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Regulation of Astroglial-Derived Dopaminergic Neurotrophic Factors by Interleukin-1β in the Striatum of Young and Middle-Aged Mice[1]

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Interleukin-1β (IL-1β) can induce dopaminergic axonal sprouting in the denervated striatum of parkinsonian animals. In order to determine whether IL-1β effects on dopaminergic axonal sprouting are mediated by the induction of astroglial-derived dopaminergic neurotrophic factors, effects of IL-1β treatment on acidic and basic fibroblast growth factor (aFGF and bFGF) and glial cell line-derived growth factor (GDNF) gene expression were examined in primary striatal astrocyte cultures and after *in vivo* administration. We found a selective induction of bFGF mRNA synthesis but not aFGF or GDNF mRNA after IL-1β treatment both *in vitro* and *in vivo*. This suggests that bFGF may be the putative endogenous dopaminergic neurotrophic factor mediating lesion-induced plasticity of dopamine neurons. In addition, to determine why recovery from injury becomes reduced with age, we examined whether there was an aging-associated decline in the ability of IL-1β to induce the synthesis of neurotrophic factors in middle-aged animals compared to young mice. Interestingly, IL-1β stimulated a greater induction in bFGF mRNA levels in the middle-aged mice compared to young mice. These results suggest that the regulation of bFGF and possibly its receptor signaling efficacy may vary as the brain ages. © 1997 Academic Press

INTRODUCTION

Interleukin-1β (IL-1β) is a cytokine that plays an important role in mediating cellular responses to injury in the central nervous system (7). IL-1β can have diverse actions on the growth and differentiation of cells (7, 68). Activated microglia are a principal source of secreted IL-1β; however, there is evidence that astrocytes could be another source of IL-1β synthesis (25, 32, 42, 48, 53, 56). IL-1β can induce reactive gliosis and neovascularization when infused into the adult brain, thus suggesting IL-1β as a regulator of wound healing (33). In contrast, while IL-1β is an astroglial growth factor in the developing CNS, IL-1β does not appear to stimulate gliosis or neovascularization in immature animals (34, 73). These studies suggest that not only does IL-1β play a role in linking the immune system with the development of the brain, but also that there are differences in cellular responses to IL-1β in the developing and adult CNS.

Recent attention has focused on the ability of IL-1β to regulate the synthesis of certain neurotrophic factors. It was first demonstrated that after sciatic nerve lesion, an increase in nerve growth factor (NGF) mRNA and protein synthesized by nonneuronal cells is induced by IL-1β (49). Moreover, IL-1β can selectively produce an induction of hippocampal NGF and basic fibroblast growth factor (bFGF) mRNA, but not acidic fibroblast growth factor (aFGF) mRNA after intraventricular injection (67, 76). These findings suggest that cytokines may differentially regulate glial hypertrophy, neurotrophic factor expression, and neuroprotection upon CNS trauma (76).

Parkinson's disease (PD) is a neurodegenerative disorder in which nigral dopamine (DA) neurons in the mesencephalon selectively degenerate, thus leading to a loss of DAergic innervation to the dorsal striatum (1, 9, 43). Therapeutic approaches such as grafting DA-producing tissues into the striatum of animal models of PD and parkinsonian patients can induce a compensatory sprouting response in the denervated striatum from endogenous DA neurons (6, 11, 14, 24, 39, 57). Several possible mechanisms may underlie the transplantation-induced compensatory sprouting in the host-denervated striatum. Neuronal grafts may release neurotrophic factors and initiate a sprouting response in spared DA axons (5, 38, 39, 58). Another possible mechanism is the activation of a cytokine-neurotrophic cascade, by which neuronal grafts initiate an inflammatory reaction (23, 35, 39, 46, 81). This inflammatory reaction mediated by microglia could release inflammatory cytokines that can stimulate reactive astrocytes to

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release neurotrophic factors, thereby promoting axonal sprouting (3, 28–32, 39, 71, 81).

Recent studies have demonstrated that intrastriatal implantation of IL-1β can enhance compensatory sprouting from residual DA neurons in the ventral tegmental area of the midbrain and can induce behavioral improvement in hemiparkinsonian rats (81). Since there is evidence showing that IL-1β does not act directly to induce the survival and plasticity of DA neurons (2, 18), it is possible that IL-1β stimulates the release of DAergic neurotrophic factors from astrocytes. Treatment with aFGF, bFGF, or glial cell line-derived neurotrophic factor (GDNF) has been shown to enhance the sprouting of DAergic fibers after a neurotoxic lesion (21, 55, 78). In addition, these specific DAergic neurotrophic factors can be synthesized by striatal astrocytes (16, 17, 44, 77). Therefore, we hypothesized that the stimulation of DAergic axonal sprouting by IL-1β is mediated by the induction of specific growth factor synthesis from astrocytes.

Previous studies have demonstrated an aging-associated decline in compensatory DAergic sprouting after neurotoxin-induced injury (20, 21, 65, 66). At the same time, other studies reported that there is an aging-associated decline in the ability of astrocytes to support process outgrowth (70, 74, 75, 79). Thus, in the present study, we also examined whether the ability of IL-1β to regulate the synthesis of astroglial-derived DAergic neurotrophic factor is altered during the aging process.

MATERIALS AND METHODS

Preparation of striatal astroglial monolayers. Breeders were paired from C57BL/6 mice (Jackson Laboratory). Postnatal day 0–3 C57BL/6 mice were decapitated, the striatum was dissected, and the meninges were removed. The tissues were trypsinized (2.5 mg/ml) and treated with DNase (10 μg/ml) for 15 min at 37°C, followed by a series of washes and centrifugations in MEM/Ham's F-12 supplemented with 10% fetal bovine serum. The resulting homogenate (cell suspension) was filtered through a sterile 37-μm nylon mesh screen. Cells were plated at $\sim 1 \times 10^6$ cells per 60-mm dish in poly-L-lysine (100 μg/ml)-coated culture petri dishes. The cultures were incubated at 37°C in an atmosphere of 8% CO_2 and 95% air.

In vitro treatment. Based on conditions previously described (76), cells at 11–13 days *in vitro* were placed into 0.5% serum for 24 h and treated with IL-1β [50 ng/ml; Intergen; dosage was based on experiments previously described from Araujo and Cotman (3) or vehicle (sterile H_2O) for 0, 2, 4, 6, 8, and 16 h ($n = 3$ for each group)]. Cells at 21–23 days *in vitro* were treated as above for 6 h ($n = 5$ for each group). Cytoplasmic total RNA was isolated and a nuclease protection assay (12) was used to quantitate aFGF, bFGF, and GDNF mRNA from the same treated cultured astrocytes.

In vivo treatments. Male C57BL/6 mice (Jackson Laboratory) of three representative age groups were compared: postnatal day 15 (young), 3 months (young-mature), and 8–10 months of age (middle-aged). The 3- and 8-month age groups were anesthetized with 287.5 mg/kg of avertin (stock of 12.5 mg/ml 2,2,2-tribromoethanol), injected intraperitoneally (ip), while the postnatal day 15 age group was anesthetized ip with 300 mg/kg of chloral hydrate. The mice were placed on a stereotactic device. A burr hole was drilled on the right side of the skull to accomodate injection. Stereotactic injections of IL-1β (100 units; Intergen) or vehicle (phosphate-buffered saline, pH 7.4) in a final volume of 0.5 μl was injected into the right lateral ventricle using a 1-μl Hamilton syringe. Coordinates for the postnatal day 15 age group were 1.5 mm caudal to the frontal nasal suture, 0.5 mm lateral from the midline suture, and 1.5 mm from the surface of the brain. Coordinates for the 3- and 8-month age groups were 2.5 mm caudal to the frontal nasal suture, 1.0 mm lateral from the midline suture, and 2.0 mm from the surface of the brain. The injection was at a rate of ~0.05 μl/min, and the needle was left in place for 2–3 min after injection. The needle was withdrawn slowly to prevent backflow of solution along the needle track. Animals were sacrificed at 0, 3, 6, 8, 24, and 48 h by cervical dislocation and decapitated. Three to five mice were used for each time period. The brains were removed and chilled in sterile saline. The right dorsal and ventral striatum were dissected and immediately frozen on dry ice and stored at −80°C until used. Cytoplasmic total RNA was isolated and a nuclease protection assay (12) was used to quantitate aFGF, bFGF, and GDNF mRNA in dissections from the same animals.

Isolation of cDNA clones. The aFGF cDNA clone was isolated by polymerase chain reaction (PCR) of mouse striatal cDNA from which a 350-bp fragment corresponding to nucleotides 33-384 was subcloned into Bluescript II. The bFGF cDNA clone was generously provided by Dr. S. Shimasaki (72) from which a 479-bp fragment corresponding to nucleotides 525-1004 was subcloned into vector Bluescript/SK+. The GDNF cDNA clone was isolated by PCR of rat genomic DNA as previously described (13) from which a 414-bp PCR DNA fragment was subcloned into Bluescript II.

Quantitative solution hybridization nuclease protection assay. Unlabeled sense and high specific activity ($\sim 1 \times 10^9$ cpm/μg) ^{32}P-labeled antisense RNA were transcribed according to the manufacturer's recommendations. A standard curve with increasing amounts (0–10 μl of a 100 fg/μl (+) strand) of sense RNA was used for quantitation. The standard and known amounts of cytoplasmic RNA isolated were hybridized with ~200 pg of antisense ^{32}P-labeled RNA probe. The samples

were heat denatured at 85°C for 5 min and hybridized overnight at 45°C. After hybridization, the samples were treated with RNase A (5 μg/ml) and RNase T1 (2 μg/ml) for 1 h at 30°C, followed by proteinase K (0.167 mg/ml) digestion at 37°C for 15 min. Samples were phenol:chloroform extracted, precipitated, resuspended in 1× TE, and electrophoresed on a nondenaturing 5% acrylamide gel. Gels were dried and quantitated by phosphor image analysis. The results were determined by linear regression analysis from the standard curve and presented as attomoles of mRNA per microgram of total RNA.

Statistical analysis. All values given are means ± SEM. Significant differences in aFGF, bFGF, or GDNF mRNA levels between control and IL-1β treatment groups were analyzed using analysis of variance followed by Fisher's protected least significant difference *post hoc* analysis. The level of significance was set at $P < 0.05$. Statistical comparisons between control and IL-1β in mature astrocyte cultures and postnatal day 15 mice that were analyzed at 6 h were made using a two-tailed Student's *t* test.

RESULTS

Effects of IL-1β on Neurotrophic Factor Gene Expression in Primary Striatal Astrocyte Cultures

Immature animals respond differently to IL-1β compared to mature animals when infused into the brain (33, 72). In addition, it has been observed that the ability of astrocytes to support process outgrowth decreases with time in culture (69, 70, 74, 75, 82). It has been characterized that as astrocytes mature *in vitro,* a decline in the growth-promoting potential is seen after 21 days, in comparison to astrocytes cultured for shorter periods of time (69, 70, 74, 75, 82). Therefore, we examined whether the *in vitro* maturational state of astrocytes altered their response to IL-1β. Thus, primary striatal astrocyte cultures at 11–13 days *in vitro* (young immature astrocytes) and cultures at 21–23 days *in vitro* (mature astrocytes) were studied. The characteristics of the cultures were evaluated by staining with an astroglial marker, glial fibrillary acidic protein (GFAP). In young and mature astrocyte cultures, more than 90% of the cells were labeled with GFAP (data not shown).

Our results show that IL-1β produced a 34% reduction in aFGF mRNA levels by 6 h ($P < 0.002$), and by 16 h, aFGF mRNA levels returned to control levels in young immature striatal astrocyte cultures (Fig. 1A). Similarly, IL-1β produced a significant 42% decrease in aFGF mRNA levels within 6 h ($P < 0.0008$) in mature striatal astrocyte cultures (Fig. 1D). In contrast, IL-1β maximally induced a 410% increase in bFGF mRNA compared to control at 6 h ($P < 0.0001$) and remained elevated by 280% at 16 h ($P < 0.0007$) in young immature astrocyte cultures (Fig. 1B). Moreover, there was an even greater increase in bFGF mRNA in mature astrocyte cultures, with an 830% increase in bFGF mRNA levels seen at 6 h ($P < 0.0008$) after IL-1β treatment (Fig. 1E). IL-1β did not change GDNF mRNA gene expression in young or mature striatal astrocyte cultures *in vitro* (Figs. 1C and 1F, respectively).

Effects of IL-1β on Neurotrophic Gene Expression in Vivo

To determine whether IL-1β effects on the induction of DAergic neurotrophic factors synthesis are altered with age *in vivo,* IL-1β was injected into the right lateral ventricle in animals of three representative age groups; postnatal day 15 (young), 3 months (young-mature), and 8–10 months of age (middle-aged). To examine if IL-1β can induce the synthesis of neurotrophic factors in the dorsal as well as in the ventral striatum, which has been shown to be the source of DA axonal sprouting to the denervated dorsal striatum in animal models of PD, both these brain regions were dissected and analyzed independently.

In the dorsal striatum of young postnatal day 15 mice, IL-1β injection caused a 41% reduction in aFGF mRNA levels by 6 h ($P < 0.02$; Fig. 2A). In contrast, IL-1β induced a 46% increase in bFGF mRNA levels by 6 h ($P < 0.02$; Fig. 2B). Similar to aFGF, IL-1β caused a 36% reduction in GDNF mRNA levels within 6 h ($P < 0.0001$) compared to control injected mice (Fig. 2C). IL-1β did not change any of the DAergic neurotrophic factors in the ventral striatum of young postnatal day 15 mice (Figs. 2D–2F).

Intraventricular injection of IL-1β into the 3-month age group produced a 34 and 54% reduction in aFGF mRNA levels by 24 h ($P < 0.004$ and $P < 0.0001$) in the dorsal and ventral striatum, respectively (Figs. 3A and 3D). In contrast, IL-1β maximally induced a 320% increase in bFGF mRNA levels within 6 h ($P < 0.0001$) and remained elevated by 24 h in the dorsal striatum (Fig. 3B). Similarly, IL-1β induced a 220% increase in bFGF mRNA levels by 6 h ($P < 0.0006$) in the ventral striatum (Fig. 3E). At the same time, we detected no change in GDNF mRNA gene expression in dorsal or ventral striatum of the young 3-month age group (Figs. 3C and 3F, respectively).

In the 8-month age group, IL-1β did not change aFGF mRNA gene expression in the dorsal and ventral striatum (Figs. 4A and 4D, respectively). In contrast, IL-1β induced a 530% increase in bFGF mRNA levels by 6 h ($P < 0.0001$) and remained elevated by 270% at 24 h ($P < 0.002$; Fig. 4B). Likewise, IL-1β induced a 220% increase in bFGF mRNA levels by 6 h ($P < 0.03$) compared to control in the ventral striatum (Fig. 4E). Moreover, although it was observed that IL-1β induced a small increase in GDNF mRNA levels in the dorsal striatum within 6 h ($P < 0.02$) when compared to

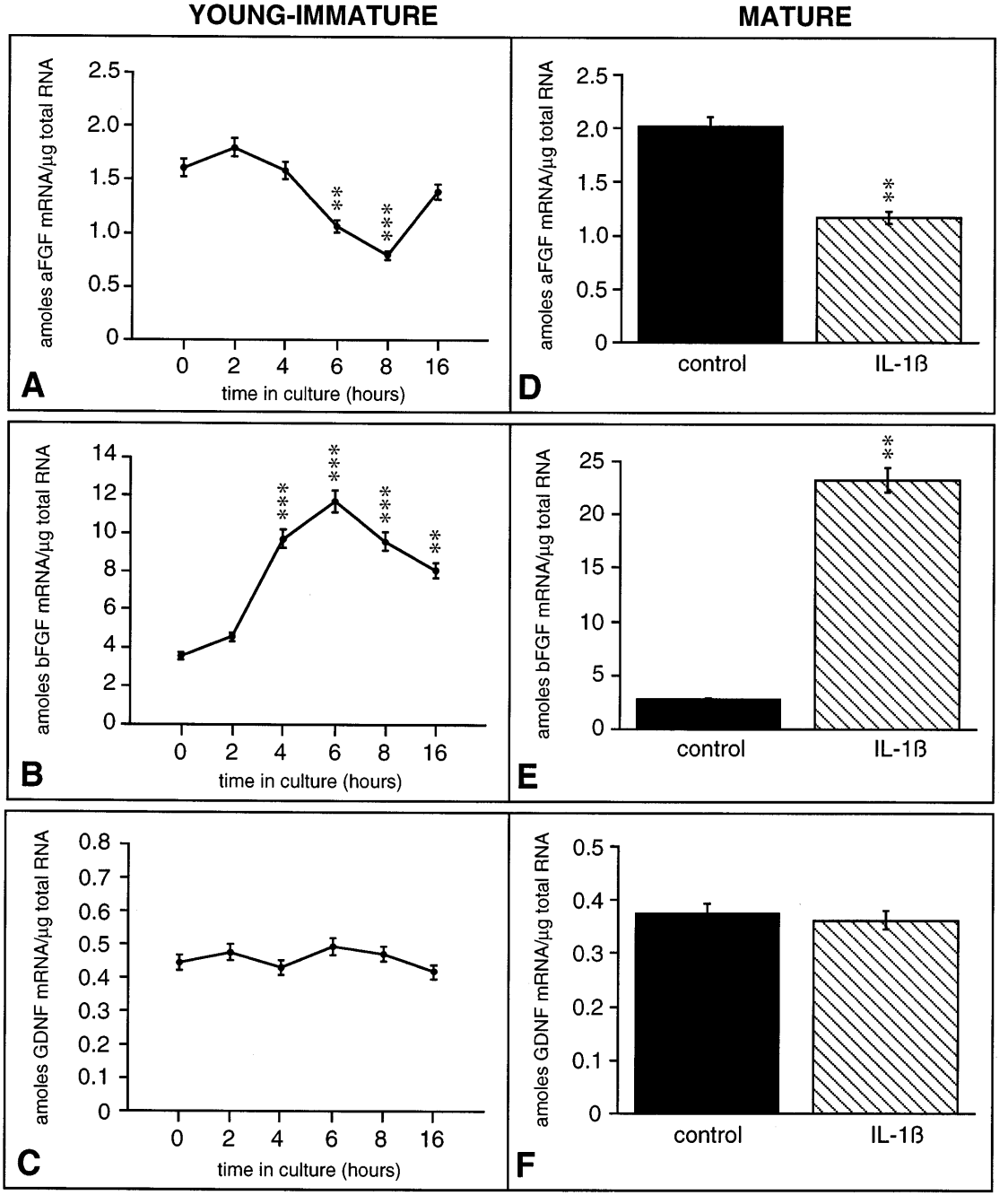

FIG. 1. Quantitative analysis of astroglial-derived dopaminergic neurotrophic factor gene expression after IL-1β treatment in primary striatal astrocyte cultures. Cells at 11–13 days *in vitro* representing young-immature astrocytes (A–C) were placed into 0.05% serum for 24 h and treated with IL-1β (50 ng/ml) or vehicle for 0, 2, 4, 6, 8, and 16 h. Cells at 21–23 days *in vitro* representing mature astrocytes (D–F) were treated as above for 6 h. A and D, aFGF mRNA levels in young-immature (A) and mature (D) striatal astrocyte cultures compared to control cultures following IL-1β treatment. B and E, bFGF mRNA levels in young-immature (B) and mature (E) astrocyte cultures after IL-1β treatment. C and F, GDNF mRNA levels in young-immature (C) or mature (F) striatal astrocyte cultures following IL-1β treatment. Values represent the mean ± SEM for $n = 3$ and $n = 5$ for each group in young and mature astrocyte cultures *in vitro*, respectively. $^{**}P < 0.005$, $^{***}P < 0.0001$ compared to control vehicle.

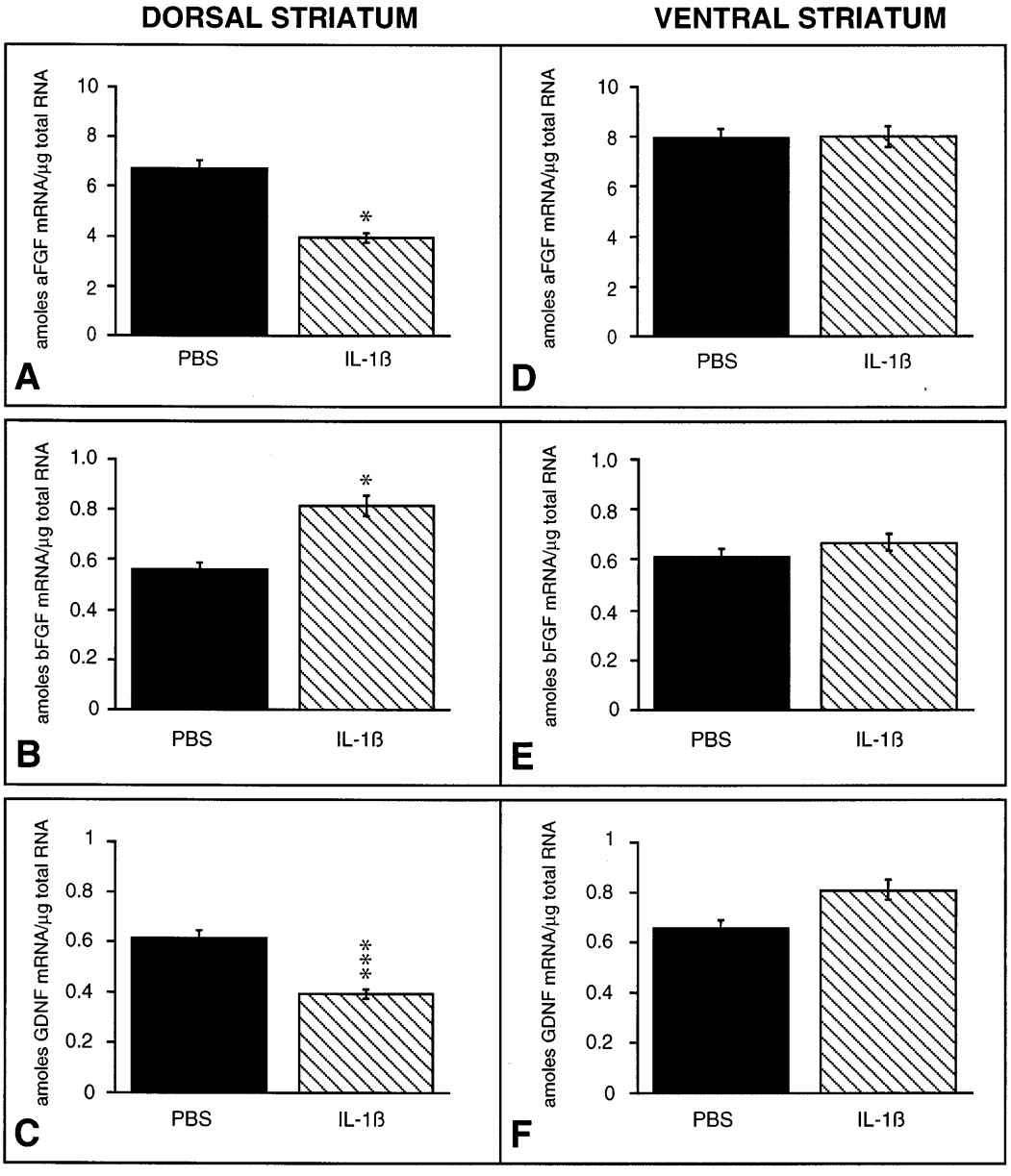

FIG. 2. Quantitative analysis of astroglial-derived dopaminergic neurotrophic factors in the dorsal (A–C) and ventral (D–F) striatum after IL-1β injection for 6 h in postnatal day 15 mice. Stereotactic injections of IL-1β (100 units) or vehicle in a final volume of 0.5 µl were made into the right lateral ventricle using a 1-µl Hamilton syringe. A and D, aFGF mRNA levels following IL-1β treatment compared to control PBS-injected animals in the dorsal (A) and ventral (D) striatum. B and E, bFGF mRNA levels following IL-1β treatment in the dorsal (B) and ventral (E) striatum compared to control PBS-injected animals. C and F, GDNF mRNA levels in the dorsal (C) and ventral (F) striatum compared to control PBS-injected animals. Values represent the mean ± SEM for $n = 5$ *in vivo*. *$P < 0.05$; **$P < 0.005$; ***$P < 0.0001$ compared to PBS injected.

control injected, this effect shortly returned to control levels by 8 h (Fig. 4C). IL-1β did not change GDNF mRNA gene expression in the ventral striatum of middle-aged mice (Fig. 4F).

DISCUSSION

The present study investigated whether astroglial-derived DAergic neurotrophic factors can be regulated by IL-1β. It was observed from the *in vitro* experiments and after intraventricular infusion of IL-1β that bFGF mRNA was upregulated by IL-1β in the striatum. In contrast, there was no sustained induction of aFGF or GDNF mRNA levels after IL-1β treatment. This therefore suggests that bFGF may be the putative DAergic neurotrophic factor mediating IL-1β effects on DAergic axonal sprouting after a neurotoxin challenge. However, in contrast to what we hypothesized, we did not

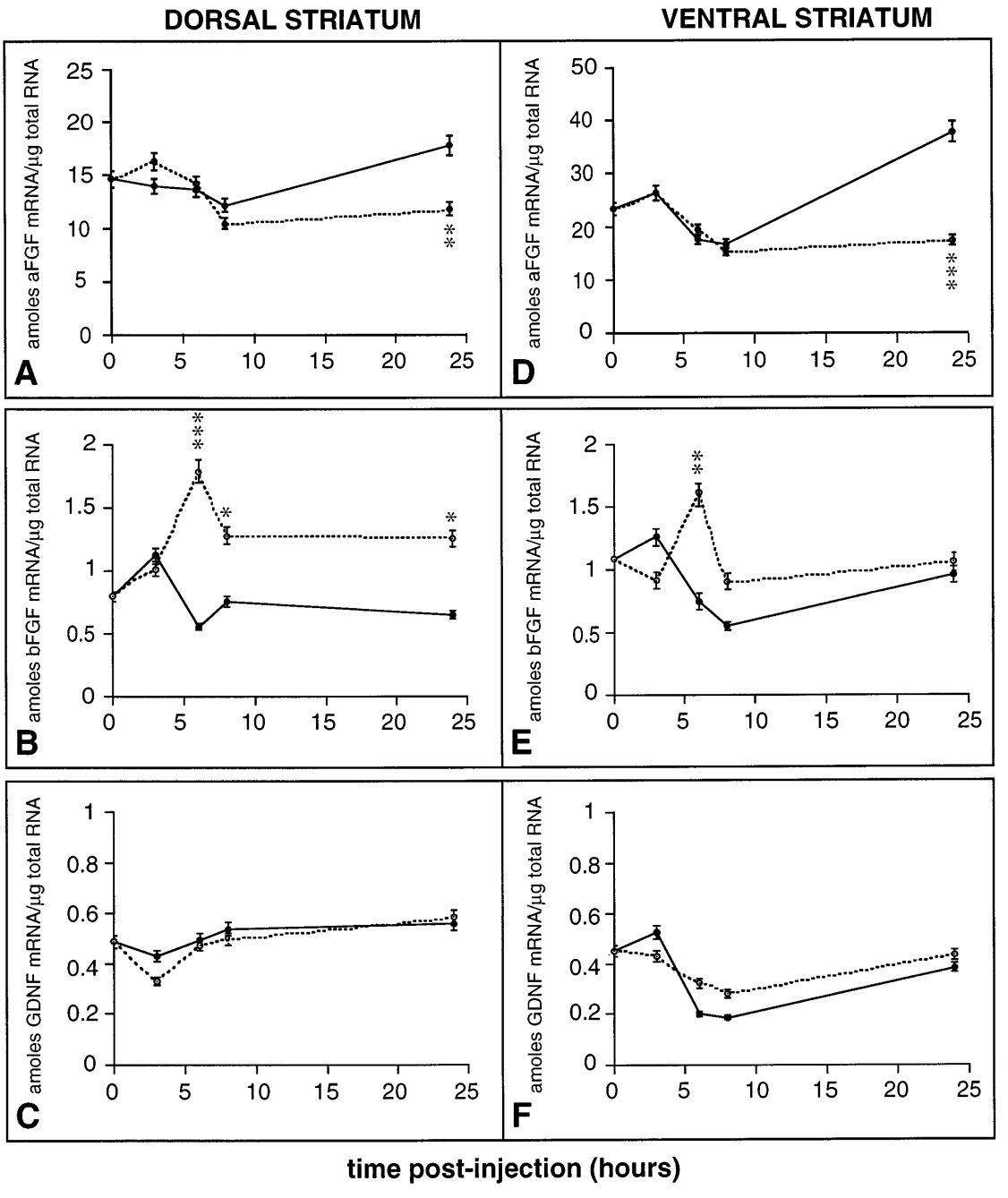

FIG. 3. Time-course quantitative analysis of astroglial-derived dopaminergic neurotrophic factors in the dorsal (A–C) and ventral (D–F) striatum at 0, 3, 6, 8, and 24 h after IL-1β treatment in 3-month-old mice. Stereotactic injections of IL-1β (100 units) or vehicle in a final volume of 0.5 µl were made into the right lateral ventricle using a 1-µl Hamilton syringe. The 0 h time point represents noninjected control. The dotted line represents IL-1β-injected and the solid line represents control PBS-injured animals. A and D, aFGF mRNA levels in the dorsal (A) and ventral (D) striatum compared to control PBS-injected animals. B and E, bFGF mRNA levels in the dorsal (B) and ventral (E) striatum compared to control PBS-injected animals. C and F, GDNF mRNA in the dorsal (C) and ventral (F) striatum after IL-1β injection. Values represent the mean ±SEM for $n = 3$ *in vivo*. *$P < 0.05$; **$P < 0.005$, ***$P < 0.0001$ compared to PBS-injected.

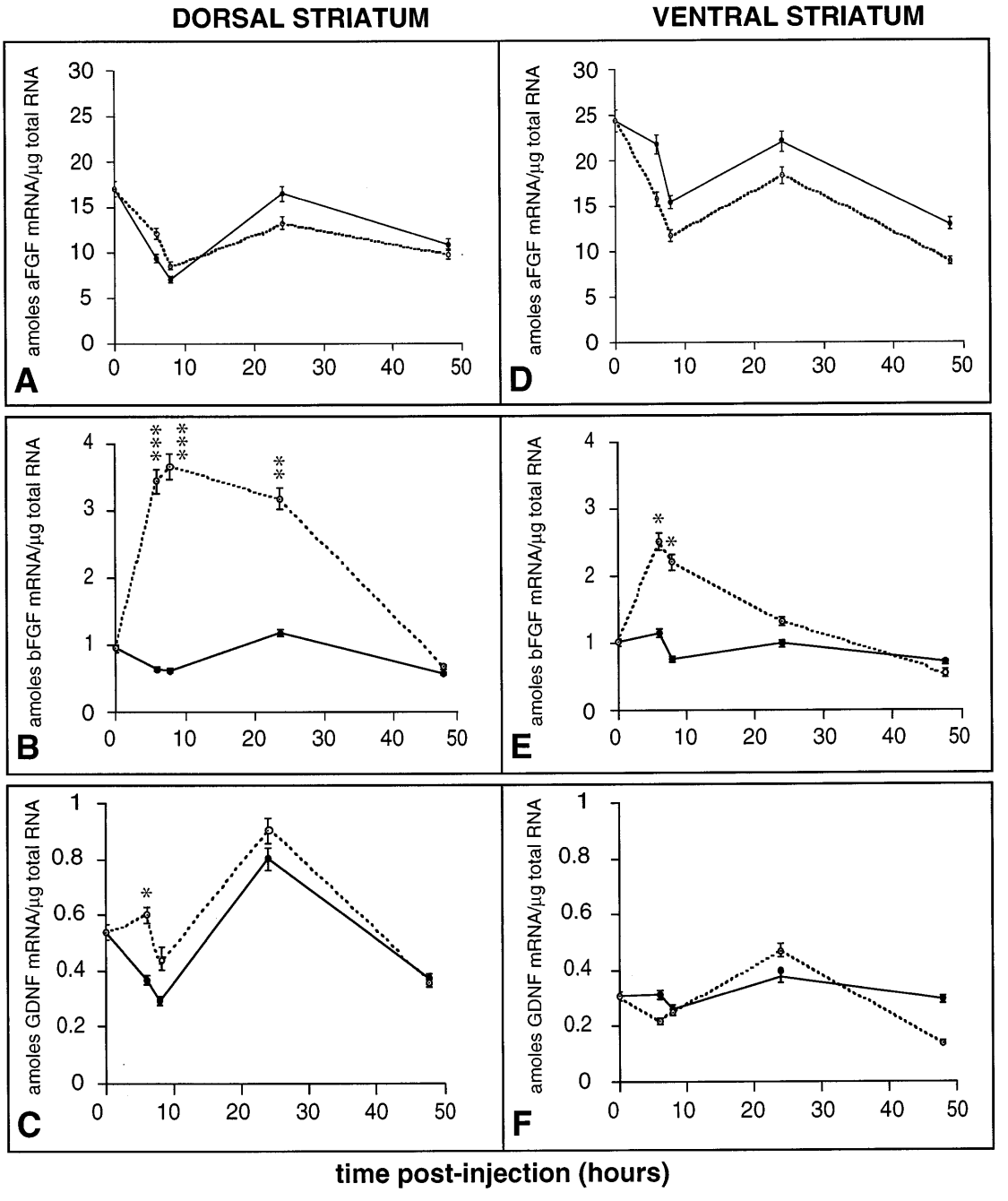

FIG. 4. Time-course quantitative analysis of astroglial-derived dopaminergic neurotrophic factors in the dorsal (A–C) and ventral (D–F) striatum at 0, 6, 8, 24, and 48 h after IL-1β treatment in 8-month-old mice. Stereotactic injections of IL-1β (100 units) or vehicle in a final volume of 0.5 µl were made into the right lateral ventricle using a 1-µl Hamilton syringe. The 0 h time point represents noninjected control. The dotted line represents IL-1β-injected and the solid line represents control PBS-injected animals. A and D, aFGF mRNA gene expression in the dorsal (A) and ventral (D) striatum compared to control PBS-injected animals. B and E, bFGF mRNA levels in the dorsal (B) and ventral (E) striatum compared to control PBS-injected animals. C and F, GDNF mRNA levels in the dorsal (C) and ventral (F) striatum compared to control PBS-injected animals. Values represent the mean ±SEM for $n = 3$ *in vivo*. *$P < 0.05$; **$P < 0.005$, ***$P < 0.0001$ cmopared to PBS injected.

observe an aging-associated decline in the ability of IL-1β to induce bFGF mRNA in the middle-aged group. In fact, IL-1β stimulated a greater induction in bFGF mRNA levels in the middle-aged compared to young mice.

IL-1 and Astroglial Cells

Several lines of evidence have indicated that the adult CNS can regenerate after lesion-induced injury. The regenerative capacity of the CNS depends not only on CNS neurons but also on the glial environment (22). Astroglia can release neurotrophic factors required for the proper maintenance and growth of nerve cells (40). During brain development, astroglia can aid neurite outgrowth, either by providing a scaffold or by providing neurotrophic support (40). In contrast, as the normal brain matures, and after injury, astroglia can be unsupportive of axon growth, especially in the vicinity of a glial scar (10, 41, 61–64, 69). However in recent years, it has been debated whether astroglia directly interfere with outgrowth of neurites in the process of regeneration (22). Previous studies have shown that astrocytes play an important role in the sprouting of NGF-sensitive damaged cholinergic neurons in the basal forebrain after septohippocampal deafferentation (26). Furthermore, it was implicated that astroglial synthesis of NGF and other neurotrophic factors which act on damaged neurons to promote regeneration were initially up-regulated by cytokines (83).

In the present study, we initially examined whether aFGF, bFGF, or GDNF, which specifically have been shown to induce DAergic axonal sprouting, can be directly regulated by IL-1β in striatal astrocytes. Our *in vitro* results indicated that IL-1β selectively induced the synthesis of bFGF mRNA but not aFGF or GDNF mRNA. IL-1β induced bFGF mRNA maximally within 6 h and sustained a significant increase for 16 h in young immature astrocytes *in vitro*. Furthermore, it was observed that there was not a decrease in the ability of mature astrocytes to respond to IL-1β treatment compared to immature astrocytes. Indeed, mature astrocytes had a relative increase in bFGF mRNA in response to IL-1β treatment.

More recently, it has become appreciated that mature astrocytes can display plasticity in their ability to support neurite extension depending on differences in the expression of cell surface and extracellular matrix (ECM) molecules. Growth-inhibiting versus growth-promoting effects of astrocytes on neurons can be mediated by the presence of chondroitin 6-sulfate proteoglycans (CSPGs) and tenascin (50, 52). It was shown that astrocytes rich in CSPGs and tenascin are inhibitory to process outgrowth whereas astrocytes that do not express these particular ECM molecules seemed to promote neurite growth (50, 52). Others have suggested that the release of cytokines during an inflammatory response to brain injury can upregulate certain ECM molecules which in turn can affect the binding and actions of trophic factors (19, 51). For example, an ECM molecule perlecan has been shown to potentiate the actions of bFGF (4, 54). Thus, our findings suggest that it is not a loss of the ability of IL-1β to stimulate neurotrophic factor synthesis in mature astrocytes that compromises their growth-promoting potential, but possibly it is due to an alteration in IL-1β regulation of growth-promoting ECM molecules. However further investigation is needed to elucidate the role of cytokines in regulating ECM molecules and their interaction with growth factors after neuronal injury.

IL-1 and the Regulation of Neurotrophic Factor Gene Expression in the CNS

The stimulation of astroglial-derived DAergic neurotrophic factor synthesis by IL-1β could be one mechanism by which IL-1β induces the survival and plasticity of DAergic neurons after injury. We performed stereotactic intraventricular injections to determine whether IL-1β can stimulate DAergic neurotrophic factor gene expression *in vivo*. We examined three representative age groups: postnatal day 15 when astrocytes have been shown to promote neurite outgrowth and 3 and 8 months of age (young adult and middle-aged, respectively) when neurons and the glial environment of the adult CNS have been shown to be less favorable for regeneration to occur after brain injury (15). IL-1β was able to induce an increase in bFGF mRNA synthesis in the striatum of postnatal day 15 mice, and this induction was even greater in young adult and middle-aged mice thus demonstrating that there was not an aging-associated loss in the capacity of IL-1β to induce bFGF mRNA. The effects of IL-1β on the induction of bFGF mRNA but not aFGF or GDNF mRNA suggests that IL-1β effects on bFGF were specific and not the result of generalized mRNA upregulation since the analyses of these neurotrophic factors were from the same animals. In addition, we observed that IL-1β can have opposite actions on the expression of two fibroblast growth factors. IL-1β induced bFGF mRNA synthesis, but at the same time reduced aFGF mRNA. This provides evidence that although aFGF and bFGF have been shown to have similar functions and actions on the brain, cytokines can have distinct influences on neurotrophic factor gene expression in the brain. This is further supported by other studies in which intraventricular injection of IL-1β induced bFGF and NGF mRNA expression but not aFGF mRNA in the hippocampus of adult rat brain (67, 76). While the present study demonstrates an effect of IL-1β on neurotrophic factor gene expression, cytokines may not necessarily alter protein levels; therefore, it is important to consider whether the changes we observed lead to alterations in

neurotrophic factor availability. However, other studies have shown that IL-1β induction of neurotrophic factor synthesis was also reflected by a corresponding increase in protein secretion (3, 49, 76).

The properties of IL-1β to stimulate reactive astrogliosis and neovascularization after infusion of IL-1β that was reported in the adult brain (33) could be mediated by bFGF. It was described that bFGF can influence cells in the CNS through its mitogenic and angiogenic properties (36, 37). Similar to IL-1β, bFGF has been shown to stimulate astrocyte proliferation as well as to induce new blood vessel growth (36, 37). In the present study, we found that IL-1β caused a significant 7- and 13-fold increase of bFGF mRNA in young-mature and middle-aged animals, respectively, when compared to the modest induction observed in the developing postnatal day 15 mice. This enhanced stimulation of bFGF mRNA in the young adult seemed to correlate well with the selective cellular responses of IL-1β effects on the adult brain compared to the developing brain (33, 73), thus suggesting a role of bFGF in mediating the actions of IL-1β in the wound healing process after neuronal injury.

Cytokines frequently act synergistically with other cytokines or neurotrophic factors to elicit an effect (8). It has been implied by others that the newly synthesized bFGF in the hippocampus after cerebroventricular injection of IL-1β may enhance the initial action of IL-1β to further stimulate bFGF expression (67). Our results of an enhanced IL-1β induction of bFGF mRNA in the striatum of middle-aged mice may be due to a similar synergistic interaction. Since we have shown that it was not the inability of middle-aged animals to synthesize neurotrophic factors, it is possible that receptor trafficking and receptor activation becomes altered with age and might be responsible for the loss of recovery seen in aged animals. Previous studies have reported that in senescent vascular cells, there are aging-associated defects in mitogenic responsiveness to FGF and epidermal growth factor (EGF; 27, 59). This was accompanied by a parallel decrease in total number of receptors and receptor binding and signalling of the EGF and FGF receptor system (27, 59, 60). However, this still remains to be explored in the aged brain.

IL-1 and Parkinson's Disease

Animal models of PD have shown that young mice can begin spontaneous recovery within 1 month after a MPTP-induced lesion of the nigrostriatal DA system compared to aged mice (65, 66). Cellular reactions after MPTP involving changes in growth factor gene expression such as bFGF mRNA observed in young mice are a possible factor mediating the recovery of striatal DA nerve terminals (47). However, it remains to be established what endogenous events may contribute to the age-associated reduction in the ability to recover from neuronal injury. There is a growing body of evidence suggesting that normal repair mechanisms associated with neurodegeneration can also contribute to the disease process if the responses are overstimulated or disregulated (19). For example, while studies have shown that bFGF can promote neurite sprouting, bFGF may also play a role in plaque formation in Alzheimer's disease (19). Since aging is often accompanied by a decline in immune homeostasis (45), possibly as the brain ages, the responses to injury may lead to a propagation of enhanced inflammatory processes which could be responsible for the aging-associated loss in the ability to recover from injury which is potentially reflected by the greater induction of bFGF synthesis by IL-1β in middle-aged mice when compared to young adults. It is possible that the enhanced stimulation of bFGF by IL-1β observed in middle-aged mice in combination with other uncharacterized factors could be triggering feedback reactions which may lead to alterations in the CNS environment that are not conducive to recovery. Perhaps, by further elucidating how cytokines, ECM molecules, and trophic factors interact and change in response to injury and whether the balance between these interactions becomes altered with age, we can gain a better understanding of how to slow neurodegenerative processes in the adult brain.

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REFERENCES

1. Agid, Y., F. Javoy-Agid, and M. Ruberg. 1987. Biochemistry of neurotransmitters in Parkinson's disease. In *Movement Disorders* (C. D. Marsden, and S. Fahn, Eds.), pp. 166–230. Butterworth, London.
2. Akaneya, Y., M. Takahashi, and H. Hatanaka. 1995. Interleukin-1β enhances survival and interleukin-6 protects against MPP+ neurotoxicity in cultures of fetal rat dopaminergic neurons. *Exp. Neurol.* **136:** 44–52.
3. Araujo, D. M., and C. W. Cotman. 1992. Basic FGF in astroglial, microglial, and neuronal cultures: Characterization of binding sites and modulation of release by lymphokines and trophic factors. *J. Neurosci.* **12:** 1668–1678.
4. Aviezer, D., D. Hecht, M. Safran, M. Elsinger, G. David, and A. Yayon. 1994. Perlecan, basal lamina proteoglycan, promotes basic FGF-receptor binding, mitogenesis and angiogenesis. *Cell* **79:** 1005–1013.
5. Bankiewicz, K. S., R. J. Plunkett, D. M. Jacobowitz, L. Porrino, U. DiPorzio, W. London, I. J. Kopin, and E. H. Oldfield. 1990. The effect of fetal mesencephalic implants on primate MPTP-induced parkinsonism. *J. Neurosurg.* **72:** 231–244.
6. Bankiewicz, K. S., R. J. Plunkett, D. M. Jacobowitz, I. J. Kopin, and E. H. Oldfield. 1991. Sprouting-induced recovery from MPTP-induced parkinsonism in primates after fetal non-dopaminergic brain implants: Histochemical and behavioral studies. *J. Neurosurg.* **74:** 97–101.

7. Bartfai, T., and M. Schultzberg. 1993. Cytokines in neuronal cell types. *Neurochem. Int.* **22:** 435–444.
8. Benveniste, E. N. 1992. Inflammatory cytokines within the central nervous system: Sources, function, and mechanism of action. *Am. J. Physiol.* **263:** C1–C6.
9. Bernheimer, H., W. Birkmayer, O. Hornykiewicz, K. Jellinger, and F. Seitelberger. 1973. Brain dopamine and the syndromes of Parkinson and Huntington. *J. Neurol. Sci.* **20:** 415–455.
10. Berry, M., W. L. Maxwell, A. Logan, A. Mathewson, P. McConnell, D. Ashhurst, and G. H. Thomas. 1983. Deposition of scar tissue in the central nervous system. *Acta Neurochir.* **32**(suppl): 31–53.
11. Blanchard, V., M. Chritin, S. Vyas, M. Savasta, C. Feuerstein, Y. Agid, F. Javoy-Agid, and R. Raisman-Vozari. 1995. Long-term induction of tyrosine hydroxylase expression: Compensatory response to partial degeneration of the dopaminergic nigrostriatal system in the rat brain. *J. Neurochem.* **64:** 1669–1679.
12. Blum, M. 1989. Regulation of neuroendocrine peptide gene expression. In *Methods in Enzymology* (P. M. Conn, Ed.), Vol. **168,** pp 618–633. Academic Press, San Diego.
13. Blum, M., and C. S. Weichert. 1995. GDNF mRNA expression in normal postnatal development, aging, and in weaver mutant mice. *Neurobiol. Aging* **16:** 925–929.
14. Bohn, M. C., L. Cupit, F. Marciano, and D. M. Gash. 1987. Adrenal medulla grafts enhance recovery of striatal dopaminergic fibers. *Science* **237:** 913–916.
15. Brodkey, J. A., M. A. Gates, E. D. Laywell, and D. A. Steindler. 1993. The complex nature of interactive neuroregeneration-related molecules. *Exp. Neurol.* **123:** 251–270.
16. Chadi, G., Y. Cao, R. F. Pettersson, and K. Fuxe. 1994. Temporal and spatial increase of astroglial basic fibroblast growth factor synthesis after 6-hydroxydopamine-induced degeneration of the nigrostriatal dopamine neurons. *Neuroscience* **61:** 891, 910.
17. Cintra, A., Y. H. Cao, C. Oellig, B. Tinner, F. Bortolotti, M. Goldstein, R. F. Pettersson, and K. Fuxe. 1991. Basic FGF is present in dopaminergic neurons of the ventral midbrain of the rat. *NeuroReport* **2:** 597–600.
18. Coelin, R. V., K. Unsicker, and K. Krieglstein. 1995. Screening of interleukins for survival-promoting effects on cultured mesencephalic dopaminergic neurons from embryonic rat brain. *Dev. Brain Res.* **89:** 150–154.
19. Cotman, C. W., A. J. Tenner, and B. J. Cumming. 1996. β-Amyloid converts an acute phase injury response to chronic injury responses. *Neurobiol. Aging* **17:** 723–731.
20. Date, I., D. L. Felten, and S. Y. Felten. 1990. Long-term effect of MPTP in the mouse brain in relation to aging: neurochemical and immunocytochemical analysis. *Brain Res.* **519:** 266–276.
21. Date, I., M. F. D. Notter, S. Y. Felten, and D. L. Felten. 1990b. MPTP-treated young mice but not aging mice show partial recovery of the nigrostriatal dopaminergic system by stereotaxic injection of acidic fibroblast growth factor (aFGF). *Brain Res.* **526:** 156–160.
22. Eddleston, M., and L. Mucke. 1993. Molecular profile of reactive astrocytes—Implications for their role in neurologic disease. *Neuroscience* **54:** 15–36.
23. Ewing, S. E., R. J. Weber, A. Zauner, and R. J. Plunkett. 1992. Recovery in hemiparkinsonian rats following intrastriatal implantation of activated leukocytes. *Brain Res.* **576:** 42–48.
24. Fiandaca, M. S., J. H. Kordower, J. T. Hansen, S. S. Jiao, and D. M. Gash. 1988. Adrenal medullary autografts into the basal ganglia of Cebus monkeys: Injury-induced regeneration. *Exp. Neurol.* **102:** 76–91.
25. Fontana, A., F. Kristensen, R. Dubs, D. Gemsa, and E. Weber. 1982. Production of prostaglandin E and interleukin-1 like factor by cultured astrocytes and C glioma cells. *J. Immunol.* **129:** 2413–2419.
26. Gage, F. H., P. Olejniczak, and D. M. Armstrong. 1988. Astrocytes are important for sprouting in the septohippocampal circuit. *Exp. Neurol.* **102:** 2–13.
27. Garfinkel, S., X. Hu, I. A. Prudovsky, G. A. McMahon, E. M. Kapnik, S. D. McDowell, and T. Maciag. 1996. FGF-1-dependent proliferative and migratory responses are impaired in senescent human umbilical vein endothelial cells and correlate with the inability to signal tyrosine phosphorylation of fibroblast growth factor receptor-1 substrates. *J. Cell. Biol.* **134:** 783–791.
28. Giulian, D. 1992. Microglia and disease of the nervous system. *Curr. Top. Neurol.* **12:** 23–54.
29. Giulian, D., and T. J. Baker. 1985. Peptides released by ameboid microglia regulate astroglial proliferation. *J. Cell. Biol.* **101:** 2411–2415.
30. Giulian, D., and L. B. Lachman. 1985. Interleukin-1 stimulation of astroglial proliferation after brain injury. *Science* **228:** 497–499.
31. Giulian, D., R. Allen, T. J. Baker, and Y. J. Tomozawa. 1986. Brain peptides and glial growth. 1. Glia-promoting factors as regulators of gliogenesis in the developing and injured central nervous system. *J. Cell. Biol.* **102:** 803–811.
32. Giulian, D., T. J. Baker, L. N. Shin, and L. B. Lachman. 1986. Interleukin-1 of the central nervous system is produced by ameboid microglia. *J. Exp. Med.* **164:** 594–604.
33. Giulian, D., J. Woodward, D. G. Young, J. F. Krebs, and L. B. Lachman. 1988. Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. *J. Neurosci.* **8:** 2485–2490.
34. Giulian, D., D. G. Young, J. Woodward, D. C. Brown, and L. B. Lachman. 1988. Interleukin-1 is an astroglial growth factor in the developing brain. *J. Neurosci.* **8:** 709–714.
35. Giulian, D., K. Vaca, and M. Corpuz. 1993. Brain glia release factors with opposing actions upon neuronal survival. *J. Neurosci.* **13:** 29–37.
36. Gospodarowicz, D., G. Neufeld, and L. Schweigerer. 1986. Molecular and biological characterization of fibroblast growth factor: An angiogenic factor which controls the proliferation and differentiation of mesoderm and neuroectoderm derived cells. *Cell. Differ.* **19:** 1.
37. Gospodarowicz, D., N. Ferrara, L. Schweigerer, and G. Neufeld. 1987. Structural characterization and biological functions of fibroblast growth factor. *Endocr. Rev.* **8:** 95–114.
38. Hansen, J. T., M. S. Fiandaca, J. H. Kordower, M. F. D. Notter, and D. M. Gash. 1990. Striatal adrenal medulla/sural nerve cografts in hemiparkinsonian monkeys. *Prog. Brain Res.* **82:** 573–580.
39. Hansen, J. T., K. Sakai, J. T. Greenamyre, and S. Moran. 1995. Sprouting of dopaminergic fibers from spared mesencephalic dopamine neurons in the unilateral partial lesioned rat. *Brain Res.* **670:** 197–204.
40. Hatten, M. E., G. Fishell, T. N. Stitt, and C. A. Mason. 1990. Astroglia as a scaffold for development of the CNS. *Semin. Neurosci.* **2:** 455–465.
41. Hatten, M. E., R. K. H. Liem, M. L. Shelanski, and C. A. Mason. 1991. Astroglia in the CNS injury. *Glia* **4:** 233–243.
42. Hetier, E., J. Ayala, P. Denefle, A. Bousseau, P. Rouget, M. Malat, and A. Prochiantz. 1988. Brain macrophages synthesize interleukin-1 and interleukin-1 mRNAs *in vitro*. *J. Neurosci. Res.* **21:** 391–397.
43. Hirsch, E., A. M. Graybiel, and Y. A. Agid. 1988. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature* **334:** 345–348.

44. Ho, A., A. C. Gore, C. S. Weickert, and M. Blum. 1995. Glutamate regulation of GDNF gene expression in the striatum and primary striatal astrocytes. *NeuroReport* **6:** 1454–1458.

45. Hodes, R. J. 1995. Molecular alterations in the aging immune system. *J. Exp. Med.* **182:** 1–3.

46. Lawrence, J. M., R. J. Morris, D. J. Wilson, and G. Raisman. 1990. Mechanisms of allograft rejection in the rat brain. *Neuroscience* **37:** 431–462.

47. Leonard, S., D. Luthman, J. Logel, J. Luthman, C. Antle, R. Freedman, and B. Hoffer. 1993. Acidic and basic fibroblast growth factor mRNAs are increased in striatum following MPTP-induced dopamine neurofiber lesion: Assay by quantitative PCR. *Mol. Brain Res.* **18:** 275–284.

48. Lieberman, A. P., P. M. Pitha, H. S. Shin, and M. L. Shin. 1989. Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotrophic virus. *Proc. Natl. Acad. Sci. USA* **86:** 56348–56352.

49. Lindholm, D., R. Heumann, M. Meyer, and H. Thoenen. 1987. Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature* **330:** 658–659.

50. McKeon, R. J., R. C. Schreiber, J. S. Rudge, and J. Silver. 1991. Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. *J. Neurosci.* **11:** 3398–3411.

51. Meiners, S., M. Marone, J. L. Rittenhouse, and H. M. Geller. 1993. Regulation of astrocytic tenascin by basic fibroblast growth factor. *Dev. Biol.* **160:** 480–493.

52. Meiners, S., E. M. Powell, and H. M. Geller. 1995. A distinct subset of tenascin/CS-6-PG-rich astrocytes restricts neuronal growth *in vitro*. *J. Neurosci.* **15:** 8096–8101.

53. Nieto-Sampedro, M., and M. A. Berman. 1987. Interleukin-1 like activity in rat brain: Sources, targets, and effect of injury. *J. Neurosci. Res.* **17:** 214–219.

54. Nurcombe, V., M. D. Ford, P. F. Wildschut, and P. Barlett. 1993. Developmental regulation of neural response to FGF-1 and FGF-2 by heparan sulfate proteoglycan. *Science* **260:** 103–106.

55. Otto, D., and K. Unsicker. 1990. Basic FGF reverses chemical and morphological deficits in the nigrostriatal system of MPTP-treated mice. *J. Neurosci.* **10:** 1912–1921.

56. Perry, V. H., and S. Gordon. 1988. Macrophages and microglia in the nervous system. *Trends Neurosci.* **11:** 273–277.

57. Pickel, V. M., E. Johnson, M. E. Carsson, and J. Chan. 1992. Compensatory changes in ultrastructure of spared dopaminergic terminals in caudate-putamen nuclei of adult rats receiving neonatal intranigral 6-hydroxydopamine. *Dev. Brain Res.* **70:** 75–86.

58. Plunkett, R. J., K. S. Bankiewicz, A. C. Cummins, R. S. Miletich, J. P. Schwartz, and E. H. Oldfield. 1990. Long-term evaluation of hemiparkinsonian monkeys after adrenal autografting or cavitation alone. *J. Neurosurg.* **73:** 918–926.

59. Reenstra, W. R., M. Yaar, and B. A. Gilchrest. 1993. Effect of donor age on epidermal growth factor processing in man. *Exp. Cell Res.* **209:** 118–122.

60. Reenstra, W. R., M. Yaar, and B. A. Gilchrest. 1996. Aging affects epidermal growth factor receptor phosphorylation and traffic kinetics. *Exp. Cell Res.* **227:** 252–255.

61. Reier, P. J. 1986. Gliosis following CNS injury: The anatomy of astrocytic scars and their influences on axonal elongation. In *Astrocytes: Cell Biology and Pathology of Astrocytes* (S. Federoff and A. Vernadakis, Eds.), pp 263–324. Academic Press, Orlando, FL.

62. Reier, P. J., and J. Houle. 1988. The glial scar: Its bearing on axonal elongation and transplantation approaches to CNS repair. In *Advances in Neurology: Functional Recovery in Neurological Disease* (S. G. Waxman, Ed.), pp 87–138. Raven Press, New York.

63. Reier, P. J., L. J. Stensaas, and L. Guth. 1983. The astrocytic scar as an impediment to regeneration in the central nervous system. In *Fundamentals of Spinal Cord Reconstruction* (C. C. Kao, R. P. Bunge, and P. J. Reier, Eds), pp. 163–195. Raven Press, New York.

64. Reier, P. J., L. F. Eng, and L. Jakeman. 1989. Reactive astrocyte and axonal outgrowth in the injured CNS: Is gliosis really an impediment to regeneration? In *Neural Regeneration and Transplantation* (F. J. Seil, Ed.), pp. 183–209. Alan R. Liss, New York.

65. Ricaurte, G. A., L. E. DeLanney, I. Irwin, and J. W. Langston. 1987. Older dopaminergic neurons do not recover from the effects of MPTP. *Neuropharmacology* **26:** 97–99.

66. Ricaurte, G. A., I. Irwin, L. S. Forno, L. E. DeLanney, E. Langston, and J. W. Langston. 1987. Aging and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced degeneration of dopaminergic neurons in the substantia nigra. *Brain Res.* **403:** 43–51.

67. Rivera, S., S. J. Gold, and C. M. Gall. 1994. Interleukin-1β increases basic fibroblast growth factor mRNA expression in adult rat brain and organotypic hippocampal cultures. *Mol. Brain Res.* **27:** 12–26.

68. Rothwell, N. J., and P. J. L. M. Strijbos. 1995. Cytokines in neurodegeneration and repair. *Int. J. Dev. Neurosci.* **13:** 179–185.

69. Rudge, J. S., and J. Silver. 1990. Inhibition of neurite outgrowth on astroglial scars *in vitro*. *J. Neurosci.* **10:** 3594–3603.

70. Rudge, J. S., G. M. Smith, and J. Silver. 1989. An *in vitro* model of wound healing in the CNS: Analysis of cell reaction and interaction at different ages. *Exp. Neurol.* **103:** 1–16.

71. Sawada, M., A. Suzumura, and T. Marunouchi. 1995. Cytokine network in the central nervous system and its roles in growth and differentiation of glial and neuronal cells. *Int. J. Dev. Neurosci.* **13:** 253–264.

72. Shimasaki, S., N. Emoto, A. Koba, M. Mercado, F. Shibata, K. Cooksey, A. Baird, and N. Ling. 1988. Complementary DNA cloning and sequencing of rat ovarian basic fibroblast growth factor and tissue distribution study of its mRNA. *Biochem. Biophys. Res. Commun.* **157:** 256–263.

73. Sievers, J., G. Struckhoff, and M. Puchner. 1993. Interleukin-1β does not induce reactive astrogliosis, neovascularization or scar formation in the immature rat brain. *Int. J. Dev. Neurosci.* **11:** 281–293.

74. Smith, G. M., R. H. Miller, and J. Silver. 1986. Changing role of forebrain astrocytes during development, regenerative failure, and induced regeneration upon transplantation. *J. Comp. Neurol.* **251:** 23–43.

75. Smith, G. M., U. Rutishauser, J. Silver, and R. H. Miller. 1990. Maturation of astrocytes *in vitro* alters the extent and molecular basis of neurite outgrowth. *Dev. Biol.* **138:** 377–390.

76. Spranger, M., D. Lindholm, C. Bandtlow, R. Heumann, H. Gnahn, M. Näher-Noé, and H. Thoenen. 1990. Regulation of nerve growth factor (NGF) synthesis in the rat central nervous system: Comparison between the effects of interleukin-1 and various growth factors in astrocyte cultures and *in vivo*. *Eur. J. Neurosci.* **2:** 69–76.

77. Strömberg, I., L. Björklund, M. Johansson, A. Tomac, F. Collins, L. Olson, B. J. Hoffer, and C. Humpel. 1993. Glial cell line-derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons *in vivo*. *Exp. Neurol.* **124:** 401–412.

78. Tomac, A., E. Lindqvist, L-F.H. Lin, S. O. Ögren, D. Young, B. J.

Hoffer, and L. Olson. 1995. Protection and repair of the nigrostriatal dopaminergic system by GDNF *in vivo*. *Nature* **373:** 335–339.

79. Tomaselli, K. J., L. F. Reichardt, and J. L. Bixby. 1986. Distinct molecular interactions mediate neuronal process outgrowth on nonneuronal cell surfaces and extracellular matrices. *J. Cell Biol*. **103:** 2659–2672.

80. Wang, J., K. S. Bankiewicz, R. J. Plunkett, J. Sheng, and D. M. Jacobowitz. 1991. Transplantation of microglia reduces experimental parkinsonism in rats. In *Intracerebral Transplantation in Movement Disorders* (O. Lindvall, A. Björklund, and H. Widner, Eds.), pp. 313–323. Elsevier, Amsterdam.

81. Wang, J., K. S. Bankiewicz, R. J. Plunkett, and E. H. Oldfield. 1994. Intrastriatal implantation of interleukin-1: Reduction of parkinsonism in rats by enhancing neuronal sprouting from residual dopaminergic neurons in the ventral tegmental area of the midbrain. *J. Neurosurg.* **80:** 484–490.

82. Wang, L-C., D. H. Baird, M. E. Hatten, and C. A. Mason. 1994. Astroglial differentiation is required for support of neurite outgrowth. *J. Neurosci.* **14:** 3195–3207.

83. Yoshida, K., and F. H. Gage. 1992. Cooperative regulation of nerve growth factor synthesis and secretion in fibroblasts and astrocytes by fibroblast growth factor and other cytokines. *Brain Res.* **569:** 14–25.