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Review

Glucocorticoids, Neurotrophins and Neurodegeneration

Jackie Leach Scully* and Uwe Otten

Institute of Physiology, University of Basel, Vesalgrasse 1, 4051 Basel, Switzerland

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INTRODUCTION

Neuronal atrophy or death occurs as part of the normal development of the nervous system, and also in aging and neurological disease. Yet individuals vary widely in their apparent susceptibility to neurodegeneration. In the affluent parts of the world, where people over 85 are the fastest growing segment of the population, it is becoming acutely important to understand the mechanisms of neurodegeneration associated with age and disease. In this review we will be looking at the effect of glucocorticoid hormones on neuronal survival and the interaction between glucocorticoids and known trophic proteins. We will be concentrating on the hippocampus, since it is a primary target for glucocorticoids, is strongly implicated in the normal functions of mood, memory and learning, and is known to be a focus of damage in several neurodegenerative conditions.

GLUCOCORTICOIDS AND THE BRAIN

Glucocorticoids (GCs) are corticosteroids that are secreted by the adrenal cortex with a circadian rhythm and, at much higher concentrations, as part of the stress response. The perception by the brain of physiological and psychological stressors triggers the release of secretagogues, including corticotropin releasing factor (CRF), by the hypothalamus, stimulating the anterior pituitary to release adrenocorticotropic hormone (ACTH), which in turn stimulates the adrenal cortex to secrete GCs. This process is controlled by a negative feedback mechanism, as GCs interact with brain and pituitary receptors to inhibit CRF and ACTH release. The overall effect of systemic GC release is to enable the organism to react to the stressor and to reestablish homeostasis once the stressful event is over. By enhancing triglyceride, glycogen and protein catabolism, increasing gluconeogenesis, blocking cellular glucose uptake and suppressing anabolic processes, GCs increase the supply of readily available energy to the organism. While this is clearly helpful over the short term, prolonged exposure to GCs can have adverse effects on many physiological systems.

Like other steroid hormones GCs produce their most significant effects by binding to intracellular receptors which can then act as transcription factors, binding to genomic hormone-responsive elements and increasing or decreasing the transcription of target genes. Two types of corticosteroid receptor have been identified: type I receptors, also known as mineralocorticoid receptors because they bind endogenous mineralocorticoids (MCs) as well as glucocorticoids with high affinity; and type II receptors, known as glucocorticoid receptors since they bind GCs preferentially, with only a small affinity for MCs. Both types of receptor are found in the brain. Type I receptors occur predominantly in the limbic system, while type II are more widely distributed; in some cases the two are colocalized on the same cell [1]. The hippocampus, which contains high levels of both receptors, is therefore a prime target for GC action in the central nervous system. Together with the hypothalamus and pituitary the hippocampus is involved in the feedback system regulating GC secretion, and both type I and type II receptors have been implicated in this, although their different affinities for endogenous GCs suggest that the type I (K_d for GCs approx. 0.5 nM) mediates control during the circadian fluctuation, type II (K_d approx. 5 nM) during the conditions of high GC concentration that follow stress in which type I receptors would be saturated [2].

PATHOPHYSIOLOGY OF GCs

Given their wideranging actions it should come as no surprise that overexposure to GCs (due to chronic stress, hypersecretion in conditions such as Cushing's

*Correspondence to J. Leach Scully.
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disease, or the administration of exogenous GCs for therapeutic purposes) can be harmful. Chronic overexposure leads to a number of pathological states including diabetes, hypertension, growth suppression, immune suppression, and infertility. Since many important brain structures contain abundant GC receptors, it is equally unsurprising that overexposure to GCs can be neurotoxic.

Pharmacological [3] and high physiological [4] concentrations have been shown to damage hippocampal pyramidal neurons, with most damage occurring in region CA3 and least in CA1 and the dentate gyrus. This has a clear physiological relevance, since a correlation can be shown between the severity of age-related GC hypersecretion and age-related hippocampal degeneration [5, 6]. Using an experimental paradigm in which rats were exposed to multiple intermittent stresses, rather than the sustained low or high GC levels that follow adrenalectomy or injected corticosterone, Kerr *et al.* [7] showed that this more physiological pattern of GC exposure could also lead to neurological damage in the hippocampus.

The story is not completely clearcut, however, since another region of the hippocampus responds differently to GCs. Adrenalectomy, which removes the major endogenous GC source, can protect against GC-mediated pyramidal cell damage; but it can also lead directly to the death of neurons in the dentate gyrus [8] in a process resembling apoptosis or programmed cell death. However, the exact magnitude and extent of this damage remains unclear [9–11]. Since these deleterious effects can be prevented by giving small concentrations of GCs it is likely that the type I receptor is involved here, and if this is the case, the ambiguity of some results could be explained if small amounts of auxiliary adrenal tissue left behind after incomplete adrenalectomy can provide enough GCs to keep dentate gyrus granule cells healthy. In terms of the functional significance of this, Conrad and Roy [12] detected both a loss of dentate gyrus granule neurons and a decrease in the learning rate for a specific memory task following long term adrenalectomy; however, the degree of damage could not be correlated with the extent of memory deficit.

The pattern of hippocampal damage seen after high GC exposure is similar to that seen in the normal aged hippocampus: a decrease in the number of pyramidal neurons especially in CA3, a loss of corticosteroid receptors probably because of preferential loss of receptor-bearing neurons, and high levels of astrogliosis. Since basal GC levels do rise with age in rats and primates (and probably also in humans; see later) it has been suggested that the "normal" hippocampal degeneration seen in aged mammals is in fact the cumulative effect of GC exposure over the course of a lifetime, increasing as basal levels rise with age. Sapolsky [13] first suggested that, since exposure to GCs apparently damages neurons responsive to them, the hippocampal feedback mechanism will fail as neuron damage increases, leading to a rise in basal GC levels and to more GC-induced damage, i.e. the GC cascade. A corollary here is that if the GC exposure–neuronal loss–decreased feedback–increased GC exposure loop could be prevented, the hippocampal damage (and possibly the cognitive changes) associated with "normal" aging would not occur.

GLUCOCORTICOID DAMAGE TO NEURONS: METABOLIC EFFECTS

The question arises as to how exactly GCs damage neurons (and why, in the case of dentate gyrus granule cells, they appear not to). The most popular model suggests that, rather than causing direct harm, GCs potentiate the damaging effects of other neurological insults.

Evidence in favour of this comes chiefly from *in vitro* and *in vivo* studies that have considered harmful agents such as neurotoxins (for example excitatory neurotransmitters at high concentration), or harmful states such as hypoglycaemia, hypoxia-ischaemia, and trauma, to see whether the known damage associated with each agent or conditon could be modified by the presence of GCs. Kainic acid, an excitotoxin, preferentially damages hippocampal CA3 pyramidal neurons, visible as dendritic atrophy, and causes seizures in rats. The extent of neuronal damage could be decreased by adrenalectomy and increased by corticosterone treatment [13]. Hypoxia-ischaemia due to an interruption in the blood flow to the brain damages predominantly CA1 pyramidal neurons. In animal models of hypoxia-ischaemia, adrenalectomy decreased and exogenous corticosterone increased the damage [14]. Similar effects were seen on the hypoglycaemia-induced damage to dentate gyrus cells [13]. Evidence that the exacerbatory effect of GCs is direct and not via some other systemic GC activity is provided by the *in vitro* studies [15, 16]. Other brain regions that contain glucocorticoid receptors, such as neocortex and striatum, and are vulnerable to the same insults, also show an increased degree of damage in the presence of elevated GCs [14], though the hippocampus remains by far the most vulnerable structure. Brain regions with lower levels of GC receptors (like the cerebellum) do not show such exacerbation. Finally, the effect of these hormones is specific to GCs. Further studies have shown that non-GC steriods do not endanger neurons [16].

To understand how GCs make neurons more vulnerable we need to know how various neurological insults cause damage in the first place. The most commonly encountered real-life sources of damage are hypoxia-ischaemia (after strokes or cardiovascular accidents), hypoglycaemia and seizures, and all three appear to include an element of neurotoxicity due to excessive release of the excitatory amino acids (EAAs)

glutamate and aspartate. The neurotoxic effects of EAAs have been known for some time (for reviews see [17–19]), and are thought to be a consequence of EAA's pathological mobilization of intracellular calcium, either directly or indirectly [20], although calcium-independent mechanisms have also been invoked [21]. All three of the above damaging states increase intrasynaptic EAA concentrations (see e.g. [22–24]). Using a number of different experimental models, such as hypoxia-ischaemia or focal ischaemia [25, 26], excitotoxin-induced seizures [27, 28], hypoglycaemia *in vivo* [29], or anoxia *in vitro* [30] it has been shown that EAA antagonists can protect against these varied insults.

It appears that the energy-dependent ion transporters of neurons and glia, which remove EAA [31–33] or calcium [34] from the extracellular space are compromised by the lack of ready energy. Each of these classic neurological insults are a form of energetic crisis; the damage can usually be ameliorated or prevented by energy supplementation [35–37]. Similarly, energy supplementation can protect from GC-mediated neuron endangerment [38, 39]. It is therefore plausible that GCs endanger via their effect on energy state, perhaps by their inhibition of glucose transport or their effect on glycogen stores [40, 41], resulting in a potentiation of EAA buildup, disturbance of calcium homeostasis, and changes in the balance between excitation and inhibition.

It would, however, be rash to assume that energetic mechanisms are the only ways in which GCs can influence neuronal health and survival. Both types of corticosteroid receptors act as pleiotropic transcription factors. GC interaction with cell-specific and developmental stage-specific factors might account for the complexities indicated above, where GCs are neurotoxic to hippocampal pyramidal neurons but apparently necessary for the survival and proper development of hippocampal granule cells of the dentate gyrus. In particular, GCs could regulate the expression of genes coding for trophic or protective proteins, providing a molecular basis for the differential induction of neurodegeneration.

THE NEUROTROPHINS

Neutotrophins (NTs) or neurotrophic factors are required for the development and maintenance of peripheral and central neurons. The first example, nerve growth factor (NGF) was identified in the early 1950s [42, 43]. Subsequently NGF was shown to promote the survival, growth, and differentiation of peripheral sympathetic and sensory dorsal root ganglion neurons, and more recently its actions in the CNS have been described. Other NTs have also been identified: brain-derived neutotrophic factor (BDNF [44]), neurotrophn-3 (NT-3 [45, 46]) and neurotrophin-4/5 (NT-4/5 [47, 48]), and cloning of their respective genes has shown them to be members of the same gene family, the NGF-related NTs. Other factors unrelated to this gene family (and in some cases identified as growth factors in other contexts) have also been found to have neurotrophic activities. These include ciliary neurotrophic factor (CNTF [49–51], leukemia inhibitory factor/cholinergic differentiating factor (LIF/CDF [52]), the fibroblast growth factors (FGFs [53]), glial cell line-derived neurotrophic factor (GDNF [54]), insulin-like growth factors [55, 56], epidermal growth factor and platelet-derived growth factor [57, 58] and transforming growth factors alpha and beta [38, 59, 60]. No doubt others remain to be recognized. Only the NGF-related NTs will be discussed further here: for a fuller review of these, see [61]).

Members of the NGF-related NT family bind with a similar low affinity to a cell surface receptor, p75 or LNGFR [62], and recent evidence also implicates a family of tyrosine protein kinases, the trk family, as the functional receptors for the NGF-related NTs. NGF is the ligand specific for trkA, BDNF and NT-4/5 for trkB, and NT-3 for trkC (although NT-3 can also interact with trkB). Current thinking is that these receptors are chiefly responsible for mediating the significant biological NT responses, with p75 playing an accessory role, although this model has not been tested in all cell types or at all stages of development [63–67], and there is evidence that, in some systems at least, p75 can signal independently of trk [68]. The family of tyrosine kinases activates intracellular signalling pathways, including those involving the gene products Ras and Raf, and MAP kinase (some details of this have recently been reviewed in [69]).

Clues to the functions of NTs have come from a consideration of where they and their cognate receptors are expressed. Originally, NTs were described in terms of their action as target-derived factors. In the peripheral nervous system NGF is thought to regulate the extent of innervation of a target by sympathetic neurons or neural crest-derived sensory neurons; during development and after peripheral nerve injury, the target source of NGF may be cells of the developing or transected nerve. *In situ* or Northern blot hybridization shows trk and p75 gene expression in the trigeminal sensory ganglia and on sympathetic neurons. Several lines of evidence suggest that NGF responsiveness is present in the adult and that NGF may have an important role in the maintenance and repair of mature neurons. Following sciatic nerve crush or transection, NGF mRNA levels in proximal stump and distal nerve segment increase [70]. The initial activation of NGF transcription by injury follows the induction of c-*fos* and c-*jun*, whose mRNAs appear within an hour [71]. NGF follows this immediate early response, appearing after 2–4 h and remaining elevated for 12–24 h. A second wave of NGF induction, at around 3 days after injury, requires the presence of Schwann cells and fibroblasts [72].

In the central nervous system, recent studies have implicated several NTs in the development and maintenance of neuronal function. NGF mRNA is found in newborn and adult rat brain: highest levels are found in the hippocampal pyramidal neurons and granule and hilar cells of the dentate gyrus, in neocortex and olfactory bulb, but also in the caudate putamen, cerebellum, hypothalamus and spinal cord [73–77]. The regions of highest expression are targets for basal forebrain cholinergic neurons, and NGF appears to act as a trophic factor for cholinergic neurons of the basal forebrain and striatal caudate putamen. Basal forebrain cholinergic neurons express p75 and trk and bind NGF with high affinity. Intraventricular administration of NGF causes an increase in the choline acetyltransferase activity of these neurons [78–80]. Most convincingly, degeneration of basal forebrain cholinergic neurons following fimbria–fornix lesion, which severs the basal forebrain neurons projecting to the hippocampus, can be prevented by infusion of NGF or grafting of NGF-producing cells [81–85], while intrastriatal infusion has a protective effect on cholinergic neurons after quinolinic acid lesions [86]. NGF mRNA and protein are also found in the caudate putamen [75] and both p75 and trk mRNA have been found in these neurons as well. All this is of particular significance because in mammals, basal forebrain cholinergic neurons have been strongly implicated in learning and memory processes, and damage here has been observed in Alzheimer's disease [87]. Recent data suggest that NGF infusion also ameliorates some deficits in memory and learning following experimental lesions [88–90]. Although studies have concentrated on basal forebrain cholinergic neurons, the possibility that NGF can act on other populations of CNS neurons (e.g. cerebellar or hypothalamic) has not been excluded.

The actions of the other members of the NGF-like NT family are much less well-characterized. BDNF encourages the survival of dorsal root ganglia and nodose ganglia of the peripheral nervous system and central neurons of the substantia nigra, retinal ganglion and basal forebrain. NT-3 has been reported to have growth-supporting effects in PNS sympathetic ganglia, dorsal root ganglia and nodose ganglia, while trkC, the high affinity receptor for NT-3, is expressed on many neuronal cells through the brain, and a role for NT-3 in the support of adult central noradrenergic neurons has recently been described [91]. BDNF, NT-3 and NT-4/5 support the survival and differentiation of mesencephalic dopaminergic neurons *in vitro* [92, 93], and GABAergic neurons from the developing substantia nigra [94]. In addition there is some evidence that BDNF and NT-3 influence mature DA neurons *in vivo*, but an unequivocal effect on survival after experimental lesion has not yet been shown. NT-4 has been reported to support trigeminal ganglion and striatal neurons in culture [95, 96]. Following fimbria–fornix transection BDNF and NT-4/5 can assist axotomized cholinergic neurons [93, 97, 98]. Several studies show that BDNF, NT-3 and NT-4/5 are trophic factors for motor neurons [99–103]. Recent experiments using transgenic mice overexpressing, or knockout mice with deletions of, specific NTs or NT receptors, promise to provide much-needed information on the physiological functions of these proteins. Early results suggest that CNS neurons are supported by a complex overlapping network of growth factors, a network that shows considerable redundancy (reviewed in [104]).

NEUROTROPHINS AND NEURONAL ACTIVITY

Recent evidence, using a variety of animal lesion models, shows that NT expression in the central nervous system can be modulated by physiological activity. Seizures induced by focal electrolytic lesions [105], convulsant doses of excitotoxins [106, 107], or electrical stimulation [108] all lead to increased NGF mRNA in limbic and cortical regions of the adult rat. Within the hippocampus, a single seizure episode causes a biphasic increase in NGF mRNA, increasing first at 6 h post-seizure-inducing lesion and again at 24 h post-lesion. In other words, the first increase is within the period of seizure activity and could correspond to the NGF response to physiological activity. The second increase is after seizure activity ends, and could correspond to a repair response by neurons or non-neuronal cells [109, 110], by processes triggered during the seizure episode. This is very reminiscent of the biphasic NGF increase seen in the PNS after peripheral nerve transection.

Like NGF, BDNF mRNA increases in dentate gyrus granule cells and pyramidal cells of the hippocampus after lesion [111, 106] and follows a similar time course. However, its rise in expression spreads beyond the hippocampus more rapidly than does NGF and a biphasic response is not seen. Kindling-induced seizures lead to a transient increase in trkB mRNA and protein in the hippocampus [112]. Unlike either NGF or BDNF, NT-3's main response to lesion is an overall decrease in expression which persists for several days [77, 113–115].

As well as seizure activity, other neuronal insults can induce changes in NT expression. Transient forebrain ischemia [116] and insulin-induced hypoglycemic coma [113] are followed by increases in BDNF and/or NGF levels and in trkB mRNA and protein [112].

One of the problems in interpreting experiments like these is the different techniques used. In a variety of ways, the normal functioning of some brain regions is somehow disturbed, frequently leading to seizures. Care must be taken to distinguish between NT responses to the seizure activity, those that might be a specific cellular response to a toxin, and those directly associated with neuronal degeneration, regeneration or synaptic plasticity. Some experimental models do not

cause seizures (e.g. [117–120]), and in some cases [121] we might be looking at processes to do with synaptic rearrangement following denervation rather than physiological activity. It should also be borne in mind that in many experiments changes in neurotrophin protein level were not examined. A change in steady-state mRNA does not automatically mean a corresponding change in protein. Nevertheless, it is significant that exogenous NTs can protect neurons subjected to metabolic or excitotoxic insults in culture [116, 122–124].

So far, the available evidence suggests that the NT response is remarkably unspecific—whatever happens, there follows an increase in NGF and BDNF mRNA (with some exceptions, e.g. Rocamora *et al.* [125], whose quinolinic acid/seizure paradigm gave a decrease in NGF and BDNF and an increase in NT-3 mRNA). Another important message is that as far as the hippocampus is concerned, in virtually all cases the most extensive neurotrophin changes are seen in the dentate gyrus. This has particular significance for the damaging role of glucocorticoids.

GLUCOCORTICOIDS AND NEUROTROPHINS

If GCs can exacerbate the harmful effects of neuronal insults such as hypoglycaemia and hypoxia-ischaemia, and these insults alter the expression of NTs, does that mean that one way in which GCs can act is by altering NT expression too? It might be, for example, that the increased expression of NGF and BDNF mentioned above in several models is a defensive mechanism against the physiological disturbance caused by the lesion, which is disrupted by the presence of GCs. An early report [126] suggested that adrenalectomy decreases NGF protein in the rat hippocampus. The experiments used both biological and immunohistochemical assays, and also looked at the low affinity neurotrophin receptor (p75) in the hippocampus. Overall, adrenalectomy reduced the level of NGF detectable in the hippocampus by half. Injection of corticosterone into adrenalectomized rats restored NGF levels and NGF receptor expression. Adrenalectomy also seemed to increase the retrograde transport of p75 from the hippocampus. Stress activation of the hypothalamic–pituitary–adrenotropic (HPA) axis has also been reported to increase levels of NGF and p75 mRNA in the rat hippocampus [127].

Barbany and Persson [128] analysed the effect of GCs on NT mRNA levels in rat cerebral cortex and hippocampus by adrenalectomizing rats with or without giving replacement GCs (in this case the synthetic GC dexamethasone). NGF, BDNF and NT-3 mRNA all showed a significant decline in both brain regions after adrenalectomy while levels were restored to normal by dexamethasone treatment. NGF and NT-3 mRNA levels could also be increased by giving dexamethasone to intact (i.e. unadrenalectomized) animals, although in this situation the time course of the increase differed from that seen in the adrenalectomized rats, and BDNF showed a slow decrease rather than an increase in mRNA. Various caveats need to be borne in mind: the adrenalectomized animals were only examined after 3 days whereas the intact + dexamethasone rats were sampled at 1, 2, 4, 10 and 24 h, so early changes in the NT levels in adrenalectomized animals might have been missed; the number of animals used in each experiment was small, and the variation between animals large; and no protein correlation was shown. Nevertheless, these results seem to support the idea that normal levels of circulating GC are required to maintain basal NT mRNA in the hippocampus and cortex, and increasing the level of GCs can modify NT expression. Other groups have reported similar results [129, 130] and in this latter report the NT changes were related to an increase in trkA phosphorylation in the septum. In other words, the induction of NGF mRNA was correlated with a "biological event". However, changes in trkA phosphorylation were not observed in the hippocampus at any time point after GC administration, even though NGF-responsive cholinergic neurons project to the hippocampus. The authors interpret this as possibly due to the relatively small increase in NGF protein in the hippocampus compared to the change in the septum.

Thus, *in vivo* the data show that, in general, GC levels are positively correlated with NT levels. Experiments *in vitro* suggest the story is more complex than this. In L929 fibroblasts, used as a model system, corticosterone reduced NGF mRNA and protein by around 80% [131–133] but not BDNF mRNA [133]. Again, in cultured rat sciatic fibroblasts in which NGF expression had been artificially stimulated by treatment with foetal calf serum or interleukin-1, dexamethasone produced a decline in NGF mRNA. When the sciatic nerve of rats is transected an increase in NGF mRNA can be observed in the non-neuronal cells surrounding the axons, and this increase can be blocked by dexamethasone [134]. Thus it looks as if, in non-neuronal cells, GCs can inhibit NGF production.

In mixed hippocampal cultures (i.e. ones containing both neurons and glial cells and not specifically enriched for either type), dexamethasone was also observed to decrease NGF mRNA overall [135]. It should be noted, however, that at the time of the experiment astrocytes outnumbered neurons by 10 to 1, and thus the physiological relevance of this observation is debatable. In addition, the authors only looked after overnight treatment with GC, and again early events, perhaps involving neurons rather than astrocytes, would not have been noticed.

Given the idea that astrocytes and neurons might respond differently to GCs, it becomes important to distinguish the responses of each cell type, alone and in combination. In the above experiments using mixed

cultures although both neurons and non-neuronal cells appeared to be expressing NGF it was not possible to say whether one or both cell type(s) responded to GCs with a decrease in expression. To get around the problem of teasing out the responses of a highly heterogeneous cell population, clonal cell lines immortalized by an oncogene or by somatic cell fusion can be used. A panel of hippocampal cell lines has been derived by somatic cell fusion of hippocampal neurons from embryonic day 18 and postnatal day 21 mice, to an immortal neuroblastoma cell line. The hybrid lines have many of the characteristics of the hippocampal parent [136, 137]. By using reverse-transcription of mRNA coupled to amplification of the resulting cDNA by the polymerase chain reaction (PCR), we were able to analyse semi-quantitatively and sensitively the changes in NGF, BDNF and NT-3 mRNA in pure populations of hippocampal neurons [138, 139]. Here we saw clearly that both NGF and NT-3 are rapidly upregulated after treatment with dexamethasone or corticosterone in a cell line derived from embryonic tissue, while in a cell line derived from postnatal tissue NT-3 was unresponsive to dexamethasone. This is in line with the known developmental changes in expression of NGF and NT-3 in the hippocampus. NT-3 expression is highest at embryonic stages and declines after birth. It may therefore be that the lack of response to dexamethasone is indicative of a general suppression of NT-3 transcription in postnatal hippocampal neurons. We saw no change in BDNF expression up to 24 h after GC application. The responses were dose- and time-dependent and seen with both dexamethasone and corticosterone. They are probably mediated by the type II receptor since the specific type II receptor antagonist RU38486 was able to block the dexamethasone- and corticosterone-induced increase [139].

In primary cultures of embryonic rat hippocampus enriched for neurons, a similar upregulation of NGF mRNA but not of BDNF mRNA was also reported [129]. This paper also looked at the effect of dexamethasone on cultures enriched for astrocytes, but because of the low basal level of NGF and relative insensitivity of Northern blotting it was necessary to raise the level of NGF mRNA with transforming growth factor beta before it could be shown that dexamethasone caused a decrease, 12 h after GC application. Therefore it was not possible to say whether GCs have an effect on basal NGF expression too. Using the more sensitive RT-PCR method, we have seen small but consistent increases in basal NGF mRNA levels in astrocytes from hippocampus, cortex and striatum, but not in cerebellum which does not have a high density of GC receptors. The difference in result could also be due to the timepoint analysed (4 h post-stimulation rather than 12 h).

Thus it seems clear that, alone or when surrounded by small numbers of astrocytes, neurons respond to GCs by increasing NGF and NT-3 expression. The fact that *in vivo* GCs cause an immediate overall increase in NGF and NT-3 mRNA [128, 130] while *in vitro* the net effect appears to be an increase in pure or enriched neuronal cultures [138, 129] and a decrease in mixed cultures [135] could be a reflection of different experimental paradigms used (method of quantification, time of analysis). Alternatively it could indicate that cell–cell interactions are of critical importance in regulating the neurotrophin responses of different cell types.

GLUCOCORTICOIDS, NEUROTROPHINS AND NEURODEGENERATION

In only a few cases has the effect of a combination of neuronal insult and presence of GCs on NT expression been monitored experimentally. Such data are crucial to answer the question of whether GCs exacerbate neuronal damage by changes in NT expression as well as by their metabolic actions, or whether the presence of GCs increase the production of protective proteins, i.e. if what we see is a response to a threat enhanced by the presence of the hormones. D'Mello *et al.* [133] showed that KA/seizure-induced increases in NGF and BDNF were not obviously affected by prior administration of dexamethasone. However, since the seizure-induced changes in NT were rather large (20-fold for BDNF), it could be that region-specific differential responses to seizure and GC were missed. Lauterborn *et al.* [109] reported that the same biphasic pattern of NGF mRNA changes occurred after seizure whether or not the animals were adrenalectomized. What is clearly needed is a careful analysis of NT changes with a variety of insult or lesion models, in the presence or absence of GCs, focusing on the isolated responses of individual cell types in specific hippocampal subfields.

An understanding of the interactions between GCs, NTs and neuronal damage and/or survival is particularly important for several reasons. First, there is the clear correlation between increasing GC levels and hippocampal degeneration. There have been a number of conflicting reports about whether or not human basal GC levels also increase with age. The ambiguity could be due to differences in what experimenters define as an aged human, and to the effect of wide individual variation. A recent report suggests that there are distinct subgroups within the aging population who not only show increases in basal GC but whose GC levels could be positively correlated to cognitive deficits [140].

Regardless of whether human GC basal levels increase with "normal" human aging, neuronal dysfunction in humans has been associated with increased levels of these hormones (examples include the abnormal cortisol responses in Alzheimer's disease linked to hippocampal atrophy [141], hypercortisolism

in depressed patients [142], hypercortisolism and neuronal abnormalities in Cushing's disease [143–145]). In some of these cases the process of neurological damage appears to be reversible and not associated with overt hippocampal neuron loss, but dysfunction could precede neuron death associated with more severe and permanent cognitive defects.

Second, GCs are widely used in medicine as immunosuppressants, anti-inflammatory agents, and most ironically in the treatment of acute spinal cord and brain injury, where it is used to reduce oedema. If GCs do in fact endanger neurons then it may be such treatment is counterproductive, at least for the hippocampus. Conversely, if one aspect of the presence of elevated GC levels is increased local production of protective NTs, this might be exploited as a means to stabilize vulnerable neurons while repair is underway.

It is also possible that GC modulation of NTs in the CNS has actions beyond that of protection. The excitotoxin experiments show that NTs respond to physiological neuronal activity. In these experiments the level of activity was usually abnormally high (in seizure states), but in some studies more moderate degrees of activity have been considered [146, 147]. It may be that NTs are involved in the maintenance and modification of neuronal circuitry during behavioural changes or learning, perhaps "fixing" new synaptic patterns [148, 149]. Here, hypo- and hypercortism may also be involved in reinforcement of synaptic plasticity. We might see here a positive aspect of GC's short-term action on the CNS, counterbalancing the damage due to long-term exposure.

REFERENCES

1. Reul J. M. H. M. and De Kloet E. R.: Two receptor systems for corticosterone in the rat brain: microdistribution and differential occupation. *Endocrinology* **117** (1985) 2505–2512.
2. Reul J. M. H. M., van der Bosch H. R. and De Kloet E. R.: Relative occupancy of type I and type 2 corticosteroid receptors in the rat brain following stress and dexamethasone treatment: functional implications. *J. Endocr.* **115** (1987) 454–467.
3. Aus der Mühlen K. and Ockenfels H.: Morphologische Veränderungen im Diencephalon und Telencephalon nach Störungen des Adenohypophyse-Nebennierenrinde, III. *Z. Zellforsch.* **93** (1969) 126–141.
4. Sapolsky R. M., Krey L. and McEwen B.: Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J. Neurosci.* **5** (1985) 1221–1227.
5. Landfield P. W., Waymire J. C. and Lynch G.: Hippocampal aging and adrenocorticoids: quantitative correlations. *Science* **202** (1978) 1098–1102.
6. Landfield P. W., Baskin R. K. and Pitler T. A.: Brain aging correlates: retardation by hormonal–pharmacological treatments. *Science* **214** (1981) 581–584.
7. Kerr D. S., Campbell L. W., Applegate M. D., Brodish A. and Landfield P. W.: Chronic stress-induced acceleration of electrophysiologic and morphometric biomarkers of hippocampal aging. *J. Neurosci.* **11** (1991) 1316–1324.
8. Solviter R. S., Valiquette G., Abrams G. M., Ronk E. C., Sollas A. L., Paul L. A. and Neubort S.: Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. *Science* **243** (1989) 535–538.
9. Sapolsky R. M., Stein-Behrens B. A. and Armanini M. P.: Long-term adrenalectomy causes loss of dentate gyrus and pyramidal neurons in the adult hippocampus. *Exp. Neurol.* **114** (1991) 246–249.
10. Sloviter R. S., Sollas A. L., Dean E. and Neubort S.: Adrenalectomy-induced granule cell degeneration in the rat hippocampal dentate gyrus: characterization of an *in vivo* model of controlled neuronal death. *J. Comp. Neurol.* **330** (1993) 324–336.
11. Sloviter R. S., Dean E. and Neubort S.: Electron microscopic analysis of adrenalectomy-induced hippocampal granule cell degeneration in the rat: apoptosis in the adult central nervous system. *J. Comp. Neurol.* **330** (1993) 337–351.
12. Conrad C. D. and Roy E. J.: Selective loss of hippocampal granule cells following adrenalectomy: implications for spatial memory. *J. Neurosci.* **13** (1993) 2582–2590.
13. Sapolsky R. M.: A mechanism for glucocorticoid toxicity in the hippocampus; increased neuronal vulnerability to metabolic insults. *J. Neurosci.* **5** (1985) 1228–1232.
14. Sapolsky R. M. and Pulsinelli W. A.: Glucocorticoids potentiate ischemic injury to neurons: therapeutic implications. *Science* **229** (1985) 1397–1400.
15. Sapolsky R. M., Packan D. R. and Vale W. W.: Glucocorticoid toxicity in the hippocampus: *in vitro* demonstration. *Brain Res.* **453** (1988) 367–371.
16. Packan D. R. and Sapolsky R. M.: Glucocorticoid endangerment of the hippocampus: tissue, steroid and receptor specificity. *Neuroendocrinology* **51** (1990) 613–618.
17. Mayer M. L. and Westbrook G. L.: Cellular mechanisms underlying excitotoxicity. *Trends Neurosci.* **10** (1987) 56–61.
18. Meldrum B. and Garthwaite G.: Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmac. Sci.* **11** (1990) 379–387.
19. Lipton S. A. and Rosenberg P. A.: Excitatory amino acids as a final common pathway for neurologic disorders. *New Engl. J. Med.* **330** (1994) 613–622.
20. Choi D. W.: Glutamate neurotoxicity in cortical cell culture is calcium-dependent. *Neurosci. Lett.* **58** (1985) 293–297.
21. Goldberg M. P. and Choi D. W.: Combined oxygen and glucose deprivation in cortical cell culture: calcium-dependent and calcium-independent mechanisms of neuronal injury. *J. Neurosci.* **13** (1993) 3510–3524.
22. Benveniste H., Drejer, J. Schousboe A. and Diemer N. H.: Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J. Neurochem.* **43** (1984) 1369–1374.
23. Drejer J., Benbeniste H., Diemer N. H. and Schousboe A.: Cellular origin of ischemia-induced glutamate release from brain tissue *in vivo* and *in vitro*. *J. Neurochem.* **45** (1985) 145–151.
24. Sandberg M., Butcher S. P. and Hagberg H.: Extracellular overflow of neuroactive amino acids during severe insulin-induced hypoglycemia: *in vivo* dialysis of the rat hippocampus. *J. Neurochem.* **57** (1986) 178–184.
25. Rothman S.: Synaptic release of excitatory amino acid neurotransmitters mediates anoxic neuronal death. *J. Neurosci.* **4** (1984) 1884–1891.
26. Simon R. P., Swan J. H., Griffiths T. and Meldrum B. S.: Blockade of *N*-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science* **226** (1984) 850–852.
27. Jones R. S. G.: Epileptiform events induced by GABA-antagonist in entorhinal cortical cells *in vitro* are partly mediated by *N*-methyl-D-aspartate receptors. *Brain Res.* **457** (1988) 113–121.
28. Raley K. M. and Lipton P.: NMDA receptor activation accelerates ischemic energy depletion in the hippocampal slice and the demonstration of a threshold for ischemic damage to protein synthesis. *Neurosci. Lett.* **110** (1990) 118–123.
29. Wieloch T.: Hypoglycemia-induced neuronal damage prevented by an *N*-methyl-D-asparate antagonist. *Science* **230** (1985) 681–683.
30. Clark G. and Rothman S.: Blockade of excitatory amino acid receptors protects anoxic hippocampal slices. *Neuroscience* **21** (1987) 665–671.
31. Bradford H. F., Young A. M. J. and Crowder J. M.: Continuous glutamate leakage from brain cells is balanced by

compensatory high-affinity reuptake transport. *Neurosci. Lett.* **81** (1987) 296–302.
32. Kauppinen R. A., Enkvist K., Holopainen I. and Akerman K. E. O.: Glucose deprivation depolarizes plasma membrane of cultured astrocytes and collapses transmembrane potassium and glutamate gradients. *Neuroscience* **27** (1988) 283–289.
33. Szatkowski M., Barbour B. and Attwell D.: Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. *Nature* **348** (1990) 443–446.
34. Andreeva N., Khodorov B., Stelmashook E., Cragoe E. Jr and Victorov I.: Inhibition of Na^+/Ca^{2+} exchange enhances delayed neuronal death elicited by glutamate in cerebellar granule cell cultures. *Brain Res.* **548** (1991) 322–325.
35. Schurr A., West C. A., Reid K. H., Tseng M. T., Reiss S. J. and Rigor B. J.: Increased glucose improves recovery of neuronal function after cerebral hypoxia *in vitro*. *Brain Res.* **421** (1987) 135–139.
36. Sapolsky R. M. and Stein B. A.: Status epilepticus-induced hippocampal damage is modulated by glucose availability. *Neurosci. Lett.* **97** (1989) 157–162.
37. Tombaugh G. C., Yang S. H., Swanson R. A. and Sapolsky R. M.: Glucocorticoids exacerbate hypoxic and hypoglycemic hippocampal injury: biochemical correlates and a role for astrocytes. *J. Neurochem.* **59** (1992) 137–146.
38. Prehn J. H. M., Peruche B., Unsicker K. and Krieglstein J.: Isoform-specific effects of transforming growth factors-β on degeneration of primary neuronal cultures induced by cytotoxic hypoxia or glutamate. *J. Neurochem.* **60** (1993) 1665–1672.
39. Sapolsky R. M.: Glucocorticoid toxicity in the hippocampus: reversal by supplementation with brain fuel. *J. Neurosci.* **6** (1986) 2240–2244.
40. Horner H. C., Packan D. R. and Sapolsky R. M.: Glucocorticoids inhibit glucose transport in cultured hippocampal neurons and glia. *Neuroendocrinology* **52** (1990) 57–64.
41. Virgin C. E. Jr, Ha T. P-T., Packan D. R., Tombaugh G. C., Yang S. H., Horner H. C. and Sapolsky R. M.: Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: implications for glucocorticoid neurotoxicity. *J. Neurochem.* **57** (1991) 1422–1428.
42. Levi-Montalcini R. and Hamburger V.: Selective growth stimulating effect of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. *J. Exp. Zool.* **116** (1951) 321–363.
43. Levi-Montalcini R. and Hamburger V.: A diffusible agent of mouse sarcoma producing hyperplasia of sympathetic ganglia and hyperneurotization of viscera in the chick embryo. *J. Exp. Zool.* **123** (1953) 233–287.
44. Leibrock J., Lottspeich F., Hohn A., Hofer M., Hengerer B., Masiakowski P. and Thoenen H.: Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* **341** (1989) 149–152.
45. Ernfors P., Ibanez C. F., Ebendal T., Olson L. and Persson H.: Molecular cloning and neurotrophic activity of a protein with structural similarity to β-nerve growth factor: developmental and topographical expression in the brain. *Proc. Natn. Acad. Sci. U.S.A.* **87** (1990) 5454–5458.
46. Maisonpierre P. C., Belluscio L., Squinto S., Ip N. Y., Furth M. E., Lindsay R. M. and Yancopoulos G. D.: Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science* **247** (1990) 1446–1451.
47. Berkemeier L. R., Winslow J. W., Kaplan D. R., Nikolics K., Geoddel D. V. and Rosenthal A.: Neurothrophin-5: a novel neurotrophic factor that activates trk and trkB. *Neuron* **7** (1991) 857–866.
48. Hallbook F., Ibanez C. F. and Persson H.: Evolutionary studies of nerve growth factor family reveal a new member abundantly expressed in *Xenopus* ovary. *Neuron* **6** (1991) 845–858.
49. Adler R., Landa K. B., Manthorpe M. and Varon S.: Cholinergic neuronotrophic factors: intraocular distribution of trophic activity for ciliary neurons. *Science* **204** (1979) 1434–1436.
50. Lin L-F. H., Mismer D., Lile J. D., Armes L. G., Butler E. T. III, Vannice J. L. and Collins F.: Purification, cloning and expression of ciliary neurotrophic factor (CNTF). *Science* **246** (1989) 1023–1025.
51. Stöckli K. A., Lottspeich F., Sendtner M., Masiakowski P., Carroll P., Götz R., Lindholm D. and Thoenen H.: Molecular cloning, expression and regional distribution of rat ciliary neurotrophic factor. *Nature* **342** (1989) 920–923.
52. Yamamori T., Fukada K., Aebersold R., Korsching S., Fann M. J. and Patterson P. H.: The cholinergic neuronal differentiation factor from heart cells is identical to leukemia inhibitory factor. *Science* **246** (1989) 1412–1416.
53. Walicke P. A.: Basic and acidic fibroblast growth factors have trophic effects on neurons from multiple CNS regions. *J. Neurosci.* **8** (1988) 2618–2627.
54. Lin L. F. H., Doherty D. H., Lile J. D., Bektesh S. and Collins F.: GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* **260** (1993) 1130–1132.
55. Aizenman Y., Weichsel M. E. Jnr and De Vellis J.: Changes in insulin and transferrin requirements of pure brain neuronal cultures during embryonic development. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 2263–2266.
56. Baskin D. G., Figlewicz D. P., Woods S. C., Porte D. and Dorsa D. M.: Insulin in the brain. *A. Rev. Physiol.* **49** (1987) 335–347.
57. Fallon J. H., Seroogy K. B., Loughlin S. E., Morrison R. S., Bradshaw R. A. and Knauer D. J.: Epidermal growth factor immunoreactive material in the central nervous system: location and development. *Science* **224** (1985) 1107–1109.
58. Morrison R. S., Kornblum H. I., Leslie F. M. and Bradshaw R. A.: Trophic stimulation of cultured neurons from neonatal rat brain by epidermal growth factor. *Science* **238** (1987) 72–75.
59. Derynck R.: Transforming growth factor alpha. *Cell* **54** (1988) 593–595.
60. Fallon J. H., Annis C. M., Gentry L. E., Twardzik D. R. and Loughlin S. E.: Localization of cells containing transforming growth factor alpha precursor immunoreactive material in the basal ganglia of the adult rat brain. *Growth Factors* **2** (1990) 241–250.
61. Lindsay R. M., Wiegand S. J., Altar C. A. and DiStefano P. S.: Neurotrophic factors; from molecule to man. *Trends Neurosci.* **17** (1994) 182–190.
62. Meakin S. O. and Shooter E. M.: The nerve growth factor family of receptors. *Trends Neurosci.* **15** (1991) 323–331.
63. Lee K. F., Li E., Huber L. J., Landis S. C., Sharpe A. H., Chao M. V. and Jaenisch R.: Targetted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral nervous system. *Cell* **69** (1992) 737–749.
64. Ip N. Y., Stiff T. N., Tapley P., Klein R., Glass D. J., Fandl J., Greene L. A., Barbacid M. and Yancopoulos G. D.: Similarities and differences in the way neurotrophins interact with the Trks in neuronal and non-neuronal cells. *Neuron* **10** (1993) 137–149.
65. Hantzopoulos P. A., Suri C., Glass D. J., Goldfarb M. P. and Yancopoulos G. D.: The low affinity NGF receptor, p75, can collaborate with each of the trks to potentiate functional responses to the neurotrophins. *Neuron* **13** (1994) 187–201.
66. Klein R., Silos-Santiago I., Smeyne R. J., Lira S. A., Brambilla R., Bryant S., Zhang L., Snider W. D. and Barbicid M.: Disruption of the neurotrophin-3 receptor gene *trk*C eliminates Ia muscle afferents and results in abnormal movements. *Nature* **368** (1994) 249–251.
67. Smeyne R. J., Klein R., Schnapp A., Long L. K., Bryant S., Lewin A., Lira S. A. and Barbacid M.: Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. *Nature* **368** (1994) 246–249.
68. Dobrowsky R. T., Werner M. H., Castellino A. M., Chao M. V. and Hannun Y. A.: Activation of the sphingomyelin cycle through the low-affinity neurotrophin receptor. *Science* **265** (1994) 1596–1599.
69. Saltiel A. R. and Decker S. J.: Cellular mechanisms of signal transduction for neurotrophins. *Bioessays* **16** (1994) 405–411.
70. Heumann R., Korsching S., Bandtlow C. and Thoenen H.: Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection. *J. Cell Biol.* **104** (1987) 1623–1631.

71. Hengerer B., Lindholm D., Heumann R., Rüther U., Wagner E. F. and Thoenen H.: Lesion-induced increase in nerve growth factor mRNA is mediated by c-*fos*. *Proc. Natn. Acad. Sci. U.S.A.* **87** (1990) 3899–3903.
72. Lindholm D., Heumann R., Meyer M. and Thoenen H.: Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature* **330** (1987) 658–659.
73. Large T. H., Bodary S. C., Clegg D. O., Weskamp G., Otten U. and Reichardt L.: Nerve growth factor gene expression in the developing rat brain. *Science* **234** (1986) 352–355.
74. Whittemore S. R., Ebendal T., Larkfors L. Olson L., Seiger A., Stranberg I. and Persson H.: Developmental and regional expression of β-nerve growth factor messenger RNA and protein in the rat central nervous system. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 817–821.
75. Mobley W. C., Woo J. E., Edwards R. H., Riopelle R. J., Largo F. M., Weskamp G., Otten U., Vallettal J. S. and Johnston M. V.: Developmental regulation of nerve growth factor and its receptor in the rat caudate-putamen. *Neuron* **3** (1989) 655–664.
76. Maisonpierre P. C., Belluscio L., Friedman B., Alderson R. E., Wiegand S. J., Furth M. E., Lindsay R. M. and Yancopoulos G. D.: NT-3, BDNF and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. *Neuron* **5** (1990) 501–509.
77. Gall C. M., Murray K. and Isackson G.: Kainic acid-induced seizures stimulate increased expression of nerve growth factor mRNA in rat hippocampus. *Molec. Brain Res.* **9** (1991) 113–123.
78. Gnahn H., Hefti F., Heumann R., Schwab M. E. and Thoenen H.: NGF-mediated increase of choline acetyltransferase (ChAT) in the neonatal rat forebrain: evidence for a physiological role of NGF in the brain? *Dev. Brain Res.* **9** (1983) 45–52.
79. Mobley W. C., Rutkowski J. L., Tennekoon G. I., Buchanan K. and Johnston M. V.: Choline acetyltransferase in striatum of neonatal rats increase by nerve growth factor. *Science* **229** (1985) 284–287.
80. Mobley W. C., Rutkowski J. L., Tennekoon G. I., Gemski J., Buchanan K. and Johnston M. V.: Nerve growth factor increases choline acetyltransferase activity in developing basal forebrain neurons. *Molec. Brain Res.* **1** (1986) 53–62.
81. Hefti F.: Nerve growth factor (NGF) promotes survival of septal cholinergic neurons after fimbrial transection. *J. Neurosci.* **6** (1986) 2155–2162.
82. Williams L. R., Varon S., Peterson G. M., Wictorin K., Fischer W., Bjorklund A. and Gage F. H.: Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbrial-fornix transection. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 9231–9235.
83. Kromer L. F.: Nerve growth factor treatment after brain injury prevents neuronal death. *Science* **235** (1987) 214–216.
84. Koliatsos V. E., Nanta H. J. W., Clatterbuck R. E., Holtzmann D. M., Mobley W. C. and Price D. L.: Mouse nerve growth factor prevents degeneration of axotomized basal forebrain cholinergic neurons in monkey. *J. Neurosci.* **10** (1990) 3801–3813.
85. Tuszynski M. H., U H. S., Amaral D. G. and Gage F.: Nerve growth factor infusion in the primate brain reduces lesion-induced cholinergic neuronal degeneration in the adult primate brain. *J. Neurosci.* **10** (1990) 3604–3614.
86. Venero J. L., Beck K. D. and Hefti F.: Intrastriatal infusion of nerve growth factor after quinolinic acid prevents reduction of cellular expression of choline acetyltransferase messenger RNA and *trk*A messenger RNA, but not glutamate decarboxylase messenger RNA. *Neuroscience* **61** (1994) 257–268.
87. Price D. L.: New perspectives on Alzheimer's disease. *A. Rev. Neurosci.* **9** (1986) 489–512.
88. Dekker A. J., Winkler J., Ray J., Thal L. J. and Gage F. H.: Grafting of nerve growth factor-producing fibroblasts reduces behavioral deficits in rats with lesions of the nucleus basalis magnocellularis. *Neuroscience* **60** (1994) 299–309.
89. Fischer W., Sirevaag A., Wiegand S. J., Lindsay R. M. and Björklund A.: Reversal of spatial memory impairments in aged rats by nerve growth factor and neurotrophins 3 and 4/5 but not by brain-derived neurotrophic factor. *Proc. Natn. Acad. Sci. U.S.A.* **91** (1994) 8607–8611.
90. Markowska A. L., Koliatsos V. E., Breckler S. J., Price D. L. and Olton D. S.: Human nerve growth factor improves spatial memory in aged but not in young rats. *J. Neurosci.* **14** (1994) 4815–4824.
91. Arenas E. and Persson H.: Neurotrophin-3 prevents the death of adult central noradrenergic neurons *in vivo*. *Nature* **367** (1994) 368–371.
92. Hyman C., Hofer M., Barde Y-A., Juhasz M., Yancopoulos G., Squinto S. P. and Lindsay R. M.: BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* **350** (1991) 230–232.
93. Knüsel B., Winslow J., Rosenthal A., Burton L. E., Seid D. P., Nikolics K. and Hefti F.: Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin-3. *Proc. Natn. Acad. Sci. U.S.A.* **88** (1991) 961–965.
94. Hyman C., Juhasz M., Jackson C., Wright P., Ip N. Y. and Lindsay R. M.: Overlapping and distinct actions of the neurotrophins BDNF, NT-3, and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. *J. Neurosci.* **14** (1994) 335–347.
95. Ibanez C. F., Ernfors P., Timmusk T., Ip N. Y., Arenas E., Yancopoulos G. D. and Persson H.: Neurotrophin-4 is a target-derived neurotrophic factor for neurons of the trigeminal ganglion. *Development* **117** (1993) 1345–1353.
96. Ardelt A. A., Flaris N. A. and Roth K. A.: Neurotrophin-4 selectively promotes survival of striatal neurons in organotypic slice culture. *Brain Res.* **647** (1994) 340–344.
97. Alderson R. F., Alterman A. L., Barde Y-A. and Lindsay R. M.: Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. *Neuron* **5** (1990) 297–306.
98. Morse J. K., Wiegand S. J., Anderson K., You Y., Cai N., Carnahan J., Miller J., DiStefano P. S., Altar A. A., Lindsay R. M. and Alderson R. F.: Brain-derived neurotrophic factor (BDNF) prevents the degeneration of medial septal cholinergic neurons following fimbria transection. *J. Neurosci.* **13** (1993) 4146–4156.
99. Sendtner M., Holtmann B., Kolbeck R., Thoenen H. and Barde Y-A.: Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature* **360** (1992) 757–759.
100. Yan Q., Elliott J. and Snider W. D.: Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature* **360** (1992) 753–755.
101. Koliatsos V. E., Clatterbuck R. E., Winslow J. W., Cayouette M. H. and Price D. L.: Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons *in vivo*. *Neuron* **10** (1993) 359–367.
102. Wong V., Arriaga R., Ip N. Y. and Lindsay R. M.: The neurotrophins BDNF, NT-3 and NT-4/5 but not NGF up-regulate the cholinergic phenotype of developing motor neurons. *Eur. J. Neurosci.* **5** (1993) 466–474.
103. Li L., Oppenheim R. W., Lei M. and Houenou L. J.: Neurotrophic agents prevent motoneuron death following sciatic nerve section in the neonatal mouse. *J. Neurobiol.* **25** (1994) 759–766.
104. Snider W. D.: Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* **77** (1994) 627–638.
105. Gall C. M. and Isackson P. J.: Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. *Science* **245** (1989) 758–761.
106. Zafra F., Hengerer B., Leibrock J., Thoenen H. and Lindholm D.: Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. *EMBO J.* **9** (1990) 3535–3550.
107. Isackson P. J., Huntsman M. M., Murray K. D. and Gall C. M.: BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. *Neuron* **6** (1991) 937–948.
108. Ernfors P., Bengzon J., Kokaia Z., Persson H. and Lindvall O.: Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. *Neuron* **7** (1991) 165–176.

109. Lauterborn J. C., Isackson P. J. and Gall C. M.: Seizure-induced increases in NGF mRNA exhibit different time courses across forebrain regions, and are biphasic in the hippocampus. *Exp. Neurol.* **125** (1994) 22–40.
110. Bengzon J., Soderstrom S., Kokaia Z., Kokaia M., Ernfors P., Persson H., Ebendal T. and Lindvall O.: Widespread increase of nerve growth factor protein in the rat forebrain after kindling-induced seizures. *Brain Res.* **587** (1992) 338–342.
111. Dugich-Djordjevic M. M., Tocco G., Lapchak P. A., Pasinetti G. M., Najm I., Baudry M. and Hefti F.: Regionally specific and rapid increases in brain-derived neurotrophic factor messenger RNA in the adult rat brain following seizures induced by systemic administration of kainic acid. *Neuroscience* **47** (1992) 303–315.
112. Merlio J. P., Ernfors P., Kokaia Z., Middlemas D. S., Bengzon J., Kokaia M., Smith M.-L., Siesjö B. K., Hunter T., Lindvall O. and Persson H.: Increased production of the TrkB protein tyrosine kinase receptor after brain insults. *Neuron* **10** (1993) 151–164.
113. Lindvall O., Ernfors P., Bengzon J., Kokaia Z., Smith M-L., Siesjo B. K. and Persson H.: Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 in the adult rat brain following cerebral ischemia and hypoglycemic coma. *Proc. Natn. Acad. Sci. U.S.A.* **89** (1992) 648–652.
114. Rocomora N., Palacios J. M. and Mengod G.: Limbic seizures induce a differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3, in the rat hippocampus. *Molec. Brain Res.* **13** (1992) 27–33.
115. Takeda A., Onodera H., Yamasaki Y., Furukawa K., Kogure K., Obinata M. and Shibahara S.: Decreased expression of neurotrophin-3 mRNA in the rat hippocampus following transient forebrain ischemia. *Brain Res.* **569** (1992) 177–180.
116. Tsukahara T., Yonekawa Y., Tanaka K., Ohara O., Watanabe S., Kimura T., Nishijima T. and Taniguchi T.: The role of brain-derived neurotrophic factor in transient forebrain ischemia in the rat brain. *Neurosurgery* **34** (1994) 323–331.
117. Ballarin M., Ernfors P., Lindefors N. and Persson H.: Hippocampal damage and kainic acid injection induce a rapid increase in mRNA for BDNF and NGF in rat brain. *Exp. Neurol.* **114** (1991) 35–43.
118. Boatell L. L., Lindefors N., Ballarin M., Ernfors P., Mahy N. and Persson H.: Activation of basal forebrain cholinergic neurons differentially regulates brain-derived neurotrophic factor mRNA expression in different projection areas. *Neurosci. Lett.* **136** (1992) 203–208.
119. Springer J. E., Gwag B. J. and Sessler F. M.: Neurotrophic factor mRNA expression in dentate gyrus is increased following *in vivo* stimulation of the angular bundle. *Molec. Brain Res.* **23** (1994) 135–143.
120. Strauss S., Otten U., Joggerst B., Plüss K. and Volk B.: Increased levels of nerve growth factor (NGF) protein and mRNA and reactive gliosis following kainic acid injection into the rat striatum. *Neurosci. Lett.* **168** (1994) 193–196.
121. Gwag B. J., Sessler F., Kimmerer K. and Springer J. E.: Neurotrophic factor mRNA expression in dentate gyrus is increased following angular bundle transection. *Brain Res.* **647** (1994) 23–29.
122. Shimohama S., Ogawa N., Tamura Y., Akaike A., Tsukahara T., Iwata H. and Kimura J.: Protective effect of nerve growth factor against glutamate-induced neurotoxicity in cultured cortical neurons. *Brain Res.* **632** (1993) 296–302.
123. Cheng B. and Mattson M. P.: NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. *Brain Res.* **640** (1994) 56–67.
124. Kokaia Z., Othberg A., Kokaia M. and Lindvall O.: BDNF makes cultured dentate granule cells more resistant to hypoglycaemic damage. *Neuroreport* **5** (1994) 1241–1244.
125. Rocamora N., Massieu L., Boddeke H. W. G. M., Mengod G. and Palacios J. M.: Neuronal death and neurotrophin gene expression: long-lasting stimulation of neurotrophin-3 messenger RNA in the degenerating CA1 and CA4 pyramidal cell layers. *Neuroscience* **53** (1993) 905–908.
126. Aloe L.: Adrenalectomy decreases nerve growth factor in young adult rat hippocampus. *Proc. Natn. Acad. Sci. U.S.A.* **86** (1989) 5636–5640.
127. Foreman P. J., Taglialatela G., Angelucci L., Turner C. P. and Perez-Polo J. R.: Nerve growth factor and p75NGFR factor receptor mRNA change in rodent CNS following stress activation of the hypothalamo-pituitary-adrenocortical axis. *J. Neurosci. Res.* **36** (1993) 10–18.
128. Barbany G. and Persson H.: Regulation of neurotrophin mRNA expression in the rat brain by glucocorticoids. *Eur. J. Neurosci.* **4** (1992) 396–403.
129. Lindholm D., Castren E., Hengerer B., Zafra F., Berninger B. and Thoenen H.: Differential regulation of nerve growth factor (NGF) synthesis in neurons and astrocytes by glucocorticoid hormones. *Eur. J. Neurosci.* **4** (1992) 404–410.
130. Saporito M. S., Brown E. R., Hartpence K. C., Wilcox H. M., Robbins E., Vaught J. L. and Carswell S.: Systemic dexamethasone administration increases septal trk autophosphorylation in adult rats via an induction of nerve growth factor. *Molec. Pharmac.* **45** (1994) 395–401.
131. Siminoski K., Bernanke J., Kay C. and Murphy R. A.: Steroids and triiodothyronine reduce nerve growth factor concentrations in medium conditioned by L-929 fibroblasts. *Endocrinology* **118** (1986) 1417–1425.
132. Siminoski K., Murphy R. A., Rennert P. and Heinrich G.: Cortisone, testosterone and aldosterone reduce levels of nerve growth factor messenger ribonucleic acid in L-929 fibroblasts. *Endocrinology* **121** (1987) 1432–1437.
133. D'Mello S. R., Jiang C., Lamberti C., Martin S. C. and Heinrich G.: Differential regulation of the nerve growth factor and brain-derived growth factor genes in L929 mouse fibroblasts. *J. Neurosci. Res.* **33** (1992) 519–526.
134. Lindholm D., Hengerer B., Heumann R., Carroll P. and Thoenen H.: Glucocorticoid hormones negatively regulate nerve growth factor expression in vivo and in cultured rat fibroblasts. *Eur. J. Neurosci.* **2** (1990) 795–801.
135. Friedman W. J., Lärkfors L., Ayer-Lelievre C., Ebendal T., Olson L. and Persson H.: Regulation of β-nerve growth factor expression by inflammatory mediators in hippocampal cultures *J. Neurosci. Res.* **27** (1990) 374–382.
136. Lee J., Hammond D. N., Large T. H., Roback J. D., Sim J. A., Brown D. A., Otten U. H. and Wainer B. H.: Neuronal properties and trophic activities of immortalized hippocampal cells from embryonic and young adult mice. *J. Neurosci.* **10** (1990) 1779–1787.
137. Wainer B. H. and Heller A.: Neuronal hybrid cell lines: generation, characterization, and utility. In *Neuronal Cell Lines: A Practical Approach* (Edited by J. N. Wood). IRL Press, Oxford (1992) pp. 1–26.
138. Scully J. L. and Otten U.: Glucocorticoid modulation of neurotrophin expression in immortalized mouse hippocampal neurons. *Neurosci. Lett.* **155** (1993) 11–14.
139. Scully J. L. and Otten U.: Modulation of neurotrophin expression in hippocampal neurons by steroid hormones. *Molec. Brain Res.* (1995) in press.
140. Lupien S., Lecours A. R., Lussier I., Schwartz G., Nair N. P. V. and Meaney M. J.: Basal cortisol levels and cognitive defects in human aging. *J. Neurosci.* **14** (1994) 2893–2903.
141. DeLeon M., McRae T., Tasi J., George A., Marcus D., Freedman M., Wolf A., McEwen B.: Abnormal cortisol response in Alzheimer's disease linked to hippocampal atrophy. *Lancet* **2** (1988) 391–392.
142. Wolkowitz O. M., Reus V. I., Weingartner H., Thompson K., Breier A., Doran A., Rubinow D. and Pickar D.: Cognitive effects of corticosteroids. *Am. J. Psychiat.* **147** (1990) 1297–1303.
143. Bentson J., Reza M., Winter J. and Wilson G.: Steroids and apparent cerebral atrophy on computed tomography scans. *J. Comput. Assist. Tomogr.* **2** (1978) 16–23.
144. Okuno T., Ito M., Yoshioka M. and Nakano Y.: Cerebral atrophy following ACTH therapy. *J. Comput. Assist. Tomogr.* **4** (1980) 20–23.
145. Starkman M. N., Gebarski S. S., Berent S. and Schteingart D. E.: Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biol. Psychiat.* **32** (1992) 756–765.

146. Lindefors N., Ernfors P., Falkenberg T. and Persson H.: Septal cholinergic afferents regulate expression of brain-derived neurotrophic factor and β-nerve growth factor mRNA in rat hippocampus. *Exp. Brain Res.* **88** (1992) 78–90.
147. Zafra F., Castren E., Thoenen H. and Lindholm D.: Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. *Proc. Natn. Acad. Sci. U.S.A.* **88** (1991) 10,037–10,041.
148. Patterson S. L., Grover L. M., Schwartzkroin P. A. and Bothwell M.: Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. *Neuron* **9** (1992) 1081–1088.
149. Lohof A. M., Ip N. Y. and Poo M-M.: Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* **363** (1993) 350–353.