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Environmental influences on brain neurotrophins in rats

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Abstract

Environmental factors can have profound influences on the brain. Enriching environments with physical, social and sensory stimuli are now established to be beneficial to brain development and ageing. A multitude of responses from cellular and molecular mechanisms to macroscopic changes in neural morphology and neurogenesis have been considered in the context for evidences that environmental inputs can regulate brain plasticity in the rat at all stages of life. Data from our laboratory have revealed that enriched environment increased nerve growth factor (NGF) gene expression and protein levels in the hippocampus, and this may contribute to events underlying environmentally induced neural plasticity. Because neurotrophic factors are essential for neural development and survival, they are likely to be involved in the cerebral consequences modified by enriched experiences. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Enriched environment; Nerve growth factor; Brain-derived neurotrophic factor; Neurotrophin-3; Neural plasticity

1. Introduction

The increasingly exciting frontiers for studying neural and behavioural plasticity have come to embrace the fact that the brain can be modified for enhanced development and functions over the lifetime of an organism. This premise resulted from studies that behaviours as well as cell structures and biochemical processes could be altered as consequences to the activity and coactivity between the environment and genetic dispositions. It has long been known that the development of an organism is characterised by the occurrence of critical periods, stages at which the outcome fate of the organism is established. External influences during these critical periods can have dramatic consequences. Therefore, the susceptibility of the developing organism to external manipulations is important to its species continuance. In an attempt to elucidate the basis of interaction between the environment and the brain, the environmental enrichment model was applied in animal studies, which is the most widely known model of experience-induced neural plasticity.

Although the interests and assertions for enriching the brain have certainly been parts of the genesis for studying behaviour and brain, the application of the concept that the structural changes in the brain are experiences dependent came only half a century ago. The pioneering work by Donald Hebb (1949) demonstrated the significance of early environmental stimulation on development. He suggested that the immature/neonatal organism's experiences during the critical periods are important in organising the neurophysiological and behavioural processes. Furthermore, these developments can be strongly modified by early stimulation, and the effects could persist throughout the entire life span of the organism. Understanding of the influences of early stimulation, hence, requires knowledge of how the organism's neurological and physiological processes interact with, and respond to, external environmental factors. Indeed, the influence of environmental factors has initiated many studies in the broad field of neuroscience concerned with the underlying brain substrates of behaviours and cognition. How the environment affects the brain at the molecular levels, which consequentially modify and shape behaviours at the macroscopic level are of great import in the field of behavioural neuroscience. In this review, we will focus on one aspect of the effects of environmental enrichment on brain, namely, the effect on brain neurotrophic factors as neural consequence of environmental enrichment.

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Results from our studies with rats raised in differential environmental conditions will be reviewed and discussed.

2. Environmental enrichment, a differential experience

Experiments with laboratory animals in the early 1960s, influenced by the initial works of Hebb, demonstrated for the first time measurable changes in the brain following training or differential experiences (Krech et al., 1960; Rosenzweig et al., 1961, 1962; Wiesel and Hubel, 1963). These researchers found that differential experience and training can cause chemical and anatomical plasticity in the brain. Animals that were exposed to environments containing abundant sensory stimulation showed changes in behaviour and neurochemical measures. These findings provided the models for the standardised enriched environmental condition that are widely used today. In the enriched environmental condition (EC), the rats are housed as groups of 6–12 per cage providing the social interaction. The EC rats also live in larger cages containing a variety of stimulating permanent objects such as ladders, running wheels, ropes, platforms, tunnels, boxes and interchangeable toy objects such as balls, figurines, building blocks, etc. These objects provide opportunities for sensory and physical manipulations as well as various learning opportunities, and the toy objects are replaced frequently. In contrast to the EC, other housing conditions commonly provided for the laboratory rodents often referred to as impoverished environmental condition (IC) and social condition (SC), where the animals are housed individually or as groups of 2–4 in regular size cages without any stimulus object. These early studies have opened up extensive follow-up studies that confirmed and expanded successively other effects of enrichment on the brain and behaviours. The evidence of enrichment influence on the brain accumulated starting from alteration of neural anatomy, neurone size, modification of gene expressions, number of spines and synapses to neurogenesis. Several neural chemical alterations were eventually identified and enhancement of behavioural responses to enrichment was measured (for reviews, see Bedi and Bhide, 1988; Mohammed et al., 1993; Rosenzweig and Bennett, 1996; Kempermann and Gage, 1999; van Praag et al., 2000; Diamond, 2001). Similar effects on brain measures following differential experience have been noted in other species such as squirrels, cats, monkeys and birds (see review by Renner and Rosenzweig, 1987). Anatomical effects of training and differential experience were also shown in specific brain regions of *Drosophila* (Heisenberg et al., 1995), as were influences on neurogenesis in crayfish brain (Sandeman and Sandeman, 2000), and changes in synaptic sites in the molluscs *Aplysia* (Krasne and Glanzman, 1995). Thus, differential experience could produce measurable changes in brain neurochemical and anatomical plasticity, and experience-dependent synaptic plasticity occurs in a wide range of species. The critical interest from all these studies aimed at transferring of findings to human studies and pertinent application to human conditions. Clearly, corroborating the fundamental idea of environmental enrichment influences on the brain and behaviour is suitable and pertinent to promote beneficial child development and successful ageing and better recovery after brain damage in human beings.

3. Neurotrophic factors

While the plethora of neural substrates showed responses to enriched environment as mentioned above, the group of plausible biochemicals with potent functions that most likely respond to external stimuli are the neurotrophic factors. We therefore, hypothesised that enriched environment might stimulate the expression of neurotrophic factors which might function in neural plasticity, neurogenesis and improved learning.

Neurotrophic factors are special endogenous signalling proteins that promote survival, division and growth, as well as differentiation and morphological plasticity of neural cells. Different neurotrophic factors are required for certain trophic functions or all of these functions in selected neural population of the nervous system. In the developing, adult and ageing nervous system, neurones are nourished and maintained by neurotrophic factors. Neurones sustain their inputs to targeted cells by producing and releasing neurotrophic factors in a retrograde manner. The best-characterised neurotrophic factor is the nerve growth factor (NGF), first described by Levi-Montalcini and Hamburger (1953). NGF is a member of the neurotrophin family of growth factors and is originally purified from the mouse submandibular gland as a complex of three dissimilar subunits: α, β and γ. Only the β subunit appears to be the neuroactive component. The NGF gene that codes for the pro β-NGF protein precursor is highly conserved across species. More recently, other members of the neurotrophin family of growth factors were identified. Using polymerase chain reaction and hybridisation screening, it was found that the brain-derived neurotrophic factor (BDNF) had 50% sequence homologue with NGF (Hohn et al., 1990; Barde, 1994). Other new members that were found include neurotrophin-3 and neurotrophin-4/5 (NT-3, NT-4/5) (Ernfors et al., 1990; Ibanez, 1996). In the brain, NGF mRNA levels are highly expressed in the hippocampus, the cerebral cortex and the olfactory bulb (Korsching et al., 1985; Whittemore et al., 1986; Ayer-LeLievre et al., 1988). These are the major sites of the basal forebrain cholinergic innervation. The cholinergic neurones in the basal forebrain (the medial septum, the nucleus basalis of Meynert and the diagonal band of Broca) provide primary action site for NGF. As a target-derived factor, NGF supports cholinergic neurones in the brain by retrograde transport to the targeted sites (Seiler and Schwab, 1984; Hefti, 1986; Krommer, 1987). Synthesized NGF in the targeted areas is taken up at cholinergic

nerve terminals and transported to the nuclei in the basal forebrain. Thus, these basal forebrain cholinergic neurones have been found to exhibit both uptake and retrograde transport of injected NGF (Seiler and Schwab, 1984; Mufson et al., 1999). Likewise, terminal regions and somas of the cholinergic neurones have receptors for NGF (Hefti et al., 1986; Taniuchi et al., 1986; Raivich and Kreutzberg, 1987). Under specific conditions, NGF is also synthesized by nonneuronal cells such as astrocytes, suggesting a nonneuronal as well as target-derived functions for the NGF.

It is well documented that at least three members of the NGF family of neurotrophic factors, NGF, BDNF and NT-3, are abundantly expressed in the hippocampus and are involved in neuroplasticity related to learning and memory (Ernfors et al., 1988; Barde, 1989). In the rat brain, the highest level of NGF is found in the target areas of the basal forebrain cholinergic neurones including the hippocampus formation, cerebral cortex and olfactory bulb (Korsching et al., 1985; Shelton and Reichard, 1986). Expressions of BDNF and NT-3 mRNAs also show regional specificity, with high levels in the hippocampus, however, differently expressed in different regions within the hippocampal formation. Thus, these three neurotrophic factors expressing neurones have nonoverlapping organisation in the hippocampus. Other regions of the brain such as the cerebral cortex and the cerebellum also show expressions of BDNF and NT-3, although in lower amounts (Ernfors et al., 1990; Hohn et al., 1990; Maisonpierre et al., 1990). All these neurotrophins have close homology, operate through partially similar receptors and have distinct trophic and tropic effects on the developing as well as the adult neurones (Ibanez, 1998). The action of neurotrophic factors is regulated by the binding to receptors in both the periphery and the central nervous system (CNS) (Ibanez, 1998). It has also been shown that the regulation of neurotrophic factors can be influenced by environmental inputs. Previously, we have shown that the expressions of NGF and BDNF could be stimulated by environmental enrichment (Falkenber et al., 1992; Mohammed et al., 1993; Torasdotter et al., 1996). The direction of these significant observations suggested to us that further progress could be made in determining the role of environmental factors such as early life stimulation, enriching experience and physical activity in regulating neurotrophins. Of particular import is the neuroprotective function of growth factors on the brain.

4. Effects of enriched environment on neurotrophic factors

It has been well established that during early development there is continuous growth and modification of neural connections. While there are differences between the events that generate and modify the developing and mature nervous systems, recent studies have shown that remodelling of synaptic connections also occurs in the adult brain. The nervous system has been found to be capable of responding to plastic changes throughout life. Hence, in order to treat and/or prevent neuropathological conditions and to understand normal brain functions, it is necessary to understand the link between neuronal plasticity and function during development and maturity. In general term, plasticity can be defined as changes observed in the nervous system or in behaviour. *Behavioural plasticity* therefore, can refer to any significant observable change in behaviour, while *neural plasticity* can refer to observed changes in the function and/or structure of the nervous system at the molecular levels that serve behavioural plasticity (James, 1890). Indeed, the importance of changes in neurones to overcome the rigidity of their connecting networks and to re-establish normal nerve paths after injuries or diseases has long been recognised (Cajal, 1928). Changes in the environment in which the organism interacts with produce various changes in cellular morphology in the brain, presumably via novel or sensory stimulation, and learning experience. One of the well-characterised sensory activation that evokes plastic response in the brain is when animals are raised in a more complex environment (discussed earlier).

We have examined the effect of exposure to environmental enrichment for 2 months on learning and NGF levels in the hippocampus of rats that were handled or nonhandled for 21 days after birth. From our previous study (Pham et al., 1997), we found that early life experience can have long-term consequences on both the neurochemical and the behavioural parameters later in life. The mechanisms mediating these effects are still unclear. However, a reasonable assumption is that such multitude of changes is at least partly mediated by endogenous trophic factors. The impacts of enriched environment have also been shown to improve cognitive function. In general, the results from several studies indicate that enriched rats perform better on tests of learning and memory than rats housed in standard or isolated conditions (Doty, 1972; Greenough et al., 1979; Janus et al., 1995; Mohammed et al., 1986, 1990; Nilsson et al., 1999). From our previous study, we showed that the nonhandled rats differed from the handled rats exposed to mild stress: they showed deficits in spatial learning and had lower hippocampal NGF levels. In this study (Pham et al., 1999a), we examined whether environmental enrichment can counteract the pernicious effects of nonhandling. We chose to study effects in the hippocampus because of the well-established evidence for the hippocampus involvement in spatial learning and memory (e.g., Barnes, 1988; McNamara and Skelton, 1993; Gallagher et al., 1994). Moreover, the highest NGF mRNA and protein concentrations are found in the cholinergic basal forebrain target regions such as the hippocampus (Korsching et al., 1985; Rennert and Heinrich 1986; Mufson et al., 1999). We found that rats that did not receive postnatal handling, when later raised in enriched environment, showed improved spatial learning and have higher hippocampal NGF levels than nonhandled isolated rats. Enrichment has been demonstrated to have

beneficial effects on the disadvantaged animal populations (see review by Will and Kelche, 1992).

An interesting finding from this study was the effectiveness of mild environmental stimulation in enhancing hippocampal NGF levels in the nonhandled rats (see Table 1). Several studies of NGF role in other physiological functions have shown that stress affects NGF levels. Alterations in NGF levels were reported following a stress-provoking situation (Aloe et al., 1994; De Simone et al., 1990). Similarly, other studies have related increased BDNF levels and induction of BDNF mRNA in the hippocampus of the rat with behavioural activation, stress and physical activity (Neeper et al., 1995; Smith et al., 1995). Interestingly, we found that the nonhandled animals raised in an enriched environment had the highest hippocampal NGF levels compared with the other groups. This may indicate that the nonhandled rats were more responsive to some of the neurochemical effects of enrichment. It could be that the handled animals had been sufficiently stimulated by postnatal handling and became habituated to the effects of further stimulation of enriched environment. Furthermore, the increase in NGF levels induced by handling condition may have reached a plateau, thus resulting in no further increase in hippocampal NGF levels following more stimulation. The nonhandled rats raised in isolated condition have lowered NGF levels and were slower in learning of the spatial task in comparison to the nonhandled rats raised in enriched condition. Both the handled and the nonhandled rats that were housed in an enriched condition showed faster acquisition in the water maze than both the respective groups housed in an isolated condition. Our results revealed that enrichment had salutary effects in both the handled and the nonhandled rats, and the novelty of enriched environment and behavioural testing exerted greater neurochemical effects on the nonhandled animals. The results could imply that stimulation early in life may preserve the neurogenic potential of the brain, and that it is the novelty of complex stimuli such as enriched environment rather than continued stimulation by complex stimuli which elicits the environmental effects on adult hippocampal plasticity. However, the potential variability of an enriched environment does not allow us to discriminate and narrow down the widespread measured effects of environmental enrichment to a single stimulus. Moreover, lack of environmental stimulation or withdrawal from the enriched environment may have negative effects. Indeed, we found that handled animals devoid of enrichment through isolation housing condition performed as poorly as nonhandled animals in the spatial learning task. This suggested that isolation housing might counteract the salutary effects of postnatal handling.

Table 1
Environmental effects on brain NGF levels

Environmental conditions	Mean value (pg/g)	
	Hippocampus	Hypothalamus
H–EC	6184.17 ± 605.71 *	575.85 ± 68.16
NH–EC	7270.83 ± 351.59 *	877.54 ± 270.73
H–IC	5875.22 ± 434.13	525.63 ± 63.83
NH–IC	4882.94 ± 500	564 ± 47.08

Enzyme immunoassay (EIA) analysis of NGF protein levels in postnatal handled (H) and nonhandled (NH) rats exposed to differential housing for 2 months: Handling plus enriched condition (H–EC; $n = 8$), handling plus isolated condition (H–IC; $n = 7$), nonhandling and enriched condition (NH–EC; $n = 8$) and nonhandling plus isolated condition (NH–IC; $n = 7$). Results presented as mean ± S.E.M. EC animals have significantly higher levels of NGF than IC animals.

* $P < .05$ compared to isolated condition.

These findings moreover emphasise the importance of characterising experience-dependent regulation of brain neurotrophins and cognitive functions over the lifetime of the animal, recognising that previous experience might influence the behavioural and neurochemical responses when the animals are exposed to new stimuli. It also seemed conceivable that extending the enriched environment with more complexity and time could further stimulate cognitive functions and adult brain plasticity. In one of our studies, we set out to examine the effects of long-term exposure to enriched environment on cognitive functions and neurotrophin levels (Pham et al., 1999b). The potential impacts of environmental enrichment and complex social stimulation on the brain have been repeatedly demonstrated and our results also confirmed that environmental factors have influences on behavioural performance and neurotrophins involved in brain plasticity, in this case, NGF in the hippocampus. These observations therefore, suggested to us that conceivably a long-term or robust exposure to complex environmental stimuli would maximise the influences of environment on brain plasticity, and hence, attenuate many degenerative aspects of ageing. In order to test this hypothesis, we raised young rats in differential housing conditions, an enriched environment and an isolated or individually housed environment for 1 year. To investigate the experience-dependent behavioural change and brain plasticity, we examined NGF levels in the hippocampus, the visual cortex, the entorhinal cortex and the hypothalamus, and the NGF receptors (NGFR), p75 and trkA, in the cholinergic neurones of the medial septal area (MSN). We also examined whether changes in expressions of hippocampal NGF and its receptors are associated with spatial and exploratory performances of the middle-aged rats. The NGF levels in all brain regions examined except the hypothalamus were significantly higher after a year of enriched housing compared to isolated housing. The highest NGF levels were detected in the hippocampus and relatively low levels were seen in the hypothalamus (Fig. 1).

In the adult rat brain, high levels of NGFR mRNA are present in the septal region, which contains the cell bodies of cholinergic neurones that project to the hippocampus, and in the cerebral cortex, whereas little or below detection level of NGFR mRNA is seen in the hypothalamus (Mobley et al., 1985; Ernfors et al., 1988). Enriched animals had significantly higher overall staining densities for both p75- and trkA-immunoreactive cell bodies in the MSN. Quantitative

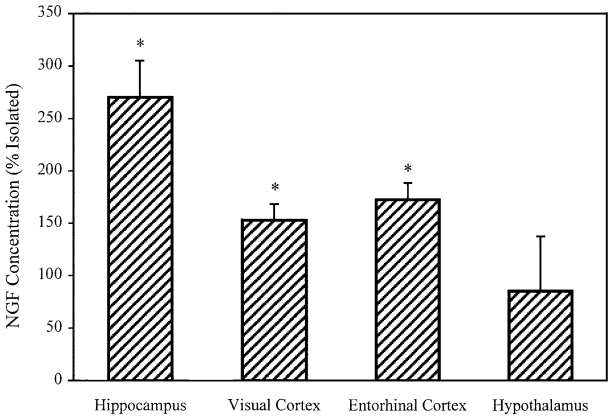

Fig. 1. The effect of living in long-term (1 year) environmental enrichment (EC, $n=8$) on the levels of NGF in selected rat brain regions (hippocampus, visual and entorhinal cortices, hypothalamus). Animals were 14 months old at time of decapitation. NGF analysis was carried out by enzyme immunoassay. Data expressed as mean ± S.E.M. percent of NGF levels versus animals housed in isolated condition (IC, $n=7$). * $P<.05$.

image analysis for cell sizes in these areas also showed significantly larger cell sizes in enriched animals as compared to isolated animals. There was a difference between the p75 and trkA antibody staining distribution. The p75 receptor appeared to be expressed along the entire cell membrane of cell bodies and neurites, while the trkA receptor was expressed primarily in the cytoplasm of cell bodies and varicosity of neurites. A summary of these findings is shown in Table 2.

The long-lasting facilitation of behavioural and neurochemical effects by enriched environment have led to extensive studies of enrichment as a model for neural plasticity and raised the possibility of the involvement of several other neurotrophins in enrichment-induced brain plasticity. A comparison of various brain regions of animals housed in an enriched environment to isolated animals with respect to NGF, BDNF and NT-3 levels showed that enriched brains have higher neurotrophin levels in all regions examined (Ickes et al., 2000). We found higher levels of BDNF, NGF and NT-3 in the medial basal forebrain of animals raised in an enriched environment compared with isolated animals. These findings further substantiated and extended our previous finding of enhanced NGF levels and cell size and density of NGFR immunoreactive neurones in the basal forebrain following enrichment. In the cerebral cortex, we also found that BDNF and NT-3 levels were significantly higher in the enriched than in the isolated animal (Fig. 2).

Using the same ELISA kits for neurotrophins, we detected significantly higher BDNF and NGF in the hippocampal formation and in the hindbrain of enriched animals. In contrast, the levels of hippocampal NT-3 were relatively low. However, this was consistent with one study reporting levels of hippocampal NT-3 in adult rats to be at 1.0 ng/g (Söderström and Ebendal, 1995), whereas during the postnatal period the levels are much higher. It is unclear why the NT-3 levels in the hippocampus were not significantly elevated in the enriched animals, which would appear to be at variance with an earlier study from our lab. In that study, NT-3 mRNA expression in adult rats was reported to be enhanced after exposure to enrichment (Torasdotter et al.,

Table 2
Environmental effects on NGF receptors in MSN

Immunoreactivity in MSN	Values in enriched (% isolated)
p75 Staining density	140.60 ± 4.51*
trkA Staining density	142.59 ± 7.12*
p75 Cell area	179.46 ± 19.95*
trkA Cell area	252.20 ± 26.08*

Mean values of NGF receptors immunoreactivity in the MSN of middle-aged rats housed in differential environments for 1 year. Values ± S.E.M. represent percent in enriched rats ($n=6$) versus isolated rats ($n=6$).
* $P<.05$ compared to isolated condition.

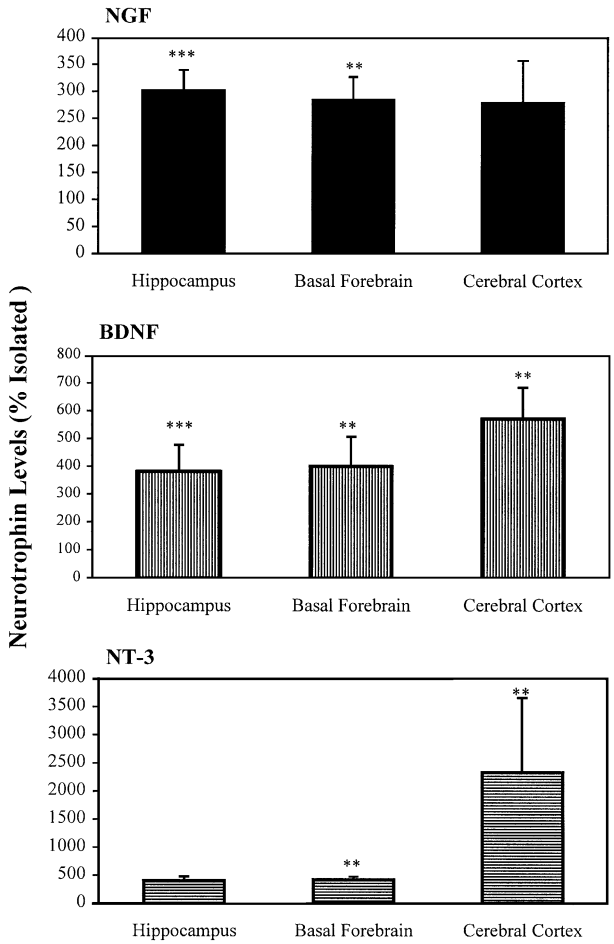

Fig. 2. Comparison of neurotrophin (NGF, BDNF, NT-3) levels in selected brain regions (hippocampal formation, basal forebrain and cerebral cortex) of middle-aged rats housed in enrichment for 1 year (EC, $n=6$) and rats housed in isolated condition (IC, $n=6$). Neurotrophin levels were analysed using commercial ELISA kits (for methods, see Hornbeck, 1994). Values represent mean percent neurotrophin levels ± S.E.M. versus IC rats. * $P<.05$.

1996). One possible explanation for our failure to see significantly higher hippocampal NT-3 levels in EC animals could be the few samples analyzed. It should also be borne in mind that the mRNA levels do not always correspond with protein levels as some studies have shown (e.g., Cirulli et al., 1998). In other experimental paradigms of plasticity, it was also found that the levels of NGF, BDNF and NT-3 levels were differentially affected. For example, electrical stimulation has been found to cause an increase in NT-3 levels, but not in NGF and BDNF levels (Ernfors et al., 1991; Korte et al., 1998; Morimoto et al., 1998). Similarly, following cerebral ischemia, mRNA levels of NGF and BDNF were increased in the dentate gyrus granule cells while mRNA levels of NT-3 were decreased (Lindvall et al., 1992). It has been suggested that NT-3, as opposed to NGF and BDNF, appears to be regulated independently of neuronal activity, its mRNA expression and secretion are dependent on hormonal levels and on BDNF levels (Hyman et al., 1994; Lindholm et al., 1994). Consequently, BDNF levels in the brain might indirectly affect NT-3 levels. There was also the intriguing observation that BDNF and NT-3 could act in an antagonistic manner in the cortex during development (McAllister et al., 1997). Thus, it was shown that during cortical development in layers 4 and 6, endogenous BDNF and NT-3 have opposing actions in regulating dendritic growth. We have noted increased dendritic growth in the aged hippocampus and the visual cortex following enrichment (see Fig. 3).

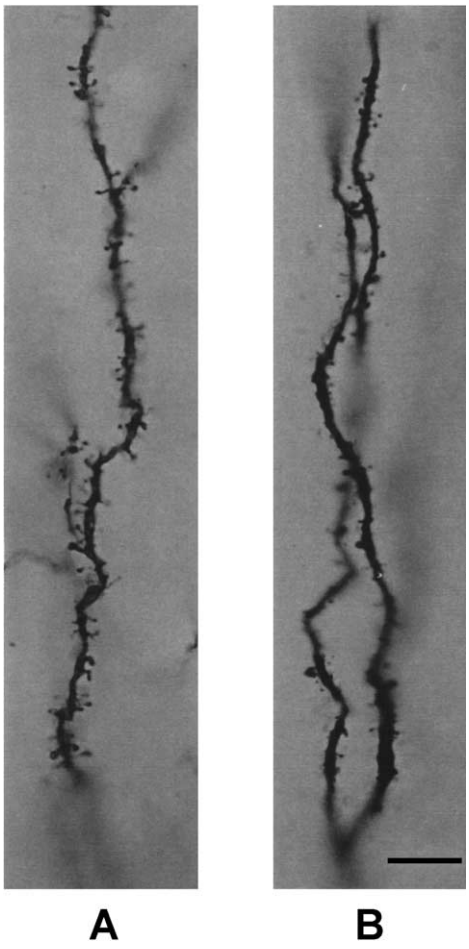

Fig. 3. Photomicrograph of dendritic tree of the dentate neurons in the (A) EC and (B) SC aged rats (20 months old). The rat brains were perfused with 4% buffer paraformaldehyde, removed and dissected in three blocks. Tissue blocks were fixed and dendritic and spine morphology were analysed by classical chrome-osmium rapid Golgi method (Scheibel, 1981). Note the longer, larger spines and the increase in the number of spines in the EC rat compared to the SC rat.

5. Environmental enrichment supports protective role of neurotrophic factors in the brain

Our results of spatial performance ability by the animals raised in an enriched environment further pointed to the abundant and lasting effects of environment on behaviours. Enriched animals performed better than the isolated animals in the spatial learning task. The shorter mean escape latency during acquisition training and retention testing identifies the environmentally enhanced learning and memory ability of the middle-aged animals. Our interest in these findings was the possibility of some direct associations between improved functions and the underlying cellular changes. The highest level of NGFR mRNA and protein has been detected in the basal forebrain (Korsching et al., 1985; Rennert and Heinrich, 1986; Richardson et al., 1986); the NGFR also has colocalisation with choline acetyltransferase in the rat forebrain (Dawbarn et al., 1988; Sobreviela et al., 1994). The basal forebrain cholinergic system is associated with cognitive functions and correlated with learning and memory in the rats. For instance, cholinergic neurones atrophy is correlated with impairment in learning and memory in rats (Fischer et al., 1989, 1992; Gallagher et al., 1990). A loss of cholinergic neurones also occurs during normal ageing and it is likely that the age-related atrophy is partly due to deficiency in supply or utilisation of endogenous neurotrophic factors (Barde, 1989, 1994; Fischer et al., 1989; Hellweg et al., 1990; Markram and Segal, 1990; Cooper et al., 1994). Indeed, reduction in basal forebrain levels of NGF mRNA and protein was found in aged and cognitive-impaired rats (Lärkfors et al., 1987; Henriksson et al., 1992) and intracerebral infusion of NGF in aged rats and implantation of a recombinant NGF producing cell line reduced basal forebrain cholinergic neurones atrophy and ameliorated spatial learning impairment in aged rats (Hefti et al., 1984; Fischer et al., 1987; Strömberg et al., 1990; Chen and Gage, 1995). In agreement with these results, we showed that long-term environmental enrichment induces changes in NGF levels and NGF receptors in brain regions recognised to be important for cognitive function. Our results also showed that the neurochemical and morphological changes were associated with improved spatial learning, and reduced novelty generated hyperactivity in middle-aged rats. Koh and Loy (1988) and Koh et al. (1989) have shown that an aged-related loss of

NGFR immunoreactivity in basal forebrain neurones was correlated with spatial memory impairment. Considering that NGF can ameliorate age-related spatial memory impairment, it is possible that NGF stimulates not only survival of neurones, but also influences fibre sprouting, architecture and synaptic organisation of cholinergic input to the hippocampus. Endogenous NGF is responsible for axonal sprouting in the periphery (Diamond et al., 1987). A similar inference of the putative NGF function for the basal forebrain cholinergic neurones can be drawn from our immunohistochemical results. We have shown that enrichment led to increased cell sizes and staining densities of the NGFR (p75 and trkA) in the medial septal brain area. Therefore, our results further substantiated findings in which grafts and administration of NGF to aged or lesioned animals amended behavioural deficits and reversed both atrophy of basal forebrain cholinergic neurones and reduction of choline acetyltransferase immunoreactivity in the rat medial septum (Chen and Gage, 1995; Chen et al., 1995; Dixson et al., 1997). Similarly, Hagg and Varon (1993) have shown vigorous sprouting and regrowth of septal cholinergic fibres following infusion with NGF.

The hippocampal system has been well documented for its involvement in learning and memory (Olton et al., 1979; Isaacson and Pribram, 1986). The abundant concentrations of trophic factors in the hippocampus suggest that they are involved in the plasticity of its function and are related to its effects on learning and memory processes. Interestingly, it was shown that regulation of NGF and BDNF mRNA expression could be mediated by excitatory amino acids receptors such as glutamatergic receptors. These receptors are mediators of synaptic transmission and are important for plasticity in the brain (Collingridge and Singer 1990). The function of the adult basal cholinergic neurones has been shown to be modulated by NGF and BDNF (Fusco et al., 1989; Lindefors et al., 1992; Bäckman et al., 1997). Confirming the importance of cholinergic functions in memory processes were results that increased cholinergic activity causes increased glutamatergic activity within the hippocampus, which resulted in a postsynaptic elevated expression of NGF and BDNF mRNA (Lindefors et al., 1992). There appears to be a bidirectional mechanism between neurotrophic-dependent neurones and their targets such that increased cholinergic activity resulted in increased neurotrophin production. Our specific findings of higher levels of NGF and BDNF in the basal forebrain of enriched animals therefore are in agreement with the previous study in which we found increased trkA receptor immunostaining and an enhanced cholinergic neurones density in the basal forebrain of enriched rats. Thus, the results of brain NGF levels in environmentally enriched animals further confirm that the relationship between the NGF levels and cholinergic morphology directly affects memory performance. Long-term promotion of neuronal activity in the limbic system, such as is suggested for the enriched animals, gives rise to increased neurotrophin production, and consequently, protection of the basal forebrain cholinergic neurones. These findings again suggested a feedback system, where more trophic factors lead to more neuronal activity, which furthermore lead to additional production of the life-essential growth factors in certain brain regions. The environmentally induced protection of the brain cholinergic system seen in the enriched animals from our studies may provide a means of alleviating age-associated dysfunction of the septohippocampal cholinergic projections by enhancing the levels of endogenous NGFs. These findings indicate a substantially important role of environment in inducing neurochemical, morphological and behavioural changes. These changes can be sustained into the later life period. Application of this knowledge in the expanding field of neuroscience research has identified many critical steps along the process from the neural stimulation to the encoding of memory and behavioural responses. Research findings will have a wide range of application in development, for successful ageing and for better recovery from nervous systems damages and in the improvement of animal welfare.

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