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Hippocampal vulnerability to stress and aging: possible role of neurotrophic factors

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Abstract

Chronic stress can accelerate age-related damage to the hippocampus. Adrenal glucocorticoids are thought to be responsible for this damage because of their ability to compromise energy metabolism and make neurons more vulnerable to glutamate excitotoxicity. Additional mechanisms by which stress or glucocorticoids could damage the hippocampus are considered in the context of recent evidence that stress regulates neurotrophic factor expression in the brain. Stress has been found to decrease brain-derived neurotrophic factor (BDNF) mRNA in the hippocampus, and this may contribute to stress-induced damage in this and other brain areas. Nerve growth factor (NGF) and neurotrophin-3 (NT-3) are increased by stress and glucocorticoids perhaps as a compensatory response to stress-induced damage. Because neurotrophic factors can protect the brain from a variety of traumatic insults, it is likely that they might also be effective in preventing or reversing glucocorticoid-induced damage to the hippocampus.

Keywords: Brain-derived neurotrophic factor; Nerve growth factor; Neurotrophin-3; Glucocorticoid; Alzheimer's disease; Aging; Excitotoxicity; Hippocampus; Stress

1. Effects of stress, glucocorticoids and aging on the hippocampus

Over the past 15 years investigators have demonstrated that aging, stress and glucocorticoids cause a variety of biochemical and morphological changes in the hippocampus which impair its function. Landfield found that the number of hippocampal neurons was markedly reduced in old compared to young Fischer rats [47]. Importantly, he showed that the culprit was not simply age but the chronic effects of adrenal glucocorticoids which are secreted into the bloodstream in response to stress, for adrenalectomy at an early age largely prevented the neuron loss in the hippocampus normally seen in older rats. Sapolsky went on to show that administration of corticosterone to rats at doses which produce blood levels similar to those induced by stress caused a down-regulation of glucocorticoid receptors (GR) which was explained in part by a loss of neurons [82]. Neuronal death was especially apparent in CA3 pyramidal layer of the hippocampus. The decrease in neurons expressing GR diminished the ability of the hippocampus to restrain corticotropin-releasing factor (CRF) neurons in the hypothalamus during stress. Thus chronic stress reduced glucocorticoid negative feedback and resulted in higher basal levels of circulating glucocorticoids which further down-regulated GR. This vicious circle most likely explains why older rats have a more prolonged hormonal response to an acute stress compared to younger rats [85]. Meaney then went on to show that neonatal handling of male rats produced a life-long increase in GR number in the hippocampus which had functional consequences – it reduced the magnitude of hypothalamic-pituitary-adrenal (HPA) axis activation in response to stress, and it prevented the age-related loss in hippocampal neurons [65]. Intermittent footshock for months damaged hippocampal neurons, and the vulnerability increased with age [41]. Immobilization and swim stress also damaged CA3 and CA4 neurons, but interestingly this damage was attenuated by testosterone, suggesting that the age-related decline in gonadal function may increase the hippocampal vulnerability to stress [67]. Evidence for stress-induced neuronal damage in the hippocampus was

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seen not only in laboratory rats but was also apparent in monkeys that had been trapped in the wild [107]. Monkeys which had died spontaneously and had gastric ulcers were found to have hippocampal degeneration in the CA3 and CA4 pyramidal layers but not in CA1 or the dentate gyrus. Finally, patients with Cushing's syndrome had decreased hippocampal size which correlated with their blood levels of cortisol [98].

Shorter periods of stress or glucocorticoid administration produce more subtle changes in hippocampal morphology. For instance, McEwen and colleagues have demonstrated that 21 days of stress or glucocorticoid administration can cause atrophy of dendrites on CA3 pyramidal neurons in the hippocampus [111,113]. Only the apical dendrites which receive mossy fiber input from the dentate granule neurons were affected. Basal dendrites which receive fewer glutamatergic inputs from the dentate gyrus had no significant changes in length or number of branch points. In addition 21 days of stress or glucocorticoid treatment also produced early signs of neuronal degeneration such as shrunken cells in the CA3 region. Dentate granule neurons and CA1 pyramidal neurons were unaffected by these treatments.

Glucocorticoids also affect hippocampal electrophysiology. Binding of the high affinity Type I or mineralocorticoid receptor by corticosterone increases the excitability of CA1 neurons [39,40]. This effect of low-dose corticosterone appears to be due to the inhibition of the slow Ca^{2+}-dependent K^+ conductance which suppresses the slow after hyperpolarization (AHP) and hence increases neuronal excitability. The stress-related neuropeptide CRF also has the ability to increase excitability by inhibiting the AHP in the CA1 pyramidal layer [1] and in the dentate gyrus (Berry and Smith, unpublished observations). High-dose corticosterone which activates the Type II glucocorticoid receptor has the opposite effect of low-dose corticosterone or CRF, it increases the AHP and hence decreases hippocampal excitability. These are probably genomic effects because they take about 1 h to become maximal and can be blocked by protein synthesis inhibitors. Of interest is the fact that the AHP is increased with aging [42] which may be due to increased levels of circulating glucocorticoids. High levels of glucocorticoids may increase the AHP by enhancing voltage-dependent Ca^{2+} conductances [43]. This could lead to chronically elevated intracellular Ca^{2+} levels and result in neurotoxicity.

Perhaps due to its ability to decrease hippocampal excitability via enhancement of the AHP, glucocorticoids impair long-term potentiation (LTP), a model of learning and memory. High doses of corticosterone for 21 days decrease LTP in the dentate granule cell layer even when LTP is measured 48 h after the cessation of corticosterone treatment [76]. Likewise inescapable stress decreases LTP in CA1 pyramidal neurons [90]. The inhibitory effect of stress on LTP may be due to more than just glucocorticoid suppression of neuronal excitability, other components of the stress response such as opioids may also be involved [89]. Stress also blocks fully developed kindled seizures [11,78].

Morphological and electrophysiological changes in the hippocampus have functional consequences. For example, stress, like aging, impairs memory tasks dependent on hippocampal function. Aged male rats which have deficits in spatial memory, assessed by the Morris swim maze in which rats must find and remember the location of a submerged platform, also have increased damage in the hippocampus and impaired glucocorticoid negative feedback [38]. In a similar fashion, a subgroup of aged humans with high basal cortisol levels showed more impairment in explicit memory and selective attention compared to a group with lower basal cortisols whereas there was no difference in long-term memory between the two groups [60]. In normal volunteers, administration of 1 mg of dexamethasone or 5 days of 80 mg/day prednisone produced more errors of commission in verbal memory tasks with no significant changes in errors of omission – in other words the subjects mistakenly included distractor words as being part of the original list they were to remember [112]. Similar deficits occurred in depressed patients who were dexamethasone non-suppressors. Another study found that 4 days of dexamethasone treatment (1 mg/day) decreased paragraph recall performance which lasted for about 1 week [72]. Moreover, neuropsychological impairments occur more frequently in patients with post-traumatic stress disorder (PTSD). For example, POWs from the Korean War (86% of whom had PTSD) had significantly more problems with short-term memory assessed by the Wechsler Memory Scale compared to combat veterans, only 14% of whom had PTSD [102]. Likewise, Vietnam veterans with combat-related PTSD scored significantly lower on the Wechsler Memory Scale compared to matched controls. These PTSD patients had problems with all aspects of short-term memory [14]. Finally, political prisoners who were physically and psychologically tortured had more problems with memory and concentration compared to non-tortured prisoners [9].

How does chronic stress cause neuronal damage? Sapolsky has persuasively articulated the idea that glucocorticoids released during stress cause energy depletion in the brain making it more vulnerable to excitotoxicity. Evidence for this hypothesis is provided by the fact that glucocorticoids block the uptake of glucose into neurons [35] and that glucocorticoid exacerbation of kainic acid-induced damage to the hippocampus can be prevented by giving the animal mannose (which can enter the neuron via a carrier that is not inhibited by glucocorticoids) [84]. Under resting conditions, glucocorticoids do not reduce energy metabolism so much that it could kill neurons. However, during situations such as ischemia, seizures, or exposure to toxins [51], glucocorti-

coids decrease energy metabolism and therefore make neurons more vulnerable to these challenges.

Glucocorticoids contribute to neuronal cell death not through an apoptotic mechanism [62] but through excitotoxicity. The excitatory amino acid, glutamate, is released not only during ischemia and hypoglycemia but also during other challenges including 'psychological' stressors [68]. Evidence that glucocorticoid endangerment of neurons is mediated via glutamate is provided by the fact that hippocampal toxicity due to the antimetabolite 3-acetylpyridine (3AP) is not dependent on glutamate neurotransmission, but glucocorticoid enhancement of 3AP neurotoxicity is dependent on glutamate binding to the NMDA receptor [5]. Glucocorticoids synergize with glutamate in a variety of ways to increase its toxicity. Glucocorticoids increase extracellular glutamate concentrations produced by kainic acid [100]. Glucocorticoids increase extracellular glutamate levels in the hippocampus by preventing glutamate reuptake into glia thus prolonging glutamate availability in the synaptic cleft for binding to NMDA and non-NMDA receptors [108]. And of particular relevance to the present discussion, glucocorticoids in the absence of stress or toxins increase extracellular glutamate concentrations in the hippocampus [101].

More evidence for glucocorticoid's effects being mediated through glutamate is provided by the fact that phenytoin which interferes with excitatory amino acid release prevents the damaging effects of stress or corticosterone in the hippocampus [109]. Other neurotransmitters may also be involved, because tianeptine which enhances serotonin uptake and therefore reduces extracellular serotonin levels also attenuates stress-induced dendrite atrophy in CA3 pyramidal neurons [110].

Ultimately glucocorticoids enhance glutamate excitotoxicity by increasing intracellular calcium levels which does damage to the neuron through cytoskeletal disruption and other means. Corticosterone greatly increases the rise in intracellular calcium concentration induced by kainic acid [23], possibly by inhibiting calcium efflux [24]. Glucocorticoids also increase kainate-induced accumulation of the microtubule-associated phosphoprotein, tau, in CA3 neurons [22]. In Alzheimer's disease tau becomes insoluble and is found in neurofibrillary tangles. GCs also exacerbate cytoskeletal disruption by increasing spectrin proteolysis. In another study, 3 days of varied stressors or 3 days of corticosterone administration exacerbated the kainate-induced reduction in microtubule-associated protein 2 (MAP2) immunoreactivity [99]. Thus, the cytoskeletal disruption indicated by accumulation of tau, loss of MAP2 and proteolysis of spectrin was probably mediated by elevated intracellular calcium levels.

2. Neurotrophic factors

While corticosterone administration at a dose of 10 mg/day per rat can substitute for stress in producing most of the hippocampal damage described above, there are a few instances where stress is more potent than glucocorticoids. For instance, there is a non-significant trend for chronic corticosterone ingestion to impair performance in the 8-arm maze test [58], while 21 days of restraint stress (6 h/day) does cause significant impairment in the acquisition of this spatial memory task [59]. Likewise, 3 days of varied stressors did exacerbate kainate-induced damage in CA1 pyramidal neurons but corticosterone did not [99].

Therefore, we considered the possibility that additional factors in the brain might be important for the stress-induced changes in brain function. Because neurotrophic factors are necessary for the normal development, survival and plasticity of neurons, we hypothesized that stress or corticosterone might decrease the expression of neurotrophic factors which would cause or exacerbate the neuropathological effects of chronic stress in the CNS.

Neurotrophic factors are humoral substances which promote the growth and differentiation of neurons [55]. Inquiry into the role of neurotrophic factors in the central nervous system has intensified during the past 5 years, since the cloning of several new members of the nerve growth factor (NGF) family, including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4). BDNF is found throughout the adult brain and may be trophic for a wide variety of neurons. NT-3 expression is particularly high during embryogenesis and may influence development of the hippocampus and cerebellum. In adult animals, removal of growth factors causes, if not neuronal death, at least atrophy and loss of neurotransmitter phenotype. Conversely, the administration of neurotrophic factors dramatically increases the expression of neuropeptides [55,70,71,86] and neurite outgrowth and branching [69,75]. Classically, neurotrophic factors are thought to be released from a post-synaptic neuron, bind to one of several tyrosine receptor kinases (Trks) on the surface of the pre-synaptic neuron, and then are retrogradely transported to the nucleus where they influence gene expression. In this regard, neurotrophic factors can be thought of as intercellular messengers which allow a target neuron to regulate gene expression in the neurons which innervate it.

Various traumatic insults induce dramatic changes in the expression of neurotrophic factors in the CNS (for review see [57]). Seizures increase NGF and BDNF mRNA in the hippocampus and cortex whereas NT-3 is decreased [25,29,30,37,79]. Similarly, ischemia or hypoglycemia induces a transient increase in BDNF mRNA in the dentate gyrus and a decrease in NT-3 mRNA in the dentate gyrus, CA1 and CA2 layers [56,105,106]. The direction of these observed changes in BDNF and NT-3 mRNA levels induced by seizures, ischemia and hypoglycemia is consistent with the notion that gluta-

mate, which is released during brain injury, positively regulates NGF and BDNF expression [114–116] and negatively regulates NT-3 [56].

A recent comprehensive review of the neuroprotective properties of growth factors argues that they may be quite useful for the prevention and therapy of a variety of brain injuries [64]. Of particular interest to the present discussion is the possibility that neurotrophic factors may counteract many of the deleterious effects of glucocorticoids and stress. In general, the cellular effects of neurotrophic factors are largely opposite of those produced by glucocorticoids or glutamate. Whereas glucocorticoids exacerbate glutamate neurotoxicity in culture, pretreatment with BDNF or NT-3 reduced cell loss due to glucose deprivation or glutamate neurotoxicity in hippocampal cell cultures [16].

3. Effects of stress on neurotrophic factors

Does stress affect the synthesis or release of growth factors? If stress causes damage in the hippocampus via growth factors, then we would predict that stress would decrease their expression. This does not seem to be the case for NGF. Serum levels of NGF increase in response to stress in rodents and humans [3,46]. Social stressors involving aggression also increase NGF mRNA in the brain, specifically in the hypothalamus but not in the hippocampus or cerebral cortex [96]. However, cold stress was found to decrease the number of NGF binding sites in the hippocampus possibly as a result of increased NGF release [104]. And indeed, 1 h of cold stress increased NGF mRNA in hippocampus as measured by Northern blotting [27]. Five consecutive days of cold stress (1 h/day) did not increase NGF. There was no change in NGF mRNA levels in the frontal cortex. The stress-induced increase in NGF did not require glucocorticoids as adrenalectomy did not prevent stress from increasing NGF in hippocampus. Likewise, exogenous corticosterone had no effect on NGF levels. These studies indicated that stress could affect neurotrophic factor secretion and synthesis not only in the periphery but also in the CNS.

Although the above studies on stress-induced changes in NGF mRNA levels were very provocative, they did not address whether neurotrophic factors might be relevant to stress-related hippocampal damage. This is because under normal conditions the high affinity receptor for NGF, TrkA, is not expressed in the hippocampus and therefore NGF probably does not influence hippocampal function directly [36]. We therefore turned to other members of the NGF family such as BDNF, NT-3 and NT-4, which along with their receptors, TrkB and TrkC, are expressed in the hippocampus, to determine if they too are affected by stress. Because BDNF affects neuronal function and morphology of cultured hippocampal neurons [75], we were interested whether stress might also affect BDNF mRNA expression in the hippocampus or elsewhere in the rat brain.

We found that immobilization stress, performed by taping the limbs of the rat to a metal board [45], decreased BDNF mRNA in the hippocampus as measured by in situ hybridization [94]. The most profound decrease occurred in the dentate gyrus granule neurons. BDNF was also decreased by stress in the CA3 and CA1 hippocampal pyramidal neurons. The reduction was not confined to the hippocampal formation as repeated stress caused decreases in BDNF in other limbic areas including the basolateral amygdala and claustrum. See Fig. 1. Even a mild stressor such as restraint in a plastic tube can cause reductions in BDNF in the dentate gyrus in as little as 45 min. The observed decrease in BDNF mRNA in various limbic nuclei suggests that widespread decreases in BDNF during stress could have a multitude of effects on nervous system function.

Another neurotrophic factor, neurotrophin-3, was increased in the dentate gyrus and hippocampus in response to stress [94]. Interestingly, NT-3 mRNA was increased only by repeated immobilization. A milder stressor such as restraint did not increase NT-3 mRNA levels.

The effects of immobilization stress on neurotrophic factor expression were specific in that we observed no changes in neurotrophin-4, trkB or trkC mRNA levels. A summary of these changes is shown in Fig. 2.

4. Effects of glucocorticoids on neurotrophic factors

Can glucocorticoids increase hippocampal vulnerability by decreasing the expression of neurotrophic factors? If damaging effects of glucocorticoids are modulated by NGF then we would predict would that glucocorticoid administration would decrease neurotrophic factor expression while adrenalectomy (ADX) would increase growth factor synthesis and release in the hippocampus. Again this prediction is not borne out for NGF. ADX has been reported to decrease NGF levels in the hippocampus which in turn decreased choline acetyltransferase in the septum (ChAT) [2,6]. Dexamethasone and aldosterone increased NGF mRNA in cultured hippocampal neurons [53]. In adult rats, corticosterone transiently increases NGF in the hippocampus and cortex, and then after 6 h levels fall below control values and return to baseline within 24 h [54]. Deletions in the NGF promoter indicate that glucocorticoids might interact with c-Fos and c-Jun at an AP-1 site to affect NGF transcription [54].

The effects of glucocorticoids on BDNF mRNA expression have been less consistent. One report has suggested that ADX also decreases BDNF as it does

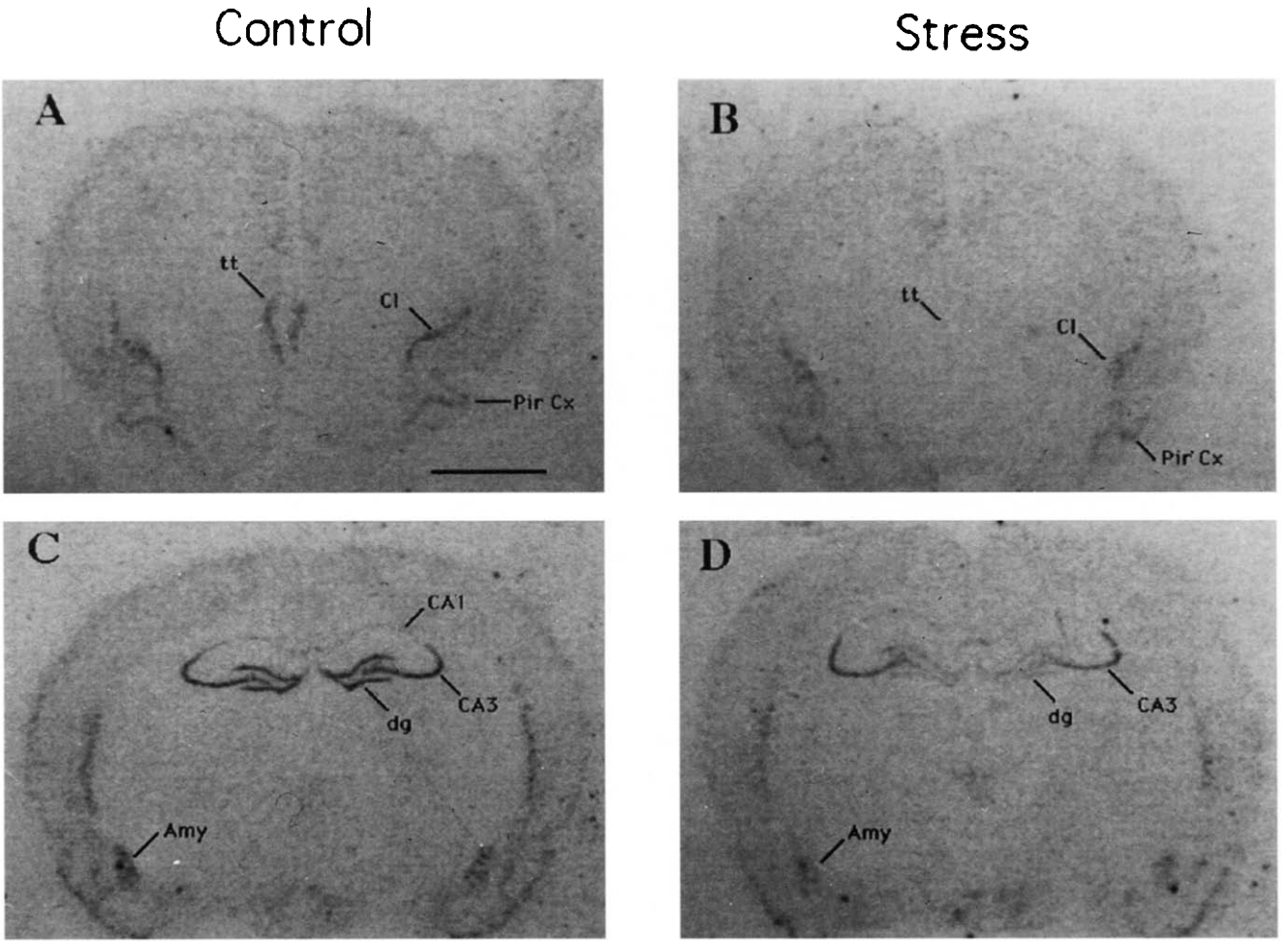

Fig. 1. Effects of stress on BDNF mRNA in rat brain. Unstressed rats (A, C) were compared to rats immobilized 2 h/day for 7 consecutive days (B, D). Autoradiographs of BDNF mRNA in situ hybridization show a decrease in BDNF expression in the tinea tecta (tt), claustrum (Cl), piriform cortex (Pir Cx), basolateral amygdala (Amy), and especially in the dentate gyrus (dg) and CA3 and CA1 layers of the hippocampus.

NGF [6]. On the other hand, basal BDNF mRNA levels were not influenced by dexamethasone in hippocampal cultures [53]. Interestingly another report also failed to find an effect of dexamethasone on basal BDNF mRNA levels but did find that dexamethasone completely blocked the ability of kainic acid or high potassium concentrations to increase BDNF mRNA in hippocampal cultures [18]. However, another report found that ADX reduced kainic acid increases in BDNF, NGF and trkB in vivo, but dexamethasone potentiated only the effects of kainic acid on NGF, not BDNF [7]. Our group did not find a significant effect of adrenalectomy on BDNF mRNA levels in the hippocampus. Instead we found that high doses of corticosterone decrease BDNF in vivo but only in the dentate gyrus [94]. Furthermore the effect of corticosterone to decrease BDNF is small in comparison to the effect of stress. When plasma glucocorticoid levels are held near baseline, stress can further reduce BDNF mRNA levels in the hippocampus. Even in the absence of adrenal glucocorticoids as during ADX, stress can decrease BDNF in the hippocampus. Thus the effects of stress on BDNF levels can be dissociated from glucocorticoids.

Evidence for a stimulatory effect of glucocorticoids on NT-3 expression is more consistent. We and others [19] have observed an increase in NT-3 mRNA levels in response to corticosterone administration and a decrease in NT-3 mRNA levels following ADX [6]. Moreover, repeated stress which increases NT-3 in intact rats fails to do so in adrenalectomized rats whose corticosterone levels are kept near baseline [94]. Thus the stress-induced increases in NT-3 expression are readily explained by high levels of corticosterone secreted during stress.

If corticosterone is not primarily responsible for the observed decrease in BDNF expression in the hippocampus during stress, what is? Glutamate is thought to be released during stress [31,68], but glutamate is known to increase, not decrease, BDNF mRNA levels [114]. It is possible that stress may increase GABA secretion more than glutamate and thus shift the balance in favor of inhibition of BDNF. Another alternative is that some

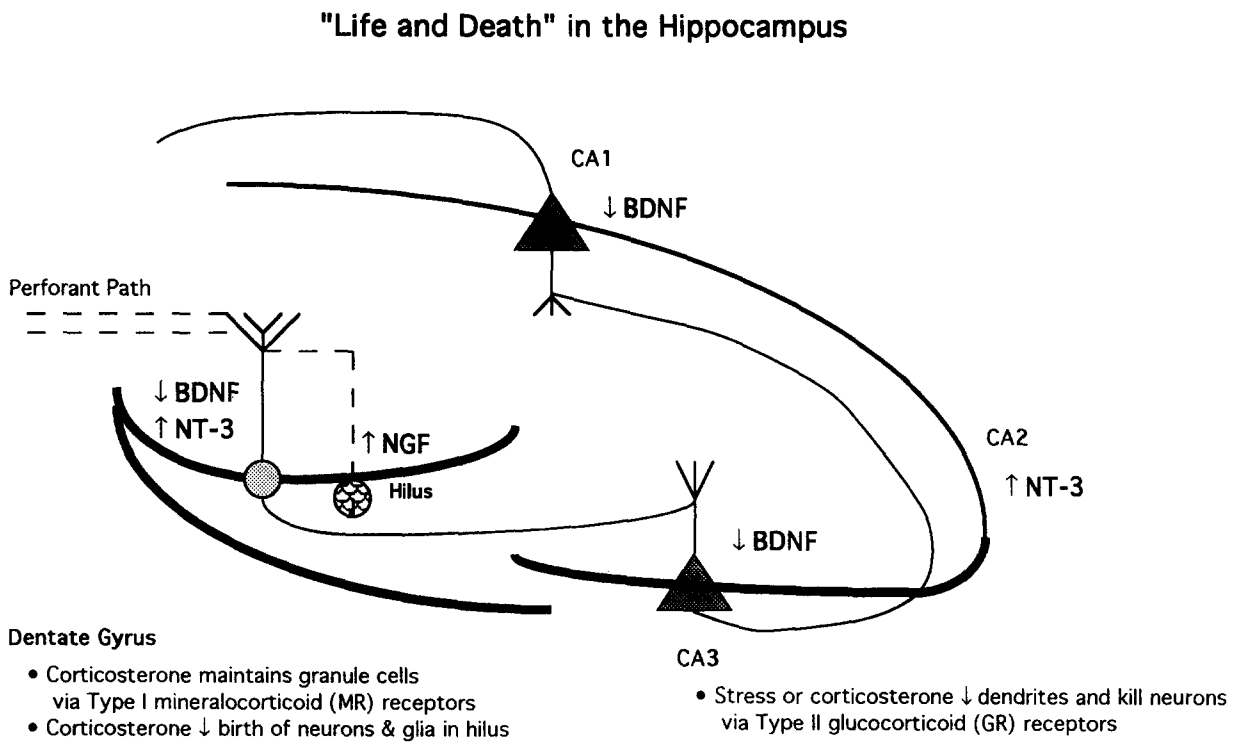

Fig. 2. Effects of stress on neurotrophin expression in the hippocampus.

other stress-induced transmitter or modulator is decreasing BDNF. A likely candidate is interleukin-1β (IL-1β). IL-1β is induced in the hippocampus by stress [66], and interestingly, high doses of systemically administered IL-1β have been shown to decrease BDNF mRNA levels in the hippocampus [50]. It is possible that this was an effect of stress as the authors did not check plasma corticosterone levels in these animals. However, IL-1β had been shown previously to increase NGF in the hippocampus [97]. Thus stress-induced increases in IL-1β might explain several of the observed changes in growth factor expression we see with immobilization stress.

5. Aging and neurotrophic factors

Normal aging produces relatively minor changes in hippocampal morphology (for review see [8]). For example, the number of granule cells does not decline with age, but there are about one third fewer synaptic contacts from the medial entorhinal cortex to the middle third of the granule cell dendritic tree. Interestingly though, hippocampal kindling takes longer in old rats and this is correlated with decreases in maze performance. Likewise LTP induction is impaired when submaximal stimulation parameters are used, and LTP does not last as long in old rats. Thus aging produces minimal morphological changes, but there is some impairment in the acquisition and maintenance of sensitization to electrical stimulation during kindling and LTP.

As hippocampal damage accelerates with age does this correlate with decreases in growth factors? Advanced age does not seem to have a profound effect on basal NGF or NGF receptor mRNAs in the brain (reviewed in [81]). However, old rats which perform well in the Morris swim maze have higher levels of NGF in the hippocampus compared to those that perform poorly [34]. BDNF and its receptor also do not appear to decrease with age in the hippocampus of male Fischer-344/N rats by Northern blotting or in situ hybridization [48]. We also do not find a consistent decrease in BDNF or NGF mRNA levels with age. However, we do have evidence that NT-3 mRNA levels decrease in the hippocampus steadily with age in Fischer-344/N rats (Smith and Cizza, unpublished observations). Whether this decrease in basal NT-3 mRNA might relate to age-related changes in hippocampal function is not known.

What seems more consistent in aging is the reduced capacity of the aged brain to induce and respond to growth factors. For example, NGF infusion has a smaller effect on ACh parameters in the hippocampus from old Fischer-344 male rats [81]. In another study, although there were no observed changes in basal NGF levels in young vs. old Fischer-344 rats, only young rats showed increases in NGF in response to medial septal lesions [88]. Thus aging attenuated the neurotrophic response

to hippocampal denervation. In a similar fashion, we have preliminary evidence that with advanced age, the ability of stress to increase NGF or decrease BDNF in the hippocampus is severely attenuated (Smith and Cizza, unpublished observations).

Neurotrophic factor levels may be decreased in Alzheimer's disease. One study found decreased NGF-receptor immunoreactivity in cholinergic neurons in brains from patients with Alzheimer's disease, but most studies find no change in NGF or NGF receptor mRNAs (reviewed in [81]). Interestingly there does appear to be a decrease in BDNF mRNA levels in the hippocampus from Alzheimer's patients [77]. Of interest in this regard is the possible role of acetylcholine in maintaining tonic levels of BDNF. Fimbrial transections which sever cholinergic afferents to the hippocampus cause significant decreases in BDNF mRNA the dentate gyrus and throughout the hippocampus [49]. NT-3 levels in Alzheimer's have not yet been reported.

If NT-3 or BDNF levels were to decrease in the dentate gyrus, neurons from the entorhinal cortex sending projections to the dentate granule neurons would likely suffer from changes in growth factor expression [20]. This might serve to 'disconnect' the entorhinal cortex from the hippocampus. This is precisely what happens in Alzheimer's disease. If afferent neurons are prevented from getting an adequate supply of growth factors from their targets, this may compromise their ability to manufacture growth factors and thus in turn compromise their afferents resulting in a 'domino effect'. Thus whatever might be the initial cause of neuronal loss in the hippocampus of Alzheimer's patients, the decrease in BDNF (and perhaps NT-3) which results may have consequences for neurons which innervate the hippocampus and depend on these growth factors. One area which is preferentially damaged in Alzheimer's is the locus coeruleus (LC) (see for review [91]). NT-3 has recently been shown to be trophic for the noradrenergic neurons in the LC based on the fact that it can rescue adult LC neurons from 6-hydroxydopamine toxicity in vivo [4]. Thus if NT-3 was reduced in the hippocampus of Alzheimer's patients, it might explain why the LC and other brainstem structures become compromised as the illness progresses.

6. Possible consequences of stress-induced changes in neurotrophic factors

These results raise the possibility that neurons which are dependent on BDNF for normal functioning may be compromised during chronic stress on account of decreased availability of this growth factor. In the mature CNS, the consequence of reduced BDNF may be atrophy rather than death of neurons. Destruction of the hippocampus, which abolishes target-derived neurotrophic factors, produces atrophy but not death of adult septal cholinergic neurons for up to 500 days after the lesion [95]. Thus, a reduction in BDNF may not be sufficient by itself to cause the death of hippocampal neurons during aging or chronic stress, but it could make neurons more vulnerable to injury. Neurotrophic factors like BDNF have trophic effects on neurons which are largely opposite to the effects of glucocorticoids. See Table 1 and discussion below.

The consequences of chronically reduced BDNF for the hippocampus might involve decreased excitability and decreased LTP. In contrast to the inhibitory effects of stress and high-dose glucocorticoids on hippocampal excitability described above, BDNF and NT-4 facilitate synaptic transmission in cultured hippocampal neurons by increasing the release of glutamate from the presynaptic terminal [52]. This finding might support the notion that BDNF or other growth factor would facilitate LTP. Although this has not been directly tested, the tyrosine kinase inhibitors, lavendustin A and genisten, blocked LTP in CA1 neurons [74]. In addition, LTP increased BDNF and NGF mRNA expression in the hippocampus but NT-4 and trkB did not change [15,21]. Thus neurotrophic factors may enable LTP and kindling which are inhibited by glucocorticoids and stress.

BDNF is another growth factor, like interleukin-1β [83], which regulates stress-related neuropeptides [71]. Thus one consequence of stress-induced decreases in BDNF may be a reduction in certain neuropeptides such as neuropeptide Y which are regulated by BDNF.

Neurotrophic factors have other effects on hippocampal physiology which are largely opposite to those produced by glutamate and glucocorticoids. For instance, fibroblast growth factor (FGF) antagonizes the inhibiting actions of glutamate on neurite outgrowth from cultured hippocampal neurons [63]. These effects of FGF required RNA transcription and protein synthe-

Table 1
Physiological effects of neurotrophic factors vs. stress or glucocorticoids

Physiological effect	Neurotrophic factors	Stress/ glucocorticoids
Increase in $[Ca^{2+}]_i$	↑	↑
Buffering of toxic $[Ca^{2+}]_i$	↑	↓
Glutamate secretion	↑	↔
Glutamate reuptake	?	↓
Long-term potentiation	↑	↓
Kindling	↑	↓
Free radical production	↑	↓
Calcium binding proteins	↑	↔,↑
Dendrite branching	↑	↓
Axonal sprouting	↑	↓
Pyramidal neuron survival	↑	↓
Hilar cell birth	↑,?	↓
Spatial memory	↑	↓

sis. Whereas glucocorticoids retard axonal sprouting of the perforant path fibers onto dentate granule neurons [87] and decrease the number of dendrite branches in hippocampal CA3 neurons [113], growth factors have opposite effects. FGF, NGF and BDNF increase axonal branching in cultured dentate granule neurons [75]. It has also been suggested that BDNF and non-catalytic trkB receptors are important in mediating axonal sprouting that occurs in the molecular layer of the dentate gyrus following combined lesions of the fimbria-fornix and perforant path [10]. Moreover, the very large increase in BDNF mRNA induced by amygdala kindling [37] is associated with axonal sprouting of the dentate mossy fibers [103]. NGF increases dendritic length and branching while NGF antiserum reduces these two dendritic parameters in the adult sympathetic ganglion [80]. NT-3 promotes neurite outgrowth and branching in cultured hippocampal neurons [69].

The fact that stress or corticosterone not only produce deleterious effects on CA3 dendrites and cell viability, but also decrease BDNF expression in the hippocampus suggests that reduced BDNF availability might contribute to the pathological effects produced by stress or high levels of corticosterone. Withdrawal of BDNF could contribute to the reduction in CA3 apical dendrites observed during chronic stress. Neurotrophic factors might also affect axonal sprouting. If the reverse were true, i.e., a reduction in BDNF causes retraction of axons, then stress might lead to a retraction of mossy fiber axons from the apical dendrites of CA3 hippocampal neurons with subsequent changes in dendrite morphology [73]. It should also be mentioned that it is conceivable that the reduction in BDNF might be protective in such a way that partially disconnecting mossy fiber pathway from the dentate gyrus to the CA3 pyramidal neurons might decrease the degree of glutamate excitation and prevent further damage. See Fig. 3.

Because of its trophic effects on acetylcholine neurons, the increase in NGF in the hippocampus produced by stress would presumably increase acetylcholine synthesis via increases in ChAT and result in improved spatial memory performance [34]. Thus the increase in NGF during stress may be compensatory to help counteract the deleterious effects of stress on hippocampal function.

The increase in NT-3 mRNA levels in response to repeated immobilization stress may also be a compensatory response designed to help preserve normal hippocampal physiology. Alternatively, it might be viewed as evidence for stress-induced neural plasticity and provide a mechanism for adapting to repeated or chronic stress. Neurotrophin-3 may play a role in the development and maintenance of the dentate gyrus. NT-3 mRNA levels in the rat brain reach their peak during the first 3 weeks of life when most of the dentate granule neurons are dividing [61]. In the hilus, progenitor neurons continue to divide and mature into granule neurons well into adulthood [32]. It is tempting to speculate that growth factors such as bFGF, NT-3 and BDNF may be important for the proliferation, differentiation and survival of these progenitor neurons in adulthood as well as in early development [28]. It is also intriguing that granule neurons, which are spared by stress, respond by increasing their expression of NT-3 mRNA. Adrenalectomy, which causes granule neuron programmed cell death [93], also decreases NT-3 mRNA in the dentate granule neurons [6,19]. It is conceivable that NT-3 regulates granule neurons via an autocrine mechanism of action based on the fact that granule neurons express both NT-3 and trkC, and that they respond to NT-3 by producing c-Fos [17]. It will be important then to determine if NT-3 is neuroprotective for dentate granule neurons or other neurons which are spared by stress.

One mechanism by which growth factors might be neuroprotective is by preventing the rise in intracellular calcium levels which occurs during metabolic insults such as glucose deprivation [16]. Another mechanism may be by inducing calcium binding proteins. NT-3 has been shown to increase the number of cultured neurons expressing calbindin-28 [17,36]. This is intriguing in light of other studies indicating that the absence of calbindin-D_{28k} is associated with vulnerability to damage in the hippocampus [92]. Corticosterone does not have an effect on calbindin-D_{28k} other than in the CA1 pyramidal layer where it too increases this calcium binding protein [44].

In apparent contradiction to their ability to prevent neurotoxicity due to high levels of intracellular calcium, BDNF and NT-3 increase cytoplasmic calcium as measured by fura-2 imaging of cultured hippocampal neurons [12]. This may occur by releasing intracellular stores of calcium via inositol triphosphate. However, this small increase in intracellular calcium levels may activate protective mechanisms within the neuron to protect it from the much larger calcium influxes due to glutamate toxicity.

Recent evidence suggests that without high levels of circulating corticosterone, stress is not sufficient to produce the functional changes in hippocampal function associated with advanced age [13]. Thus corticosterone seems to be the primary factor in mediating this damage. As discussed here, with the possible exception of BDNF, the effects of corticosterone on growth factor regulation are either small or in the opposite direction expected. However, regardless of the physiological relevance of corticosterone and stress-induced changes in neurotrophic factors, these substances may be of immense therapeutic importance in the treatment of traumatic injuries to the brain. Neurotrophic factors have therapeutic efficacy in models of spatial memory impairment. For example, in aged rats with memory impairments in the Morris swim maze which were given intracerebroventricular injections of neurotrophins for 4 weeks,

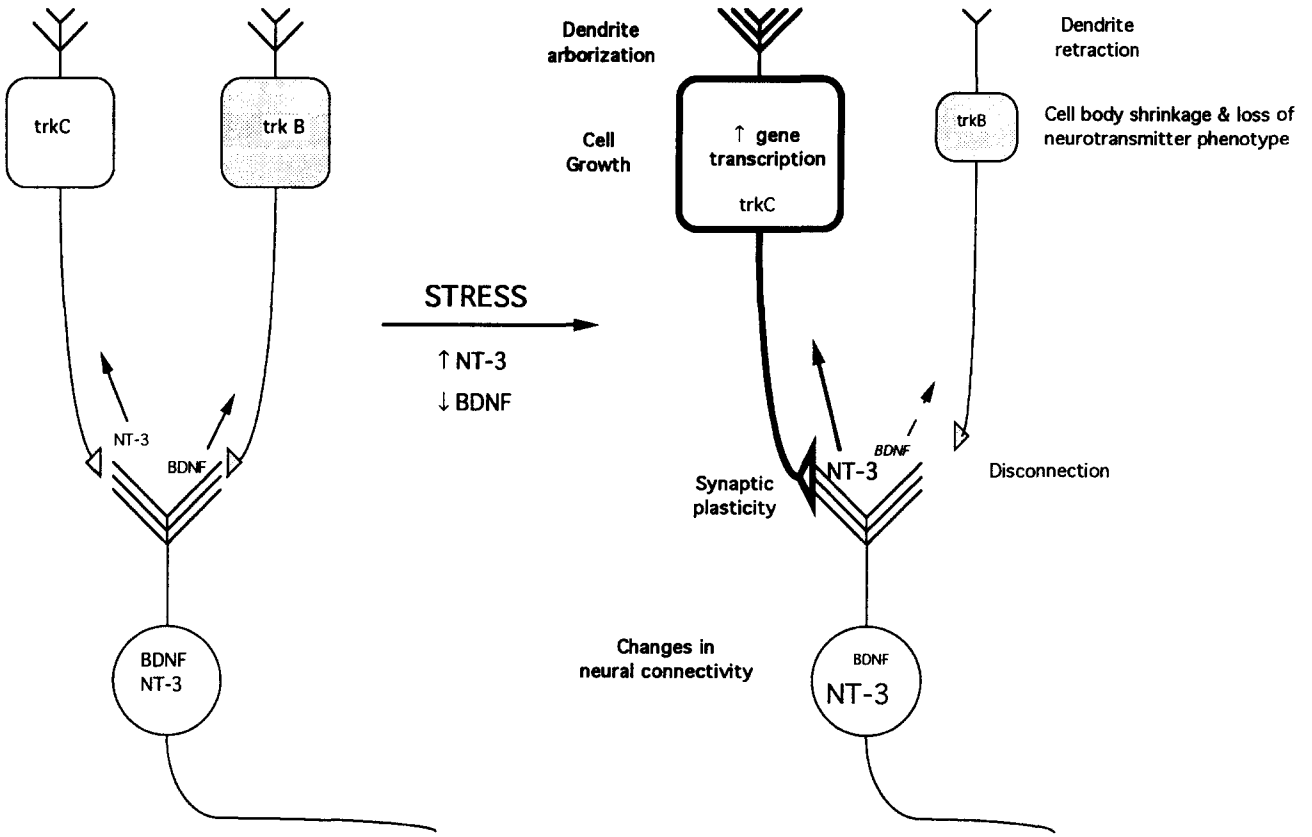

Fig. 3. Effects of stress-induced decrease in BDNF and increase in NT-3 on neuronal plasticity.

NGF, NT-3 and NT-4 improved acquisition and retention of spatial memory [26]. BDNF was not effective, but this may be because most of the BDNF was trapped in the CSF by non-catalytic TrkB receptors which prevented its entry into brain parenchyma. Based on their neuroprotective effects, growth factors might also be useful in preventing or ameliorating stress-induced damage to the hippocampus. This hypothesis can be tested by delivering growth factors to animals which have been exposed to chronic stress or glucocorticoids and assessing whether the growth factors can prevent or reverse the morphological and functional damage. The advent of lipophilic alkaloids which inhibit or stimulate neurotrophic factor receptor function increase the likelihood that agents which cross the blood brain barrier and modulate growth factor function may be a reality in the not too distant future [33].

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