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Growth Factors in behavioral teratology

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Summary. - Polypeptide Growth Factors are protein molecules which regulate cell proliferation and/or differentiation. A number of different Growth Factors (GFs) have been identified and characterized in recent years, and they have been shown to control several physiological processes, such as growth, repair, differentiation, and development of specific cell populations. In particular Nerve Growth Factor, the best characterized among the about 30 GF molecules, is endowed with specific activities on cholinergic and peptidergic CNS neurons. Several GFs originally named according to their biological activity (Epidermal Growth Factor, EGF; Fibroblast Growth Factor: FGF; Transforming Growth Factor: TGF; Insulin-like Growth Factor: IGF) have been recently found in the central nervous system. The effects of *in vivo* GF administration on the ontogenesis of altricial rodents are reported. Indexes of neurobehavioral maturation are accelerated upon neonatal NGF and bFGF exposure, while a similar treatment with EGF exerts both growth-promoting and growth-inhibiting effects on mouse somatic and behavioral development. Administration of IGF appears to affect ultrasonic vocalization in mouse pups. Moreover, NGF given intracerebroventricularly to newborn mice anticipates both the appearance of the scopolamine-induced hyperactivity and the maturation of behaviours under cholinergic control. The present findings are in agreement with a model where different GFs can "switch on" developmental events leading sometimes to dramatic changes in the normal ontogenetic pattern.

Key words: Growth Factors, neurobehavioral development, behavioral teratology.

Riassunto *(Fattori di crescita nella teratologia comportamentale)*. - I fattori di crescita polipeptidici sono agenti proteici multifunzionali che regolano la crescita e il differenziamento cellulare. Numerosi fattori di crescita (FC) sono stati di recente identificati e caratterizzati. In particolare il fattore di crescita della cellula nervosa o Nerve Growth Factor (NGF) ha un'azione trofica su neuroni peptidergici e colinergici del sistema nervoso centrale dei mammiferi. Molti dei fattori di crescita originariamente caratterizzati per la loro attività biologica in tessuti di origine non nervosa (Epidermal Growth Factor: EGF; Fibroblast Growth Factor: FGF; Transforming Growth Factor: TGF; Insulin-like Growth Factor: IGF) sono stati successivamente scoperti anche nel sistema nervoso centrale. Sono qui riportati gli effetti della somministrazione *in vivo* di differenti FC sullo sviluppo ontogenetico di roditori altriciali. Alcuni parametri di maturazione neurocomportamentale sono accelerati dalla somministrazione precoce di NGF e FGF, mentre un trattamento simile con EGF ha effetti sia di accelerazione che di ritardo della crescita somatica e comportamentale di piccoli di topo. Il trattamento con IGF sembrerebbe piuttosto influenzare il pattern di vocalizzazione ultrasonica dei piccoli. La somministrazione intracerebrale di NGF anticipa la comparsa della ipercinesi indotta dalla scopolamina, un antagonista dei recettori muscarinici, e favorisce la maturazione di comportamenti sotto controllo colinergico. Nel complesso, quindi, questi dati suggeriscono che differenti FC possono innescare importanti eventi ontogenetici producendo talvolta alterazioni notevoli del normale pattern di sviluppo.

Parole chiave: fattori di crescita, sviluppo neurocomportamentale, teratologia comportamentale.

Growth Factors are major regulators of somatic and neurobehavioral development

Polypeptide Growth Factors are protein molecules endowed with regulations of cell proliferation and/or differentiation. Since the discovery in the early fifties of Nerve Growth Factor (NGF), the best characterized member of this molecular family, about 30-40 of different GFs have been isolated and purified (Table 1). Most of them have been named according to their biological activity, examples being Epidermal Growth Factor (EGF), Fibroblast Growth Factors (FGFs), and Transforming Growth Factors (TGFs). Literature has greatly increased since then, and is now evident that many of these peptides exert a much wider range of action than the biological property for which they were originally named. Many GFs are considered multifunctional agents, controlling several relevant physiological processes, such as growth, repair, differentiation, and development of specific cell lines [1-3]. Moreover, the specificity of GFs' action has been seriously questioned by new findings showing that cell lines of very distant embryological origin can react in a similar way to the same GF molecule. For example, for decades it was supposed that the NGF molecule exerted a specific trophic role limited to sympathetic and sensory nerve elements. Since 1979, such a view has been thoroughly changed by the finding of a specific and prominent growth-promoting activity of NGF on both

Table 1. - Taxonomic status of GF molecules

Growth Factor "Superfamily"	Members	
Epidermal GF	EGF Transforming GF-α (TGF-α) Vaccinia GF	
Insulin-like GF	IGF-I IGF-II	
Transforming GF-β	TGF-β (Type 1, 2, and 1.2) Inhibin-A Inhibin-B Activin-A Activin-B Mullerian inhibiting substance	
Heparin-Binding GF	Acidic (a) HBGH	- aFibroblast GF - Endothelial Cell GF
	Basic (b) HBGF Products of the proto-oncogenes *int2, hst,* and of Kaposi's sarcoma	
Neurotrophic factors	Nerve GF (NGF) NeuroTrophin - 3 (NT-3) Brain Derived GF (BDGF) Ciliary NeuroTrophic Factor (CNTF) Basic (b) Fibroblast GF (bFGF)	

Assignment of a taxonomic status to some GF molecules. They are usually grouped in five superfamilies, according to sequence homology and/or biological effects.

central cholinergic neurons and specific cell lines of the immune system, namely mast cells and B and T lymphocytes [4-6].

Molecular, biochemical, anatomical and behavioral evidence points to a major biological role for GFs in the control of development. However, the regulatory role of GFs in mammalian ontogenesis is still far from being fully accounted for GFs not only exert different effects on different cell lines, but can also act in a different (even opposite) way on the same cell type depending on the developmental stage of the cell [7]. An example is β-TGF acting on fibroblast cells either as a growth-promoting agent or as a growth-inhibiting factor [8]. Moreover, the stage-dependency in the GFs' effects could represent one of the most relevant variable both in the course of ontogenesis and in processes of phylogenetic differentiation [9].

In recent years, a number of research groups (including ours) have begun to investigate the effects of *in vivo* GF administration on the physical and behavioral development of altricial rodents, mainly mice. Most of the findings on somatic and neurobehavioral development following GF administration are in agreement with a model where different GFs can "switch on" developmental events leading sometimes to dramatic changes in the normal ontogenetic pattern.

Nerve Growth Factor, the best characterized growth factor

In the developing nervous system of both vertebrates and invertebrates, neurons are generated in excess and following the arrival of axons in the target areas, a large portion of them undergo naturally occurring cell death. This important developmental process (i.e. a series of armonically concerted developmental events, finely tuned by environmental events occurring early in development). Raff [10] recently provided an exhaustive introduction on *apoptosis*, a brand-new term used in the last years to indicate naturally-occurring-cell death. The hypothesis that neuronal survival and connectivity during development are dependent on and regulated by the availability of trophic factors in target fields has received support from work on NGF (see historical review in [11]).

As mentioned, NGF is a specific neurotrophic factor for developing sensory and sympathetic neurons of the peripheral nervous system require NGF for development, *survival* and maintenance of function [12, 13]. Recent data indicate that NGF also exerts its trophic action on neurons of the central nervous system (CNS) [14-19].

NGF is involved in cholinergic regulatory functions in the rodent CNS. Specifically, both NGF and NGF mRNA are particularly evident in zones with dense

cholinergic innervation. High NGF levels have been found both in regions innervated by the magnocellular cholinergic neurons of the basal forebrain (hippocampus, olfactory bulbs, neocortex) and in regions containing the cell bodies of these neurons, such as the septum [16, 20]. NGF mRNA has been detected in the hippocampus and the neocortex, but not in the septum. This suggests that cholinergic neurons in the basal forebrain are supplied with NGF by retrograde axonal transport from their target regions [21, 22] a process lasting to the adult stage.

From a developmental viewpoint, both NGF mRNA expression and NGF protein synthesis reach the adult levels at the end of the third postnatal week in rats, when some portions of the cholinergic system undergo a rapid maturational transition [22, 23]. NGF accumulation in the basal forebrain parallels that in the hippocampus and the neocortex, and preceding an increase in ChAT activity. This suggests that the neurochemical differentiation of magnocellular cholinergic neurons is regulated by retrogradely transported NGF. Moreover, NGF receptor distribution in the CNS resembles the distribution of cholinergic neurons of the forebrain [24]. Finally, a marked increase in the activity of the enzyme choline acetyltransferase (ChAT) is observed in septum, hippocampus, nucleus basalis, neocortex, and caudate-putamen of neonatal rats receiving repeated intracerebroventricular (i.c.v.) NGF administrations [15, 18].

These findings indicated that NGF is involved in survival and differentiation of the basal forebrain cholinergic neurons. However, NGF receptors have recently been identified in several areas of the mammalian brain (midbrain, cerebellum, and diencephalon) not innervated by magnocellular neurons and do not contain neurons known to be NGF-sensitive but expressing mRNAs encoding both NGF and its receptor. Thus, NGF may exert some regulatory or growth promoting function in a much wider spectrum of brain regions than originally thought [25].

Neurotrophic Growth Factors and "suicide proteins"

In the course of normal embryonic development most neuronal populations undergo a period of cell death in which a substantial fraction of the cell population dies. This process matches a number of neurons to the requirements of their biological targets. Death seems to result from the failure of neuronal cells to acquire an adequate supply of neurotrophic inputs from the target areas. In recent years, other molecules besides NGF that support the survival of different kinds of embryonic neurons in culture have been identified. These include Brain Derived Neurotrophic Factor (BDNF), Ciliary Neurotrophic Factor (CTNF), and Neurotrophines (NT3, NT4, NT5). Furthermore, molecules previously known for their effects on non-neural tissues have recently been found to display neurotrophic effects in cell culture assays. A good example is EGF, acting on specific areas of rat CNS.

Neurotrophic factors were initially distinguished from ubiquitous metabolites necessary for cell maintenance and growth by their specificity, each neurotrophic factor promoting survival of specific neuronal types at a particular developmental stage. They are also involved in a wide spectrum of developmental events ranging from axonal guidance to regulation of neurotransmitter synthesis [26].

The prototypic case of NGF as an instrument of target-controlled neuronal survival is described by Davies *et al.* [27] in a review characterizing two early phases (trophic effects on growing axons and subsequent target field innervation and neuronal death), and focusing on the most densely innervated cutaneous target field of the mouse embryo, the snout region in which whiskers develop. Recently Martin *et al.* [28] reported that the natural death of NGF-deprived sympathetic neurons is not a passive event, but appears to depend on the synthesis of new RNAs and proteins ("suicide proteins"). In fact, inhibition of either RNA or protein synthesis is effective in preventing death of NGF-starved sympathetic neurons *in vitro*, suggesting that under normal conditions NGF may promote the survival of sympathetic cells by suppressing an endogenous-acting death program. However, not all neurons require NGF supply for survival; for example, trigeminal mesencephalic neurons, a population of neural-crest derived proprioceptive neurons, is supported by BDNF *in vitro* [29]. Conversely, ciliary ganglion neurons, a population of parasympathetic neurons requires CNTF for survival. In both cases, inhibition of RNA and protein synthesis results in increased neuronal survival [30]. So, at least three neurotrophic factors (NGF, BDNF, CTNF) promote survival by an apparently similar mechanism, possibly involving the suppression of an endogenous cell death program.

Molecular genetic studies have indicated that NGF, BDNF, and NT3 are structurally related and sustain the survival of distinct, but overlapping, sets of neurons *in vitro* [31]. They are expressed at comparably high levels in the adult rat hippocampus, and the pattern of brain localization of BDNF mRNA significantly overlaps the distribution of NGF mRNA [32]. The expression of these three related neurotrophic factors in the rat embryo appears to coincide with the onset of neurogenesis. However, the levels at which the three factors are expressed throughout the CNS development differ dramatically. NT3 is the most highly expressed in late-maturing regions of the brain (cerebellum, hippocampus, and neocortex), and its expression decreases with maturation of these regions. Conversely, BDNF

expression is low in developing regions and increases as these regions mature, while the variations in NGF expression do not follow a consistent pattern [31, 33]. Thus, both the spatial and temporal differences in the expression pattern of these three related neurotrophic molecules may indicate that they play a parallel as well as a reciprocal role in CNS development [33].

Behavioral teratology effects of exogenous Nerve Growth Factor administration

While the morphological and biochemical effects of neonatal NGF treatments have been well characterized in the past [11-13, 15] only limited data are available on the repercussions of these effects on behavioral regulations at early developmental stages. The involvement of central cholinergic pathways in learning and memory processes suggested a role for NGF in cognitive functions [34]; NGF administration at the early postnatal stage, however, has proven unable to modify the very limited retention performances of neonatal mice in an odor-aversion learning task [35, 36].

We found that repeated systemic NGF administrations during the first ten days of postnatal life affect the neurobehavioral maturation of mice [37]. However, the use of the systemic route makes it difficult to distinguish between direct effects at the CNS level and effects at the peripheral nervous system (PNS) level. In fact, it is not clear whether some NGF molecules or bioactive NGF fragments manage to cross the blood-brain barrier, which is still largely immature and highly sensitive to the pituitary-adrenal changes produced by daily maternal separation, unavoidable during treatment procedures.

The behavioral syndrome caused by repeated subcutaneous neonatal admnistration of NGF consists of a marked anticipation in the appearance of righting and grasping reflexes, and of a slight acceleration in the appearance of cliff aversion and response to tactile stimulation of the perioral area. Behavioral parameters involving more sophisticated neuromotor coordination, such as Vertical Screen Test or Bar Holding, are apparently not affected by the NGF treatment. In general, NGF seems to be particularly effective in accelerating neurobehavioral maturation during the very early postnatal stages, while later-developing responses are less sensitive or insensitive. Similarly, the pattern of ultrasound emission, which is considered a sensitive indicator of the pup's maturational level, is not influenced by NGF treatment [37].

Administration of NGF by the intracerebral route at a later stage of development appears to influence the maturation of central cholinergic regulations in weanling mice [38]. In fact, we found that the hyperkinetic effect of scopolamine (a centrally-active muscarinic cholinergic blocker) previously characterized in weanling mice [39], is markedly enhanced at the time of its first appearance (end of the third postnatal week) by a single i.c.v. administration of NGF given 24 h prior to the activity test (Fig. 1). This result represented initial evidence of a change in behavioral reactivity obtained via a specific NGF effect on the central cholinergic (muscarinic) system.

These findings are in full agreement with the molecular and biochemical evidence which points to the third postnatal week as the ontogenetic stage when the production of brain NGF mRNA is at its maximum. A study carried out in our laboratory confirms that NGF plays a crucial role in the functional maturation of central cholinergic regulations. In fact, i.c.v. NGF administration to mice on postnatal days 2 and 4 enhances the scopolamine blocking effect on suckling behaviour recorded on postnatal day five. More strikingly, NGF-treated pups already show marked scopolamine hyperactivity at this age; as was mentioned previously, this effect normally appears only around weaning time (Fig. 2). Our findings [40] are in accordance with the recent report by Hess and Blozovski [41], which described a four-day acceleration of spontaneous alternation in a T-maze following a single intrahippocampal NGF injection on day 8 or thirteen. In normal animals, spontaneous alternation appears at about 15 days of age, increases rapidly until day 17, then undergoes a temporary decrease (to about 50%) between days 20 and 30, and finally approaches adult performance at about day forty. Previous studies from the same group showed a close parallelism between the developmental pattern of spontaneous alternation and the ontogeny of the septohippocampal cholinergic system [42].

Finally, Bengt Meyerson's group at Uppsala, Sweden, recently reported long-term behavioral repercussions of neonatal NGF treatment. Specifically, i.c.v. admini-

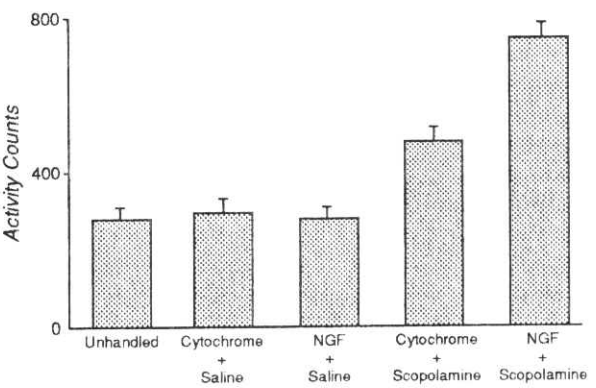

Fig. 1. - Proactive NGF effects on scopolamine hyperactivity in 21-day old mice. The hyperactivity response to the muscarinic cholinergic blocker scopolamine (2 mg/kg) is enhanced after a single NGF injection (20 μg) given i.c.v. 24 h before testing. Activity levels (means ± SEM) were measured in Varimex Activity Meter apparatus during a single 15 min session.

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stration of NGF to mice from postnatal day 2 to postnatal day 12 can influence their sociosexual preferences as assessed on postnatal day thirty.

The role of Epidermal Growth Factor in developmental processes: from epithelial to brain tissues

Since the discovery of EGF, the body of knowledge of the functional role of this molecule has been considerably extended. EGF was initially characterized as a powerful growth-promoting agent for epidermal and epithelial cells. Recent findings showed that EGF causes an enhancement of survival and process outgrowth in primary cultures of subneocortical telencephalic neurons of neonatal rats, an effect blocked by EGF-antibodies [43]. Other lines of evidence suggesting the presence of EGF in the CNS come from immunohistochemical and biochemical studies [44]. It has also been reported that EGF is mitogenic for glial cells [45]. With regard to the *in vivo* effects of EGF, Cohen [46] originally reported that systemic EGF caused a 5-day advancement of eyelid opening and a 2-day acceleration of incisor eruption. However, new data, indicate that EGF exerts both accelerating and retarding effects on rat somatic growth. Endogenous EGF may act as a modulator for growth-regulating hormones, such as thyroid hormones and glucocorticoids [47].

We compared the effects of systemic administration of NGF and EGF [48], and found that EGF exposure delays the appearance of a number of neurobehavioral responses, while exerting marked "accelerating" effects on physical maturation (Fig. 3).

Our results have shown that such an accelerated maturation of somatic parameters goes hand in hand with a general delay in the full appearance of several behavioral responses, indicating that a specific regulatory agent can selectively and markedly influence some developmental parameters (and in opposite ways), without altering others. In particular, the appearance of righting, forelimb placing, and grasping responses was significantly retarded by the EGF treatment, as was the appearance of a number of responses involving more complex motor capabilities.

The only exception in this picture appears to be the marked ontogenetic advance in the appearance of visual placing response. In the visual placing test, the mouse is suspended by the tail and lowered toward a solid surface; the animal normally raises its head and extends the forelimbs in a placing response. This response may obviously depend both on the development of visual capabilities and on the maturation of specific motor components of the forelimb placing response. It should be mentioned here that systemic administration of NGF and EGF significatively retards the pups' body weight gain. This effect could be attributed either to the interaction of GFs with growth-regulating systems (e.g., EGF and thyroid hormones seem to be functionally related) and/or to the earlier phenotypic differentiation of target cell lines, which often inhibits mitotic and migratory cell activities.

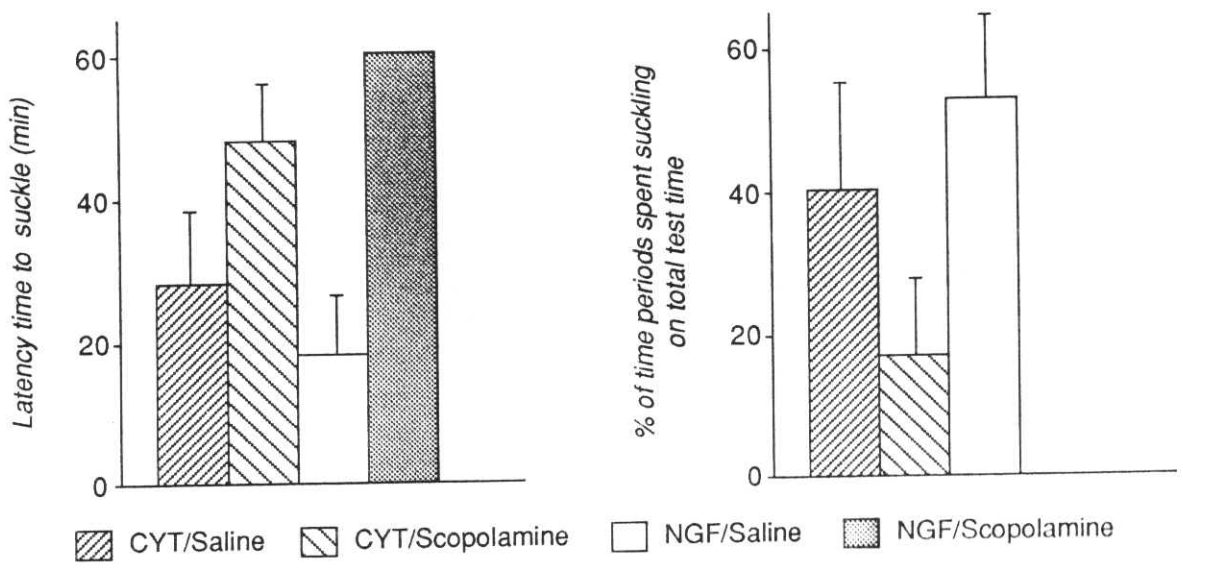

Fig. 2. - Latency time to attach to a nipple (left) and percentage of time periods spent suckling over total test time (right) by 5-day old male mice given either NGF or cytochrome c on postnatal days 2 and 4 and either scopolamine or saline 15 min before the suckling test on day 5. Values are means ± SEM.

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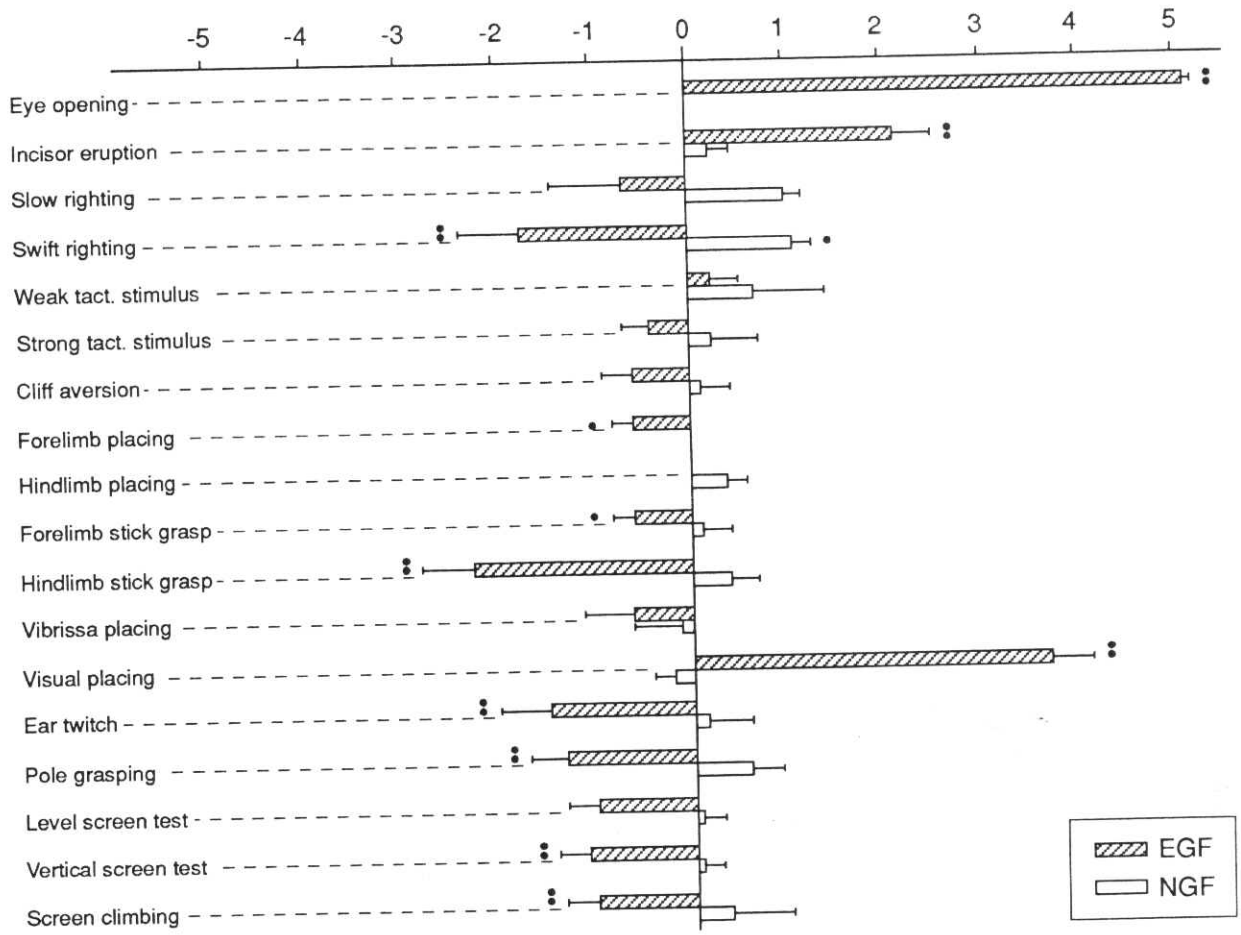

Fig. 3. - Comparison between systemic NGF or EGF administration (from postnatal day 2 to day 10) on somatic and neurobehavioral development of male mice. Data are mean differences (± SEM) between the mean day of adult-like response of EGF- or NGF-treated pups, and the corresponding value in the respective cytochrome c control littermates. (Cytochrome c is used as control treatment since it is physicochemically similar to NGF, but lacks its growth-promoting properties). Significance levels refer to comparisons between GF-treated pups and control littermates: * $p < 0.05$; ** $p < 0.01$.

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Also Transforming Growth Factors regulate normal development

In recent years another group of polypeptide GFs has been characterized, namely the Transforming Growth Factors, which also exert a powerful regulatory role on cell development, both *in vivo* and *in vitro* [49]. Two distinct sets of TGFs have been identified. Alfa-transforming growth factor, a hyperplastic agent produced by a wide variety of rodent and human tumor cells, is structurally and functionally homologous to EGF [50], causing precocious eyelid opening and incisor eruption in neonatal rodents [51, 52]. Beta-TGF, which is chemically distinct from both α-TGF and EGF, can either stimulate or inhibit cell proliferation and differentiation, depending both on the cell type and on the entire set of growth factors operating within the cells. *In vivo*, β-TGF inhibits mammary growth and morphogenesis in mice while accelerating wound healing in rats [8].

The growth-promoting or growth-inhibiting effects of β-TGF on a particular cell subpopulation are not a function of the peptide itself, but rather of the total set of growth factors and GF receptors that are operant in the cell at a given time. For example, in certain cases β-TGF stimulates anchorage-independent growth, while in others it actively inhibits growth. It can also act synergically either with GFs such as EGF and Platelet Derived Growth Factor, or with other serum factors [8].

We compared the effects of repeated administration of α-TGF and EGF on neurobehavioral development of neonatal mice. We observed that α-TGF shares a marked accelerating effect on somatic growth (significant acceleration of eye opening and lower incisor eruption) with EGF. By contrast, the already reported retarding effects of EGF on neurobehavioral development do not occur upon α-TGF exposure. Although the two GFs share about 30% sequence homology (and are usually listed in the same family of EGF-like polypeptides, see

also Table 1), their effects on early postnatal development of altricial mammals indicate that their role in the normal maturational pattern may be rather different.

Insulin-like Growth Factors and abnormal brain development

Insulin is a major endocrine regulator of the uptake, cellular transport and intermediary metabolism of small nutrient molecules, such as aminoacids, fatty acids and glucose. In the past, the CNS was considered to be largely insulin-insensitive, despite reports about insulin physiological functions in the CNS dating back to the 1960s, and the impressive body of literature accumulated since then. The biological functions exerted by the insulin molecule at the CNS level are still obscure, and the recent discovery that Insulin-like Growth Factors (IGFs) and their receptors are also present in the CNS has further complicated this picture [53]. The two IGFs, or somatomedins, are polypetide mitogens which apparently play fundamental roles in mammalian growth processes. IGF-I is the major mediator through which growth hormone exerts its biological effects on postnatal growth [54, 55], while IGF-II seems to perform similar growth-promoting functions during fetal life [54]. Somatomedin and insulin receptors are already present in the brain of the human fetus before the end of the first trimester of gestation [57].

The actual *in situ* production of insulin-like mRNA has been limited to a few cells of the periventricular regions of the hypothalamus, while peptides with immunological properties similar to those of IGF-I and IGF-II have been detected in extracts of brain by radioimmunoassay.

IGF-II seems to be the predominant immunoreactive form present at the CNS level. Cultured CNS explants synthetize IGFs and, more interestingly, the uptake of plasma IGF-II, but not IGF-I, into the CNS by transport across the blood brain barrier has been suggested. The brain seems to show the highest concentration of IGF-II mRNA of all major organs, with up to fifty times that of the liver. In comparison, brain IGF-I mRNA levels are about four times lower than in the liver.

Binding sites endowed with the properties of receptors for insulin, IGF-I, and IGF-II, are evident in brain cell cultures and in membrane preparations from all major brain regions from adults and fetuses. Two types of IGF receptors (type 1 and type 2) have been characterized. Type 1 IGF receptor is structurally similar to the insuline receptor, has high affinity for IGF-I but also recognizes IGF-II; it also binds insulin. Type 2 receptor is a monomer with highest affinity for IGF-II; it also recognizes IGF-I but not insulin. IGF receptors are much more abundant than insulin receptors in the CNS [53].

Both insulin and IGFs are known to elicit diverse developmental and behavioral effects. To focus on behavioral changes, the inhibition of feeding is the most striking alteration they cause. With the exception of obese Zucker rat, insulin infusion into brain cerebrospinal fluid (CSF) inhibits feeding and results in body weight loss in several mammalian species. Food intake is also reduced by IGF-I and II, the former exerting a much more pronounced effect than the latter. However, the results from experiments in which insulin and IGFs are infused into the CSF are difficult to interpret because of the limited specificity of the different receptors and for the lack of information concerning their actual concentration around the receptor site. These results strongly suggest a relationship between CSF levels of insulin or IGFs, and food intake and body weight. The nature of such an interaction and its neural mechanism(s) are presently unknown. It has to be taken into account that receptors for insulin and the IGFs are located in specific regions of the hypothalamus and the olfactory bulb possessing neurotransmitters and peptides reportedly associated with feeding behavior and appetite. Thus, it is conceivable that the effects of insulin and of IGFs on food intake and body weight may be due to specific interactions with neurons present in these regions. The well-known feedback effect of plasma IGF on growth hormone secretion appears to operate (at least partially) by IGF-I action at the hypothalamic level [56]. The question is whether abnormalities in brain development depend on an interference in normal IGF regulation.

A disturbance in IGF production may be involved in disorders of CNS development originating from both genetic and environmental factors [58]. Specifically, it has been suggested that one of the secondary effects of the Down's syndrome is a delay in the onset of IGF production during early fetal life and an IGF deficit during a critical period of neuronal proliferation. The somatic growth retardation observed in many patients affected by Down's syndrome may be explained by the low levels of IGFs found in serum. Altered IGF serum levels have also been observed in children with unclassified mental retardation [59] or with minimal brain dysfunction [60]. Moreover, the growth retardation resulting from malnutrition is paralleled by reduced IGF levels in serum [61].

In developing rodents, the maintenance of the normal IGF biosynthesis is controlled by both caloric and proteic intake. In preweanling rats, malnutrition resulting in brain growth retardation is accompanied by IGF deficiency [62]. However, administration of recombinant IGF-I is not sufficient to restore growth impairment in malnourished animals [63]. These apparently conflicting results suggest the occurrence of an accompanying alteration in receptor sensitivity to IGF. An elevation in the threshold of cellular response to IGF may be part of an adaptive mechanism evolved to regulate cell growth according to nutrient availability.

It has been hypothesized that there is a critical period for imprinting of the IGF "hormonostat" during early development. In rats maintained under malnutrition conditions for the whole preweaning period, a permanent reduction of IGF biosynthesis accompanied by growth retardation throughout life has been reported [64]. From this point of view, administration of teratogenic agents during early development may induce permanent alteration in the setting of the IGF hormonostat. Such an early change could have important implications for later brain functions. In conclusion, IGFs synthesized in the adult brain exert an anabolic action as maintenance hormones [59], and long-term alteration in their biosynthesis may result in impairment of neuronal and glial cell metabolism throughout life.

Very recent data also identify IGF-I as a potential mediator of EGF-induced growth retardation in neonatal rats. A single EGF injection was shown to rapidly decrease (within few h) circulating IGF-I levels in 1 and 8 day old rats, while 12 day pups were unaffected. A four day exposure to EGF resulted in lowered IGF-I levels in pups aged 1 to 11 days [65]. Finally, transgenic mice overexpressing the growth hormone gene had neither accelerated growth nor increased IGF-I expression during the early neonatal period (first week of postnatal life) [66].

In our experiments, aimed at interfering with endogenous availability of IGF-I during early postnatal development, IGF-I was administered to neonatal mice daily on postnatal days 2, 4, and 7. Bioactive IGF-I fragments 24-41 or 57-70 (numbers refer to the aminoacidic chain) were also used to evidence which sequence could be responsible for the developmental alteration observed. As expected, repeated intraventricular administration of IGF-I induced a significant increase in body weight gain. However, the behavioral development of the pups receiving IGF-I was not affected, since both early (righting, grasping, cliff aversion) or late responses (screen climbing, bar holding, visual and vibrissa placing) did not differ from control (vehicle-injected) littermates. Quite interestingly, the pattern of ultrasonic call emission was increased by IGF-I. This specific IGF-I effect on ultrasound emission was also produced by the 24-41 fragment, while the 57-70 fragment had practically no effect (Fig. 4).

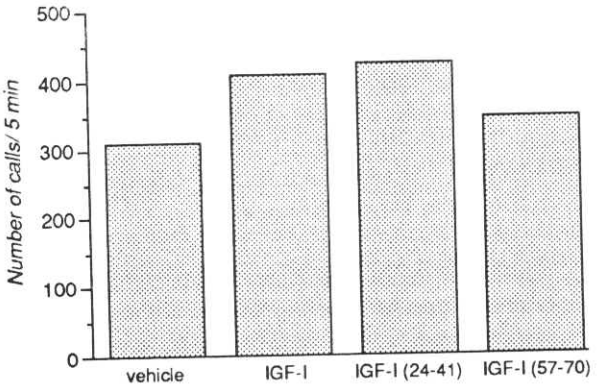

Fig. 4. - Ultrasonic calling rate of mouse neonates receiving on postnatal days 2, 4, and 7 an i.c.v. injection either of IGF-I, or of bioactive IGF-I fragments 24-41 or 57-70.

Basic Fibroblast Growth Factor: a molecule mimicking NGF effects

Basic Fibroblast Growth Factor (bFGF) was originally discovered as a growth factor stimulating proliferation of non neural tissue, specifically mesenchimal cells. However, the CNS was subsequently shown to contain very high concentrations of this protein [67]. bFGF exerts a strong mitogenic action on neural precursor cells of PNS and CNS [68, 69], while promoting survival and fiber outgrowth of rodent hippocampal and cortical neurons in culture [70, 71]. The finding that bFGF also increased choline acetyltransferase (ChAt) activity levels in cultured chick ciliary neurons [72] led to the hypothesis that bFGF might be endowed with an NGF-like activity in the CNS, exerting its neurotrophic role on central cholinergic neurons of the septohippocampal system. This hypothesis has since been supported by the discovery that, like NGF, exogenous bFGF may prevent death of cholinergic neurons in the medial septum following interruption of their projection to the hippocampus [73, 74].

This neurotrophic role may very likely be exerted early in development, as indicated by *in vitro* findings [75]. A study by Heuer *et al.* [76], aimed at characterizing the temporal pattern of FGF and NGF receptor expression in the developing chicken nervous system, has showed that the action of NGF and FGF may be sequential rather than additive during development. Specifically, the FGF receptor (FGF-R) is expressed first in proliferative neuroepithelial cells, and then in postmitotic neuroblasts late in development. Conversely, NGF-R expression increases as FGF-R expression decreases. Thus, FGF might have a crucial role in the initial stages of neuronal differentiation of CNS neuroepithelial cells, generating immature neurons that are subsequently induced by NGF to survive and grow. However, FGF-R expression reappears late in development, indicating that in differentiated neurons FGF may take over a neurotrophic role previously filled by NGF.

A recent study by Yoshida and Gage [77] has suggested an interesting model for the concerted action of FGFs and NGF in the CNS. In fact, both acid and basic forms of FGF are able to stimulate NGF secretion by newborn rat astrocytes and fibroblasts. The NGF secretion might be determinant to support the survival of damaged neurons in brain injury. To further support the interplay among different GFs in the course of neuronal development, Cattaneo and McKay [78] found that neuronal precursor cells proliferate in response to NGF, but only after they have been exposed to bFGF. On NGF withdrawal, the proliferative cells differentiate into neurons.

Table 2. - A summary of short- and medium-term effects of i.c.v. administration of GF on mouse physical and neurobehavioral development

i.c.v. treatment	Tests				
	Fox's scale	**UVR**	**Homing**	**Open field**	**Olfactory preference**
EGF	PND 2, 4, 6, 8, 10		PND 12	PND 16	PND 19
NGF	"		"	"	PND 10,19
IGF-I	PND 2-12	PND 8	PND 10	"	"
IGF-I (24-41)	"	"	"	"	"
IGF-I (57-70)	"	"	"	"	"
bFGF	"	"	"	"	"

	Results	
Body weight		
- EGF:	significant reduction *vs* control	
- NGF:	significant reduction *vs* control	
- IGF-I:	significant increase *vs* control	
UVR		
- IGF-I: ultrasonic vocalization rate higher than control		
- IGF-I (24-41): similar trend as IGF-I		
Fox's scale	**Acceleration**	**Delay**
- EGF:	Eye opening, incisor eruption	righting, placing, grasping
- NGF:	righting, tactile stimulation, cliff aversion	
-bFGF:	righting, tactile stimulation, placing	
-IGF-I:	no effect	
Homing		
- EGF:	homing latency longer than control	
- NGF:	no effect	
- IGFs:	no effect	
- bFGF:	no effect	
Open field	in no case an effect was evident	
Olfactory preference		
- EGF:	no effect	
- NGF:	enhanced preference for the familiar odor on PND10	

Schematic representation of the effects of various GFs, given intracerebroventricularly (i.c.v.) to mice at the neonatal stage. PND = Postnatal day; UVR = ultrasonic vocalization rate; Fox's Scale = battery for assessment of neurobehavioral development; homing = homing test, in which newborn rodents have to reach the nest area in a given time; open field = test assessing exploratory behavior; olfactory preference = test assessing the establishment and the retention of a preference through choice of a familiar odor *vs* a novel one.

Some preliminary results from our laboratory have shown that intracerebral bFGF administration exerts an NGF-like effect on early behavioral development of newborn mice, significantly accelerating the appearance of the righting and placing reflexes (see also Table 2).

Such a finding is in good agreement with the series of very recent data indicating strict functional analogies between NGF and bFGF action. Finally, a summary table (Table 2) of the *in vivo* effects of a variety of GFs on mouse development is given, indicating both analogies and differences in the way they produce short- and medium-term alterations in ontogeny when administered directly at the CNS level.

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