Stress, Depression and Hippocampal Apoptosis

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Abstract: In this review, we summarize and discuss recent studies on structural plasticity changes, particularly apoptosis, in the mammalian hippocampus in relation to stress and depression.

Apoptosis continues to occur, yet with very low numbers, in the adult hippocampal dentate gyrus (DG) of various species. Stress and steroid exposure modulate the rate of apoptosis in the DG. Contrary to earlier studies, the impact of chronic stress on structural parameters of the hippocampus like cell number and volume, is rather modest, and requires prolonged and severe stress exposure before only small reductions (< 10 %) become detectable.

This does not exclude other structural parameters, like synaptic terminal structure, or dendritic arborization from being significantly altered in critical hippocampal subregions like the DG and/or CA3. Neither does it imply that the functional implications of the changes after stress are also modest. Of interest, most of the structural plasticity changes appear transient and are generally reversible after appropiate recovery periods, or following cessation or blockade of the stress or corticosteroid exposure.

The temporary slowing down of both apoptosis and adult proliferation, i.e. the DG turnover, after chronic stress will affect the overall composition, average age and identity of DG cells, and will have considerable consequences for the connectivity, input and properties of the hippocampal circuit and thus for memory function. Modulation of apoptosis and neurogenesis, by drugs interfering with stress components like MR and/or GR, and/or mediators of the cell death cascade, may therefore provide important drug targets for the modulation of mood and memory.

INTRODUCTION

This review aims to summarize and discuss recent studies on structural plasticity changes, particularly apoptosis, in the mammalian hippocampus in relation to stress and depression. As stress effects on adult neurogenesis will be addressed already in a separate chapter in this issue, this topic will be discussed only when in relation to apoptosis or when of particular relevance for the model of stress involved.

Ever present as it may be in e.g. the modern Western society, stress represents an old, yet essential alarm system for any individual organism. By definition, stress systems are activated whenever a discrepancy occurs between an organism's expectations and the reality it encounters, that generally involves a threat to the organism's homeostasis [1]. Lack of information, loss of control, unpredictability or uncertainty when faced with predator threat, or physical perturbations of homeostasis, like food or water shortage, blood loss, injury, or inflammation e.g., but also psychosocial demands can all produce stress signals. These signals activate a complex regulatory system in the body and brain, that is comprised of various adaptive physiological and psychological processes.

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In mammals, the stress response is mediated by the limbic-hypothalamo-pituitary-adrenal (HPA) system, a classic neuroendocrine circuit in which limbic and hypothalamic brain structures integrate emotional, cognitive, neuroendocrine, and autonomic inputs, that eventually determine the magnitude and duration of behavioral, neural and hormonal responses to stress. Together with other neuro-hormonal components such as the sympathico-adrenomedullary system, stress induced activation of the HPA system triggers the production of corticotropin-releasing hormone (CRH) in parvocellular neurons of the hypothalamic paraventricular nucleus (PVN). This in turn induces adrenocorticotroph hormone (ACTH) release from the pituitary, which causes glucocorticoid (GC) release (cortisol in primates including man and pigs, corticosterone in rodents) from the adrenal cortex into the blood.

Glucocorticoid plasma levels are carefully kept within physiological limits through GC-mediated feedback inhibition at specific steroid receptors in the pituitary and PVN. Also the hippocampus, that at least in rat, contains high densities of glucocorticoid (GR) and mineralocorticoid (MR) receptors, is sensitive to GC action. Upon steroid binding to MR or GR, the activated receptor translocates to the nucleus, where gene transcription and electrophysiological properties can be altered. Amongst other brain areas, the hippocampus is furthermore thought to exert an (indirect) tonic inhibitory control on HPA system activity, and is important in emotional processing and in key aspects of learning and memory [2-7].

Even though the stress response is considered harmless in itself, prolonged and sustained hyperactivity of the HPA system can result in maladaptation following e.g. alterations in HPA setpoint or feedback, that can cause prolonged (over)exposure of the brain and body to aberrant levels of glucocorticoids and can induce pathological conditions. A wide range of studies have addressed the consequences of chronic stress and prolonged glucocorticoid exposure for hippocampal structural integrity and function [3, 8-51]. In rodents and man, GC excess is generally associated with deleterious functional changes in e.g. hippocampal excitability, longterm potentiation and learning [19, 23, 24, 29-31, 34, 35, 52-62]. The deleterious effects of glucocorticoids on structural parameters, on the other hand, comprise initial, and still reversible, atrophy of the dendritic tree of particularly CA3, and to a lesser extent, also of CA1 neurons [36, 39, 59, 63-73], and reversible remodelling of synaptic terminal structures [39, 66]. In later stages, the hippocampus as a whole shrinks, and an increased vulnerability to metabolic insults and even neuronal death of CA3 neurons have been reported [74-85], that appears glutamate receptor mediated [86, 87], and can extend into regions CA1 and CA4 if severe stress persists.

Effects of chronic stress are assumed to be largely mediated through the GR. However, chronic stress may also alter the function of the mineralocorticoid receptor (MR) that is implicated in tonic inhibitory control of the HPA axis, suppresses adrenalectomy-induced hippocampal apoptosis and modulates neurogenesis [2, 88-91]. In view of its proposed inhibitory role in HPA activity control [4, 5, 7, 92, 93], neuronal loss as initially reported in stressed laboratory animals and later also in primates, was expected to cause a disinhibition of the HPA axis, leading to a positive feedforward cascade of increasing glucocorticoid levels over time, that was hypothesized to cause e.g. an age-related accumulation of hippocampal damage. At the time, in disorders like Alzheimer's disease and major depression, reductions in hippocampal volume were reported, that were paralleled by elevated basal glucocorticoid plasma levels. As also other data suggested similar correlations between hippocampal volume changes, cognitive impairment and changes in HPA axis activity, the 'glucocorticoid cascade concept of stress and hippocampal damage" was put forward [84, 85, 94-98] that has been further rephrased and adapted in later papers [48, 80, 99-104].

Stress induced neuronal loss in the hippocampal CA3 region was initially expected to be mediated through an apoptotic type of cell death [75-77, 80, 101, 105-110]. Of note, also in the hippocampal dentate gyrus, apoptosis occurs, even in control animals not subjected to stress, and notably in close association with adult neurogenesis in this region [73, 107, 111-113]. Unlike CA3, where no adult neurogenesis is known to occur, both cell birth and cell death are closely correlated in the dentate gyrus (DG), albeit with different time kinetics. Whereas neurogenesis can be monitored over time after Bromo-deoxy-uridine (BrdU) incorporation in dividing cells in S-phase, apoptosis is extremely rare in tissue sections, at least in non-acute disorders, due to its shortlasting presence, i.e. hours, during which the cell death process is completed. For the rat brain e.g., steroid related apoptosis was detectable for a maximum of 72 hours [108]. Hence, the chance of "trapping" ongoing apoptosis in thin tissue sections obtained from a chronic brain disorder, is very low [78, 114-116]. Yet, accumulating over time, the contribution of (shifts in) apoptotic rate to structural hippocampal changes, e.g. after an altered balance between DG apoptosis and neurogenesis after stress, can be considerable.

The present review aims to provide a summary and update on the effects of stress on apoptosis and cellular integrity of the hippocampus in various animal models for stress, and in major depression. These include psychosocial conflict in the tree shrew, chronic unpredictable multiple stress in rat, chronic restraint in the pig, and the human hippocampus of major depressed and of steroid treated patients. The tree shrew was further studied for additional modulation of apoptosis by antidepressant treatment.

HPA CHANGES AND THE HUMAN HIPPOCAMPUS IN MAJOR DEPRESSION

Numerous clinical and preclinical studies have shown that hyperactivity and disturbance of HPA axis function are implicated in the pathogenesis of depression [6, 22, 70, 71, 78, 117-129]. Although other factors are involved as well, HPA feedback abnormalities occur frequently in patients with depressive symptomatology and often resemble a subset of the changes seen in chronically stressed animals. In humans, depressive disorders are a collection of symptoms, that together constitute a recognizable clinical condition. Patients suffering from major depression frequently show psychomotor retardation, a phase shift in circadian activity patterns, early morning awakenings, appetite disturbances, weight loss and a loss of libido. Hyperactivity of the HPA system is common and reflected by the high percentage of dexametha-

sone non-suppressors in this population, hypertrophy of the adrenals and pituitary, increased plasma levels of ACTH and cortisol, and increases in CRH and vasopressin expression in PVN neurons [38, 119, 121, 124, 125, 130-135]. Moreover, significant decreases in hippocampal volume, as measured by magnetic resonance imaging (MRI), occur frequently in depressed patients [70, 136-142]. On basis of these data, one would expect hippocampal damage in this condition as well.

To address the neuropathological correlates of cortisol exposure for the human hippocampus, hippocampal tissue was studied of a well characterized series of major depressed patients. From a large part of these patients, changes in the HPA axis at the level of the hypothalamus had been demonstrated previously [124, 125, 130], yet their adrenal status or cortisol level was unknown. To address the impact of high steroid levels per se, an additional group of non-depressed individuals was included that had been treated with high dosages of synthetic steroids, like prednisone or dexamethasone, for various reasons. Despite their important peripheral effects on e.g. inflammation and the fact that they suppress the endogenous HPA axis, these drugs are likely to reach the brain as well, where they may affect hippocampal structure and function [24, 87, 113, 143-155], and were even reported to inflict a rare condition like steroid-induced dementia [156-158].

As glucocorticoids may increase susceptibility to apoptosis through calcium- and reactive oxygen species pathways, *in situ* end labeling (ISEL) was applied, that identifies fragmented DNA associated with both apoptotic and necrotic cell death, processes that can be discriminated using morphological criteria. Additional indices were markers for oxidative damage and cellular stress, such as inducible heat shock protein 70 (HSP70), that is strongly upregulated in response to insults and cell death, and nuclear transcription factor kappa B (NFkB), a GC-regulated transcription factor implicated in protection against apoptosis or oxidative stress.

The results revealed that the hippocampus of major depressed patients is structurally intact, as no indications for massive cell loss were observed in either the depressed or the steroid treated group. Even though all patients in the former group were established to have suffered from severe depression for a prolonged period, no significant structural, synap-

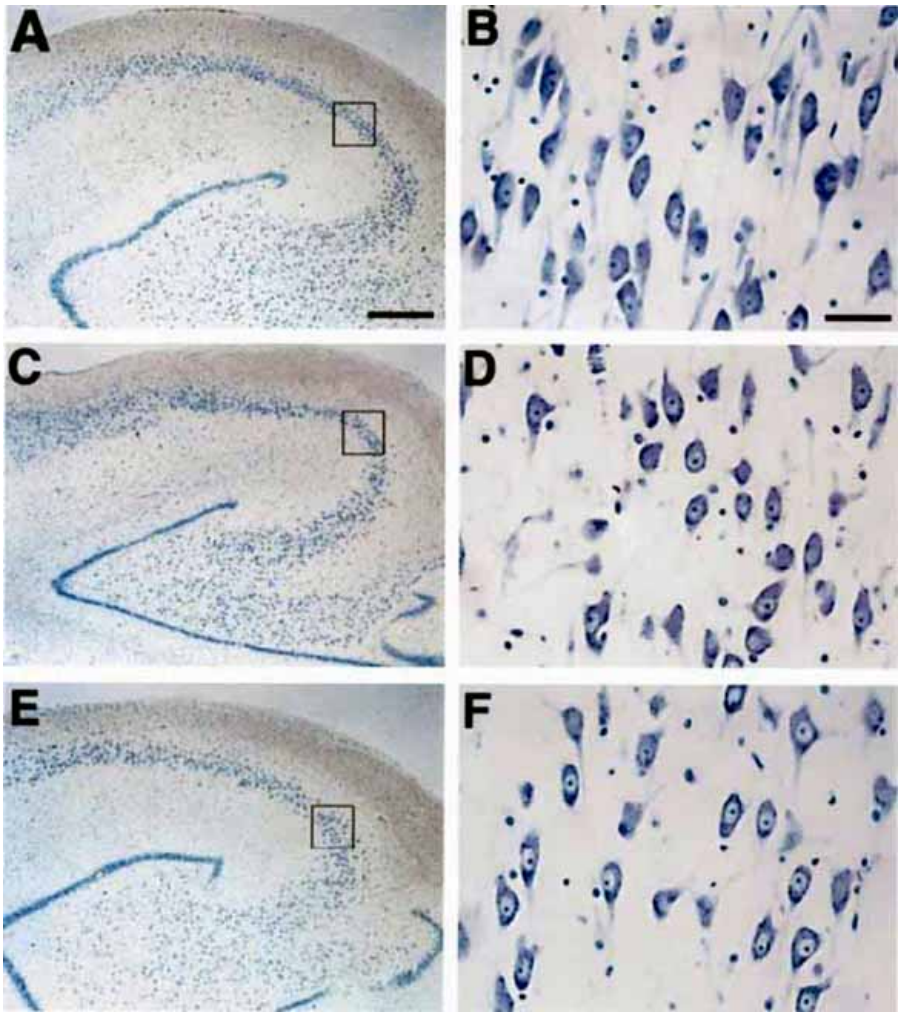
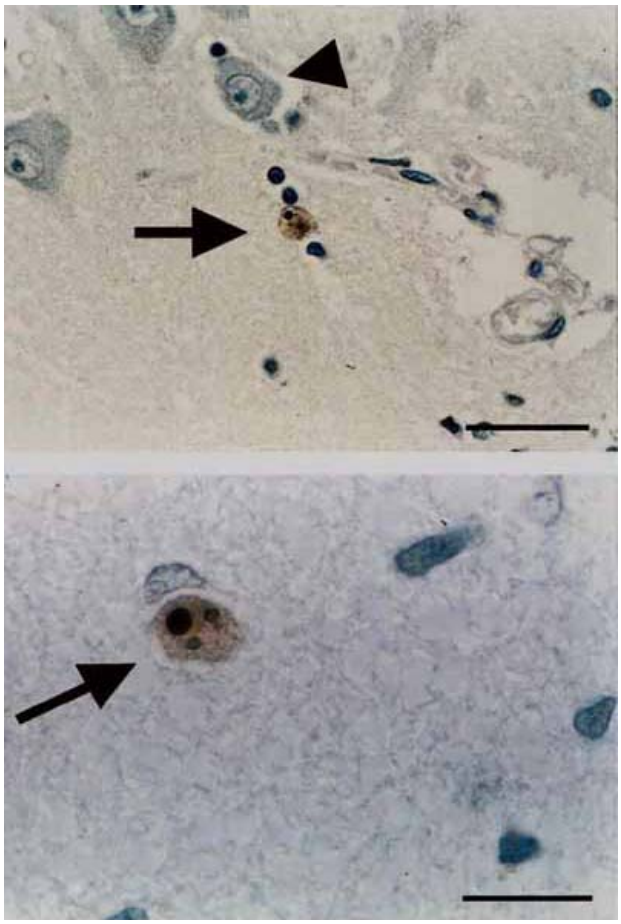


Fig. (1). Structural integrity of the hippocampus in major depression.

Representative photomicrographs (Nissl staining) from the hippocampus of A and B) a depressed patient, (C and D) a steroid-treated patient and (E and F) a control subject. B, D and F show the CA3 area of the same patients at higher magnification. No morphological evidence for neuronal damage or major cell loss can be observed in either the depressed or the steroid-treated patient. Scale bars, 710 mm (A, C and E), 45 mm (B, D and F). From [160], with permission).

tic or neurodegenerative alterations could be detected using Alz-50, GFAP, Nissl (Fig. **1A-F**) or Bodian Silver stain, nor with synaptophysin or B-50, markers for synaptic density and plasticity in these patients. In 11 out of 15 depressed patients as well as in 3 steroid-treated patients, very rare, but convincing apoptosis was found only in the entorhinal cortex, subiculum, dentate gyrus and CA4 (Fig. **2A**,**B**). Except for some of the steroid-treated patients, HSP70 staining was generally absent, nor were indications for NFkB activation found [159, 160].

A



B

Fig. (2). Apoptotic cells in the hippocampus of depressed patients.

A: Apoptotic cell (arrow) just below CA1 of a depressed patient with apoptotic bodies clearly visible as well as a smaller appearance than healthy neurons (triangle).

B: Clearly ISEL-positive, apoptotic cell (arrow points to the apoptotic bodies) in the subiculum of a depressed patient (From [159], with permission).

The detection of apoptosis in 11 out of 15 depressed patients, in 3 steroid treated and in 1 control subject, suggests that apoptosis is involved in steroid-related changes also in the human hippocampus. However, apoptosis was absent from areas at risk for GC damage like CA3 and mostly found in the dentate gyrus. In absence of major pyramidal loss or any obvious, lasting, neuropathological alteration, and as supported by other observations as well [161] the anatomical consequences of GC overexposure for the structural viability or the induction of neuropathology in the human hippocampus appear modest. They also indicate that apoptotic cell death probably only contributes to a minor extent to the volume changes in depression. However, it can not be excluded that increases in apoptosis had occurred already in earlier stages, e.g. at the onset of the disorder, or that the present apoptosis reflects interneuron or glia death. Moreover, almost all patients studied here received antidepressant therapy during their lives, and often continued until death. From many antidepressant drugs, it is known they can profoundly influence neurogenesis and apoptosis as well [162-176] (see below). However, in these patients, a clear relation between the type of antidepressant medication and either of the present markers studied, could not be established.

Finally, it is important to point out that although more information on the neuropathology of depression becomes known [177-179], a detailed (stereological) analysis of hippocampal cell number, preferably in a non-medicated patient cohort with previously established volume changes [69, 71], has not been performed yet. Hence, subtle structural changes too small to be detected with the present techniques, may have gone unnoticed. Either way, the prominent morphological changes in previous animal experiments, that were already apparent at low power morphological examination of conventionally stained sections, were clearly absent in the present cases that had suffered from major depression for prolonged periods of time. Although subtle changes at the level of the dendritic tree arborization [63, 64, 67] may have gone unnoticed, our results, and those of others [161], do not support the notion that stress or (endogeneous) corticosteroid overexposure actually causes major and permanent hippocampal damage or induce cell loss in the human hippocampus.

Additionally, modern stereological methods for unbiased neuronal counting have recently been applied to estimate changes in cell number in the hippocampus of chronically stressed tree shrews, stressed or GC exposed rats or chickadees, and GC-treated primates [73, 87, 103, 180-184]. Interestingly, all failed to find major reductions in neuron number in the main hippocampal subareas, consistent with the present observations in depression. Interestingly, studies in which the effects of synthetic steroid treatment, i.e. dexamethasone, were investigated in rats appear to be an exception to this. Since dexamethasone poorly penetrates the bloodbrain barrier, these effects may be different from exposure to the endogeneous hormone [146, 185]. In rats, clear increases were found in apoptosis in the striatum and in all hippocampal regions, but not the septal nucleus, after acute treatment [152], that appeared more severe in aged animals [113]. Despite the fact that no parallel changes on neurogenesis were reported, these effects could, interestingly, be attenuated by oestradiol and antidepressant treatment [152, 186, 187]. Similar analyses of dexamethasone effects on the human brain indicate at least clear feedback effects on CRH and AVP expression in the hypothalamus [188, 189], while the consequences of dexamethasone treatment for the integrity of the human hippocampus await further study (Lucassen PJ, Kuipers A, Bauer J, Boekhoorn K, Ravid R, Swaab DF, De Kloet ER and Joels M. Dexamathasone induced changes in cell death and proliferation in the adult human hippocampus, FENS Forum 2002; Abstract# 088.17).

In vivo MRI studies suggest a correlation between hippocampal or brain atrophy, memory deficits and cumulative GC exposure during e.g. aging and depression, although also exceptions have been reported [24, 59, 70, 71, 82, 137, 141, 142, 190-192]. However, such studies do not provide conclusive evidence for permanent changes, such as cell loss. Hippocampal volume reductions during high steroid doses, or in Cushing's disease, were reversible after a decrease or cessation of the steroid exposure. This further agrees with the general clinical experience with depressive or Cushing patients, where treatment with GR antagonists or surgery can relieve depressive symptoms, normalize several of the HPA alterations and even the hippocampal atrophy as recently demonstrated [164, 191, 193-200]. Hence, reversible and adaptive, rather than neurotoxic phenomena are expected in this subarea. Furthermore, as CA3 in man constitutes only a relatively small part of the hippocampus proper, it awaits further study whether GC-induced volume changes in this particular subarea contribute significantly to the atrophy of the entire hippocampus, that is already detectable at the NMR level.

One possible explanation for the inverse correlation between hypercortisolemia and brain volume may be a reduction in water content or balance caused by high levels of corticosteroids [201-203]; an effect also observed in the clinic when patients are treated with corticosteroids to reduce oedema. A remarkable discrepancy further exists between the relatively intact neurological and psychiatric status of most patients treated with steroids and their obvious ventricular enlargements. These enlargements and the parallel cerebral atrophy may normalize following cessation of corticosteroid administration. Also, it was suggested that significant reductions in neuropil in major depression may contribute to the decreased hippocampal volume detected by neuroimaging [204]. Such mechanisms involving nonstructural alterations like changes in water balance, are consistent with the observations that the hippocampal atrophy and the depressive symptoms that occur after glucocorticoid over exposure, are reversible and may normalize following a decrease or cessation of steroid administration [198-203, 205, 206] or e.g. after operation in Cushing's disease [198, 200, 207-209] that, interestingly, in Cushing's disease, has been associated with functional improvements [199].

Another possibility is that GCs affect glia cells, that not only possess GRs, but are also sensitive to steroid action, and can even undergo apoptosis, e.g. following exposure to oxidative stress [168, 210-212]. Indeed, previous work in the tree shrew points to a role for glia, at least at the endstage of the stress period [168, 172, 213-217]. Interestingly, recent stereological analysis of hippocampal subareas of rats subjected to stress or GC treatment, failed to reveal any change in neuron number, whereas volume reductions were found in the neuron-sparse subareas of the hippocampus, that contain mainly glia [73, 87]. Also in patients with major depression and bipolar disorder, reduced glial cell densities have been reported in restricted brain regions, or propose glia as a target for antidepressant drugs [211, 218].

Aside from the obvious differences listed above, primary alterations in feedback regulation through e.g. changes in GR or MR affinity, function or number could create a relative insensitivity of hippocampal neurons to GC excess. Studies on GR polymorphisms so far do not indicate that these have a major role in brain, nor do alterations in feedback sensitivity occur after chronic corticosterone treatment e.g. in rat [12, 219, 220]. Studies on GR polymorphisms in depression, have been inconsistent and incomplete until now [2, 165, 221-224]. Furthermore, RNA studies have been performed in some brain areas [221, 225-229], but localization and quantification of GR and MR *protein* levels in human brain awaits further studies [230], particularly in the main HPA feedback areas in depressed patients. So far, however, unpublished observations indicate that GR protein levels in primate and human hippocampus are rather low. In a very recent study, stereological analysis revealed a significant reduction of neuron number in the PVN of depressed patients extending earlier data indicating that in depression structural changes apparently also occur in non-hippocampal, important components of the HPA system [231-233].

PSYCHOSOCIAL STRESS IN THE TREE SHREW

In order to improve our knowledge on the mechanisms of stress-related structural alterations, reliable / suitable animal models are a prerequisite that should at least meet the criteria of predictive, face and construct validity for mood disorders. In order to find possible parallels and further validate the changes observed in human depressed cases, psychological stress was studied in the non-rodent, day-active tree shrew, an established animal model for aspects of major depresion with considerable clinical relevance [164, 168, 234-236]. Whereas studies on stress are often performed in rats, applying physical stressors like restraint, most stressors and stress-related disorders in humans, such as depression, are rather psychological in nature. A considerable number of the symptoms seen in depression are comparable to the stress responses observed in subordinate tree shrews. These animals reveal prominent changes in behavior and stress response depending on their social status.

The induction of psychosocial conflict is carried out according to standard procedures described earlier in detail [236-239]. Briefly, one naive male is introduced into the cage of a socially experienced male, which results in active competition for control over the territory. After establishment of a clear dominant/subordinate relationship, the two animals are separated by a wire mesh barrier that is removed every day for approximately 1 hour, thereby allowing physical contact between the two males during this time only. Using this procedure, the subordinate animal is protected from repeated attacks, but is constantly exposed to olfactory, visual and acoustic cues from the dominant animal. Under these conditions, subordinate animals produce high and non-adapting cortisol levels and display characteristic subordination behavior as well as selective atrophy of the CA3 region and the hippocampus.

Interestingly, these stress-induced changes can normalise after treatment with some, but not all antidepressants [168, 234, 242, 243]. For instance, treatment of subordinate animals with the antidepressants clomipramine and fluvoxamine, counteracted the behavioral and endocrine effects of chronic psychosocial stress. Of note, the time course of recovery corresponded closely to that observed when treating depressed patients. In contrast, treatment of subordinate animals with the anxiolytic drug diazepam had no beneficial

effects, supporting the view that the stress-induced behavioral and neuroendocrine responses in psychosocially stressed tree shrews are depression related.

The tree shrew model has besides its obvious 'face validity', also a 'predictive validity' for depression which makes it an interesting non-rodent model for research on the etiology and pathophysiology of depressive disorders. Until recently, little was known about the neuropathological consequences of this stressor, or the involvement of apoptosis in this model. When studying the adult tree shrew brain using ISEL, clear apoptosis was found in the hippocampus and related cortical areas. Twenty one days of psychosocial stress caused prominent rises in cortisol and reductions in body weight, reductions in CA3 surface area, and affected apoptosis in an unevenly and even opposite manner in discrete hippocampal subregions and cortex. Although a significant decrease in apoptosis in the CA1 stratum radiatum and increase in the DG hilus were found after stress, no significant increase was found in the CA3 pyramidal cell layer, an area predicted to be at risk and rather a trend was found even towards a reduced apoptosis after stress [239].

In view of the trisynaptic hippocampal circuit, apoptosis that was initially induced in CA3 shortly after stressor onset, could, in theory, have contributed, through anterograde or retrograde projections, to subsequent apoptosis in other subregions at a later timepoint. The apoptosis in the CA1 stratum radiatum area could be an example of this. Conversely, the CA3 receives strong, direct projections from the DG. Since the DG is a highly plastic brain area, where adult neurogenesis and apoptosis occur together [107, 112], it would have been interesting to establish whether stress has preferentially increased death of the newborn, rather than of the residing, adult granular or glia cells. However, this would require phenotyping of the dying cells by e.g. double labeling, which is currently technically not possible on this material. The ISEL technique does not allow identification of the cell type that dies by apoptosis, due to possible formalin fixation induced epitope masking [244, 245], and/or to the fact that apoptotic cells in this last phase of their demise, have lost their protein markers. Based on the criterium of anatomical location, primarily glial cells or interneurons are expected to be involved, as supported by other observations as well [210, 211, 218, 246].

Furthermore, as indicated above, large numbers of cells may have died already shortly after stressor onset, leaving less cells available to engage in apoptosis at later time points. This could have resulted in lowered apoptosis at the end of stress exposure. Indeed, also in chronically stressed rats (see below), apoptosis (Fig. **3**) was found to be decreased [112] (see below). Interestingly, in an earlier stereological study with an identical design, no reductions were found in the total numbers of CA1 and CA3 neurons [182], which agrees with the present failure to find significant changes in apoptosis in these pyramidal cell layers in the stressed tree shrew. It also is consistent with the significant reduction found in surface area of the CA3 radiatum, confirming previous studies that show retraction of the dendritic tree in this area after stress, and suggests that alterations in CA3 may indeed contribute to the volumetric reductions of the hippocampus as a whole [64, 168, 181, 247, 248].

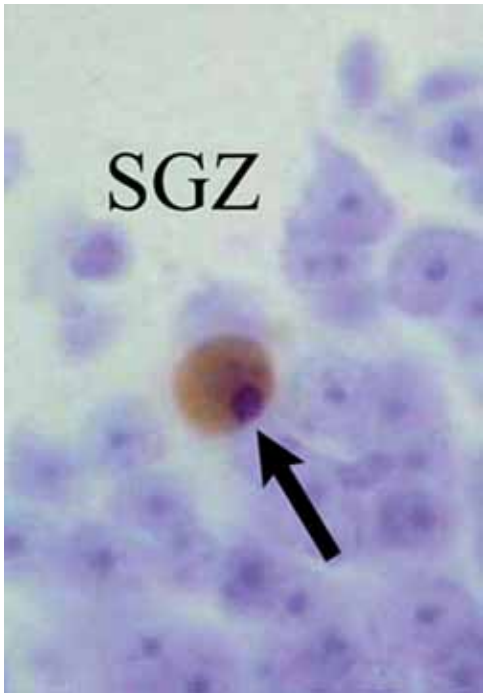


Fig. (3). Example of an apoptotic cell in the subgranular zone (SGZ) of the tree shrew hippocampus.

Recent MRI studies in tree shrew have indicated relatively mild reductions in hippocampal volume, i.e. around 10 %, already shortly after stress, but prior to cognitive disturbances [55, 164, 236, 237, 248, 249]. This is consistent with the 7.6 % reduction in total hippocampal volume found morphometrically after prolonged psychosocial stress exposure in the tree shrew [168, 236, 248, 249]. Admittedly, the initial concept that chronic stress inexorably causes hippocampal ageing and cell loss [48, 77, 80, 84, 95, 101-103, 190, 250, 251], is not necessarily in contrast with the results in tree shrew, as the designs were clearly different. Yet, even though the nature of the psychosocial stress differed considerably from the physical stressors applied before, its duration and resulting cortisol levels were similar.

In conclusion, effects of psychosocial stress on hippocampal volume and overall structure, i.e. hippocampal cell numbers in the tree shrew are relatively mild while marked changes were observed in the dendritic morphology of CA3 neurons [64]. They are, importantly, subregion specific. Also, differential changes in apoptosis were induced in different subfields of the adult tree shrew hippocampus and entorhinal cortex. As no loss in the principal CA and DG neuronal layers was previously found, it was concluded that despite robust and long-lasting cortisol increases, the stress-related change in apoptosis must reflect other parameters, like interneuron or glial cell death that contribute to the hippocampal changes in this experimental model [164, 168, 172, 210, 218, 243, 252].

PSYCHOSOCIAL STRESS IN THE TREE SHREW: EFFECTS OF ANTIDEPRESSANT TREATMENT

Despite the fact that antidepressant drugs have been used for several decades, their underlying mechanisms of action are still poorly understood. Earlier hypotheses have focused

on altered transmitter availability in the synaptic cleft, and assumed antidepressants to act by readjusting the aberrant intrasynaptic concentrations of serotonin and/or norepinephrine. Recent clinical and preclinical studies now suggest that major depressive disorders may also involve impairments of structural plasticity and cellular resilience [160, 165, 171, 253, 254]. If stress-induced changes in dentate granule cell turnover are indeed an important factor in the hippocampal volume reductions found in these disorders, antidepressants may act by restoring altered rates of cell birth or death. Consistent with this hypothesis, many different antidepressant treatments including lithium and electroconvulsive therapy were shown to stimulate dentate cytogenesis [165, 166, 168, 171, 172, 174, 253, 255-257] or exert neurotrophic/protective effects, by e.g. modulating factors involved in cell survival and growth, such as CREB, BDNF and bcl-2 a.o. [78, 258, 259].

Previously, it was found that the antidepressant tianeptine [216, 217, 260-262] normalized both stress-induced hippocampal volume reductions as well as the suppressed rate of cytogenesis in stressed animals [164]. An overview on the actions of tianeptine is given in a recent review [263]. Although most studies investigated the effects of antidepressants on cell viability in naive, unchallenged animals, these drugs are mainly effective within the context of a clinical condition—for example, a stress history—, and after chronic application. Therefore, possible protective effects of chronic antidepressant treatment was studied in the tree shrew model [213]. Animals were subjected to a seven-day period of psychosocial stress before the onset of daily administration of tianeptine (Servier, Courbevoie, France, 50 mg/kg per day), and stress continued throughout the 28-day treatment period.

The results show that both stress and tianeptine treatment had a region-specific effect [168, 172, 239]; stress increased apoptosis in the temporal cortex, while it reduced it in the Ammon's Horn (Fig. **4**). No significant effect was observed in the dentate gyrus. Interestingly, tianeptine treatment significantly reduced apoptosis in the dentate gyrus, both in control and stressed animals, but had no effect in the Am-

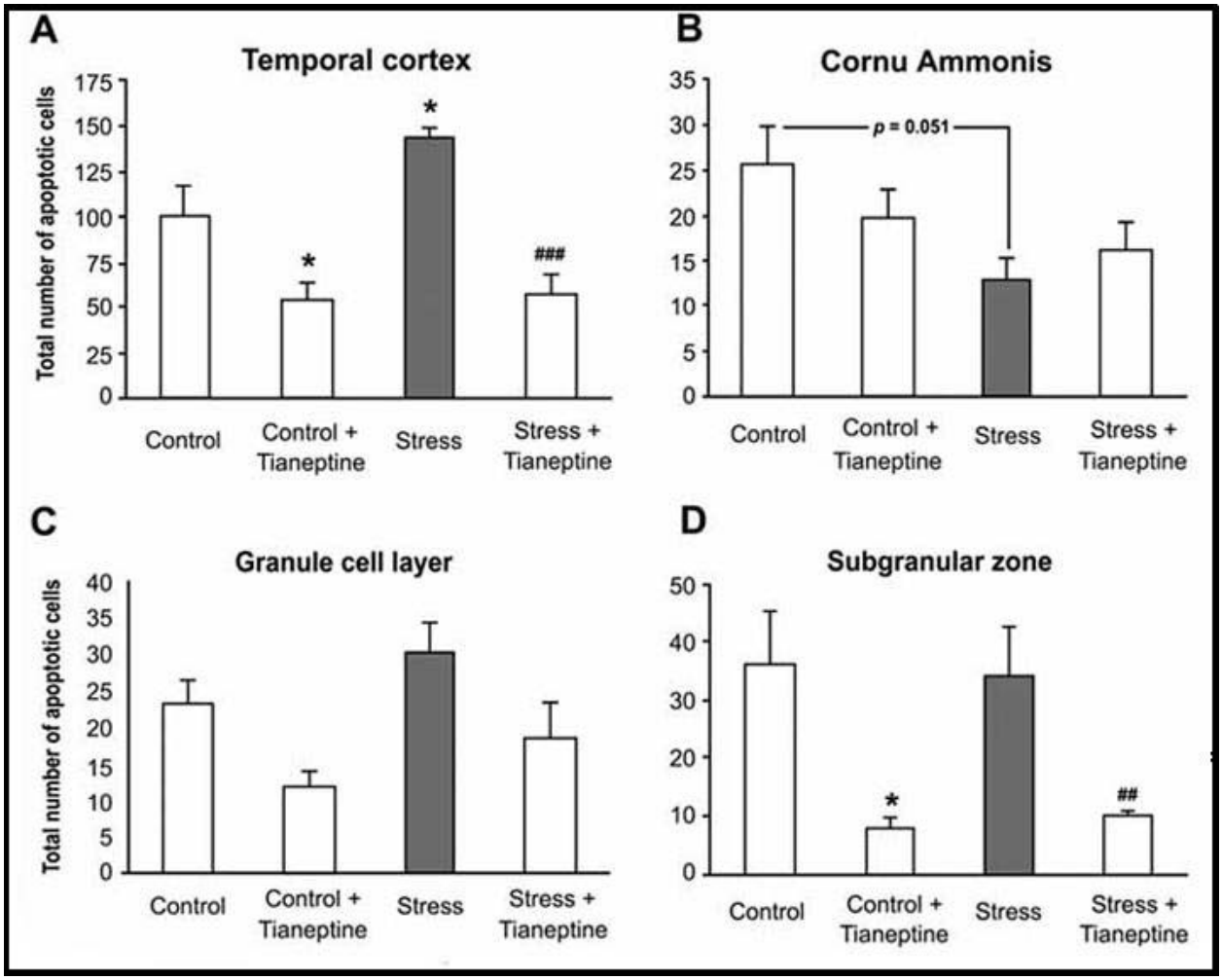


Fig. (4). Effects of chronic psychosocial stress and concomitant tianeptine treatment on apoptosis in the temporal cortex **(A)**, Cornu Ammonis **(B)**, dentate granule cell layer **(C)**, and subgranular zone **(D)** of the tree shrew. **(A)** In the temporal cortex, chronic stress resulted in a significantly increased occurrence of apoptotic cells, whereas anti-depressant treatment had a significant antiapoptotic effect in both control and stressed animals. **(B)** In the Ammons Horn, the frequency of apoptosis was significantly suppressed after 5 weeks of psychosocial stress. **(C, D)** In both the granule cell layer and the subgranular zone of the dentate gyrus, drug treatment significantly decreased the incidence of apoptosis (a 2-way ANOVA revealed significant main effect of drug treatment, and the results of the Newman-Keuls post hoc analysis are: * $p < 0.05$, versus Control. ## $p < 0.01$, ### $p < 0.001$, versus Stress). From [172], with permission).

mon's Horn. Similar antiapoptotic changes were observed in the associated temporal cortex, indicating that the effect of tianeptine was not restricted to the hippocampus alone. To address the type of cell that dies, parallel Fluoro-Jade (FJ) staining for neurodegeneration on adjacent sections indicated that the apoptosis detected with ISEL, at this stage after stress, most likely represents non-neuronal cells [172].

Collectively, this shows that in addition to the cytogenic effects [164], tianeptine exerts an antiapoptotic effect both in hippocampal subfields as well as the temporal cortex [168, 172] (Fig. **4**). These data agree with recent studies on rats where hippocampal apoptosis after dexamethasone treatment was also reduced by antidepressant treatment [152, 186]. In general, the results are consistent with current theories that ascribe enhanced general cell survival to antidepressant action [163, 166, 168, 171, 173, 174, 253, 255, 265, 266].

CHRONIC UNPREDICTABLE MULTIPLE STRESS EFFECTS ON THE RAT HIPPOCAMPUS

To address whether stress affects apoptosis also in a rodent model, we turned to the rat. Stress induced volume and functional changes in the rat hippocampus are generally attributed to synaptic, axonal or dendritic tree changes, although also exceptions have been reported. In addition, indirect adaptation through modulation of DG structural plasticity has been proposed [63, 65, 72, 87, 181, 182, 267-270]. Regarding the latter option, acute stress was known to suppress neurogenesis, but relatively little was known on how *chronic* stress affects the turnover, i.e. proliferation and apoptosis, of the rat dentate gyrus.

Structural plasticity changes were studied in the rat DG following 21 days of exposure to multiple unpredictable stressors [63, 112, 271-275] that consisted of a mixture of different psychosocial as well as physical stressors twice daily, including cold immobilization, forced swim, crowding and isolation. This paradigm not only reduces the risk of adaptation, but also better mimics the variability of stressors encountered in daily life, especially when compared to chronic restraint. As described earlier, this paradigm is furthermore associated with the classic parameters of chronic stress exposure such as increased adrenal weight, reduced thymus weight, reduced body weight gain, basal corticosterone hypersecretion and reduction in CA3 volume. Another question we addressed was whether the structural DG changes after stress are lasting, or rather reversible over time, which is why an additional group was included that was allowed to recover for 3 more weeks after the stress exposure. Effects of acute stress and recovery were studied as well [112].

Exposure to unpredictable stress differentially affected apoptosis in the rat hippocampus (Fig. **5**). Acute stress caused a significant increase in apoptosis in the hilus (h), subgranular zone (SGZ), granular cell layer (GCL) and consequently also over the whole DG in rats examined 24 h later. These effects were already absent again when the rats were allowed to recover for one more day. After chronic exposure to stress, a rather heterogeneous picture of cell death appeared with lower numbers of dying cells occurring in the SGZ, and increased numbers in the GCL, but overall less cell death in the DG compared to controls. This reduction in cell death rate was still present after a one-day recovery but had normalized after 3 weeks. Overall, apoptosis thus decreased in the whole dentate gyrus after chronic stress (h + SGZ + GCL), but normalized after 3 additional weeks of recovery (Fig. **5**).

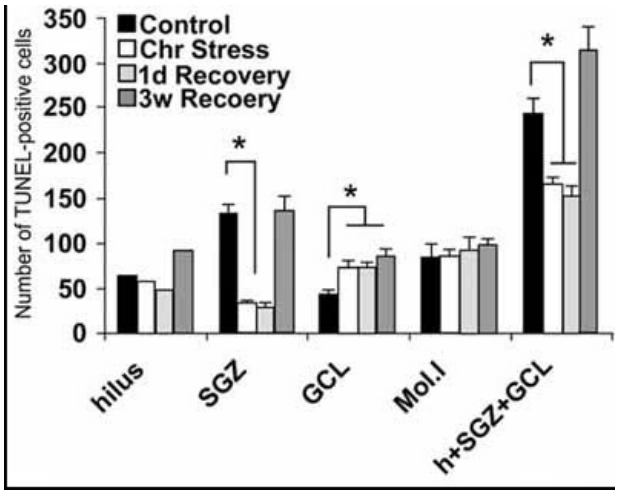


Fig. (5). Apoptotic changes in the DG of the rat hippocampus after chronic, multiple unpredictable stress exposure (From [112], with permission).

In view of the intimate association with cell death in the DG, stress induced changes in cytogenesis are interesting in this model as well; both acute as well as chronic stress decreased new cell proliferation in the rat DG. The reduced proliferation rate found after acute stress had already completely normalized after one day of recovery, whereas after chronic stress, the suppression of proliferation had partially normalized after a subsequent stress free period of 3 weeks. Of interest, in a related model of chronic stress, i.e. restraint, others found 6 weeks to be required for full recovery from stress induced structural changes [112, 276]. In the chronic unpredictable paradigm furthermore, migration and the percentage of newborn neurons generated, was not affected by 3 weeks of chronic stress. Others, in a different model, have recently shown also changes in newborn cell survival to depend on the corticoid environment during this survival period [277].

Together, this suggests that the acute stress effects on apoptosis in different hippocampal subregions are short lasting; chronic stress however, has longer-lasting effects on the main structural-dynamic hippocampal parameters, but most are almost normalized after 3 weeks (Fig. **5**). Since apoptosis was increased and cytogenesis decreased after acute stress, a rapid and short-lasting reduction in DG turnover probably takes place, or, alternatively, increased cell death is paralleled by a reduction in new cell birth. This latter possibility is consistent with the close association of the two processes in the adult DG [107] and with observations showing that around 50 percent of the newborn cells die between 1 and 2 weeks [278, 279] after their generation. Also in adult tree shrews, apoptosis was decreased after 3 weeks of psychosocial stress in the hilar region of the dentate gyrus [239, 244], suggesting this effect is not limited to rats alone.

Several explanations can be given for the heterogeneity in apoptotic changes. In view of the trisynaptic hippocampal

circuit, initial apoptosis induced in e.g. CA3, could have contributed to apoptosis in other sub-regions at later time-points. However, no such increases in apoptosis were found in CA3 in the acute stress group. After chronic stress, there is clearly less apoptosis in the SGZ, which could be explained by a decreased presence of young proliferating cells due to preferential death of this population in response to corticosterone exposure. Even though it remains to be proven whether stress preferentially increases death of newborn, rather than residing, adult granular cells, or possibly even glia or interneurons, differential susceptibility to cell death may also depend on the age of individual cells, or on the extent and type of their already established connections and synaptic input. In view of this heterogenous nature of the DG, it is most likely that distinct types of cells in the GCL react differently to acute and chronic stress, a phenomenon also seen after adrenalectomy or NMDA receptor blockade [107, 113, 278, 280].

Clearly, the effects of chronic unpredictable stress on structural plasticity are rather modest and adaptive for the rat DG, rather than neurotoxic for the CA3, a result that is in full agreement with the human data obtained in patients with longterm depression or treatment with corticosteroids. The temporary suppression in turnover following chronic stress, predicts that the age composition of the DG population, their connectivity and the resulting circuit properties, are quite different from control situations. Indeed, clear changes in various physiological parameters were found, a.o., in a parallel series of electrophysiological and molecular studies on the same model [3, 12, 14, 16-19, 54, 281].

As chronic stress and abnormalities in HPA axis activity exist already prior to the onset of clinical symptoms in depression [2, 120, 126-128, 282, 283], it is worth mentioning that the clinical symptoms of patients with psychotic depression quickly ameliorated upon treatment with a high dose of a GR antagonist [193, 194, 284, 285]. As suggested by the normalization of the corticosterone-induced suppression of neurogenesis after already a short treatment with the GR antagonist RU486 [333]. It will be of interest to address whether GR antagonist treatment can also reverse the structural and physiological changes induced in the chronic unpredictable stress model as well.

Similarly, an interesting candidate in this respect is the MR that is implicated in tonic inhibitory control of the HPA axis. MR activation is known to suppress adrenalectomy-induced apoptosis and is involved in (maintenance of) neuronal viability and implicated in neurogenesis [2, 88, 185, 286-288]. Moreover, MR activation modulates hippocampal calcium currents and serotonin responses [286, 289, 290] and its expression is altered after stress or antidepressant treatment and in brain regions in depression [2, 88, 90, 91, 226, 228, 291-293].

STRESS EFFECTS ON APOPTOSIS IN NON-RODENT SPECIES: THE PORCINE HIPPOCAMPUS

Although the consequences of stress and hypercortisolaemia for the rodent and tree shrew DG are well described, relatively little is known about other mammals. To address whether the effects in rodents are comparable to other species, we choose to study the porcine hippocampus, as these social and intelligent animals not only share many features with humans with regard to their brain (e.g. larger brain size than rodents, a more mature stage of brain development at the moment of birth), they are, like humans, also very sensitive to stress. Recently, corticosteroid receptors have been identified in the porcine hippocampus [150, 294-299]. Furthermore, in husbandry practice, breeding pigs are often individually housed in narrow boxes, tethered with a short chain around the neck to the floor. As this prevents them from interactions with other animals or explorative behaviour, tethered housing in fact resembles a chronic restraint stress condition [300-305], which is supported by the characteristic alterations in behaviour, autonomic and endocrine regulation, documented previously. Tethered housed pigs develop behavioural stereotypies, increased sympathetic reactivity, increased adrenocortical steroidogenic capacity and sensitivity to ACTH, chronic hypercortisolaemia and a flattened diurnal cortisol rhythm [300, 306-310]; all conditions that were shown to affect hippocampal viability in rat.

In order to address whether the porcine hippocampus was affected structurally by chronic stress, we studied structural parameters of the porcine DG after 5 months of tethered housing and investigated the possible relation between saliva cortisol measured antemortem and neuronal number or volume of the DG in the individual animal. Also neuropathological correlates of this chronic stressor were examined in this species, or whether a relation would be present between cortisol and apoptosis. Conventional Nissl, ethylgreen, H&E, silver staining and Alz-50 immuno-cytochemistry were used to visualize early degenerative or neuropathological alterations.

Stereological analysis revealed high correlations between DG volume and neuron number in individual animals in both hemispheres. Notably, basal cortisol was negatively correlated with volume and neuron number of the left, but not right DG (Fig. **6**). Although obvious neuropathology was completely absent, apoptosis was present in DG and alveus and less so in CA areas. The stereologically estimated numbers of apoptotic cells in the DG were negatively correlated with cortisol, but this was not found for other hippocampal subregions [311] (Fig. **7**).

These data indicate for the first time a profound lateralization in the relationship between DG structure, apoptosis and basal cortisol after stress in pigs. Even though five months of chronic stress failed to induce any lasting neuropathology, the accumulation of changes in apoptosis over time could have contributed to the structural alterations observed in the DG although this obviously also depends on putative parallel changes in neurogenesis. Clearly, the inverse correlation, i.e. higher numbers of apoptosis with lower levels of cortisol, agrees with similar correlations in e.g. the adrenalectomy model of DG apoptosis in rat [312-314]. Further studies should reveal whether stress has been instrumental in this or whether such differences were present from early life onwards. Recent studies in rodents at least indicate that early life stress can permanently affect cell birth and death rates. These effects can last throughout adult life of the offspring in rodents, often in parallel to alterations in hippocampal functioning [315-322], but also affect e.g. adult DG size in a sex specific manner [323]. A lateralization after stress is furthermore consistent with reports on lateralized hippocampal volume changes in stress-related human disor-

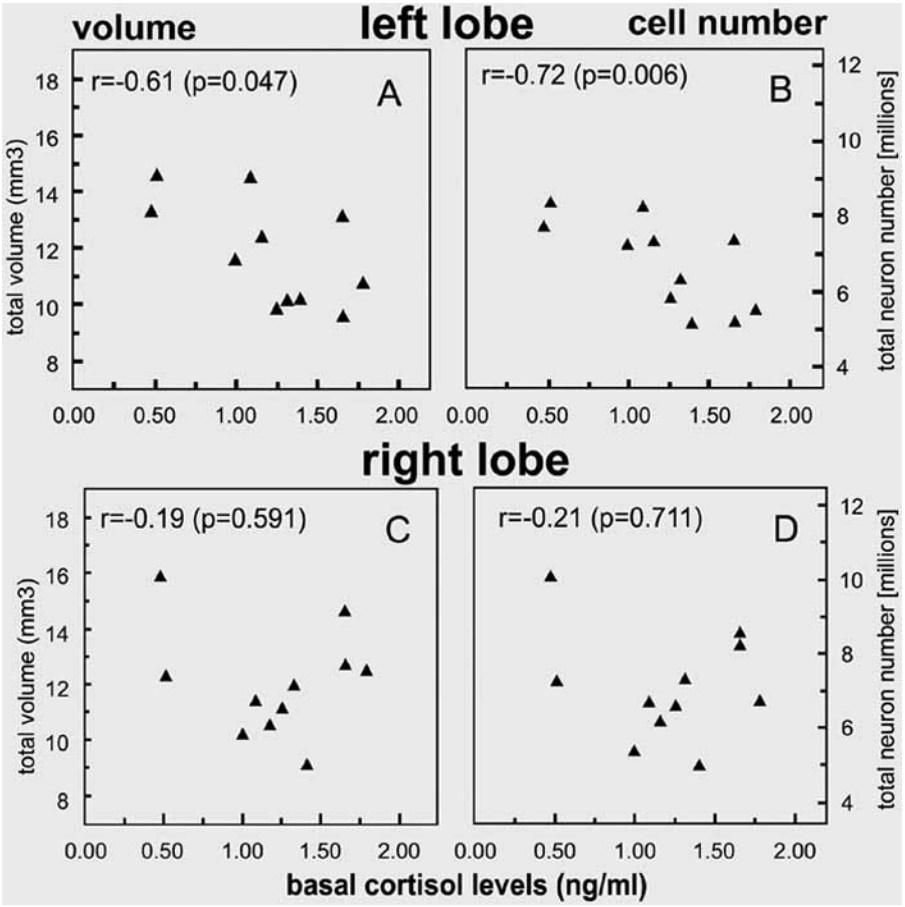


Fig. (6). Correlation of averaged, basal (prefeeding) salivary cortisol levels with total volume (A,C) and neuron number (B,D) in the left (A,B) and right (C,D) dentate gyrus (DG) of the porcine hippocampus. Cortisol concentrations were negatively correlated in a highly significant way with total neuron number (r = -0.72, *P* =0.006) and volume (r = -0.61, *P*= 0.047) of the left hippocampal lobe. No such correlation was found between cortisol and these structural parameters in the right DG (*P* > 0.05) (From [311], with permission).

ders [69, 71, 137, 139, 141, 142, 190, 324, 325] suggesting that these effects are not limited to this species alone.

CONCLUDING REMARKS

Apoptosis continues to occur in the adult mammalian hippocampus. Even though its occurrence in tissue sections is low due to its rapid time kinetics, apoptosis is clearly modulated by stress and steroids in several conditions and models. Rather than inducing apoptosis in CA areas, stress and steroid exposure appear mainly to interfere with the rate of ongoing apoptosis in the DG area. Since chronic stress induces parallel decreases in adult proliferation in the DG, stress in fact influences the turnover rate of DG cells, which, in turn, may affect its main projection area, the CA3 region and, together, could induce functional alterations.

Contrary to the conclusion from previous studies, the impact of chronic stress on the main structural parameters of the main hippocampal areas like cell number, appears rather modest, and may require more prolonged and severe exposure before only small reductions (< 10 %) will become detectable [112, 276]. Even though these overall anatomical changes are small, this does not exclude that still other parameters, e.g in critical hippocampal subregions like the DG and/or CA3, are significantly altered, such as synaptic terminal structure, or dendritic arborization [39, 63, 64, 67], nor do they imply that the functional implications of such changes are also modest. Clearly, the temporary slowing down of structural DG turnover by chronic stress changes the overall composition, average age and identity of DG cells, which is likely to have considerable consequences for the connectivity and properties of the circuit and hence for hippocampal function [12].

Regarding the discrepancy between hippocampal volume reductions in the absence of clear reductions in neuron number, it is important to realize that most of the structural plasticity changes are transient and generally reversible after appropiate recovery periods or following cessation of the stress or corticosteroid exposure.

In view of their established contributions to hippocampal network properties and the proposed correlations with learning and memory performance [315, 317, 326-330], modulation not only of neurogenesis, but also of hippocampal apoptosis, either through pharmaceutical or environmental means, may thus have important consequences for the composition and function of the DG and the hippocampus as a whole. A possible candidate in this respect is the MR that is implicated in tonic inhibitory control of the HPA axis and involved in neuronal viability and neurogenesis [2, 88, 185, 286, 331].

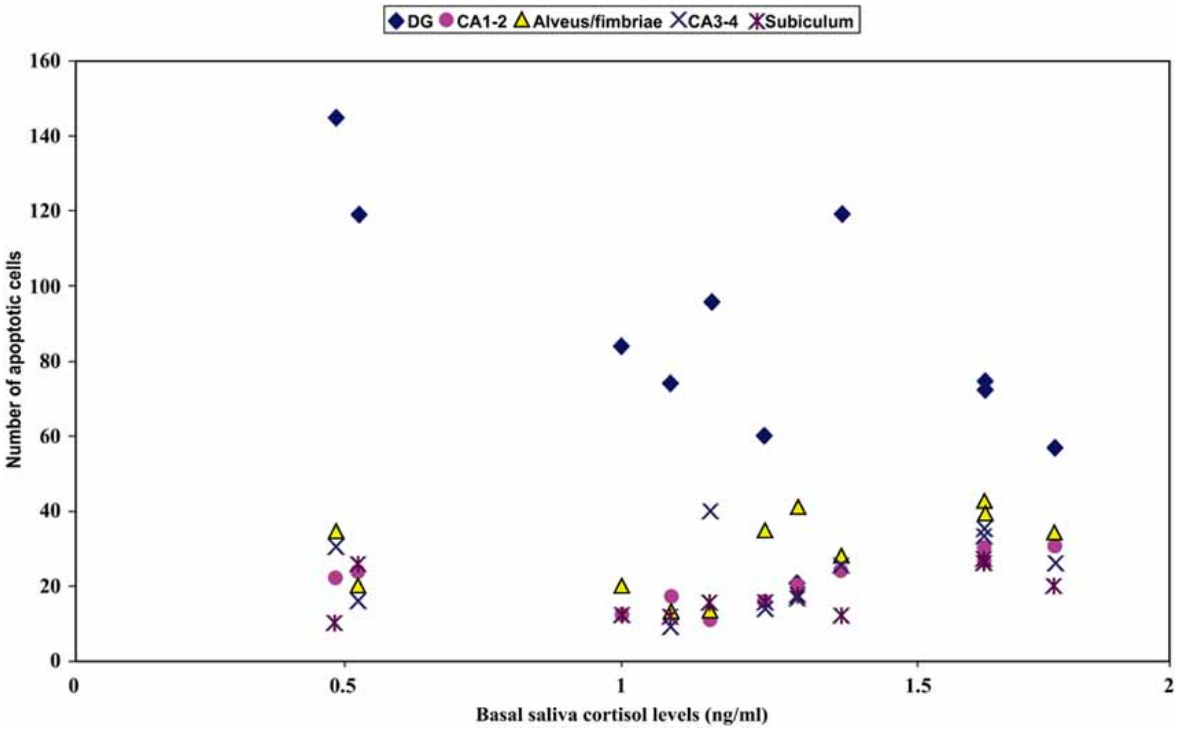


Fig. (7). Representation of basal saliva cortisol levels (ng/ml) plotted versus the stereologically determined numbers of apoptotic cells in the main subregions of the porcine hippocampus. *P* Pearson linear correlation test revealed a significant correlation (r = -0.6356, *P* = 0.0356) for the dentate gyrus (DG), but not for the other subregions (e.g., CA1–2; r = 0.0366, *P*= 0.915) (From [311], with permission).

Moreover, MR activation modulates hippocampal physiological properties [286, 289, 290] and its expression is altered after stress, antidepressant treatment and in depression [2, 88, 90, 91, 226, 228, 291-293]. Also blockade of the GR seems, at least in clinical studies, a promising tool to effectively treat psychotic forms of depression normalize or neurogenesis after stress [2, 22, 193, 194, 332]. The changes in apoptosis after stress summarized here, suggest that modulation of hippocampal structural plasticity by drugs interfering with MR and/or GR action [331], and/or with mediators of the cell death cascade, may provide important drug targets for the modulation of mood and memory.

ABBREVIATIONS

ACTH = Adrenocorticotroph hormone

BDNF = Brain derived neurotrophic factor

BRDU = Bromo-deoxy-uridine

CA = Cornu ammonis

CREB = Cyclic AMP response element binding protein

CRH = Corticotropin-releasing hormone

DG = Dentate gyrus

FJ = Fluoro-Jade

GC = Glucocorticoids

GFAP = Glial fibrillary acidic protein

GR = Glucocorticoid receptor

GCL = Granular cell layer

H&E = Haematoxylin eosin

HPA = Hypothalamo-pituitary-adrenal

HSP70 = Heat shock protein 70

ISEL = *In situ* end labeling

MR = Mineralocorticoid receptor

MRI = Magnetic resonance imaging

NFkB = Nuclear transcription factor kappa B

NMDA = N-methyl-D-aspartate

PVN = Paraventricular nucleus

SGZ = Subgranular zone

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