- Bomze HM, Bulsara KR, Iskandar BJ, et al. Spinal axon regeneration evoked by replacing two growth cone proteins in adult neurons. *Nat Neurosci* 2001;4:38–43.
- Schnell L, Schwab ME. Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. *Nature* 1990;343:269–272.
- Richardson PM, McGuinness UM, Aguayo AJ. Axons from CNS neurons regenerate into PNS grafts. *Nature* 1980;284:264–265.
- David S, Aguayo AJ. Axonal elongation into peripheral nervous system “bridges” after central nervous system injury in adult rats. *Science* 1981;214:931–933.
- Benfey M, Aguayo AJ. Extensive elongation of axons from rat brain into peripheral nerve grafts. *Nature* 1982;296:150–152.
- Sawai H, Clarke DB, Kirtlerova P, et al. Brain-derived neurotrophic factor and neurotrophin-4/5 stimulate growth of axonal branches from regenerating retinal ganglion cells. *J Neurosci* 1996;16:3887–3894.
- Bregman BS, Kunkel-Bagden E, Schnell L, et al. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. *Nature* 1995;378:498–501.
- Richardson PM, Issa VM. Peripheral injury enhances central regeneration of primary sensory neurones. *Nature* 1984;309:791–793.
- Abercrombie M. Estimation of nuclear population from microtome sections. *Anat Rec* 1946;94:239–247.
- Coggeshall RE, La Forte R, Klein CM. Calibration of methods for determining numbers of dorsal root ganglion cells. *J Neurosci Methods* 1990;35:187–194.
- Smith DS, Skene JH. A transcription-dependent switch controls competence of adult neurons for distinct modes of axon growth. *J Neurosci* 1997;17:646–658.
- Gruner J. A monitored contusion model of spinal cord injury in the rat. *J Neurotrauma* 1992;9:123–128.
- Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 1995;12:1–21.
- Basso DM, Beattie MS, Bresnahan JC, et al. MASCIS evaluation of open field locomotor scores: effects of experience and teamwork on reliability. Multicenter Animal Spinal Cord Injury Study. *J Neurotrauma* 1996;13:343–359.
- McDonald JW, Liu XZ, Qu Y, et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med* 1999;5:1410–1412.
- Bonilla IE, Tanabe K, Strittmatter SM. Small proline-rich repeat protein 1A is expressed by axotomized neurons and promotes axonal outgrowth. *J Neurosci* 2002;22:1303–1315.
- Condic ML. Adult neuronal regeneration induced by transgenic integrin expression. *J Neurosci* 2001;21:4782–4788.
- Ramer MS, Priestley JV, McMahon SB. Functional regeneration of sensory axons into the adult spinal cord. *Nature* 2000;403:312–316.
- Cai D, Deng K, Mellado W, et al. Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro. *Neuron* 2002;35:711–719.
- Neumann S, Bradke F, Tessier-Lavigne M, Basbaum AI. Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. *Neuron* 2002;34:885–893.
- Pasterkamp RJ, Anderson PN, Verhaagen J. Peripheral nerve injury fails to induce growth of lesioned ascending dorsal column axons into spinal cord scar tissue expressing the axon repellent Semaphorin3A. *Eur J Neurosci* 2001;13:457–471.
- Bradbury EJ, Moon LD, Popat RJ, et al. Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* 2002;416:636–640.
- Davies SJ, Goucher DR, Doller C, Silver J. Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. *J Neurosci* 1999;19:5810–5822.
- Kruman II, Kumaravel TS, Lohani A, et al. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer’s disease. *J Neurosci* 2002;22:1752–1762.
- Kruman II, Culmsee C, Chan SL, et al. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. *J Neurosci* 2000;20:6920–6926.
- Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol Genet Metab* 2000;71:121–138.
- Hernandez-Diaz S, Werler MM, Walker AM, Mitchell AA. Folic acid antagonists during pregnancy and the risk of birth defects. *N Engl J Med* 2000;343:1608–1614.