Neuroscience 152 (2008) 594–600

ACQUISITION OF CONDITIONED TASTE AVERSION IS IMPAIRED IN THE AMYLOID PRECURSOR PROTEIN/PRESENILIN 1 MOUSE MODEL OF ALZHEIMER'S DISEASE

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Abstract—Research into the underlying mechanisms of cognitive dysfunction in Alzheimer's disease (AD) has relied traditionally on tasks such as the water maze which evaluate spatial learning and memory. Since non-spatial forms of memory are also disrupted by AD, it is critical to establish other paradigms capable of investigating these deficits. Utilizing a non-spatial learning task, acquisition of conditioned taste aversion (CTA) was evaluated in a mouse model of AD. This line of transgenic mice encode a mutated allele of the human amyloid precursor protein (APP) and presenilin 1 (PS1) genes and exhibit extensive amyloid plaque deposition in the brain by 6–7 mo of age. Compared with wild-type mice, 10–17 month old APP/PS1 mice failed to acquire CTA to saccharin. Mice that only possessed one of the two mutations were able to acquire CTA to the saccharin. In 2–5 month old APP/PS1 mice acquisition of CTA was disrupted despite the lack of extensive plaque deposition. However, further analysis indicated a potential gender difference in both the CTA deficit and onset of plaque deposition with females showing greater conditioned aversion. Published by Elsevier Ltd on behalf of IBRO.

Key words: Alzheimer's disease, learning, transgenic mice, conditioned taste aversion.

Alzheimer's disease (AD) is one of the most prominent neurodegenerative disorders associated with aging. The hallmark symptom of AD is impaired learning and memory function that progresses from mild to severe. AD is characterized pathologically by extra-cellular deposition of beta-amyloid (Aβ) plaques and intra-cellular neurofibrillar tangles consisting of aggregates of hyperphosphorylated protein tau (Selkoe, 1989; Mandelkow and Mandelkow, 1998). The pathology is first observed in the hippocampal and cortical regions and has been shown to be correlated with deficits in learning and memory (Convit et al., 1997; De Leon et al., 1997), although this linkage remains controversial (Morgan, 2003).

Over the past decade the use of mice expressing human familial mutations of AD has provided insight into the relationship between the pathological mechanisms and memory impairments (Ashe, 2001). Mice expressing mutations in amyloid precursor protein (APP), presenilin 1 (PS1), presenilin-2 (PS2), tau or apolipoprotein (apoE) have been generated to model various behavioral, physiological, pathological and biochemical aspects of AD (Corder et al., 1998; Ashe, 2000; Lewis et al., 2001; Gordon et al., 2002; Hwang et al., 2002; Jolas et al., 2002; Teter et al., 2002). The mice developed thus far to investigate the pathology and learning and memory associated deficits in AD do not include a mouse that possesses all of the pathophysiology of AD. However, even though they do not model all of the symptoms, these mice have proven useful tools for evaluating the contribution of specific genes to learning and memory deficits as well as potential therapeutics for alleviating these impairments.

The evaluation of learning and memory deficits in these mice to date has focused primarily on hippocampal-dependent spatial tasks, such as the water maze. However, the deficits associated with AD are not limited to spatial information (Pepin and Eslinger, 1989; Hom, 1992). A review of the human literature indicated AD patients display deficits in explicit memory as well as implicit memory deficits for verbal and visuoperceptual information (Carlesimo and Oscar-Berman, 1992). Therefore, it is important to evaluate if particular mouse models exhibit deficits on non-spatial learning and memory tasks involving brain regions other than the hippocampus.

Conditioned taste aversion (CTA) is a simple pavlovian conditioning paradigm evaluating non-spatial learning and can be rapidly acquired in even a single trial. CTA involves pairing the taste of a novel conditioned stimulus, such as a saccharin solution, with an aversive unconditioned stimulus such as nausea. One advantage of CTA is that the pathways for processing of the gustatory conditioned stimulus and unconditioned stimulus information are relatively well-known (for a detailed review see Welzl et al., 2001). Parallel processing of both the conditioned and unconditioned stimulus occurs at the nucleus of the solitary tract, parabrachial nucleus of the pons, parvicellular thalamic ventral posteromedial nucleus, amygdala and agranular insular cortex, indicating that an association between the conditioned stimulus and unconditioned stimulus could form at any of these locations. Other structures that have been implicated in the formation of a CTA include the

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Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, beta-amyloid; CTA, conditioned taste aversion; PS1, presenilin 1.

0306-4522/08$32.00+0.00 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2008.01.025

hypothalamus (Roth et al., 1973), basal forebrain (Lopez-Garcia et al., 1993) and potentially the noradrenergic system (Dunn and Everitt, 1987). Lesions of the hippocampus impact CTA only mildly, or not at all (Best and Orr, 1973; Murphy and Brown, 1974; Yamamoto et al., 1995). Therefore, CTA appears to be an ideal paradigm for evaluating non-spatial forms of learning, not dependent on hippocampal function, in mouse models of AD.

A recent study investigating CTA in TgCRND8 mice with a double mutation in human APP genes (Janus et al., 2004) found that TgCRND8 mice exhibited a deficit in the acquisition of CTA. Another study reported no acquisition deficits in CTA in P301L transgenic mice exhibiting neurofibrillary tangles by 6 months of age; however, they did note accelerated extinction of the CTA (Pennanen et al., 2004). More recently a study has shown that mice with reduced levels of neprilysin, a major Aβ degrading enzyme, exhibit weaker CTA that extinguished faster than age-matched controls (Madani et al., 2006). Given these findings, CTA may be a useful model for assessing learning and memory deficits in mouse models of AD, but may depend on the AD-related genes involved.

To further validate CTA as a model to evaluate learning and memory deficits in mouse models of AD, we conducted a series of experiments to evaluate CTA in another mouse model of AD. The mice utilized were double transgenic mice expressing a human familial mutation in both APP_{swe} and $PS1_{\Delta e9}$. These mice exhibit Aβ deposition as well as learning and memory deficits by 6 months of age (Savonenko et al., 2005). The initial experiment was designed to replicate the findings reported by Janus et al. (2004) utilizing 10–17 month old mice after the reported onset of Aβ deposition. The second experiment examined the contribution of each transgene alone in 9–17 month old mice to assess if the deficit in CTA could be attributed to a single mutated gene, and the final experiment evaluated CTA in mice 2–5 months of age, prior to reported plaque deposition, in an attempt to assess if a deficit in CTA was correlated with Aβ deposition.

EXPERIMENTAL PROCEDURES

Animals

The mice used for this study were male and female APP_{swe}/$PS1_{\Delta E9}$ dtg (+/+), heterozygous for APP (+/−) or PS1 (−/+), or wildtype (−/−) mice on a mixed strain background (primarily C57BL/6 and C3He/J), derived from founder mice originally developed and donated by D. Borchelt and colleagues at the Johns Hopkins School of Medicine, Baltimore, MD, USA (Borchelt et al., 1997). The mice were raised in the vivarium at the Gerontology Research Center under controlled experimental conditions (22±1 °C, 70±10% humidity) with a 12-h light/dark cycle. All of the procedures were approved by the Animal Care and Use Committee of the Gerontology Research Center and followed the NIH guidelines for the Care and Use of Laboratory Animals. Every effort was made to minimize the number of animals used and their suffering.

Conditioning procedures

At the initiation of the experiments, mice were moved to individual hanging wire cages with solid Plexiglas inserts placed on the bottom of the cage, and paper bedding was provided to cover the bottom of the cage. The mice had *ad libitum* access to food for the duration of the experiment. Water was provided through two 15 ml tubes attached to the outside of each cage. Access to water was limited to 7 h each day from 08:00–15:00 h. The water intake of mice for the first 30 min was measured, and on average the mice consumed around 1 ml of water during the first 30 min each day. The mice were also weighed each day prior to the bottles being attached to the cages. After 6 days of habituation where only water was provided, mice were given a conditioning day where a 0.5% saccharin solution serving as the conditioned stimulus (saccharin sodium salt, Sigma Chemical Co., St. Louis, MO, USA) was provided in one bottle during the first 30 min period. For the remainder of the 7-h period, water was available in both bottles. The location of the saccharin bottle on the conditioning day was counterbalanced across experimental groups. One hour after the saccharin solution was removed, mice were given an i.p. injection of either lithium chloride (LiCl: 0.14 M, 2% of the body weight; Sigma Chemical Co.: L7026), or a corresponding amount of saline. The LiCl served as the unconditioned stimulus by producing a behavioral malaise. The following day consisted of a recovery period where mice had normal access to water in both bottles. Two days following conditioning, mice were given a two-bottle choice test in which the 0.5% saccharin solution was presented in one bottle with water in the other. The location of the saccharin on the cage was pseudo-randomized across experimental groups and the location of the saccharin solution on the conditioning day. Saccharin intake was expressed as a percentage of the total fluid intake (ml saccharin/(ml saccharin−ml water)×100).

The group numbers for the three experiments were: experiment 1 (10–17 month old APP/PS1 or wild-type mice; mean age=14.1): APP/PS1–saline (N=13; mean age=13.6); APP/PS1–LiCl (N=13: mean age=14.2); wild-type–saline (N=6: mean age=15.0); wild-type–LiCl (N=7: mean age=14.0): experiment 2 (9–17 month old APP alone, PS1 alone or wild-type mice; mean age=13.4): APP–saline (N=8: mean age=13.6); APP–LiCl (N=9: mean age=13.5); PS1–saline (N=8: mean age=13.6); PS1–LiCl (N=8: mean age=13.0): experiment 3 (2–5 month old APP/PS1 or wild-type mice; mean age=3.5): APP/PS1–saline (N=11: mean age=3.5); APP/PS1–LiCl (N=11: mean age=3.6); wild-type–saline (N=11: mean age=3.5); wild-type–LiCl (N=12: mean age=3.4). The numbers of males and females were roughly equivalent within groups, and the age range of mice was counterbalanced across groups., e.g. the mean ages for the mice in experiment 1 were: APP/PS1–saline=13.6 months; APP/PS1–LiCl=14.2 months; wild-type–saline=15.0 months; wild-type–LiCl=14.0 months.

Histology

At the completion of the behavioral testing, mice were perfused with phosphate-buffered saline followed by paraformaldehyde. The brains were removed and frozen for sectioning. The brain slices were stained with Congo Red solution and counterstained with Cresyl Violet. The hippocampus, amygdala and cortex were visually examined for the presence of congophilic amyloid deposits, but the number of plaques was not quantified.

Data analysis

Planned comparisons were conducted to evaluate saccharin preference after treatment with saline versus LiCl within each genotype. The critical α level was set at 0.05 for all statistical tests. The values in the figures represent the means±S.E.M. The data were analyzed using the SPSS statistical program version 11.0.