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Longitudinal Brain Corticotropin Releasing Factor and Somatostatin in a Transgenic Mouse (TG2576) Model of Alzheimer's Disease

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Abstract

Neuropeptides corticotropin releasing factor (CRF) and somatostatin (SRIF) are substantially decreased in cortical regions of Alzheimer's disease (AD) post-mortem brain tissue. The accumulation of amyloid- β ($A\beta$) in AD brain has been postulated to be neurotoxic. Using male Tg2576 mice transgenic over-expressing amyloid- β protein precursor (APP), we examined brain concentrations of CRF and SRIF at 12, 18 and 24 months. Mice were evaluated for locomotor activity and spatial memory. The APP mice had continued increased locomotor activity from 6 months of age compared to controls. Spatial memory was impaired beginning at 12 months in the APP mice relative to controls. APP mice at 24 months had a significantly higher number of amyloid plaques when compared to the 12 and 18 month time points. Brain concentrations of SRIF and CRF were significantly altered in a number of cortical and sub-cortical brain regions relative to controls, but in most regions were increased rather than decreased as in clinical AD. This data shows that although the insertion of the APP gene does cause age dependent increase in plaque load, it does not cause a change in regional neuropeptides consistent with AD, suggesting that neuropeptide changes in AD are not solely due to $A\beta$ load.

Keywords

Amyloid peptide; Alzheimer's disease; CRF; somatostatin; longitudinal; transgenic mice

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease that occurs in the later stages of life and is estimated to encompass 40% of the population reaching 80 years of age [7]. More than 90% of AD cases are termed sporadic and appear to have no familial linkage. In order to treat AD effectively, an early diagnosis is crucial. This would require a diagnosis some eight to ten years earlier than current methods allow [13]. Current treatments for AD that are approved by the FDA focus on neurotransmitter systems thought to mediate symptoms of dementia. However examination of brain tissue from patients dying at various stages of AD have shown that acetylcholine (ACh)-containing neurons do not decrease

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significantly until the patients have reached a dementia rating scale (DRS) of 4 out of 5 stages [11,15]. Where and which neurotransmitter deficiencies occur in post mortem brains of AD are known with some certainty, but when and why this degenerative cascade occurs remains relatively uncertain.

Two neuropeptide neurotransmitters, corticotropin releasing factor (CRF) and somatostatin (SRIF), are also confirmed to be decreased in AD brains [28]. These neuropeptides regulate anterior pituitary hormone release but exist also as neurotransmitters in extra hypothalamic circuits in the brain. In 1985 Bissette et al. [4] discovered that CRF was markedly decreased in AD in cortical regions where it is predominantly found in interneurons. In 1986 Behan et al determined that CRF receptors are upregulated in proportion to the decrease of CRF [3]. Further studies indicate that CRF decreases are significant at a DRS of 2 [12], much earlier than the DRS of 4 reported for ACh deficits. This early decrease in CRF could present not just a possible diagnostic tool for AD, but with the upregulation of receptors could also provide a possibility for treatment.

Decreases in SRIF protein have been reported in a number of diseases associated with cognitive impairment [5]. In AD there is a correlation between decreases in SRIF, cognitive impairment and plaque density. However, there is no upregulation of SRIF receptors [2, 25] and longitudinal studies have shown that decreases in SRIF occur at a similar stage of AD as ACh [11]. Therefore, it is unlikely that SRIF agonists would be successful in the treatment of AD.

Basic research on the loss of these neurotransmitter-containing neurons has focused recently on the toxicity of the amyloid- β ($A\beta$) protein, a cleavage product of amyloid precursor protein (APP), and its putative link to the cognitive impairment seen in AD. The $A\beta$ protein accumulates in the senile plaques of AD patients, however elderly patients without dementia or any sign of cognitive impairment have been found on autopsy with levels of $A\beta$ exceeding that of mild cognitive impairment or AD patients [27]. This finding, among others, provides support for the dissociation of $A\beta$ levels and loss of cognitive function.

Transgenic mice are widely used in research to determine the cascade of events in familial AD. One of the more popular models is the Tg2576, a mouse that over expresses a mutant form of human APP developed by Karen Hsiao in 1996 [18]. These mice have been reported to have greatly increased numbers of amyloid plaques than controls beginning as early as 10 months of age and have spatial memory deficits relative to nontransgenic control mice. Using this mouse model, we proposed that, should $A\beta$ in fact be causing the cognitive impairment and the reduced CRF and/or SRIF levels seen in the human form of the disease, these mice, upon aging and sacrifice, should have decreased levels of CRF and SRIF in their brain tissue as is seen in human subjects.

METHODS

Subjects

The animals used were male, Tg2576 mice from Taconic Laboratories that over- express a mutated form of human APP695 [18]. The mutation is derived from a large Swedish family

with early onset AD, consists of a double mutation of Lys⁶⁷⁰-Asn and Met⁶⁷¹-Leu inserted into the APP sequence and uses a hamster prion promoter to express high levels of the mutant APP protein. Due to the aggressive nature of the male transgenic mice, all subjects were singly caged on a 12/12 light cycle and at ambient temperature. Animals were allowed free access to food and water. As the Tg2576 mice are reported to be hypoglycemic [30], all animals were fed fruit loops cereal three times per week in order to sugar supplement their diet.

Subjects were divided into three groups and sacrificed at 12, 18 and 24 months of age respectively. Each experimental time point had its own set of age-matched controls. All experimentation on animal subjects was approved by the Institutional Animal Care and Use Committee at the G. V. "Sonny" Montgomery VA Medical Center, Jackson, MS (Protocol Number GB-2).

Morris water maze

The Morris water maze consists of a 1.0 meter diameter pool surrounded by several visual cues and filled to a depth of 10 cm with water at 23–25°C.

A circular platform with diameter of 5 cm, which is either visible (above the surface) or invisible (below the surface) to the subject, is placed in the water for escape purposes. The pool was monitored by Noldus Ethovision package which allows the observer to track and record the animal's progress around the pool. The water maze task was conducted over three days.

On **pre-training day (day 1)** a visible platform was placed in the north quadrant of the pool. Subjects were placed in the west quadrant facing the wall and allowed to swim freely in the pool for 2 minutes or until they located the platform and mounted it. If the platform was not located within this two minute period the subject was placed on the platform for 15 seconds and allowed to observe the visual cues in the room. The subject was then removed from the pool and placed in its resting cage for a minimum period of 10 minutes. Subjects continued to swim trials until they located the platform under 60 seconds in three consecutive trials. Upon completion of this task successful subjects moved on to the training day. Subjects who did not complete this task in fewer than ten trials were deemed unsuccessful and had to repeat trial one on a subsequent day (pre-training – day 2). **Training Day:** Similar to day one except that the visible platform was placed in the east quadrant. Only successful subjects moved on to the probe day. **Probe Day:** On the day after the training day a submerged platform was placed in the pool in the east quadrant. Subjects were placed in random quadrants facing the tank wall and allowed to swim freely in the pool for 2 minutes or until they located the platform and mounted it. If the platform was not located within this two-minute period the subject was not placed on the platform but just returned to its resting cages to await the next trial.

The subjects continued swim trials until they located the platform under 60 seconds in three consecutive trials. Subjects who did not complete this task in fewer than ten trials were deemed unsuccessful but did not repeat the probe day test. At the end of the "Probe Day"

testing each animal completed one trial without the presence of the platform. The data was analyzed for time spent in the target quadrant as well as entries into the target quadrant.

Locomotor activity

The locomotor activity system consists of 8 Optovarimax photocell monitoring apparatus (Columbus Inst, Columbus OH) with a clear Perspex box set into each. The Opto-varimax monitors the animal's movements with a set of 15 cm × 15 cm infrared beams that traverse the floor of the monitoring box. Each time a beam is broken by the animal's progress throughout the box the ambulation score is incremented by one. Animals were tested for 15 minutes each.

Tissue preparation

Euthanasia was accomplished by decapitation without anaesthesia and after prior habituation to euthanasia procedure to reduce activation of the HPA axis. Brains were rapidly removed and frozen for subsequent dissection of the following regions on a Petri dish cover placed upon wet ice: Anterior caudate (AC), Anterior Septum (AS), Cerebellum (CB), Cingulate Cortex (CC), Dorsal Brain Stem (DBS), Entorhinal Cortex (EC), Frontal Cortex (FC), Hippocampus (HIP), Hypothalamus (HYP), Nucleus Accumbens (NA), Posterior Caudate (PC), Piriform Cortex (PCX), Ventral brain Stem (VBS), and Substantia Nigra (SN).

A tissue block containing the left hippocampus and the entorhinal cortex was placed in Zamboni's fixative and used for the immunohistochemical staining of beta amyloid. The blocks of tissue in the fixative were submerged in 10% sucrose for 24 hours then in 30% sucrose for a further 24 hours. Subsequently each block was cut into 30 micron sections on a cryostat, dried for 10 minutes and then stored frozen at -80°C until stained.

The remaining brain regions were weighed, homogenized in cold 1.0 M HCl and centrifuged to separate the protein (pellet) and the neuropeptides (supernatant). The pellet was reconstituted in 1 M NaOH and protein content was measured using a Folin phenol reagent in a Technicon® automated protein analyzer. With the exception of the hippocampus, regions were not separated into left and right hemisphere.

Radioimmunoassay

CRF and SRIF were measured in the acid extracts by radioimmunoassay.

Radioactive tracers for the SRIF and CRF assays were synthesized by Bolton-Hunter chloramine T reaction using synthetic Tyr1-SRIF (Bachem, Torrance CA) and Tyr0-CRF (Peninsula, Belmont, CA) and Iodine 125 (New England Nuclear, Boston, MA). Tracer was purified by HPLC and fractions were characterized for peak immunoreactivity using two or more concentrations of primary antibody.

Assays were conducted with primary rabbit antisera for CRF (Peninsula Labs Belmont CA, final concentration 1:20 K, IC₅₀ = 35 pg) and an antibody to SRIF raised in sheep (W.Vale, Salk Institute, La Jolla CA, final concentration 1:75 K, IC₅₀ = 30 pg). Standard curves were constructed from synthetic peptides by serial dilution and ranged from 5120 pg to 0.625 pg.

The CRF and SRIF radioimmunoassay were performed as displacement assays over a period of 4 days: Day 1 – Assay buffer was added to lyophilized tissue samples, primary antisera was also added and the samples were incubated at 4°C for 24 hours, Day 2 – Radioactive tracer (20,000 cpm) was added to all tubes which were then incubated for 16-18 hours at 4 °C , Day 3 – The 2nd antibody (goat – anti rabbit for CRF or rabbit – anti – sheep for SRIF) was added to all tubes followed by incubation for 24 h at 4°C, Day 4 – All tubes were centrifuged to separate bound from free tracer, The supernatant was aspirated and the remaining radioactivity was counted on an LKB Rack gamma counter. Total sample amounts of neuropeptide were calculated from aliquot concentration and were divided by total sample protein to allow reporting regional neuropeptide concentrations as pg/mg protein.

Corticosterone assay

Trunk blood was collected at the time of euthanasia and centrifuged. The supernatant was assayed for corticosterone levels using an I¹²⁵ RIA kit from MP Biomedical (formerly ICN Biomedical). The kit was used according to manufacturer's instructions except that antisera, tracer and precipitant were used at half strength.

Immunohistochemical staining

Slides containing 8 sections from the middle of the tissue block were used in the immunostaining procedure. This process was accomplished over two days. Day 1 – The slides were incubated for thirty minutes at room temperature in a pre-incubation solution and stored in pre-incubation solution containing a primary antibody against A β (1-42) (from Chemicon, dilution 1:100) at 4°C for 24 hours. Day 2 – the slides were washed in 0.1 M phosphate buffer, pH 7.6, containing 0.9% NaCl (PBS) and then incubated for 90 minutes in a biotinylated secondary antibody also diluted with pre-incubation solution. Following three more washes in PBS the slides were then treated with an avidin-biotinperoxidase complex for 60 minutes (ABC Kit from Vector Laboratories). After three additional washes with PBS, the antibody complexes were visualized by incubating for 5 minutes in PBS containing 0.05% diaminobenzidine tetrahydrochloride (DAB) and 0.005% H₂O₂. The procedure was concluded with three additional washes in PBS and dehydration of the sections in a series of increasing grades of ethanol followed by two xylene washes before coverslipping.

As controls for immunohistochemical staining we omitted either the primary or the biotinylated secondary antibody, and either omission resulted in absence of immunostaining. In sections incubated with primary antibodies pre-adsorbed with A β (1-42) staining of plaques was prevented.

Plaque quantification

Pictures encompassing the entorhinal cortex were taken at the microscope from each of the stained slides at 10X magnification. From each of these pictures a span of 1 mm tangential to the brain surface and centered on the entorhinal cortex section was selected and used to quantify the % of area covered by amyloid plaques with image analysis software Image J (version 1.32). This quantification procedure involved drawing a box spanning all cortical layers from the subpial edge of the cortex on the outside of the section to the boundary between grey and white matter. The box measured 1mm along the length of the EC

tangentially to the brain surface. Each of the plaques within this box was circumscribed and its area measured. The total area of plaques was calculated for each tissue section in this manner and the data expressed as a % of the box area in that section. Three sections equally spaced were quantified per animal and the average area % for these three sections was the variable included in statistical analysis.

Reagents

Unless otherwise noted all reagents were obtained from SIGMA-ALDRICH.

Statistical analysis

Data was analysed by ANOVA followed by FLSD posthoc test for significance. Significance was determined by $p < 0.05$.

RESULTS

A +20% mortality occurred in the first two months in our facility forcing us to order a second group of animals that became the 12 month time point. This was necessary to preserve the statistical power in the 18 and 24 month time points from the initial mouse order. Thus, it is important that when viewing the following data to remember that the 12 month time point was from a different set of transgenic mouse cohorts relative to the 18 and 24 month group.

Water maze

The proportion of APP animals that could not achieve the criteria in Trial 1 of the water maze training in the first day increased as the animals aged (Table 1A). Most of these mice were tested up to thirty times over a three day period and never mastered the task criteria, either ignoring the platform altogether or pushing off of it in order to swim in the opposite direction. The percent of control mice that could not complete day one averaged 15% throughout the course of the experiment and did not increase with age. A noticeable difference in learning performance was apparent at 12 months when the number of trials needed to train the APP mice increased significantly as compared to controls. The latency to target on trial three of the task when an invisible platform was in place was also increased but this did not reach statistical significance until the APP mice reached 24 months (Table 1B). There was no significant difference between successfully trained controls and APP mice in the memory probe trials at any time point. Nor was there any statistically significant difference in the number of entries into the target quadrant during this probe trial between transgenic and control mice.

Locomotor activity

There was no difference in the locomotor activity of the APP animals compared to controls in the 12 month age group. The APP cohort that made up the 18 & 24 month groups had increased activity from 6 months of age when compared to controls. This increase was maintained to 24 months of age. However, none of these results reached statistical significance due to the high individual variability in the APP animals' activity (Table 2).

Plaque quantification

The number of amyloid immunoreactive plaques in the APP mice increased as the animals aged (Fig. 1). The 24 month group averaged 59 ± 7 plaques per section with the maximum number being 134. This average was significantly different from the number present at 12 months (2.2 ± 0.7) and 18 (16.7 ± 1.7) months. The percentage area of the tissues containing plaques was also increased at 24 months compared to the 12 and 18 groups due to the increased plaques size and number. This result was only significant when comparing the 24 and 12 month groups (Table 3). One of the 12 month old control mice presented with an stained structure with round morphology similar to a small plaque. Two control mice at 18 months and four at 24 months also were found to contain only one or two of these very sporadic structures after examining all sections. These isolated structures may represent artifacts or regions of the brain with particular affinity for the reagents used since they also appeared in control sections after pre-adsorption of the antibody to A β with the A β peptide, while pre-adsorption when processing sections from transgenic animals prevented the labeling of virtually all plaque-like structures. Also there was no significant correlation between performance in the water maze task and plaque load either in the control or the APP transgenic mice.

Regional brain content of CRF and SRIF

Relative to their age-matched controls, the APP transgenic mice had increased levels of CRF and somatostatin in the majority of cortical regions examined. This was true for all time point groups. In no cortical region examined was there a continual decline of either neuropeptide in the transgenic mice as is seen in AD patients when compared to age matched controls. A summary of the results as percent of control is presented in Table 4. The hypothalamus was the only region that showed age- dependent decline in the concentrations of both peptides (Fig. 3A & B). Several of the cortical regions (frontal (Fig. 4A), cingulate and piriform) exhibited increased CRF at 18 months that were either statistically significant at that point (frontal and cingulate) or became so at 24 months (entorhinal and piriform). SRIF levels in the piriform cortex were decreased at 18 months but this deficit was not sustained to 24 months. Similar SRIF results were observed in the entorhinal cortex (Fig. 2B). In the hippocampus, the CRF (Fig. 5A) levels remain decreased relative to controls at all three time point, but is only statistically significant at 18 months. There appears to be a relative decrease in CRF from 12 to 18 months in the APP transgenic mice, but this level rises again to control concentrations by 24 months. SRIF in the hippocampus (Fig. 5B) is significantly decreased in the 12 month transgenic mice relative to their aged-matched controls. These levels are increased at 18 months but decreased again at 24 months. There is no indication that changes in CRF are occurring earlier than SRIF in these transgenic mice other than the greater number of regions with CRF increases at 24 months.

Corticosterone levels

Results for corticosterone levels are represented in Table 5. The 12 month APP transgenic mice had a significantly lower level of corticosterone than their aged matched control counterparts ($p = 0.03$). There was no significant difference between the APP mice and the control mice at either of the other two time points. The 24 month APP animals had a

significantly higher corticosterone levels than the younger APP mice. The control mice at the 24 month time point also had higher corticosterone levels than the younger control mice, but statistical significance was only reached when compared to the 12 month old controls.

DISCUSSION

The actual cause of neuronal death in AD remains controversial. Several theories promote mechanisms associated with neuropathological changes such as $A\beta$ neurotoxicity due to pathological metabolism of APP, hyperphosphorylation of tau proteins and the formation of paired helical filaments of the neurofibrillary tangles and, more recently, oxidative damage associated with free radical formation due to neuroinflammation and triggering of apoptotic pathways. However, no tested or proposed treatment has been demonstrated to either arrest clinical AD progression or to restore functional capacity once it has become devastated. One major obstacle is that AD cannot be diagnosed early enough to begin treatment at a stage of the disease before degeneration of a particular neuronal system outstrips the capability of the remaining neurons to compensate for this loss of function.

The $A\beta$ cascade theory of AD is currently the most popular and has not yet been proved invalid. However, a number of problems with this theory have not been adequately addressed. The neurotoxicity of $A\beta$ was first demonstrated in cultured neurons, but only following significant aging of the $A\beta$ solution forming fibrillary structures similar to mature plaques seen in the clinical disease [45]. Microinjection of soluble amyloid (with or without aging) into intact laboratory animal has not shown any overt neurodegeneration [14,16,23,24,29,37,41,44], although there is a report on “aged” non-fibrillary forms of infused amyloid that may produce neuronal degeneration in the rat hippocampus [26]. Furthermore, in the clinical disease there is no halo of degeneration in the area surrounding amyloid plaques that would be expected if the fibrillary amyloid was toxic to the surrounding neurons [36]. Finally, there is little to no loss of neurons in Tg 2576 mice as reported by Irizarry et al. [20]. Thus, the loss of neurons containing CRF or SRIF would not be presumed in these mice, but synaptic availability could still be altered by changes in the synthesis or degradation of these neuropeptide neurotransmitters. *In vitro* data suggests that CRF may protect neurons from $A\beta$ induced damage [31] and the early loss of CRF may accelerate $A\beta$ toxicity in clinical AD.

Should $A\beta$ load be responsible for the eventual neurodegeneration and loss of cognitive function in AD, we proposed that these transgenic mice over expressing APP should not only have increased $A\beta$ load, but should also display the characteristic neuropeptide changes seen in the clinical disease.

The present findings reveal that there were in fact few regions showing decreased amounts of either CRF or SRIF occurring at more than one of the three time points. The significant decrease in hypothalamic CRF (Fig. 3A) at 24 months seen in these transgenic mice are similar to previously reported decreases in hypothalamic CRF in animals subjected to various stressors [8]. In human AD, levels of CRF protein in the hypothalamus are not generally changed [6], although it has been reported that numbers of CRF neurons in the hypothalamus [32] as well as the numbers of CRF mRNA containing neurons [33] were

often increased in post mortem AD tissue relative to aged normal controls. Immunohistochemical staining for CRF neuron distribution and in situ hybridization for CRF mRNA would be necessary to determine if this was the case in this model, but decreased hypothalamic CRF content in Tg2576 does not model post-mortem AD.

A major distinction between these neuropeptide alterations in transgenic mice and those seen in clinical post-mortem AD tissue are the concentrations in cortical regions of the transgenic mice. Post-mortem entorhinal cortex tissue (EC) of AD patients exhibits the most robust decreases in both CRF and SRIF [6]. However, in these APP transgenic mice there is no continuity in the peptide levels for this region. The CRF level in the EC shows a significant increase at 24 months while the SRIF level appears to return to normal at 24 months although at 12 and 18 months it was significantly increased (Fig. 2A). The majority of the other cortical regions assayed showed similar increases inconsistent with the clinical disease. Furthermore, in clinical AD CRF decreases are seen at a DRS of 2 compared to a DRS of 4 for SRIF or acetylcholine [11] changes. These results do not provide any indications of this earlier involvement of CRF. There are a larger number of areas with altered CRF at 24 months compared to SRIF. However, unlike the clinical disease, the SRIF changes are not seen consistently in the same areas or the same direction as CRF in these mice.

The data regarding the Tg2576 mice is conflicted in regards to both behaviour and biochemistry. For example Barnes and Good [1] found impaired freezing elicited by a tone in these mice. Comery et al. [9] ameliorated this impairment by acute gamma secretase inhibition. In contrast, Corcoran et al. [10] showed normal levels of conditional freezing to an auditory conditional stimulus and that learning and memory deficits in old Tg2576 mice were limited to hippocampus-dependent tasks, despite amyloid deposition in cortex, hippocampus, and amygdala. King and Arendash [22] found that these mice do not exhibit widespread cognitive impairment even up to 19 months of age.

Discrepancies are apparent also in the characterization of the nature of the amyloid deposits in these mice. In 2001 Terai et al. [39] examined beta-amyloid deposits in the cerebral cortex of Tg2576 mice and found them to be similar to those in AD patients. However, in 2002 Kalback et al. [21] found that the A β peptides accumulated in plaques in the mice are physically and chemically different to those seen in AD. Westerman et al. [42] found an inverse correlation between water maze performance and insoluble beta amyloid in young and old Tg2576 mice stratified by age.

While the latency to find the platform was somewhat increased in the transgenic mice, there was no difference in the time spent in the target quadrant in the probe trial. There were a number of possible reasons for this. The control mice tended to seek the missing platform elsewhere and so only spent a few seconds in the target quadrant at any one time. The APP mice took longer on average to reach the target quadrant, either through slower swim speed or through taking a longer route, so their time in the target quadrant was slightly higher than the controls. However, the APP mice often kept swimming in the same direction once they passed where the target should have been, while the control mice would double back to re-

check their position in the pool. The time in target quadrant therefore averaged the same for both groups.

There are a number of possible explanations for the difference in the neuropeptide changes seen in this model compared to clinical AD. It may be that there is a differential response of mouse neurons to the overproduction of $A\beta$ when it occurs throughout development and adulthood, although this is also experienced by familial AD subjects. The differences could also be due to the transgene insertion site itself. As previously mentioned, there were two separate sets of animals used in these experiments. The first set became the 18–24-month groups while the second set was sacrificed at 12 months but was ordered 5 months after the first set. These later mouse orders that become the 12-month time point cohort of transgenic mice were noticeably different in their behaviour from the first group. Subjectively these animals seemed more anxious when entering the water maze and locomotor activity monitors. We contacted Taconic Labs about this difference and they informed us that it was probably because the breeding stock used to generate the second group of animals was a different generation of transgenic founders with possibly different non-specific transgene effects. It is also conceivable therefore that non-specific transgene effects are also responsible for the memory deficits and the reported neuropeptide changes. This putative scenario is supported by the fact that there were no statistically significant correlations between plaque number and either behavioral measurements and/or peptide concentrations. There were no significant correlations between CRF or SRIF concentrations in the substantia nigra or caudate nucleus and the locomotor activity scores and there were no significant correlations with either peptide in the hippocampus and the water maze performance parameters. As can be seen from the corticosterone levels, the behavioral discrepancies cannot be attributed to stress. The APPs at 12 months had a lower corticosterone levels than their aged matched counterparts and there was no significant difference detected between the APP mice and their corresponding controls at either the 18 or the 24 month time points.

There is also the possibility that $A\beta$ does not produce neuropathology in these transgenic mice and possibly not in clinical AD either. The insertion of the APP gene into the genome of these animals did cause an age dependant increase in plaque number and size. That is undisputed. These mice also had learning deficits that worsened as the animals aged. However, performance in the swim maze task did not correlate with hippocampal/ entorhinal cortex plaque content. If over production of $A\beta$ is the cause of the neurodegeneration in AD, then these mice should have exhibited neuropeptide changes at least in the same direction as seen in the clinical setting.

Studies involving mice with a double mutation for APP and Presenilin 1 have shown a substantial enhancement of AD-like pathology [17]. Rutten et al. [35] observed that the PS1 mutation is involved in neurode-generation that is distinct from its contribution to alterations in $A\beta$ generation. Wong et al. [43] observed a reduction in the density and size of cholinergic synapses in several cortical regions in these double PS1/APP transgenic mice consistent with clinical AD. In 2003 this same group found decreases in vesicular acetylcholine transporter immunoreactive button density in the double transgenic mice but not in the APP single transgenic mice [19]. The APP single transgenic mice have dendritic abnormalities similar to those seen in clinical AD [38] but a paper by Tsai et al. [40] saw breakage of dendrites in

the same time frame using the double transgenic mutant (PS1/APP) mice suggesting again the enhancement of pathology with the double mutation. A recent paper by Ribe et al. [34] examining an APP/tau transgenic mouse implies a reciprocal interaction between the alterations of BA and tau *in vivo*.

Finally, it is possible that had the transgenic mice died later of “natural causes” instead of decapitation at 24 months, the cortical neuropeptide concentrations would have decreased further than the controls. As 24 months in mice is equivalent to approximately 70–75 years in humans, deficits may not have fully developed at this time point. In order to model AD in a way that is useful for the development of successful treatments it is essential to model the neurochemical as well as the behavioural aspects. The fact that these animals did not produce the decreases in peptides known to be adversely affected in clinical AD poses a serious challenge to the current hypothesis that A β overproduction alone initiates the cascade of pathology consistently seen in human patients with AD.

In summary, while we did observe the previously reported increase in locomotor activity and decreased spatial memory performance for these Tg2576 mice, the previously reported lack of neurodegeneration and current findings of increased neuropeptide content in cortical areas indicate that beta amyloid overproduction and deposition that increases with age in this transgenic strain do not translate into some of the neurochemical changes seen in human AD. Neuropeptide deficits in clinical AD are therefore not successfully modelled in these transgenic Tg2576 mice with increased A β production and spatial memory defects.

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Fig. 1.

Representations of Tissue sections. Photomicrographs of entorhinal cortex sections immunostained for $A\beta$ demonstrating an age-related increase in amyloid deposition. Amyloid plaques appear as oval brown deposits. A – 12 month old APP mouse, B – 12 month old control mouse, C – 18 month old APP mouse, D – 18 month old control mouse, E – 24 month old APP mouse, F – 24 month old control mouse. (Calibration bar: 500 μ m).

Fig. 2.

(A) CRF measurements in the Entorhinal Cortex (EC) at each time point. All data is expressed as percent control. The dotted line at zero represents the point at which the amount of CRF in the APP would be equal to the control animals. (B) SRIF measurements in the EC at each time point. Significance at individual time points refers to a significant difference between the APP and Control groups at that time point. (p values shown on the graph).

Fig. 3.

(A) CRF measurements in the Hypothalamus (HYP) at each time point. (B) SRIF measurements in the HYP at each time point.

Fig. 4.

(A) CRF measurements in the Frontal Cortex (FC) at each time point. (B) SRIF measurements in the FC at each time point.

Fig. 5.

(A) CRF measurements in the Hippocampus (HIP) at each time point. (B) SRIF measurements in the HIP at each time point.

Table 1A

Presents the data from day three of the final swim for each of the time points which took place two days before sacrifice. Data shown is the average latency to target for each group as well as the average number of trials it took to successfully complete the task

AGE (MONTHS)	APP %	CONTROLS %
8	17	17
11	24	18
15	49	19
17	61	20
20	45	13
22	50	13
24	44	10

Table 1B
Shows the % of animals that could not complete day one of the water maze task at various ages

TIME POINT	TREATMENT	Trials		Latency	
		MEAN	STD ERR	MEAN	STD ERR
12 MONTHS	APP (<i>n</i> = 12)	3.25	0.13	18.24	4.88
	CONTROL (<i>n</i> = 14)	3.00	0.00	11.52	2.25
18 MONTHS	APP (<i>n</i> = 8)	4.50	1.00	29.13	9.90
	CONTROL (<i>n</i> = 20)	3.65	0.42	18.75	3.48
24 MONTHS	APP (<i>n</i> = 8)	3.13	0.13	26.125**	6.05
	CONTROL (<i>n</i> = 18)	3.00	0.00	8.41	1.28

**Significant with respect to control latency *p* = 0.0005.

Table 2

Shows the data from the final locomotor activity test for each time point which occurred one day prior to sacrifice. Data represents the average distance travelled over a 15 minute period

TIME POINT	TREATMENT	Distance	
		MEAN	STD ERR
12 MONTHS	APP ($n = 16$)	1061.34	129.25
	CONTROL ($n = 16$)	909.79	89.99
18 MONTHS	APP ($n = 18$)	1509.32	298.73
	CONTROL ($n = 25$)	941.01	106.82
24 MONTHS	APP ($n = 17$)	3379.64	628.48
	CONTROL ($n = 21$)	2417.18	222.16

Table 3

Represents the quantitative data from the stained tissue sections. Data is presented as mean and SEM of the number of plaques per section (NO plaques) or the area % covered by plaques (% AREA)

TIME POINT	NO PLAQ		% AREA	
	MEAN	STD ERR	MEAN	STD ERR
12 months ($n = 16$)	2.167 ^{**}	0.66	0.14	0.04
18 months ($n = 18$)	16.67	1.70	3.47	1.93
24 months ($n = 16$)	59.14 ^{***}	7.32	6.077 [*]	0.99

* = significant with respect to 12 month group ($p = 0.003$),

** = significant with respect to 18 month group ($p = 0.018$),

*** = significant with respect to 12 & 18 month groups ($p < 0.001$).

Table 4

%Control CRF and SRIF. All data is expressed as % control $\pm$ SEM. **(A)** Presents a summary of the % control data from both the CRF and SRIF assays in each of the regions in the 12 month time point animals. ($n = 16$ for APP group and 17 for control group) **(B)** Presents a summary of the % control data from both the CRF and SRIF assays in each of the regions in the 18 month time point animals. ($n = 18$ for APP group and 25 for control group) **(C)** Presents a summary of the % control data from both the CRF and SRIF assays in each of the regions in the 24 month time point animals. ($n = 16$ for APP group and 20 for control group)

Brain Region	%Control CRF	p-value	%Control SRIF	p-value
A				
AC	64.4 $\pm$ 19.2	0.017 *	93.6 $\pm$ 10.4	0.610 (ns)
AS	104.3 $\pm$ 9.0	0.780 (ns)	103.3 $\pm$ 6.2	0.700 (ns)
CB	131.2 $\pm$ 6.5	0.004 **	132.5 $\pm$ 13.1	0.100 (ns)
CC	82.7 $\pm$ 23.2	0.630 (ns)	129.0 $\pm$ 9.5	0.050 *
DBS	115.8 $\pm$ 7.3	0.240 (ns)	89.4 $\pm$ 7.3	0.170 (ns)
EC	112.1 $\pm$ 6.7	0.420 (ns)	138.5 $\pm$ 8.7	0.030 *
FC	95.4 $\pm$ 9.1	0.730 (ns)	73.7 $\pm$ 7.5	0.007 **
HIP	74.0 $\pm$ 28.7	0.450 (ns)	75.2 $\pm$ 8.8	0.030 *
HYP	152.3 $\pm$ 11.8	0.010 **	107.8 $\pm$ 7.1	0.170 (ns)
NA	117.0 $\pm$ 11.3	0.330 (ns)	96.4 $\pm$ 4.8	0.670 (ns)
PC	115.8 $\pm$ 13.6	0.530 (ns)	99.2 $\pm$ 7.4	0.940 (ns)
PCX	101.6 $\pm$ 16.7	0.920 (ns)	101.2 $\pm$ 10.5	0.930 (ns)
VBS	98.2 $\pm$ 8.7	0.880 (ns)	82.1 $\pm$ 4.9	0.007 **
B				
AC	87.4 $\pm$ 9.1	0.180 (ns)	91.9 $\pm$ 14.1	0.560 (ns)
AS	96.1 $\pm$ 11.4	0.820 (ns)	97.9 $\pm$ 6.8	0.820 (ns)
CB	75.4 $\pm$ 8.6	0.014 *	99.5 $\pm$ 9.5	0.980 (ns)
CC	176.2 $\pm$ 13.8	0.002 **	119.3 $\pm$ 10.6	0.190 (ns)
DBS	82.4 $\pm$ 8.8	0.080 (ns)	79.7 $\pm$ 5.5	0.005 **
EC	90.7 $\pm$ 10.8	0.430 (ns)	145.7 $\pm$ 9.1	0.003 **
FC	137.9 $\pm$ 12.7	0.030 *	114.6 $\pm$ 5.9	0.230 (ns)
HIP	14.6 $\pm$ 18.7	0.001 ***	119.0 $\pm$ 5.7	0.100 (ns)
HYP	86.0 $\pm$ 15.3	0.290 (ns)	92.0 $\pm$ 7.2	0.360 (ns)
NA	139.5 $\pm$ 10.7	0.510 (ns)	79.1 $\pm$ 8.9	0.040 *
PC	125.4 $\pm$ 16.7	0.310 (ns)	123.7 $\pm$ 10.5	0.170 (ns)
PCX	112.1 $\pm$ 6.3	0.300 (ns)	78.7 $\pm$ 5.4	0.007 **
VBS	94.4 $\pm$ 9.4	0.630 (ns)	77.6 $\pm$ 6.5	0.007 **
C				
AC	89 $\pm$ 16	0.6400 (ns)	80 $\pm$ 9	0.2200 (ns)
AS	120 $\pm$ 16	0.3000 (ns)	74 $\pm$ 10	0.0520 (ns)

Brain Region	%Control CRF	p-value	%Control SRIF	p-value
CB	81 ± 7	0.0300 *	58 ± 8	0.0020 **
CC	145 ± 6	0.0008 ***	104 ± 12	0.7700 (ns)
DBS	111 ± 7	0.2000 (ns)	67 ± 4	0.0008 ***
EC	125 ± 7	0.0080 **	93 ± 7	0.4400 (ns)
FC	135 ± 7	0.0020 **	94 ± 6	0.4400 (ns)
HIP	103 ± 9	0.8200 (ns)	79 ± 7	0.1100 (ns)
HYP	82 ± 5	0.0400 *	66 ± 4	0.0001 ***
NA	151 ± 10	0.0001 ***	90 ± 5	0.2300 (ns)
PC	60 ± 29	0.4300 (ns)	107 ± 9	0.6600 (ns)
PCX	174 ± 7	0.0001 ***	107 ± 10	0.5500 (ns)
PO	75 ± 5	0.0300 *	85 ± 6	0.1100 (ns)
VBS	78 ± 4	0.0060 **	91 ± 7	0.4100 (ns)

* = $p < 0.05$,

** = $p < 0.01$,

*** = $p < 0.001$,

ns = not significant.

Table 5

Represents the corticosterone values from each time point measured by RIA from trunk blood. Data is represented as mean and standard error of the mean. The p values represent the comparison between the APP mice and the respective controls at each time point

TREATMENT	N	Mean	Std Err	P value
12 MONTH TIME POINT				
A	15	6.7	6.5	0.03
C	15	11.9	5.5	
18 MONTH TIME POINT				
A	14	7.2	2.1	0.07
C	20	16.9	4.0	
4 MONTH TIME POINT				
A	13	47.3	17.9	0.17
C	16	23.9	3.8	