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Research Report

Habituation, discrimination and anxiety in transgenic mice overexpressing acetylcholinesterase splice variants

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ABSTRACT

TgS and TgR transgenic mice overexpress different splice variants of acetylcholinesterase and serve as models for genetic disruption of the cholinergic system. Whereas the TgS mouse overexpresses synaptic AChE, the TgR mouse overexpresses the rare readthrough variant whose C-terminal lacks the cysteine residue which permits adherence to the membrane. The two genotypes were compared to the parent strain, FVB/N mice on locomotion, discrimination learning and anxiety behavior following two exposures to the elevated plus maze. Male TgS mice were slower to acquire a simple odor discrimination, failed to habituate to a novel environment but were not impaired on reversal or set shifting compared to the FVB/N or TgR mice. In addition, TgS mice showed less avoidance behavior on the first exposure and but less exploration on the second exposure to the EPM. TgR mice were not impaired on discrimination learning; however, the females showed excessive running in circles in the activity meter. The findings suggest that the effects of overexpression of AChE are unique to different splice variants and may be sex-dependent.

1. Introduction

Cholinergic projections play a vital role in modulating activity of the cerebral cortex, thalamus and hippocampus, as well as mesencephalic limbic structures (Mesulam, 1994). Forebrain and brainstem cholinergic projections influence behaviors related to attention (Sarter and Bruno, 1997), and discrimination learning in rodents (Davidson et al., 1999; Levin et al., 2001) and monkeys (Davidson et al., 1999; Fine et al., 1997; Muggleton et al., 2003). Sustained visual attention tasks are particularly sensitive to deficits in forebrain cholinergic transmission, whereas spatial memory tasks are less susceptible (Balducci et al., 2003; Everitt and Robbins, 1997; Humby et al., 1999). In addition to their influence on cognition, forebrain cholinergic neurons are involved in the hormonal and behavioral responses to stress in adult rodents (Zhu et al., 2001; Meshorer et al., 2002). Hippocampal and cortical ACh release is enhanced as a consequence of novelty, pain or stress (Acquas et al., 1996; Kaufer et al., 1998; Ceccarrelli et al., 1999). In response to excessive release of ACh following stress, there is also an increase in the rare readthrough splice variant of AChE (designated AChE-R) (Kaufer et al., 1998; Meshorer et al., 2002; Nijholt et al., 2004; Perrier et al., 2005). AChE-R, like the synaptic acetylcholinesterase (AChE-S), hydrolyzes acetylcholine (ACh), but the C-terminal of AChE-R lacks the cysteine residue, preventing its adherence to the membrane (Soreq and Seidman, 2001). In humans, elevated AChE-R cleavage was correlated with attenuation of the elevated AChE activity, reduced secretion of inflammatory cytokines, an increase in cortisol and impaired memory after an injection of endotoxin

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(Cohen et al., 2003), suggesting that AChE-R cleavage counteracts the stress-induced elevation in ACh and affects the HPA axis.

The influence of stress on alternative mRNA splicing is thought to be one mechanism for coping with stress, although the functional significance has yet to be determined in some cases. Social stress in shrews significantly decreased the expression of the STREX (stress regulated exon) variant of the calcium-activated potassium channel (BK), perhaps in order to promote passive coping (McCobb et al., 2006). The STREX splice variant has been associated with enhanced ability for repeated firing compared to the other splice variant ZERO (no insert). During early stages of gestation, the STREX variant is more abundant than the ZERO variant, but by postnatal day 35, the proportions have reversed and most BK is expressed as ZERO (MacDonald et al., 2006).

Another example of alternative splicing related to stress may be seen in the D3 dopamine receptor, which has seven splice variants. The D3nf variant, which was associated with decreased binding of dopamine, was identified in the cortex of brains from schizophrenic patients. D3 agonists reduce locomotion and the D3nf variant leads to less efficient binding to the receptor (Pritchard et al., 2006). Alternative splicing of the D3 receptor has been shown to account for approximately one third of the variance between high (HR) and low (LR) responders to a novel environment. HR tend to show more activity in a novel environment, a greater tendency to self-administration of stimulant drugs and higher stress-induced release of dopamine, compared to LR and a lower D3/D3 nf ratio than LR in the substantia nigra/ventral tegmental area (SN/VTA). Moreover, a negative correlation was found between novelty stimulated locomotion and the D3/D3nf ratio in prefrontal cortex, and SN/VTA (Pritchard et al., 2006).

Social stress was also shown to affect splicing of the gene for Brain Derived Neurotrophic factor (BDNF). Although the functional significance of the different splice variants of the Bdnf gene is unknown, they are thought to affect histone remodeling. Chronic social stress downregulated expression of splice variants III and IV of the Bdnf gene, without affecting variants I, II and V and this downregulation was reversed by chronic, but not acute antidepressant treatment. In addition, antidepressants in unstressed mice upregulated the expression of the same splice variants of the Bdnf mRNA (Tsankova et al., 2006).

AChE also has non-catalytic functions in development and in cell maintenance (Soreq and Seidman, 2001). In the nervous system, AChE-R is associated with increased cytokine production (Grisaru et al., 2006), altered function of cell adhesion proteins, increase in glucocorticoids (reviewed in Meshorer and Soreq, 2006), stimulation of protein kinase C (PKC) and recruitment of the PKC1 receptor, RACK1 (Sklan et al., 2006). Neurite sprouting in the central nervous system (Day and Greenfield, 2002) and axonal regeneration in the peripheral nervous system (Keymer et al., 1999) are considered to be non-catalytic effects of AChE.

During gestation, the synaptic and readthrough variants of the enzyme have interactive effects on neuronal migration and proliferation (Dori et al., 2005). In adults, AChE-R mRNA was elevated in the cortex for as long as 14 days after closed head injury, and neurological sequelae of injury, such as excessive neurite sprouting and hippocampal cell loss, were prevented by an antisense oligonucleotide targeted to AChE-R (Shohami et al., 2000). Mice transgenically overexpressing AChE-S show early neuronal degeneration (Sternfeld et al., 2000) and are more susceptible to neuronal loss and mortality following closed head injury (Shohami et al., 2000). TgS and TgR transgenic mice overexpress primarily AChE-S and AChE-R, respectively. The TgS mice were found to be more active than FVB/N mice in an open field (Erb et al., 2001), but not in their home cage and slower to adapt to changes in the light/dark cycle (Cohen et al., 2002). TgS mice showed a learning deficit in the social recognition (Cohen et al., 2002), and Morris water maze spatial tasks (Beeri et al., 1995), and performance of a procedural learning task, whereas TgR mice showed enhanced performance on the latter task. In parallel, the TgS showed impaired, and the TgR improved LTP maintenance (Farchi et al., 2007). However, in the Morris water maze task, TgS mice swam more slowly even to a visible platform (Beeri et al., 1995), suggesting that water maze impairment was not related to spatial learning. Several studies tested anxiety behavior in these transgenic mice, with inconsistent results. Less anxiety was reported in the TgS (Erb et al., 2001) and more stress-induced anxiety reported in the TgR mice (Birikh et al., 2003); however, a recent study found no difference among TgS, TgR and the FVB/N parent strain (Farchi et al., 2007). In order to characterize the behavioral phenotype of TgS and TgR mice, locomotion, discrimination learning and double exposure to the EPM were tested.

Mice were tested on odor discrimination tasks that involved higher order learning such as reversal or set shifting, in contrast to the sensorimotor learning studied by Farchi et al. (2007). Tasks that are not dependent on vision were chosen as the parent strain of FVB/N mice shown to have retinal degeneration (Broide et al., 1999). In the present study, the dependent variable was not dependent on motor strength or speed. It was predicted that TgS mice would be impaired in initial and higher-order learning, whereas TgR mice would make fewer errors on these tasks.

Re-exposure to the EPM has been shown to measure different forms of anxiety than the first exposure (Espejo, 1997; File et al., 1998), to be sensitive to cholinergic drugs in adults (File et al., 1998) and to developmental exposure to an AChE inhibitor (Kofman and Ben-Bashat, 2006). Therefore, the effect of double exposure to the EPM on TgS and TgR mice was tested. Sex differences were also investigated as effects of early exposure to AChE inhibitors can be sex-dependent (Aldridge et al., 2005; Kofman and Ben-Bashat, 2006) and these have never been reported in the studies describing the phenotype of TgS and TgR mice.

2. Results

2.1. *Experiment 1. Analysis of locomotor activity in an open field*

Fig. 1 shows a significant interaction between sex and genotype $F(2,63)=3.26$, $p<.05$ in the analysis of covariance (ANCOVA) of the locomotor activity of TgS, TgR and FVB/N mice, with weight as a continuous variable. Contrast analysis

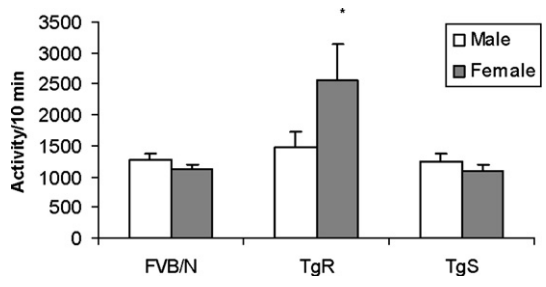

Fig. 1 – Activity in an automated activity meter (mean + SEM). The females from the AChE-R group showed significantly greater activity. No significant differences were found among the males.

revealed that the TgR female mice had significantly greater activity than the females from the FVB/N control and TgS genotype $F(1,63)=6.49$, $p=.01$. The most striking finding is that the female TgR mice tended to run rapidly in circles in the middle of the arena throughout the session, rather than exploring the environment. No differences were found among the males. Weight differed significantly between the two sexes, $F(1,64)=105.4$, $p<.000001$, such that females weighed less than males and among the genotypes, $F(2,64)=54.48$, $p<.000001$, such that TgS mice weighed more than FVB/N mice and TgR (post hoc Scheffé test, $p<.000001$), and TgR mice weighed less than FVB/N mice as well ($p<.05$) but the results of the analysis of sex by genotype interaction remained significant even if weight was not used as a covariate, $F(2,64)=3.26$, $p<.05$.

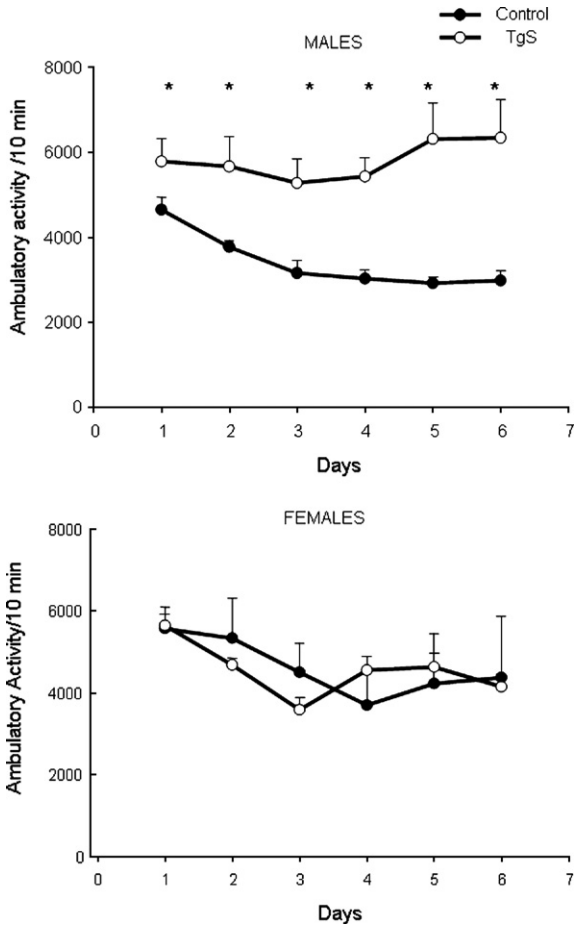

Fig. 2 – Ambulatory activity (mean + SEM) measured for 10 min per day in male (top) and female (bottom) TgS and FVB/N control mice. In male mice the TgS mice were significantly more active from days 1 to 6.

2.2. *Experiment 2. Habituation of exploratory locomotor activity*

Fig. 2 shows significant interactions between sex and genotype (FVB/N and TgS), $F(1,28)=6.70$, $p<.05$ and day and genotype $F(5,140)=2.94$, $p<.05$. Further analysis showed that the male TgS mice showed higher activity than the male FVB/N mice on days 1–6, indicating absence of habituation to the novel environment, $F(1,28)=13.96$, $p<.001$, in contrast to the previous experiment. The female TgS mice did not differ from the female FVB/N mice, $F(1,28)=.01$, $p=.91$. In fact, the females of both genotypes showed significant habituation by the third day, $F(1, 28)=20.84$, $p<.0001$, and every day thereafter.

2.3. *Experiment 3. Odor discrimination and set shifting*

The number of days to reach criterion was measured for the effects of type of switch (ID, ED), genotype (FVB/N control, TgS and TgR) as between subject variables and type of discrimination (pre-shift, post-shift) and criterion (discrimination, learning) as within subject variables. Data were transformed to logarithms (log 10), following the Levene's test of homogeneity of variance. There was a significant interaction between shift, criterion and group, $F(2,44)=3.67$, $p<.05$, as well as the expected significant interaction between shift and criterion, $F(1,44)=251$, $p<.000001$. Since there was no significant effect of the type of shift (ID, ED), $F(1, 44)=1.13$, $p=.29$, and no interactions between shift and genotype, $F(2,44)=.34$, $p=.72$., switch and criterion, $F(1,44)=.17$, $p=.68$, or shift × genotype × criterion, $F(2,44)=1.93$, $p=.16$, the data were reanalyzed combining the two types of shift tasks. This analysis revealed a significant interaction between shift and criterion, such that the "rule learning" criterion was attained more quickly post-shift than pre-shift, $F(1,47)=265$, $p<.000001$. Fig. 3 illustrates a significant

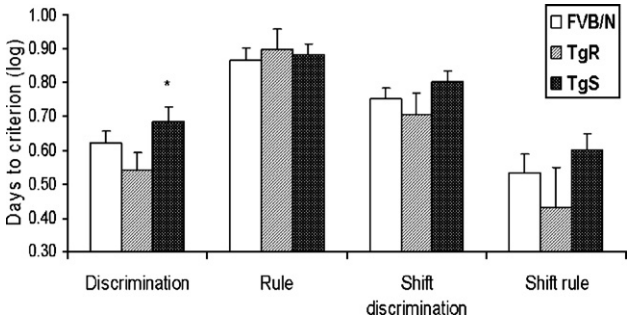

Fig. 3 – Number of days to reach discrimination and rule learning criterion in the simple odor discrimination and in the set-shifting paradigm (mean + SEM). AChE-S mice were slower to learn the initial discrimination, but were not impaired on shifting. *$p<.05$.

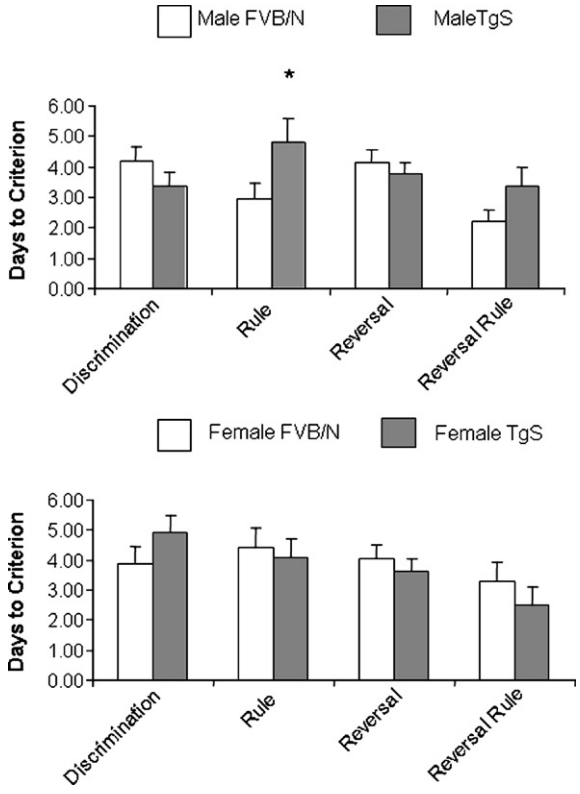

Fig. 4 – Number of days to reach learning criteria (mean + SEM) in male (top) and female (bottom) TgS and FVB/N control mice. The TgS male mice took longer to reach the rule learning criterion than did the FVB/N group. *($p < .05$).

interaction between group, learning criterion and shift (pre-post), $F(2, 47) 4.07$, $p < .03$. Analysis of contrasts between the groups showed that the TgS mice were slower to attain the discrimination criterion in the first odor discrimination compared to the TgR and the FVB/N control mice, but once they attained the criterion, they were not slower to attain rule learning. Following the shift, there was no difference between groups in the rate of learning.

2.4. *Experiment 4. Odor discrimination and reversal*

The number of days to reach each criterion was analyzed for the effect of genotype (FVB/N vs. TgS), criterion (initial discrimination, initial learning, reversal discrimination, reversal learning as a repeated measure) and sex. The reversal criterion (3 consecutive days) was learned more quickly than the first discrimination, consistent with the suggestion that rule learning is involved, as revealed by a main effect for criterion, $F(3,189) = 3.80$, $p < .01$, in the three-way analysis of variance for sex, genotype and criterion (repeated measure). The interaction between sex and genotype was close to significant, $F(3,180) = 2.52$, $p = .058$, suggesting that further analysis was warranted. Fig. 4 shows that the TgS males were slower to attain the rule learning criterion than the FVB/N males ($p < .05$) on the first discrimination, but not on the reversal learning. Retention errors for the day after reaching the first learning criterion for both the initial discrimination and the reversal of the discrimination were analyzed with task as a repeated measure. There was a significant interaction between treatment group and sex, $F(1,60) = 4.46$, $p < .05$), indicating that male TgS mice made more errors than the FVB/N males, whereas there was no difference between TgS females and FVB/N females (Table 1).

2.5. *Experiment 5. Double exposure to the elevated plus maze*

Fig. 5 illustrates the effects of genotype for the Exploration Index and Avoidance Index as detailed below.

2.5.1. *Exploration Index*

As expected, there was a significant effect of exposure, $F(1,54) = 27.43$, $p < .000005$, indicating less exploration on the second exposure. There was a significant effect of genotype, $F(2,54) = 3.23$, $p < .05$, indicating less exploratory behavior by the TgS mice. No main effect of sex or interactions between sex and other variables was found. The near-significant interaction between Exposure and Genotype, $F(2, 54) = 2.95$, $p = .06$, was analyzed further in order to further clarify the main effect of genotype. As shown in Fig. 5, a contrast analysis showed that the TgS mice had lower exploration on the second exposure compared with the FVB/N and TgR groups, $F(1,54) = 17.50$, $p < .0005$, but not on the first exposure, $F(1,54) = .62$, $p = .43$

2.5.2. *Avoidance Index*

Fig. 5B shows that there was a significant effect of exposure, $F(1,54) = 23.31$, $p < .00005$, and a significant interaction between genotype and exposure, $F(2,54) = 4.39$, $p < .02$. Contrast analysis indicated that the TgS group showed less avoidance on the first exposure compared to the other 2 genotypes, $F(1,54)$ 4.63, $p < .05$. There were no differences among the genotypes on the second exposure.

2.5.3. *Risk Assessment Index*

There was a significant effect of exposure, $F(1,54) = 39.77$, $p < .000001$. The main effect of sex approached significance,

Table 1 – Retention errors on the day following the initial discrimination or reversal criterion

Treatment/sex	FVB/N mean (SD)		TgS mean (SD)	
	Discrimination	Reversal	Discrimination	Reversal
Male*	2.86 (1.88)	2.60 (1.72)	4.35 (1.80)	4.00 (2.06)
Female	4.06 (1.68)	3.47 (2.85)	4.40 (1.88)	2.60 (2.35)

*TgS males > FVB/N males, $p < .05$.

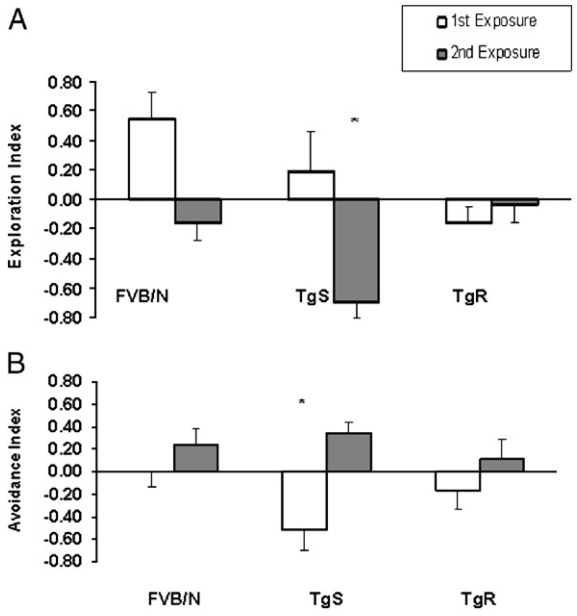

Fig. 5 – (A) Exploration Index (mean + SEM) composed of average *Z* score of time in open arms, entries into open arms and head dips from open arms for mice on the first and second exposure to an elevated plus maze. TgS mice showed a significantly greater decrease on the second exposure. *$p < .005$. (B) Avoidance Index (mean + SEM), composed of average *Z* score of time in closed arms and entries into closed arms, for mice on the first and second exposure to an elevated plus maze. TgS mice showed an increase in avoidance on the second exposure. *$p < .05$.

$F(1,54) = 3.95$, $p = .05$, indicating more risk assessment on the part or the females, as did the main effect of genotype, $F(2,54) = 2.92$, $p = .06$. The interaction between sex and genotype was not significant $F(2,54) = 2.47$, $p < .09$ (data not shown).

3. Discussion

Male TgS mice overexpressing AChE showed deficits in both non-associative and associative learning tasks. Short daily sessions of exploratory behavior suggested that the male TgS mice did not habituate to the novel environment and continued to show high levels of exploration. No learning deficits were observed in the TgR mice; however, the female TgR mice demonstrated rapid circling in the arena in Experiment 1. This behavior was also observed in the home cage. TgR mice were previously shown to have an exaggerated response to a mild stressor (saline injection) in cage emergence (Birikh et al., 2003) and the elevated plus maze, which manifested itself in a tendency to behavioral inhibition. Stress in an open field is usually associated with freezing and thigmotaxis; however, in TgR females, the exaggerated non-exploratory running behavior suggested that these mice may show a deficit in behavioral inhibition in stressful situations. The behavior was not accompanied by rearing or sniffing, or any other evidence of exploration or goal-directed behavior. The increased activity and slower habituation in the male TgS mice in Experiment 2, contrasts with Experiment 1, in which there was no difference between FVB/N and TgS males, even when the data were analyzed separately for peripheral and central activity (not shown). Although there is no obvious explanation for the difference between the two experiments, others have also observed hyperactivity in TgS mice (Erb et al., 2001).

Despite the fact that they did not readily habituate to a novel environment, the TgS mice showed less exploration than the FVB/N and TgR mice on the second exposure to the elevated plus maze, suggesting that although they did not demonstrate the classic pattern of anxious behavior upon first exposure to the elevated plus maze, they did show excessive learned fear (Espejo, 1997). Moreover, the female TgR mice, which showed a hyperactive response to an open field, did not show increased activity on the elevated plus maze.

The dissociation between activity in the EPM, an apparatus that permits withdrawal to a secure (closed) environment, and the elevated activity in the open field in TgS male and TgR female mice emphasizes the need for caution in inferring anxious or anxiolytic effects from a single measure. In the EPM, a genotype that showed elevated open field activity may show no elevation in activity, but may show enhanced avoidance on re-exposure to the maze. The male TgS mice had a slower learning curve on a simple odor discrimination task, but not on more complex learning, such as reversal learning or set-shifting. Although it is not clear why the female TgS mice were spared, the sex difference might be related to the protective effect of estrogen on cholinergic-dependent cognitive tasks (Tinkler and Voyko, 2005). The deficit observed in the TgS males was in the initial stages of odor discrimination, but not after shifting to a new discrimination. In the Experiment 3, more errors were made in the initial stages of learning, whereas in Experiment 4 more errors were made after attaining the first criterion of 8/10 correct responses. This poor performance pattern on a task governed by discrimination of extroceptive cues is reminiscent of the increased number of errors made by TgS mice on maintenance performance of a linear maze task involving proprioceptive cues and reversal (Farchi et al., 2007). Previous studies found that the cholinergic system is critical for encoding novel or conditioned stimuli (Acquas et al., 1996), and conditioning induced cortical plasticity (Sarter and Bruno, 1997) in rodents. In humans, an AChE inhibitor speeded reaction time on a working memory task for faces and reduced activation of right prefrontal cortex, suggesting that less effort was required to perform the task (Furey et al., 2000). Older subjects, who showed greater task-related increases in activity in the left and right frontal regions during placebo administration, also had a more noticeable decrease in the physostigmine condition in the right prefrontal cortex and right anterior ventral temporal cortex (Freo et al., 2005). The discrimination learning impairment in the TgS mice suggests that excess AChE may impair the ability of the mice to enhance cholinergic output in a timely manner while learning the task. TgR mice may have been spared by mechanisms that were previously referred to, such as enhanced neuronal plasticity (Farchi et al., 2007).

ACh in the TgS mice appears to be regulated by compensatory mechanisms, such as augmented choline uptake (Beeri et al., 1997), and indeed hippocampal ACh efflux was found to

be similar in TgS and FVB/N mice, despite the enhanced AChE activity (Erb et al., 2001). However, the behavioral changes in the TgS mice might be related to anomalous morphological neuronal and astrocyte features (Sternfeld et al., 2000; Cohen et al., 2002), reduced dendritic arborization and dendritic spines (Beeri et al., 1997) and impaired plasticity (Farchi et al., 2007), or inability to elevate ACh levels according to task requirements. Interestingly, a social memory deficit in these mice was partially reversed by an AChE inhibitor (Cohen et al., 2002). Deficits in early phases of learning and in habituation could also result from enhanced anxiety in a novel situation. Therefore, in the present study, the elevated plus maze anxiety test, using double exposure, was used. The second exposure in this study, as in previous studies (Espejo, 1997), is characterized by reduced exploration and augmented avoidance. In contrast to a recent finding which reported no difference among TgS, TgR and FVB/N mice on the elevated plus maze (Farchi et al., 2007), in the present study the TgS mice showed a tendency toward less avoidance on the first exposure, but less exploration upon re-exposure to the maze. The enhanced re-exposure anxiety is consistent with findings that show that TgS mice are more vulnerable to novel and stressful situations and to stress-related neuropathology (Meshorer and Soreq, 2006). ACh is involved in the regulation of the hormonal and behavioral stress responses in adult rodents (Zhu et al., 2001; Meshorer et al., 2002). AChE activity in the hypothalamus increased following mild stress, and this was partially reversible by an antisense oligonucleotide to AChE-R (Birikh et al., 2003; Nijholt et al., 2004). Constitutively high AChE may prevent an organism from increasing AChE activity and put it at risk for debilitating effects of stress. Human participants with low serum paraoxonase (PON) and high AChE activity had higher state anxiety scores (Sklan et al., 2004).

To conclude, the different behavioral profiles of TgS and TgR mice suggest that there are interactions between overexpression of AChE splice variants and sex. The previous behavioral studies on these transgenic mice tested either males or females only, or combined data from both sexes, without analyzing possible differences (Beeri et al., 1995; Birikh et al., 2003; Farchi et al., 2007). TgS and TgR mice provide a model with which developmental changes in the cortex induced by perturbation of the cholinergic system (Dori et al., 2005) can be explored to further understand the role of interaction with AChE splice variants and gonadal hormones on higher order learning and emotional reactivity. It is not known whether the behavioral deficits in discrimination learning or habituation are related to the catalytic or non-catalytic effects of AChE. However, Farchi et al. (2007) reported that the enhanced LTP decay was similar in slices from transgenic mice overexpressing an enzymatically inactive form of human AChE (AChE SIn) and those ovexpressing the active form of human AChE-S. Thus, ex vivo, the deficits in hippocampal plasticity appear to be related to non-catalytic effects of AChE. Moreover, neither carbachol nor physostigmine attenuated the rapid decay of LTP in hippocampal slices derived from TgS and TgSIn mice, suggesting that the enzymatic activity per se was not involved. On the other hand, an acetylcholinesterase inhibitor was able to partially reverse memory deficits in recognition of a conspecific in TgS mice (Cohen et al., 2002). Further research comparing TgS to TgSIn on behavior is required to unravel the relevance of the multi-faceted mechanisms of AChE to behavior.

4. Experimental procedures

4.1. *Animals*

TgS and TgR and FVB/N mice from the parent strain were received from the laboratory of Hermona Soreq, Yissum Ltd., Hebrew University, Jerusalem. Mice were subsequently bred and housed in the colony of the Health Science Faculty of Ben-Gurion University of the Negev under constant temperature of 23 °C, with *ad lib* water and food, except in the discrimination experiments that involved food deprivation. Adult mice were tested at age 4–5 months. The experimental procedures were approved by the Institutional Committee for Ethics in Animal Experimentation, according to NIH guidelines.

4.2. *Experiment 1. Analysis of locomotor activity in an open field*

Male and female mice from FVB/N control, TgS transgenic mice and TgR transgenic mice were placed for 10 min in an automated activity (Optivarimax, Model 92480, Columbus Instruments) measuring 40×40×20 cm. Ambulatory activity was recorded by sensors spaced 2.6 cm apart in a single session. The ambulatory activity was analyzed for the effects of sex (male, female) and genotype (FVB/N, TgS and TgR) with weight as covariate. The number of mice in each group was: male FVB/N (10), TgR (13), TgS (10) and 12 mice in each of the female groups.

4.3. *Experiment 2. Habituation of exploratory locomotor activity*

In this experiment, TgS genotype males and females were compared to FVB/N mice.

Ambulatory activity was monitored, as described above, for 10 min/day for 6 consecutive days. The experiment included 32 mice: FVB/N male (11), FVB/N female, TgS male, TgS female (7 each).

4.4. *Experiment 3. Odor discrimination and set shifting*

Fifty male mice were tested in 6 different groups, according to genotype and type of shift that was to be tested after the initial discrimination learning. The groups with number of mice in parentheses were as follows: FVB/N intradimensional shift (ID) (11), FVB/N extradimensional shift (ED) (10), TgS ID (10), TgS ED (10), TgR ID (4), TgR ED (5). The lower number of TgR mice was due to lower number of male births in the cohort.

The Y maze consisted of a corridor, 60 cm length×1 cm width×26 cm height, with a guillotine door located in the middle. Each of the 2 goal boxes (25×15×19 cm) was constructed with 3 walls and an open end facing the corridor at an angle of approximately 30°, such that the mice had to enter one of them when it reached the end of the corridor. Each goal box contained 2 plastic receptacles in which a piece of filter paper,

infused with .2 ml of a commercial vanilla or rum essence, was placed. The goal box with the correct odor contained a chocolate flavored 10 mg food pellet (Bio Serv Inc.).

Mice were placed on a restricted diet (2.5–3 gm/day) during the learning and testing period. First, they were habituated to the maze in pairs for 2 days and then individually for 2 more days. During this period, the mice explored and learned to find food in the goal boxes.

Thereafter, they were tested for 20 trials/day or until they reached a criterion of 8 correct responses in 10 consecutive trials. For each animal (counterbalanced between groups and sexes), either vanilla or rum was the correct choice (S+) and the other essence was the S–. The side of the S+ and S– was altered quasi-randomly for the 20 daily trials such that each stimulus appeared on one side in 50% of the trials and the S+ did not appear on the same side in more than 3 consecutive trials. Animals were tested daily. Two learning criteria were used as dependent measures: (1) the day that 8/10 correct responses were completed within the first 12 trials (discrimination criterion) and (2) the day that criterion 1 was attained for 3 consecutive days (learning criterion).

The day after the second criterion was reached, the mice were taught another discrimination. Half the mice in each group received a second odor discrimination between almond and lemon odors (intra-dimensional shift—ID) while the other half were given a spatial-left right discrimination (extra-dimensional shift—ED), in which the almond and lemon odors were present but irrelevant to the task. For the spatial task, each odor was associated in half the trials with the correct spatial choice and for the other half trials with the incorrect choice, whereas for the odor task, the correct odor was on the left for half the trials and on the right for the other half.

4.5. *Experiment 4. Odor discrimination and reversal*

Thirty-two FVB/N control (17 female, 15 males) and 32 TgS (15 female and 17 male) were tested in a Y maze with removable goal boxes, using the same habituation and training procedure as described above. Two learning criteria were used as dependent measures: (1) the day that 8/10 correct responses were completed within the first 12 trials (discrimination criterion) and (2) the day that criterion 1 was attained for 3 consecutive days (learning criterion). The day after the second criterion was reached, the rule was reversed for each animal, that is, if the S+ had been rum, it was changed to vanilla. The same 2 criteria were applied. In addition to measuring the number of days required to reach the criterion, we measured retention errors (number of errors within 20 trials) on the days after reaching the second criterion for the discrimination and reversal learning.

4.6. *Experiment 5. Double exposure to the elevated plus maze*

Male and female mice derived from 3 genotypes: FVB/N, TgS and TgR were tested at age 4 months on the elevated plus maze, as described above. Each group consisted of 10 mice. The elevated plus maze consisted of 2 transparent closed arms (47 cm length × 5 cm width × 20 cm height) and 2 open arms (47 cm length × 5 cm width, with a 3 cm ledge, to prevent falling), in the form of a plus, elevated .5 m above the floor. The mice were placed in the maze for 6 min and behavior was videotaped. Forty-eight hours later, the mice were re-exposed to the EPM in the same manner. The maze was cleaned with a dilute solution of ethanol and water after each mouse. The tapes were analyzed by an observer blind to the treatment condition. The following behaviors, described in previous studies (Espejo, 1997; Rodgers and Dalvi, 1997), were recorded: entries into open and closed arms, time spent in open and closed arms, time spent in the center square (5 × 5) at the juncture of the arms, head dips made when the mouse was entirely (all 4 paws) in the open arms (unprotected head dips) and head dips when the body was in central juncture (protected head dips). The multiple variables that are traditionally analyzed were combined into three indices of exploratory, risk assessment and avoidance based on localization of the behavior in the maze. Because the measures included counts of behavior and time measurements, the values of both exposure were converted to standard scores for each group. The Exploration Index (EI) consisted of the mean Z score for the time spent in open arms, entries to open arms and unprotected head dips. The Risk-Taking Index (RTI) was the mean Z score of the time spent in the central area and protected head dips and the Avoidance Index (AI) was the mean Z score of the time spent in closed arms and entries into closed arms. Each of the three indices was analyzed for genotype (FVB/N, TgS, TgR) and sex with Exposure (first, second) as a repeated measure.

4.7. *Statistical analysis*

Data were analyzed using the GLM module of Statistica (Statsoft, Version 7.0). In Experiments 1 and 2, weight was included as a covariate, as the size of the animal could affect the distance covered by the mouse. Experiments 3 and 4 analyzed the effects of genotype and sex, considering each stage of learning (criteria) as a successive within-subject variable. In Experiment 5, each index of the elevated plus maze behavior was analyzed using a three-way ANOVA for the effects of sex (male, female), and genotype (FVB/N, TgS, TgR) as between subject variables and exposure (first and 48 h later) as a within-subject measure.

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