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Chronic psychosocial stress exposes Alzheimer's disease phenotype in a novel at-risk model

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1. ABSTRACT

Because of the extensive individual variations in the time of onset and severity of the prevalent sporadic form of Alzheimer's disease (AD), a patient-related external factor must be assumed to play a significant role in the development of the disease. Since stress is increasingly recognized as an external factor in the development of AD, a number of labs, including this lab, have shown that chronic stress or corticosterone administration worsens the AD phenotype in both transgenic and non-transgenic models of the disease. Recently we develop a novel at-risk model that correlates with seemingly normal individuals who are predisposed to develop AD. This review is a summarized recount of the findings we have reported on the effect of chronic psychosocial stress in this at-risk model of AD. Behavioral (learning and memory tests), electrophysiological and molecular findings indicated that even mild chronic psychosocial stress clearly transforms this seemingly normal rat model to a full-fledge AD phenotype.

2. INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder characterized by accumulation of high levels of neurotoxic amyloid-beta (Aβ) peptides, intracellular aggregation of hyperphosphorylated tau protein, and neuronal death resulting in a gradual loss of cognitive abilities (1,2). The early symptom of the disease is a slow and insidious destruction of memory and cognitive skills. Molecular studies have shown that the rare early-onset familial form of AD is due to mutations in genes for amyloid precursor protein (APP), presenilin 1 (PS1) or presenilin 2 (PS2) (3,4). However, the overwhelming majority of AD cases are of the late onset, sporadic type. The sporadic nature of the disease suggests an environmental link that may trigger AD pathogenesis. In addition to its late-onset, the variation in susceptibility to and time of onset of the disease suggests that aside from genetic factors, environmental determinants, such as chronic stress, may play a critical role in the severity of sporadic AD.

The cognitive deficits observed in AD patients are widely believed to result from progressive synaptic dysfunction and neurodegeneration probably initiated by soluble oligomeric Aβ peptides, in particular the $A\beta_{1-42}$ form. During AD, a progressive failure of synaptic transmission occurs; it begins as a localized decrease in synaptic function, and over time, progresses to global impairment of neurotransmission in the brain (3,5,6). Because of the poor correlation of the severity of dementia with the extent of neuronal loss and degree of fibrillar Aβ load in the AD brain, the original amyloid cascade hypothesis has been substantially revised. It is now thought that certain oligomeric forms of soluble Aβ can cause cognitive impairment in animals in the absence of neurodegeneration (7). Additionally, synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), the cellular correlates of learning and memory, are highly vulnerable to disruption by soluble Aβ species (8).

Based on clinical reports of elevated plasma cortisol levels in individuals with dementia and in AD patients (9,10,11,12), it has been postulated that stress may be associated with this disease (13,14). Further support of this hypothesis comes from epidemiological findings that stressed individuals are more likely to develop mild cognitive impairment, or even AD, than non-stressed individuals (15). Clinical reports of hypercortism in AD patients (16,17) and animal studies (18,19) have shown that glucocorticoids participate in the regulation of APP levels suggesting involvement of the stress hormones in the pathogenesis of AD. Exposure to severe and/or prolonged mental stress causes over-activation and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, inflicting negative changes on the brain morphology and function (20,21). The hippocampal formation is particularly susceptible to the deleterious effects of stress and is one of the first brain regions to succumb to the onslaught of AD by showing degenerative symptoms including significant impairments of the hippocampus-dependent cognitive abilities. Moreover, chronic stress is known to exacerbate the negative changes associated with various brain disorders including schizophrenia (22), Cushing's disease (23), hypothyroidism (12, 24), and AD (25).

Reports show that impairment of neurogenesis and the timing of Aβ plaque deposition are significantly influenced by stress or stress hormones in transgenic mouse models of AD (26,27,28). Moreover, it has been shown that chronic psychosocial stress in Aβ rat model of AD has a markedly more devastating effect on the hippocampus than either chronic stress or infusion of a pathogenic Aβ dose alone. The presence of chronic stress exacerbates Aβ-induced learning and memory impairment and further diminishes long-term potentiation (LTP) in the hippocampal CA1 region of anesthetized rats (25,29).

Previously, we have shown that combination of chronic psychosocial stress and chronic infusion of pathogenic doses of Aβ peptides impairs learning and memory and severely diminishes LTP in the hippocampal cornu Ammonis (CA1) region of brains of anesthetized rats (25,29). The combination proved to be markedly more devastating than either chronic stress or Aβ infusion alone. We then wanted to determine whether chronic stress could hasten the development of AD in normal individuals who are susceptible to the disease. To answer this question, we developed a novel model intended to represent normal individuals who may have traits that predispose them to AD (at-risk) but do not yet display AD-associated symptoms.

3. THE SYMPTOM-FREE AT-RISK Aβ RAT MODEL OF AD

As a complementary alternative to transgenic animal models, non-transgenic models of AD are valuable tools for studying the specific pathogenesis induced by icv infusion of Aβ. Exogenous Aβ administration model of AD has its own limitations as do transgenic models. For example, similar to transgenic models, exogenous Aβ administration does not reproduce the full complexity of the human pathology. However, studies involving exogenous administration of Aβ have reported neurodegeneration and microglial activation, proximal to Aβ deposits (30,31). Additionally, injection/infusion of Aβ peptides is an invasive procedure, particularly when the peptides are infused via osmotic pump. It introduces inevitable injury at the site of injection/infusion, which possibly contributes to the induction of inflammatory processes. However, these limitations can be overcome to a significant degree by adjusting the infusion rate, the vehicle, the volume of injection, and the recovery time before examination of the animal to minimize the confounding effect of the procedure involved in administering Aβ.

During normal cellular metabolism, neurons secrete low levels of soluble Aβ peptides into cerebrospinal fluid (CSF) and plasma (32,33). It has been proposed that the extent and the rate at which the pool of soluble Aβ reaches the pathogenic levels are dependent on the rates of Aβ catabolism and clearance (33,34,35,36). Various studies have shown that Aβ exerts a dose-dependent effect both in vitro (37,38,39,40) and in vivo (41,42). This suggests that Aβ neurotoxic effects may not be harmful until it reaches a threshold concentration. We have devised a unique rat model created by icv infusion of a sub-pathogenic concentration that produces no significant effect on the rat performance in the hippocampus-dependent memory task of the radial arm water maze. The results of various tests indicate that the at-risk rat model of AD is not significantly different than control rats. The model is the first non-transgenic rat AD model to imitate a preclinical condition in which there is a heighten susceptibility to AD without any observable cognitive deficits. However, when this pre-clinical (at-risk) AD rat model is generated in chronically stressed rats, they exhibit AD phenotype similar to that previously reported in a full-fledged model of AD (25,29) (figure 1). This model may represent individuals who are seemingly normal but prone to develop AD phenotype on exposure to offending external factors. In this report we will review the impact of chronic psychosocial stress in this model utilizing various experimental approaches.

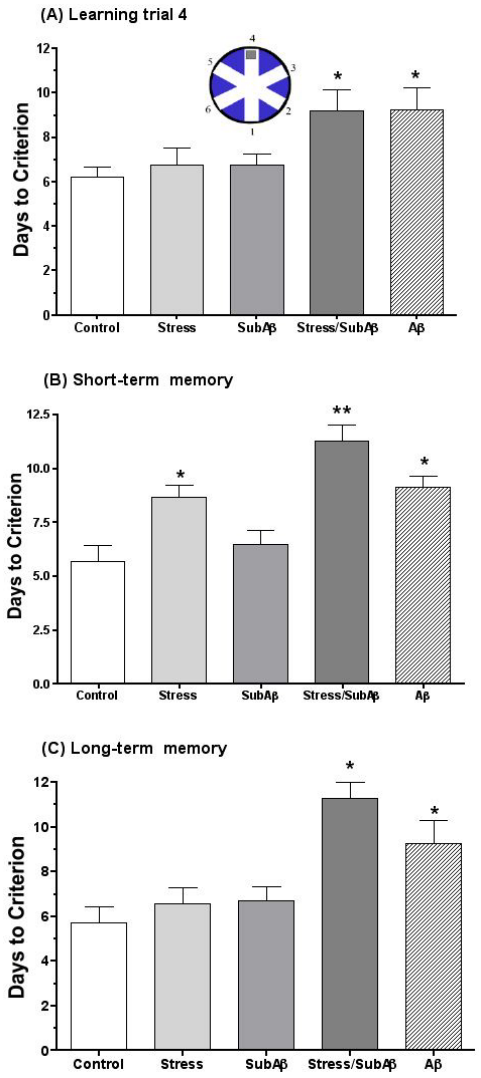

Figure 1. Effect of chronic psychosocial stress on the cognitive function of the subthreshold (subAβ, subclinical, at-risk for AD) rat model of Alzheimer's disease. Days to criterion (DTC) value is reached when a rat makes a maximum of one error in three consecutive days of testing in the RAWM (inset in panel A). DTC indicates that the combination of stress and subAβ (stress/subAβ) impairs learning (panel A), short-term memory (panel B) and long-term memory (C) more than stress or subAβ alone. Values from full-fledged AD model (Aβ) are shown for comparison. All values are mean + S.E.M. (n=12-15 rats/group); *p<0.05 compared to control and subAβ groups; **p<0.05 compared to all groups. Modified from references (25,29,46,47,48,50).

4. TREATMENTS AND ANIMAL GROUPS

For studying the impact of chronic psychosocial stress on the at-risk AD model, four experimental groups were used: control, stress, subAβ-treated, and stress/subAβ. The stress and stress/subAβ groups were subjected to psychosocial stress for 6 weeks. In addition, the Aβ and stress/subAβ groups were infused, icv, with $Aβ_{1-42}$ (160 pmol/day) during the fifth and sixth week. This dose was determined by constructing a dose-response (performance in the radial arm water maze) relationship. The control and stress groups were infused with 160 pmol/day $Aβ_{42-1}$, an inactive reverse peptide. The validity of this model has been ascertained by three different experimental approaches. In the following discussion of the results of the effect of chronic psychosocial stress on the subAβ model comparison will be drawn between the findings seen with stress/subAβ group and those seen with the full pathogenic dose of Aβ (300 pmol/day icv) in an earlier study.

4.1. Chronic psychosocial stress

A form of "intruder" stress model was used, where rats were initially kept with the same cage mates for a period of one week to establish a social hierarchy. Then, two rats from one cage were switched randomly with two rats from a second cage every day for a period of 6 weeks. This model of psychosocial stress displays two salient indicators of stress: marked increase (by 50%) in blood corticosterone level and up to 40mm Hg elevation of blood pressure (20).

5. BEHAVIORAL TESTS: THE RADIAL ARM WATER MAZE TASK

A reliable and sensitive behavioral test for analyzing hippocampus dependent learning and memory, the radial arm water maze (RAWM) is a hybrid of the radial arm maze and the Morris water maze. It combines the variable spatial complexity of the radial arm maze with the rapid motivated learning of the Morris water maze while minimizing their disadvantages (13,43,44,45).

The RAWM training protocol consisted of a learning phase of four 1-min consecutive learning trials followed by a short-term and a long-term memory tests, 20 min and 24 hr, respectively, after the last learning trial. The animals had to locate a black platform submerged 1 cm below the water level near the end of one of the 6 swim arms, designated as the "goal arm". A correct selection occurred when the rat swam directly to the goal arm, while error was scored each time the rat entered into an arm other than the goal arm. This procedure was conducted for a minimum of 8 consecutive days or until the rat satisfied the "days to criterion" (DTC), which is defined as the number of days in which the rat commits a maximum of one error in three consecutive days in the fourth learning trial and memory tests (25,29,46,47,48).

5.1. Learning trials

During the four acquisition trials, the ability of rats of the subAβ (at-risk) group to learn the location of the survival platform was not significantly different from that of the stress or control group throughout the days of testing. However, the learning ability of the stress/subAβ rats was markedly impaired as days went by, particularly in the fourth trial, compared to the other three experimental groups. This was confirmed by the results of the fourth trial DTC, which showed that the stress/subAβ rats required significantly ($p<0.05$) more days to reach the learning criterion than the other three experimental groups (48). The learning DTC of the stress/subAβ group was similar to that of rats infused with a full pathogenic dose of Aβ peptides (25) (Figure 1A).

5.2. Short-term memory trial

In the 20 min memory tests, again the subAβ group performance was not different than that of control rats. On days 6-8, the stress/subAβ rats continued to commit significantly greater number of errors, than all other groups, including the stress group, which, itself, committed more error than the control and subAβ groups. These findings were further confirmed by the DTC for short-term memory, which showed that the stress/subAβ rats required significantly ($p<0.05$) more days to reach the criterion than the other three experimental groups (48) and was not significantly different than that required by rats infused with full pathogenic dose of Aβ peptides (25)(Figure 1B). As reported previously, stressed rats also required significantly more days than the control and subAβ groups to reach DTC. (48,49).

5.3. Long-term memory trial

By days 6-8 in the 24 hr memory test, the differences in long-term memory between the stress/subAβ rats and the other experimental groups became more evident. The stress/subAβ animals committed significantly more errors than the control, stress, and subAβ groups in the long-term memory test. In confirmation, the DTC test showed that the stress/subAβ rats required significantly more days to reach the criterion than the other three experimental groups (50). Similar number of days was required by rats receiving the full pathogenic dose of Aβ peptides (25) (Figure 1C) indicating that stress transformed the symptomless subAβ rat into a full-fledged AD phenotype.

5.4. Summary

These findings showed that our at-risk AD model's cognitive ability was not different from that of the control rats, however, when these rats were exposed to chronic psychosocial stress they exhibited clear signs of impaired cognitive ability similar to those seen in rats infused with a full pathogenic dose of Aβ (25,29,46,47). Together these findings may be considered as validation of the subAβ as a model for seemingly normal individuals who are at-risk for developing AD.

6. SYNAPTIC PLASTICITY

The strength of synaptic connections among neurons changes substantially during encoding of memory, where some synapses exhibiting a decrease in synaptic strength and others showing an increase (51,52). This bidirectional modification in synaptic strength improves the accuracy, capacity, and flexibility of memory storage (53). The decrease in synaptic strength is known as long-term depression (LTD), whereas the increase in synaptic strength is termed long-term potentiation (LTP) with at least two major phases an early (E-LTP), and a late phase (L-LTP). The E-LTP and L-LTP differ from each other in a number of facets. One short train of stimuli at 100 Hz can induce the E-LTP, a marked potentiation of synaptic responses lasting 30 min to 3 hr. In contrast, L-LTP requires four trains of stimuli and lasts more than 3 h (54). It is well recognized that protein kinases are essential intermediates in the induction and/or maintenance of LTP/memory. Whereas E-LTP mainly depends on phosphorylation of existing calcium-dependent calmodulin kinase II (CaMKII) (55), L-LTP requires new protein synthesis through kinases-induced activation of transcription factors such as cyclic-AMP response element binding protein (CREB). These kinases include protein kinase A (PKA) (56), mitogen-activated protein kinases (MAPK) and calcium-calmodulin-dependent protein kinase IV (CaMKIV) (57).

To relate cognitive impairment in subAβ revealed by chronic stress to possible changes in the cellular correlates of memory, we evaluated synaptic plasticity in the hippocampus. We recorded population spikes (pSpike) from area CA1 of the hippocampi of anesthetized rats and determined changes in the slope of field excitatory postsynaptic potential (fEPSP: a measure of synaptic strength) and pSpike amplitude (a measure of the number of neurons firing action potentials) (24).

6.1. Early phase long-term potentiation (E-LTP)

It has been shown that postsynaptic calcium-dependent CaMKII activity is necessary and sufficient to generate and maintain LTP in area CA1 of the hippocampus (55,58,59,60,61,62). Early phase LTP is believed to be a cellular correlate of short-term memory (63,64). In the control and subAβ animals, high-frequency stimulation (HFS) induced a robust E-LTP manifested as a marked increase in the slope of fEPSP and pSpike amplitude of area CA1. In both the stress and stress/subAβ groups the slope of the fEPSP and amplitude of pSpike were markedly lower than those of the control and subAβ groups at all time points after application of HFS. Furthermore, at all time points, the slope of fEPSP in stress/subAβ animals was significantly lower ($p<0.05$) than that of the stress animal group at 60 min after HFS (48) (fig. 2A). The magnitude of impairment of E-LTP in the stress/subAβ rats is similar to that seen in rat infused with a pathogenic dose of Aβ peptides (25,48) (Figure 3A).

6.2. Late-phase long-term potentiation (L-LTP)

Late phase LTP is believed to be a cellular correlate of long-term memory (63). New protein synthesis is required for the expression of L-LTP. Multiple high frequency stimulation (MHFS) causes a large and highly localized Ca^{2+} influx, which activates adenylyl cyclase type I (ACI) (65). This, in turn, activates PKA, which by itself or through MAPKp44/42 (extracellular signal-regulated kinase 1 and 2; ERK1/2) can activate (phosphorylate) CREB (66). The phosphorylated CREB (P-CREB) activates multiple genes essential for L-LTP expression (63). Calcium calmodulin kinase IV (CaMKIV) is also activated by Ca^{2+} and can directly phosphorylate CREB (57).

Multiple high frequency stimulation (MHFS) evoked L-LTP as a marked increase in the slope of fEPSP and amplitude of pSpike in the control, stress and subAβ animal. However, in the stress/subAβ animals, the same MHFS produced a greatly diminished L-LTP ($P < 0.05$) at all time points (Figure 2B; 48). The degree of reduction of L-LTP in the stress/subAβ rats is similar to that seen in rat infused with pathogenic dose of Aβ peptide (25,48).

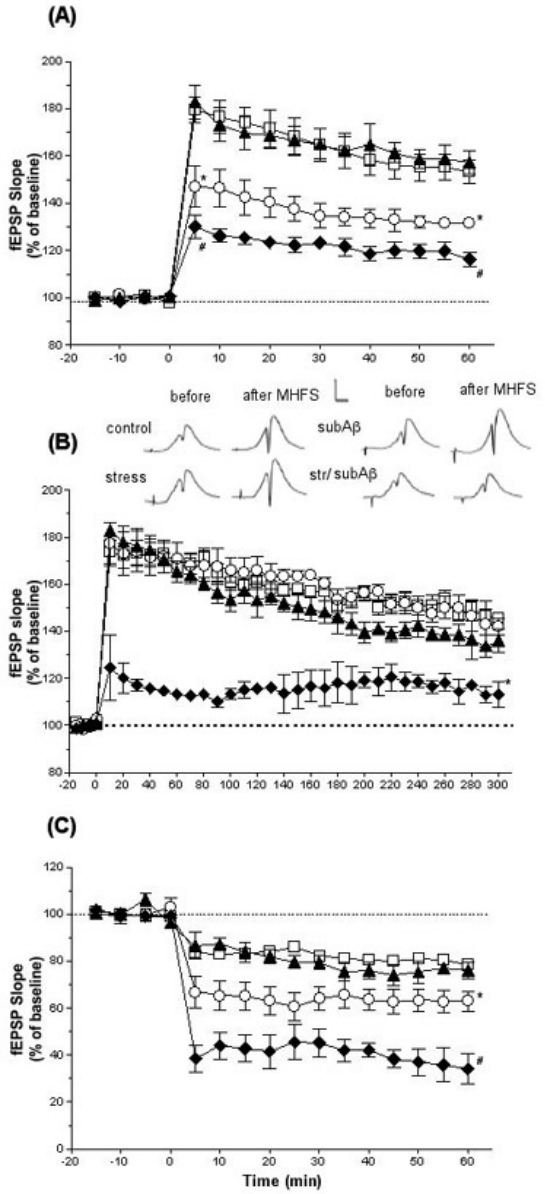

Figure 2. Hippocampal CA1 synaptic plasticity in stress/subAβ rats. Long-term potentiation (LTP) and long-term depression (LTD) of area CA1 evoked by repetitive stimulation (applied at time=0) of the Schaffer collaterals/commissural pathway and measured as changes in the slope of fEPSP in urethane-anesthetized rats. (A) Early phase (E-LTP) is normal in the subAβ group, but significantly decreased in the stress and more so in stress/subAβ groups. (B) Late-phase (L-LTP), is significantly decreased in the stress/subAβ but not in the stress or subAβ groups. (C) LTD is normal in the subAβ group, but significantly decreased in the stress and more so in stress/subAβ groups. Values are mean + S.E.M. from 5-8 rats (*$p<0.05$ compared to control animals; #$p<0.05$ compared to all animals). Insets are traces from representative experiments. Modified from references (25,29,46,47,48,50).

6.3. Long-term depression (LTD)

Prolonged low frequency stimulation depresses hippocampal synaptic transmission. The long-lasting decrease in synaptic strength, known as long-term depression (LTD), has been described in many regions of the brain, including the hippocampus. LTD is thought to be a fine-tuning mechanism for learning and memory processes (53,67,68,69,70). It has been shown that behavioral learning in a novel environment enhances the induction of LTD in vivo (71). In early studies, the induction of LTD by low-frequency stimulation was observed only in hippocampal slices of young animals (72,73,74,75). Later, however, LTD was induced in anesthetized or freely moving adult animals by using the paired-pulse protocol (24, 76,77,78790). The expression of LTD requires postsynaptic depolarization, activation of N-methyl-D-aspartate (NMDA) receptors (72,80), and a moderate increase in the concentration of intracellular calcium ion (Ca^{2+}). This elevation of Ca^{2+} in the postsynaptic region leads to the formation of calcium/calmodulin complex, which in turn leads to the activation of several protein phosphatases required for the expression of LTD.

We examine the magnitude of NMDA receptor-dependent LTD expressed in the Schaffer collaterals pathway using paired pulse protocol in anesthetized animals. This stimulation protocol produced robust LTD, which was measured as decreases in the slope of fEPSP and pSpike amplitude in control and subAβ groups. The LTD magnitude of the stress rat group was significantly greater than that seen in control or subAβ animals. The magnitude of LTD in stress/subAβ animals was significantly greater than that in control, stress, and subAβ animals (Figure 2C). The magnitude of LTD in the stress/subAβ rats is similar to that seen in rats infused with a full pathogenic dose of Aβ peptides (25,48) (Figure 3C).

6.4. Summary

Electrophysiological experiments indicate that synaptic plasticity in the subAβ rat model of AD is not significantly different than that in control animals, which provides further validation of this model as a representative of sub-clinical cases of AD. Additionally, findings from these experiments provide a significant correlation with behavioral tests, clearly showing that exposure of the subAβ rats chronic stress causes severe impairment of synaptic plasticity equivalent to that seen in unstressed rats treated with a full pathogenic dose of Aβ peptides.

7. BASAL LEVELS OF MEMORY- AND SYNAPTIC PLASTICITY-RELATED SIGNALING MOLECULES ESSENTIAL

To elucidate the potential molecular mechanisms by which stress reveals impairment of cognitive ability and synaptic plasticity in the at-risk, subAβ rats and to correlate these findings with the results from the behavioral and electrophysiological studies, we evaluated the effect of chronic stress and subAβ on the basal levels of signaling molecules essential for these processes by immunoblot

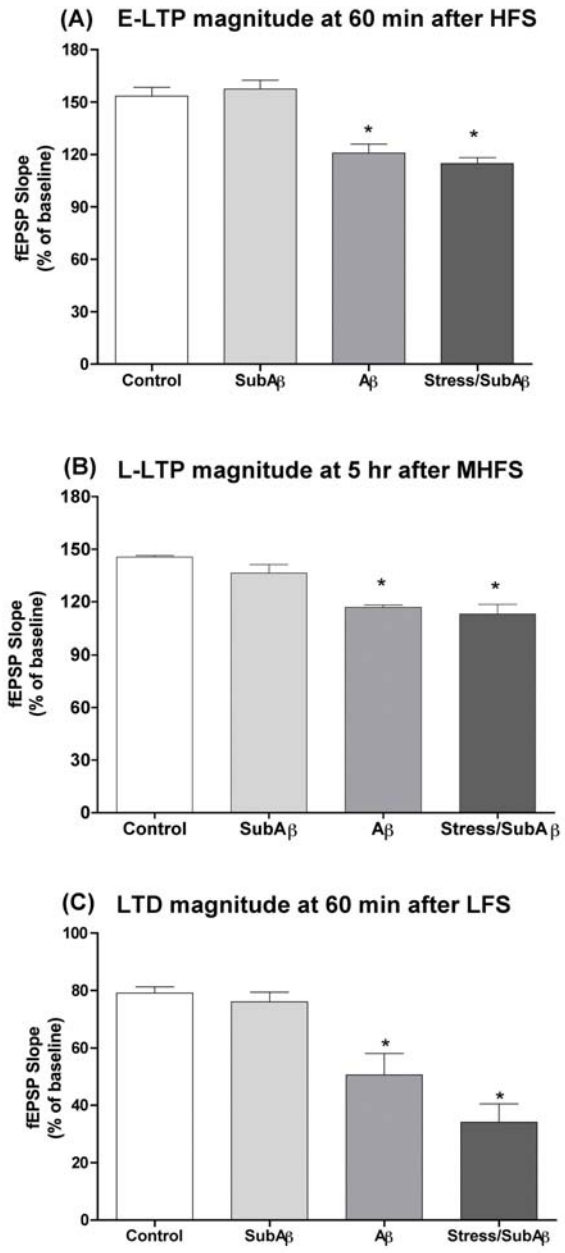

Figure 3. Synaptic plasticity in stress/subAβ rats is impaired to a similar extent as that of the full-fledged AD model (Aβ). Values, summarized from figure 2, are mean + S.E.M. from 5-8 rats (*p<0.05 compared to control animals) (25,29,46,47,48,50).

analysis. Table 1 summarizes the results of these experiments.

7.1. Basal Levels of CaMKII

Calcium calmodulin kinase II plays a critically important role in the memory processes and synaptic plasticity. The molecular mechanisms responsible for the expression of LTP have been thoroughly studied. Normally, the putative cascade of events that leads to the formation of the active phosphorylated CaMKII (P-CaMKII) and, ultimately, expression of LTP is initiated by HFS. It is widely accepted that HFS causes presynaptic release of glutamate, which activates glutamate receptors on the postsynaptic membrane, producing calcium influx (55,81,82). This transient increase of intracellular calcium leads to activation of protein kinase C gamma (PKCγ), which phosphorylates neurogranin, causing dissociation of the neurogranin-calmodulin complex (14,83). The released calmodulin forms a calcium/calmodulin complex, which binds to and activates CaMKII, triggering its autophosphorylation (84,85), resulting in a constitutively active CaMKII (P-CaMKII), which phosphorylates and activates α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) receptors and the synaptic vesicle-specific protein, synapsin, which are important for LTP expression. (82,86,87). The activation of these substrates by P-CaMKII is sustained; even when calcium returns to basal levels, until it is dephosphorylated by protein phosphatases including calcineurin (84,88,89). It is proposed that activation of CaMKII serves as a molecular switch that converts transient Ca^{2+} signals into long lasting biochemical changes that trigger synaptic plasticity (90).

Immunoblot analysis showed that chronic stress or the pathogenic dose of Aβ peptides markedly reduced the basal levels of phosphorylated (p)-CaMKII without affecting the levels of total CaMKII (25). Similar results were obtained in the stress/subAβ rats. The basal levels of both p-CaMKII and total CaMKII in the subAβ rats were not significantly different than those in control rats (48). However, in both the stress and stress/subAβ rat groups, the p-CaMKII levels were markedly reduced while those of the total CaMKII were not affected (48). The ratios of the levels of p-CaMKII/total CaMKII levels in the stress and stress/subAβ rats were significantly decreased (Figure 4A) suggesting impairment of the mechanism of CaMKII phosphorylation or enhanced dephosphorylation or both. Enhanced dephosphorylation is supported by the finding that the basal levels of the dephosphorylating enzyme, calcineurin are markedly increased in the stress and stress/subAβ groups (Figure 4B).

7.2. Basal Levels of calcineurin

Phosphorylated CaMKII is normally dephosphorylated by a protein phosphatase, principally calcineurin, which is a negative modulator of cognitive memory and seems to be involved in AD pathogenesis. For example, inhibiting calcineurin with tacrolimus (FK506) reversed cognitive impairment in Tg2576 mice (91). Several different phosphatases are present in the hippocampus of which calcineurin is thought to be a key phosphatase in the regulation of synaptic plasticity. Calcineurin reduces postsynaptic activity in the hippocampus (92) and has been shown to be responsible for induction and maintenance of LTD (77). Calcineurin also dephosphorylates inhibitor 1 protein, the natural regulator of protein phosphatase 1 (93), which is effective in dephosphorylation of CaMKII (94). Furthermore, over-expression of calcineurin in the hippocampus blocks LTP (95) and impairs hippocampal-dependent memory

Table 1. Summary of the effects of stress and/or SubAβ on the basal levels of AD- and cognition-related signalling molecules in CA1 area of the hippocampus compared to the control

	Stress	SubAβ	Str/subAβ	Full Aβ
p-CaMKII	↓	↔	↓	↓
t-CaMKII	↔	↔	↔	↔
Calcineurin	↑	↔	↑	↑
BDNF	↔	↑	↑	↑
p-CREB	↔	↔	↓	↓
t-CREB	↔	↔	↔	↔
CaMKIV	↔	↔	↓	↓
BACE	↔	↔	↑	↑
APP	↔	↔	↔	NT

↔ no change, ↓ decreased, ↑ increased, NT not tested. Stress: rats were subjected to chronic psychosocial stress before and during infusion of Aβ peptide. SubAβ: rats were intracerebroventricularly infused with non-pathogenic dose of Aβ (160pmol/day) for 14 days (by osmotic pump). Str/SubAβ: chronically stressed SubAβ rats. Full SubAβ: unstressed rats infused with pathogenic dose of Aβ (300 pm/day) for 14 days.

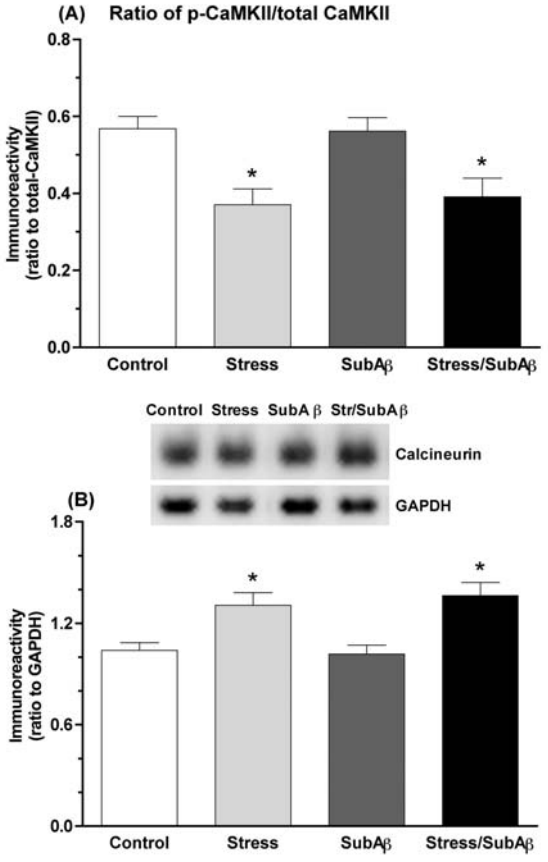

Figure 4. (A) Ratio of basal levels of phosphorylated-calcium-calmodulin-dependent protein kinase II (p-CaMKII) to total (t)-CaMKII in area CA1. Values are mean + S.E.M. from 5-7 rats/group. (B) Basal levels of calcineurin in area CA1. Values are mean + S.E.M. from 5-7 rats (*p<0.05 compared to control). *Insets:* representative immunoblot images. Modified from references (25,29,46,47,48,50).

formation in mice (96). In fact, it has been suggested, "if phosphatases go up, memory goes down" (97).

The role of calcineurin in AD pathology is well documented (98,99,100). The mRNA of calcineurin was upregulated in pyramidal neurons of the hippocampus in the AD brain, indicating that calcineurin may play a critical role in the pathophysiological mechanisms of AD (101). In cultured cortical neurons, the disruption in APP function that resulted in neurite degeneration, oxidative stress, nuclear condensation and eventually cell death was due to a calcineurin-dependent proteolysis of CaMKIV and its nuclear target CREB (102). Moreover, in the astrocyte, calcineurin activation was associated with formation of reactive inflammatory processes related to AD (103). Calcineurin activation was also associated with Aβ-induced apoptotic cell death (104). In primary hippocampal cultures, calcineurin mediated the endocytosis of soluble oligomeric assemblies of Aβ that involved Aβ-induced synaptic disruption (105). In fact, exogenous Aβ was shown to increase calcineurin activity in primary cultures of rat brain cortical neurons (106). Additionally, it was reported that the enzymatic activity of calcineurin was upregulated in the CNS of a transgenic animal model for Aβ over-production (107,108). Furthermore, acute treatment of these transgenic mice with a calcineurin inhibitor rescued memory function (107), especially those related to long-term memory (91). In the same transgenic mice model, Aβ was shown to promote calcineurin-dependent CREB and Bcl-2 associated death (BAD) protein dephosphorylation and cell death (108). These mice showed reduced pCREB in the CNS, which was return to normal by calcineurin inhibition (108).

The levels of calcineurin in area CA1 of subAβ at-risk rats were normal as they were not significantly different than those in the control rat group (Figure 4B). However, area CA1 of both the stress and the stress/subAβ rats showed significantly increased levels of calcineurin (Figure 4B). The increase in calcineurin levels of the stress/subAβ is similar to that seen in the CA1 area of rats treated with a full pathogenic dose of Aβ peptides (25).

7.3. Basal levels of cAMP response element binding (CREB) protein

Several studies have implicated a role for CREB in AD-associated impairment of memory and synaptic plasticity. For example, high intracellular concentrations of Aβ block nuclear translocation of phosphorylated CREB resulting in a decrease of cyclic adenosine monophosphate response element (CRE)-mediated responses (109). Furthermore, Aβ reduces CREB phosphorylation by decreasing NMDA receptor activation (110). Since active CREB is a necessary component for hippocampus-dependent long-term memory formation in mammals (111,112) we measured basal levels of phosphorylated and total (phosphorylated and non-phosphorylated) CREB. Although the basal levels of p-CREB and total CREB in area CA1 of control, stress and subAβ rat groups were normal, those of the stress/subAβ group were significantly reduced (Figure 5A & B). The ratios of the p-CREB/total CREB levels for all groups were similar (Figure 5C)

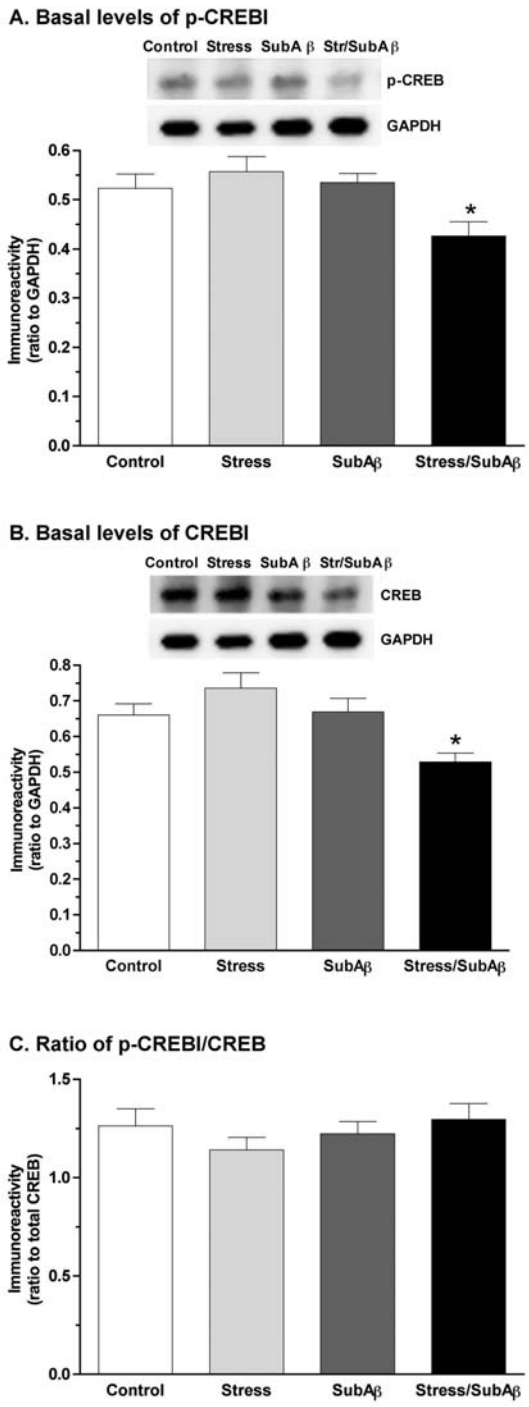

Figure 5. Basal levels of phosphorylated cyclic AMP response element binding protein (p-CREB) and total CREB in the hippocampal area CA1. The combination of chronic stress and subAβ infusion caused a significant decrease in the basal levels of p-CREB (A) and total CREB (B). There is no difference in the ratio of p-CREB to total CREB among the four groups (C). Results are expressed as mean ± S.E.M. (*) Indicates significant difference ($p < 0.05$, n = 5-6 rats/group) from control, stress, and subAβ values. Insets are representative bands. (25,29,46,47,48,50).

suggesting curtailed synthesis of the CREB protein in the CA1 area of stress/subAβ. Given the critical and highly conserved role CREB plays in the generation of protein synthesis-dependent long-term changes in the brain (113) including long-term memory and synaptic plasticity (111), the reduced basal levels of CREB may be responsible for the impaired long-term memory in the stress/subAβ animals. Infusion of pathogenic dose of Aβ caused significant reduction of p-CREB in area CA1 of rats (46). These findings further demonstrate that chronic psychosocial stress can reveal AD phenotype in at-risk individuals.

7.4. Basal levels of brain derived neurotropic factor (BDNF)

BDNF is a member of a neurotrophic family of target-derived growth factors, including nerve growth factor, neurotrophin-3, and neurotrophin-4/5. BDNF improves survival of cholinergic neurons of the basal forebrain (114), as well as neurons in the hippocampus (115,116) and cortex (117). BDNF participate in certain forms of neuronal plasticity, including cognition (118,119). In addition to its action on neuronal survival and differentiation, BDNF has a role in the regulation of synaptic strength. It can act as an activity-dependent modulator of neuronal structure, and its release after tetanic stimulation modulates the induction and maintenance phase of LTP in the hippocampus (120,121). Experimental evidence supports the role of BDNF in memory processes. For example, BDNF mRNA expression has been shown to correlate with behavioral performance in various cognition tests (122). Furthermore, studies reveal that genetic manipulation or pharmacological deprivation of BDNF or its receptor, tyrosine kinase B (TrKB), in the hippocampus results in severe impairment of memory and LTP (123). Due to the critical role of BDNF in memory and synaptic plasticity, it is possible that its loss may also contribute to cellular demise and memory deficits associated with neurodegenerative diseases such as AD. Studies have shown that beta-amyloid oligomers can inhibit BDNF-induced increase of activity-regulated cytoskeleton-associated gene (ACR) expression in cultured cortical neurons. Inhibition of ACR protein synthesis have been shown to impair both the maintenance of LTP and consolidation of long-term memory, thus may contribute to the memory deficits observed during the early stages of AD (124,125). Furthermore, marked downregulation of BDNF mRNA and protein has been reported in the hippocampus and temporal cortex of autopsied AD brains (126). However, there is seemingly inconsistent reports about the levels of BDNF in AD and animal models of the disorder. The reason for these conflicting findings is unclear; it may be the consequence of a variety of factors including different stage of the disease at which BDNF was determined in AD brain, the type of AD model used and the dose level of Aβ administered.

Immunoblot analysis showed that the basal levels of BDNF were not changed in stress and subAβ animals compared to control animals. However, when chronic stress was coupled with subAβ infusion (in the stress/subAβ group), there was a significant ($p < 0.05$) decrease in the

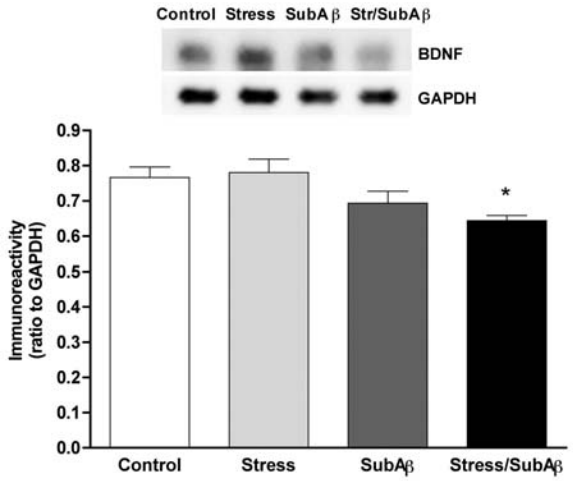

Figure 6. Basal levels of brain-derived neurotrophic factor (BDNF) in area CA1 of the hippocampus. There is a significant decrease in the basal levels of BDNF in animals exposed to both chronic stress and subAβ infusion. Results are expressed as mean ± S.E.M. (*) Indicates significant difference ($p < 0.05$, n = 6-7 rats/group) from control and stress values. Insets are representative bands (25,29,46,47,48,50).

basal level of BDNF compared to the control and stress groups (Figure 6). Similar findings were reported on the effect of sublethal dose of Aβ in cultured cortical neurons (127). In this lab we reported significantly ($p<0.05$) higher levels of BDNF in rats infused with a pathogenic dose of Aβ (47).

7.5. Summary

Molecular experiments measuring the levels of signaling molecules involved in learning and memory and synaptic plasticity provide further evidence of the deleterious impact of chronic psychosocial in subjects prone to AD by accelerating the development of the symptoms of the disease. These experiments also show that subAβ rat model is normal, which correlates with both the behavioral and electrophysiological findings.

8. POSSIBLE MECHANISMS OF THE EFFECTS OF STRESS

We have shown previously that chronic stress reduces the magnitude of HFS-induced LTP and decreases basal levels of p-CaMKII in hippocampal area CA1 (25,49). Similarly, from both *in vivo* and *in vitro* studies, the presence of abnormal levels of Aβ peptides is known to disrupt phosphorylation of CaMKII and interfere with LTP induction (29, 128,129). Based on findings from our model, we propose that reduction of CaMKII-dependent protein phosphorylation by stress may accentuate the loss of p-CaMKII caused by Aβ, thus contribute to the mechanism by which chronic stress impairs memory and LTP in this model of AD.

In general, activation of mineralocorticoid (type-I) receptor by low levels of corticosteroids produce a small Ca^{2+} influx, which has an excitatory effect on hippocampal CA1 pyramidal cells, whereas activation of glucocorticoid (type-II) receptor by high levels of corticosteroids during stressful conditions, enhances Ca^{2+} influx and inhibit CA1 pyramidal cell excitability (130,131). Given the stress-induced glucocorticoid effects on Ca^{2+} dynamics, it is not surprising that stress worsens Ca^{2+}-dependent signaling processes in Aβ rats. This finding is in line with previous reports suggesting that Aβ perturbs intracellular Ca^{2+} signaling (26, 132,133) and inhibits Ca^{2+}-dependent post-translational protein phosphorylation (35). For example, studies using acute application of $A\beta_{1-42}$ during HFS showed inhibition of LTP in the dentate gyrus, with corresponding reductions in p-CaMKII levels (129).

Brain-derived neurotrophic factor (BDNF) plays a major role in neuronal survival (134,135). The levels of neurotrophic factors, including BDNF, are increased in specific brain regions in response to various types of insults, including ischemia, seizure, traumatic brain injury, and neurotoxins (136,137). Earlier reports that show an increase in the BDNF mRNA in the hippocampus (138) and in the protein levels of BDNF in the forebrain in APPsw mice (139) as well as area CA1 in Aβ-treated rats (29), suggest that in early AD, a protective mechanism may be activated to counter the Aβ-induced neurotoxicity. In contrast, chronic stress has been reported to significantly decrease BDNF levels in area CA1 of the hippocampus (20). Therefore, by limiting the availability of BDNF, stress interferes with the repair process and consequently exacerbating the effect of Aβ.

Interestingly, recent reports have shown that the expression of nerve cell adhesion molecule (NCAM) is increased in the brains of AD patients suggesting probable neurogenesis (140). This could be an attempt by the brain to repair or replace neurons lost to the disease. In contrast, chronic stress is known to cause severe reduction in NCAM levels (10,140). We speculate that the neurotoxic effect of Aβ in the brain might be initially countered through repair as suggested by the reported increased levels of NCAM. However, in the presence of chronic stress, the ability of NCAM to repair is severely limited by the stress-induced reduction in the concentration of these protein molecules.

Another possible mechanism for the stress effect is that it may alter the processing and production of various AD-related proteins. It has been shown that exposure to stress or glucocorticoids increases the levels of APP, C99, and the beta site APP cleaving enzyme (BACE), suggesting that stress is driving the processing of APP toward the amyloidogenic pathway which may account for the increased levels of Aβ (25,29,141) and the increased amount of plaque formation (142) that are also observed with stress.

In summary, the presence of chronic stress can have a profound effect on the course of development of AD. Using various experimental approaches in a number of studies, this lab has reported the negative effect of chronic psychosocial stress on the development of diminished

cognitive abilities in a novel rat model that relates to seemingly normal individuals that are predisposed to AD. The results of these studies suggest that the coincidence of chronic stress in these subjects hastens the appearance mental deficiencies.

9. ACKNOWLEDGEMENT

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Abbreviations: Ab: amyloid-beta , ACI: adenylyl cyclase type I, ACR: activity-regulated cytoskeleton-associated gene , AD: Alzheimer's disease , AMPA: a-amino-3-hydroxy-5-methyl-4-isoxazole , APP: amyloid precursor protein , BACE: beta site amyloid precursor protein cleaving enzyme, CSF: cerebrospinal fluid , CREB: cyclic-AMP response element binding protein , CaMKIV: calcium-calmodulin-dependent protein kinase IV, CaMKII: calcium-calmodulin-dependent protein kinase II, DTC: days to criterion, E-LTP: early phase LTP, ERK1/2: extracellular signal-regulated kinase 1 and 2 , fEPSP: field excitatory postsynaptic potential , HFS: high-frequency stimulation, HPA: hypothalamic-pituitary-adrenal , icv: intracerebroventricular, L-LTP: late phase LTP, LTP: long-term potentiation , LTD: long-term depression , MHFS: multiple high frequency stimulation , MAPK: mitogen-activated protein kinases , NCAM: nerve cell adhesion molecule , NMDA: N-methyl-D-aspartate , PKA: protein kinase A, PKC: protein kinase C, PS1)presenilin 1 , PS2: presenilin 2 , RAWM: radial arm water maze , TrKB: tyrosine kinase B

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