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Performance of galanin transgenic mice in the 5-choice serial reaction time attentional task

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Abstract

The neuropeptide galanin impairs learning and memory in rodents. The mechanism underlying the cognitive effects of galanin may be related to inhibitory effects of galanin on cholinergic transmission. As cholinergic function is thought to modulate sustained attention, the present study examined whether galanin-overexpressing transgenic mice have impairments in sustained attention. Galanin transgenic (GAL-tg) mice and wild-type (WT) littermate controls were trained in a 5-choice serial reaction time task, modified to assess sustained attention. GAL-tg and WT mice performed similarly during acquisition with respect to accuracy, total omissions, and response speed. Attentional mechanisms were challenged by parametric changes including increased event rate, event asynchrony, or decreased stimulus duration. Singly, these challenges did not differentially affect performance between genotypes. Concurrent administration of these challenges, which represents an optimal test of sustained attention, also had similar effects on GAL-tg and WT mice. When stimulus discriminability was reduced by constant illumination of the house light, GAL-tg mice omitted more trials than WT mice, but other measures of performance did not differ by genotype. Moreover, intraventricular injection of galanin in WT mice did not affect sustained attention. These data indicate that previously reported learning and memory effects of galanin are not secondary to attentional dysfunction.

Keywords: Galanin; Attention; Mice; Five-choice; Operant; Transgenic; Neuropeptide; Acetylcholine

1. Introduction

The neuropeptide galanin has widespread distribution in the mammalian central and peripheral nervous systems (Hökfelt et al., 1998; Tatemoto et al., 1983). On the cellular level, galanin inhibits adenylate cyclase activity (Karelson and Langel, 1998), carbachol-stimulated phosphotidyl inositol (PI) hydrolysis (Consolo et al., 1991; Palazzi et al., 1991) and the release of other neurotransmitters including acetylcholine, norepinephrine, serotonin, and glutamate (Consolo et al., 1991; Fisone et al., 1987; Kehr et al., 2002; Kinney et al., 1998; Robinson et al., 1996; Tsuda et al., 1992; Yoshitake et al., 2003; Zini et al., 1993). On the behavioral level, galanin stimulates feeding (Corwin et al., 1993; Crawley, 1999; Crawley et al., 1990; Kyrkouli et al., 1986, 1990), modulates anxiety- and depression-like behavior (Holmes et al., 2002), and impairs learning and memory (Wrenn and Crawley, 2001).

The deleterious effects of galanin on learning and memory are well documented in a range of behavioral paradigms. In rats, both intraventricular (McDonald and Crawley, 1996; Robinson and Crawley, 1993) and intrahippocampal (Robinson and Crawley, 1994) galanin impaired working memory in an operant delayed non-matching to position task. Intraventricular (Sundström et al., 1988) and intrahippocampal (Ögren et al., 1996) injection of galanin also impaired the acquisition of spatial memory as assessed in the Morris water maze. In other spatial navigation tasks, intraventricular galanin impaired acquisition in the starburst radial maze (Malin et al., 1992), and intraseptal galanin impaired spatial working memory in the T-maze (Givens et al., 1992). More recently, post-training

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intraventricular injection of galanin blocked consolidation of spatial memory in the water maze (Kinney et al., 2003).

The cognitive effects of galanin are of clinical interest because of the observation that galanin is overexpressed in the basal forebrain of Alzheimer's disease (AD) patients (Beal et al., 1990; Bowser et al., 1997; Chan-Palay, 1988). This pathological observation coupled with the pharmacological data from the rat studies, described above, has led to the hypothesis that galanin contributes to the cognitive dysfunction that is characteristic of AD (Counts et al., 2001; Hökfelt et al., 1987; Wrenn and Crawley, 2001). This hypothesis has been recently tested using transgenic mice that overexpress galanin (GAL-tg). Reported functional changes in GAL-tg mice have included an increased resistance to seizures induced by perforant path stimulation, systemic kainic acid, or pentylenetetrazol administration (Mazarati et al., 2000) and reduced basal acetylcholine release in the ventral hippocampus (Laplante et al., 2004). Similar to the spatial deficits produced by galanin administration to rats, GAL-tg mice were impaired in the probe trial of the Morris water maze (Steiner et al., 2001). Additionally, GAL-tg mice were impaired in olfactory memory in the social transmission of food preference task (Wrenn et al., 2003) and in emotional memory in a trace version of fear conditioning (Kinney et al., 2002). These impairments were seen in the absence of changes in critical control measures ruling out the possibility that they were artifacts due to changes in sensory or motor function. However, the hypothesis that galanin exerts its detrimental effects on learning and memory by interfering with attentional function has not been addressed directly. Addressing this alternative interpretation of the GAL-tg phenotype is necessary because attention (which refers to the detection of stimuli) and memory (which refers to the recall of stimuli) are likely to be closely associated (Sarter et al., 2003). This issue can only be addressed using attentional paradigms such as the 5-choice serial reaction time task (5-CSRTT) because the available learning and memory paradigms may not adequately tax attentional processes (Sarter et al., 2003).

The possibility that galanin affects attentional processes is based on the considerable literature that galanin inhibits cholinergic function (McDonald and Crawley, 1997) and that cholinergic activity regulates attentional processes (described below). The inhibition of central cholinergic function by galanin is evident in studies from several different levels of analysis. On the biochemical level, galanin inhibited carbachol-stimulated PI hydrolysis (Consolo et al., 1991; Palazzi et al., 1991). In physiological studies using slice preparations of the hippocampus, galanin blocked the slow excitatory post-synaptic potential induced by acetylcholine in CA1 pyramidal neurons (Dutar et al., 1989). Further, galanin inhibited the evoked release of acetylcholine as demonstrated by both in vitro and in vivo studies. In hippocampal tissue slices from the rat (Fisone et al., 1987) and the monkey (Fisone et al., 1991), galanin inhibited K^+-stimulated acetylcholine release. In complementary work using in vivo microdialysis, both intraventricular (Fisone et al., 1987) and intraseptal (Robinson et al., 1996) galanin inhibited scopolamine-stimulated release of acetylcholine, and genetically overexpressed galanin reduced basal release of acetylcholine in the hippocampus (Laplante et al., 2004).

A substantial body of evidence shows that central cholinergic activity is a critical mediator of attentional function. This evidence comes from a number of experimental approaches including pharmacological and lesion studies, as well as assays of acetylcholine release during performance of attentional tasks (reviewed in (Robbins, 2002) and (Sarter et al., 2003)). For example, the disruption of the central cholinergic system either by scopolamine or by selective lesioning impairs accuracy in the 5CSRTT (Dalley et al., 2004; Humby et al., 1999; Jones et al., 1995; Jones and Higgins, 1995; Lehman et al., 2003; McGaughy et al., 2002), while only omissions increased in other studies (Chudasama et al., 2004; Risbrough et al., 2002). Moreover, evidence for a cholinergic regulation of attention is not limited to the 5CSRTT task. Highly specific cholinergic lesions of the nucleus basalis and of the prefrontal cortex using the immunotoxin 192 IgG-saporin (Wiley et al., 1991) have impaired performance in attentional tasks that assess vigilance (McGaughy et al., 1996), cross-modal divided attention (Turchi and Sarter, 1997), cued target detection (Bushnell et al., 1998), incremental attention (Bucci et al., 1998; Chiba et al., 1995), and decremental attention (Baxter et al., 1997).

Given the well documented role of central cholinergic function in the mediation of attention and the evidence of galanin's inhibitory modulation of central cholinergic function, we hypothesized that attentional dysfunction might contribute to the learning and memory deficits observed in GAL-tg mice. The rationale for this hypothesis is that even small effects of galanin overexpression on cholinergic signaling may produce attentional deficits because small, circumscribed lesions of cholinergic nuclei have successfully produced attentional dysfunction (see above). This hypothesis was tested by assessing the performance of galanin-overexpressing transgenic mice (GAL-tg) (Mazarati et al., 2000; Steiner et al., 2001) in the 5CSRTT, modified in various ways to tax sustained attention processes. As a corroborative approach, the effect of intraventricular injection of galanin on 5CSRTT performance was assessed in wild-type mice of the C57BL/6J strain.

2. Materials and methods

2.1. Mice

Experimental subjects were singly-housed male galanin transgenic (GAL-tg) mice ($n=15$) and wild-type (WT) littermate controls ($n=15$). Single-housing has been used in other studies of mice in the 5CSRTT and is not known to obscure genotype effects (van Gaalen et al., 2003; Greco et al., 2005). Mice were approximately 3 months of age at the start of the experiment. Prior to 5CSRTT training the GAL-tg mice were shown to have a deficit in trace fear conditioning, replicating a previous finding (Kinney et al., 2002) and confirming a known behavioral effect of galanin in these mice. The generation of the GAL-tg mice has been described previously (Mazarati et al., 2000; Steiner et al., 2001). Briefly, the overexpression of galanin was conditionally localized to adrenergic neurons by using a DNA construct in which the mouse galanin gene was coupled to the human dopamine β-hydroxylase promoter. Mice were generated on a mixed C57/DBA background and backcrossed for 7 generations

into C57BL/6J. The line was then rederived at the Jackson Laboratory (JAX) in Bar Harbor, ME and backcrossed for >3 generations. The line is maintained at JAX using alternate heterozygote matings and backcrosses into C57BL/6J. Mice were genotyped by PCR and identified by scanner and subcutaneous microchip. Identified mice were shipped to NIH and maintained in a temperature controlled vivarium with lights on from 6:00 AM to 6:00 PM. Food and water were available ad libitum except during training and testing in the 5CSRTT, as described below. All methods were approved by the National Institute of Mental Health Animal Care and Use Committee and followed the NIH Guidelines "Using Animals in Intramural Research."

2.2. Apparatus

Mice were trained in five operant boxes (Med Associates, St. Albans, VT), each of which was interfaced to a Dell Optiplex PC and enclosed in a sound attenuating chamber (height 42 cm, width 64.5 cm, depth 40 cm). These operant boxes were different chambers located in a different room than that used in the fear conditioning experiment mentioned above. We saw no evidence of stress sensitization, such as excessive freezing in the operant chambers, during 5-choice acquisition. The operant boxes (height, 15 cm, width 18 cm, depth 20 cm) consisted of an array of five nose poke holes (see Fig. 1A) on one panel. The nose poke holes were 1 cm in diameter and contained a recessed LED stimulus light that illuminated the hole when turned on. An infrared photocell beam was used for the detection of nose pokes into the holes. The house light was located approximately 7 cm above the central nose poke opening. The panel opposite the nose poke holes contained an aperture into which a dipper cup (10 μl) containing a liquid reward could be raised. Environmental contingencies for the various stages of training and testing were programmed using MED-PC software.

2.3. Food restriction and dipper training

Prior to training mice were acclimated to the food reinforcer (vanilla-flavored powdered Ensure, Abbott Laboratories, Columbus, OH, diluted 1:8 in water) by an overnight exposure

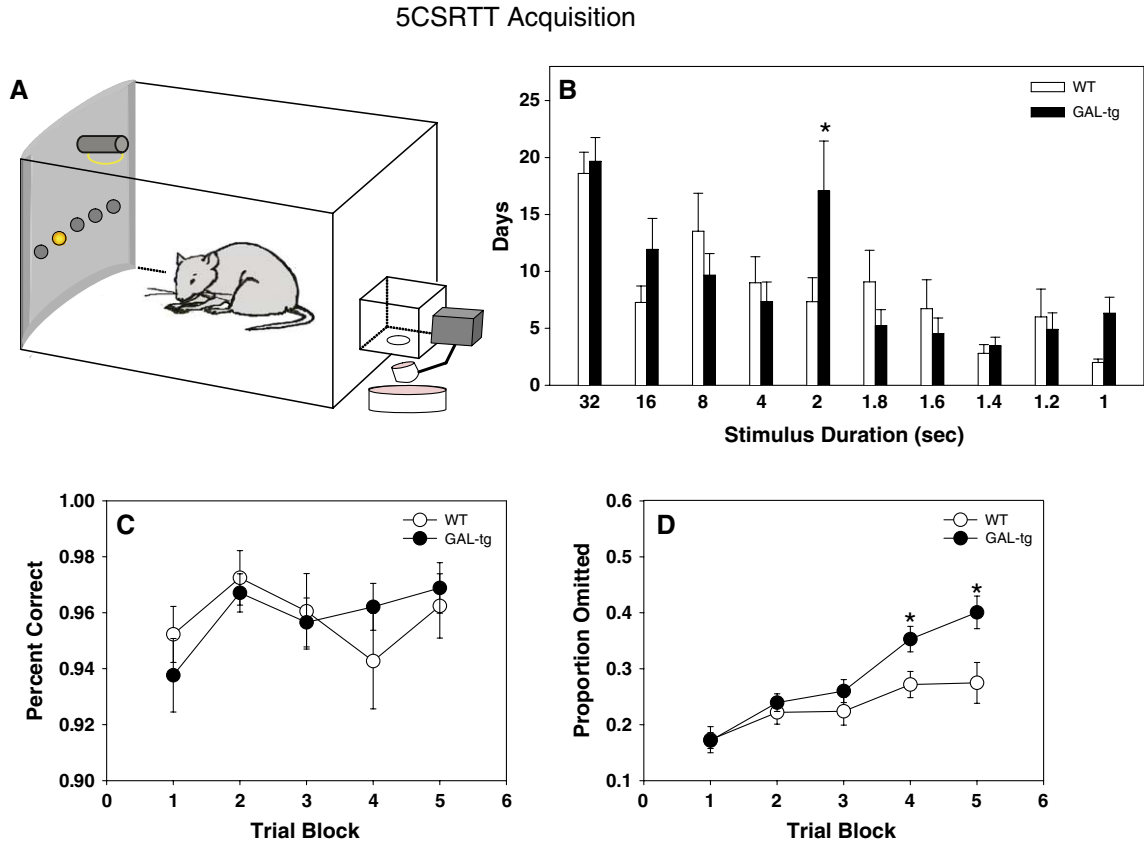

Fig. 1. (A) The 5-choice serial reaction time task. One panel (on the left in the figure) of an operant box contained an array of five nose poke holes and a house light. The opposite panel contained an aperture in which a dipper cup could be presented to the mouse by the raising of the dipper arm from a trough containing the liquid food reinforcer (Ensure, 10 μl, 1:8 dilution). Trials began with the random illumination of one of the nose poke holes. If the mouse executed the correct response by poking its nose into the illuminated hole, the liquid food reinforcer was presented. (B) Acquisition of the 5-choice serial reaction time task. Data are shown as the number of days (*y*-axis) required to reach criteria performance (>80% correct responses, <20% omission rate) at each stimulus duration (*x*-axis). WT and GAL-tg mice did not differ in the number of days needed to reach criteria at any stimulus duration except 2 s. At 2 s the GAL-tg mice required significantly more days to reach criteria than WT mice (*, $p<0.01$). (C) Choice accuracy data, expressed as proportion of correct responses, are shown in 10 trial blocks collapsed across all of the 2 s stimulus duration sessions. There was no effect of genotype on accuracy. (D) Omission data, expressed as proportion of the total trials that were omitted, are shown in 10 trial blocks collapsed across sessions that used a stimulus duration of 2 s. GAL-tg mice omitted significantly more trials than the WT mice in blocks 4 and 5 (trials 30–50; *, $p<0.05$).

to the reinforcer in the absence of food and water. After one overnight exposure to the Ensure, mice were given a daily ration of rodent chow such that body weight was maintained at approximately 80% of free-feeding body weight. Dipper training in the 5-choice boxes began on the third day after exposure to the Ensure, and consisted of dipper cup presentations for 10 s durations at random intervals over a 40 min session. Head entries during the dipper presentations were taken as evidence of learning to associate the dipper cup with the liquid food reinforcer.

2.4. 5-choice training

The 5-choice trials in the present experiment were not self-paced in order to provide the task with greater construct validity as a test of sustained attention. Each trial began with the random illumination of one of the nose poke holes. Initially, trials were separated by an intertrial interval (ITI) of 5 s. If the mouse poked its nose into a hole while the hole was illuminated, or within 5 s of illumination offset, the dipper cup was raised, giving the mouse access to the liquid food reinforcer. Nose pokes into an incorrect dark hole were recorded as errors and resulted in a 10 s timeout that was signaled by the illumination of the house light. Timeouts were also imposed when the mouse failed to nose poke into any hole (omissions) and when the mouse nose poked during the ITI (anticipatory responses). Multiple nose pokes into a correct hole (perseverative responses) were recorded but had no scheduled consequences.

Training was performed as a step-wise progression in which stimulus duration was incrementally decreased as the mice reached the performance criteria of >80% correct responses and <20% omissions. The sequence of stimulus durations was 32, 16, 8, 4, 2, 1.8, 1.6, 1.4, 1.2, 1.0, and 0.8 s. The primary measure of task acquisition was the number of once-daily sessions required to reach criteria performance. Training sessions were performed 5 days per week for 23 weeks. Overtraining is unlikely to be a confound since we are assessing the ability of the mice to detect stimuli in the context of various attentional challenges rather than the ability to learn the operant contingencies of the task.

2.5. Baseline parameters

At the end of the task acquisition period, mice were run for 20 once-daily sessions at baseline parameters in order to attain stable performance values. The baseline parameters consisted of a 1.4 s stimulus duration and an ITI of 5 s. This stimulus duration was chosen to define baseline performance because it was the shortest stimulus duration at which all the mice reached performance criteria during training.

2.6. Attentional challenges

After stable baseline performance was established, the parameters were adjusted in a series of challenges designed to tax mechanisms of sustained attention. These dynamic alterations of the signal event rate, duration, salience, and temporal predictability were chosen because they satisfy conceptual constraints for valid measurement of sustained attention (Parasumaran et al., 1987; Parasuraman, 1986; Parasuraman and Giambra, 1991; Parasuraman and Haxby, 1993). These manipulations are described below in the sequence that they were conducted. All mice experienced each dynamic alteration. Possible experience effects on performance were minimized by not performing the attentional challenges until baseline performance was stable and asymptotic and by progressing through the challenges from simple to more complex.

(a) *high, variable event rate (HRVITI)* — Stimuli were presented at a faster, variable rate (variable ITI=3+2). Mice were tested under these parameters for one session per day for 4 days, 54 trials per session.
(b) *high, variable event rate with variable stimulus duration (HRVSD)* — High, variable event rate was coupled with a dynamic range of stimulus durations (0.4, 0.8, 1.2 s) that were shorter than those of baseline. Mice were tested under these parameters one session per day for 20 days, 54 trials per session.
(c) *tone distracter* — A tone distracter (2.9 kHz, 75 dB) was presented simultaneous with the stimulus light within the context of high, variable event rate and short, variable stimulus duration. This challenge was performed for one, 54-trial session.
(d) *no house light (NHL)* — Prior to this manipulation, erroneous response strategies resulted in the imposition of a timeout period demarcated by illumination of the house light. For this challenge, the house light remained off during the timeout periods in the context of high, variable event rate and variable stimulus duration. Mice were run under these conditions for 5 once-daily sessions.
(e) *constant house light (CHL)* — The house light was left on constantly in the context of high, variable event rate and variable stimulus duration in order to remove demarcation of the timeouts and reduce stimulus discrimination. This challenge was performed for 5 once-daily sessions.
(f) *no reward* — Mice were tested in the context of high, variable event rate and variable stimulus duration. Correct responses resulted in the presentation of an empty dipper cup.

2.7. Testing of cannulated mice after intraventricular galanin

As the transgenic animals may have developed compensatory mechanisms that could occlude the effects of galanin overexpression, a final experiment was conducted to test the effects of pharmacologically administered galanin on performance in the 5-choice serial reaction time task. After the training and challenges described above, the WT mice received surgery to implant guide cannula (31 gauge, Plastics One, Inc., Roanoke, VA) into the left lateral ventricle (following Paxinos (Franklin and Paxinos, 1997): A/P −0.2, ML 1.0, D/V −2.5). Anesthesia was induced using a 5% isoflurane (Baxter Healthcare Corp., Deerfield, IL) mixture and was maintained during surgery with a 2.5% isoflurane mixture. Mice were

placed in a stereotaxic frame (Cartesian Research, Inc., Sandy, OR) and the skull was exposed using a mid-line incision. Bregma was identified and a small hole was drilled for cannula placement. The cannula was secured first by a layer of Slow Jet adhesive (Carl Goldberg Models, Inc., Chicago, IL) followed by dental acrylic (Stoelting, Wood Dale, IL). In order to maintain patency of the cannula, a dummy cannula (33 gauge) was inserted into the guide cannula. The mice were allowed to recover from surgery for 7 days with food and water available ad libitum. After recovery food restriction was reinstated as described above and behavioral training resumed using combined high, variable event rate and variable signal durations (54 total trials per session, 16 trials per stimulus duration). After stable performance was regained (14 days), the effects of galanin infusions or vehicle control infusions were assessed. The schedule of infusions and testing was comprised of 6 injection days with 1 or 2 non-injection days intervening between the injection sessions. Injection sessions were arranged such that each mouse received each treatment condition twice in counter-balanced order. The treatments were distilled water vehicle, 0.5 nmol galanin, and 1.0 nmol galanin (rat galanin, 1–29, American Peptide Co., Inc., Sunnyvale, CA). This lot of galanin was seen to give consistent effects in previous behavioral studies in our laboratory. The doses were chosen based on effectiveness that we have observed in these other behavioral studies (unpublished data). For data analysis, data were collapsed across the two sessions for each dose in order to provide an adequate number of trials for statistical analysis. Thus, Dose × Stimulus Duration analyses were comprised of 32 trials at each stimulus duration. All infusions were 0.5 μl in volume, administered at a rate of 0.1 μl/5 s, 5 min prior to task onset. The injector was left in placed for approximately 30 s after infusion was complete. After removal of the injector, the dummy cannula was replaced. Five mice were removed from the experiment because of an unidentified illness characterized by lethargy and refusal to eat.

2.8. Verification of cannula placement

At the completion of testing, the cannulated mice were sacrificed by cervical displacement, and the brains were immediately removed and placed in a 3% formaldehyde solution. Cannula track placement was verified in 50 μm coronal sections stained with thionin (data not shown). Cannula tracks in two mice were not on target, and data from these mice were removed from the analysis (final $n=8$).

2.9. Statistical analyses

All data were analyzed by two-way repeated measures analysis of variance (ANOVA). In the analysis of the acquisition of the task, stimulus duration and genotype were the independent variables, and the number of days required to reach criteria performance was the dependent variable. Measures of performance that were collected and analyzed during all phases of training and testing included proportion correct (correct responses/correct responses + incorrect responses), proportion of trials omitted, correct response latency, dipper latency, anticipatory responses, and perseverative responses. In the analysis of data from the attentional challenges, the factors in the two-way ANOVAs were genotype and either block or stimulus duration, depending upon the attentional challenge being analyzed. In the experiment with the cannulated mice, the factors were galanin dose and stimulus duration. The Tukey test was used for all post hoc analyses. The threshold for significance was $p<0.05$.

3. Results

3.1. Task acquisition

The primary measure of task acquisition was the number of days of training required to reach the performance criteria for each stimulus duration (Fig. 1B). There was a significant main effect of stimulus duration on the number of days of training required to reach criteria ($F_{(9,\ 28)}=6.30$, $p<0.001$). This main effect reflected a progressive decrease in the number of training days required for the mice to attain criteria as the stimulus durations decreased. There was not a significant effect of genotype on days of training needed, but there was a significant interaction between stimulus duration and genotype ($F_{(9,\ 211)}=1.97$, $p=0.04$). Post hoc analysis determined that the GAL-tg mice required significantly more days of training at the 2 s stimulus duration only ($p<0.01$).

To address the disparity in training requirements at the 2 s stimulus duration versus other stimulus durations, choice accuracy (Fig. 1C) and occurrence of omissions (Fig. 1D) were expressed in 10-trial blocks collapsed across the 2 s stimulus duration sessions. These data were then analyzed for main effects of genotype and trial block. No significant effect of genotype or trial block on accuracy was detected; however, there were significant effects of genotype ($F_{(1,\ 28)}=7.28$, $p=0.01$) and block ($F_{(4,\ 28)}=18.65$, $p<0.001$) on omissions, as well as a significant interaction between genotype and block ($F_{(4,\ 112)}=2.70$, $p=0.03$). Post hoc analysis determined that the GAL-tg mice had significantly higher omissions in blocks 4 ($p=0.02$) and 5 ($p<0.001$). Thus, the GAL-tg mice required more training at the stimulus duration of 2 s because they omitted more trials than WT mice in those sessions.

All behavioral measures taken during acquisition of the task were analyzed within each stimulus duration (data not shown). Analysis of accuracy data showed that for all stimulus durations, there was no effect of block or genotype. Likewise, no effect of genotype on any other performance measure (omission rate, correct latency, anticipatory responses, perseverative responses, or dipper latency) for any stimulus duration was noted (except for the effect of genotype on omissions at the 2 s stimulus duration, as described above). However, each of these measures tended to change with trial block (i.e. time on task). With the exception of the sessions using a 32 s stimulus duration, omissions, correct latency, and dipper latency increased with time on task ($p<0.05$). Conversely, anticipatory and perseverative responses decreased as the session progressed for all signal durations ($p<0.05$), except the 32 s stimulus

duration sessions. This pattern in the various performance measures indicates a general decrease in responsiveness with session progression.

3.2. Baseline performance

After the acquisition phase, the mice were trained for 20 once-daily sessions at baseline parameters defined as a static stimulus duration of 1.4 s, an ITI of 5 s, and a session length of 50 trials. The data from these sessions were analyzed in 10-trial blocks collapsed across all 20 sessions (Fig. 2). Similar to the acquisition data, the attentional measures of accuracy ($F_{(4, 28)}=4.40, p<0.01$; Fig. 2A), omission rate ($F_{(4, 28)}=124.67, p<0.0001$; Fig. 2B), and correct latency ($F_{(4, 28)}=60.37, p<0.0001$; Fig. 2C) significantly increased with training block. Dipper latency also increased with trial block ($F_{(4, 28)}=11.89$, $p<0.0001$; data not shown). In contrast, anticipatory responses ($F_{(4, 28)}=35.45, p<0.0001$; data not shown) and perseverative responses ($F_{(4, 28)}=9.60$, $p<0.0001$; data not shown) significantly decreased with training block. There was no effect of genotype on any measure of baseline performance.

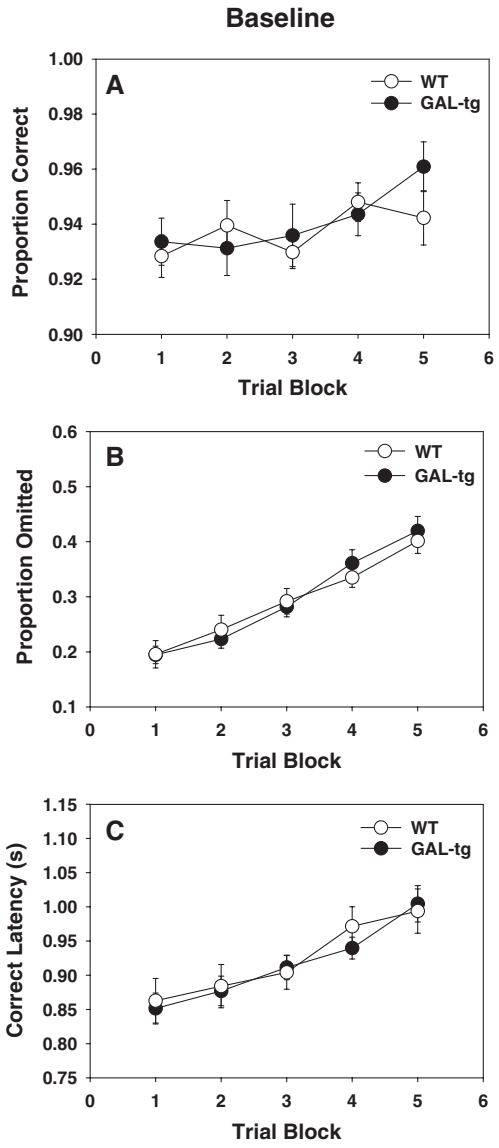

Fig. 2. Baseline performance in the 5-choice serial reaction time task using a 1.4 s stimulus duration and 5 s intertrial interval. Choice accuracy (A), omissions (B), and latencies on correct responses (C) are shown as a function of blocks of 10 trials collapsed across 20 once-daily baseline sessions (50 trials per session). There was no effect of genotype on these measures.

3.3. High event rate/variable ITI (HRVITI)

Immediately after stable performance was established for baseline task conditions, the mice were challenged with dynamic alterations of various task parameters in an effort to tax mechanisms of sustained attention. The first of these challenges was to increase event rate and event asynchrony by presenting the stimuli at a fast, variable rate (variable ITI=3+2 s). Mice performed under these conditions for one session per day for 4 days and 54 trials per session. Data were analyzed in 9-trial blocks collapsed across the four sessions (data not shown). The effect of block on the various measures was the same as that observed under baseline conditions. Accuracy ($F_{(5, 28)}=4.16$, $p<0.01$), omission rate ($F_{(5, 28)}=55.80$, $p<0.0001$), correct latency ($F_{(5, 28)}=7.27, p<0.0001$), and dipper latency ($F_{(5, 28)}=7.51$, $p<0.0001$) all significantly increased with block. Anticipatory responses ($F_{(5, 28)}=6.73, p<0.0001$) and perseverative responses ($F_{(5, 28)}=4.79$, $p<0.001$) significantly decreased with time on task. There was no effect of genotype on any measure, indicating that GAL-tg mice and WT responded similarly to this challenge.

In order to confirm that the increases in event rate and event asynchrony affected performance, measures from the HRVITI challenge were compared with those from baseline sessions. Increasing event rate and event asynchrony had no effect on accuracy or dipper latency; however, this dynamic alteration significantly increased omissions ($F_{(1, 28)}=75.66, p<0.0001$) and correct latency ($F_{(1, 28)}=16.50$, $p<0.001$) while significantly decreasing anticipatory ($F_{(1, 28)}=14.41$, $p<0.001$) and perseverative responses ($F_{(1, 28)}=28.77, p=0.03$). These effects did not differ between the genotypes.

3.4. High variable event rate with variable stimulus duration (HRVSD)

Coupling a high, variable event rate (ITI: 3+2 s) with variable stimulus durations (0.4, 0.8, or 1.2 s) served as the platform for several subsequent attentional challenges, each with additional parameter adjustments intended to maximize the attentional demands placed on the animals. Mice were tested for 20 days with these conditions of dynamic signal duration and high, variable event rate (single session per day, 54 trials per session). Data were expressed and analyzed as a function of stimulus duration collapsed across the 20 sessions (Fig. 3). Accuracy ($F_{(2, 28)}=207.00, p<0.001$; Fig. 3A), correct response latencies ($F_{(2, 28)}=91.69, p<0.001$; Fig. 3C), and perseverative responses ($F_{(2, 28)}=37.32$, $p<0.001$; Table 1) all significantly increased with increasing stimulus duration, reflecting signal-length dependent performance. Omission rate ($F_{(2, 28)}=407.43$, $p<0.001$; Fig. 3B), dipper latency ($F_{(2, 28)}=6.38$, $p=0.003$;

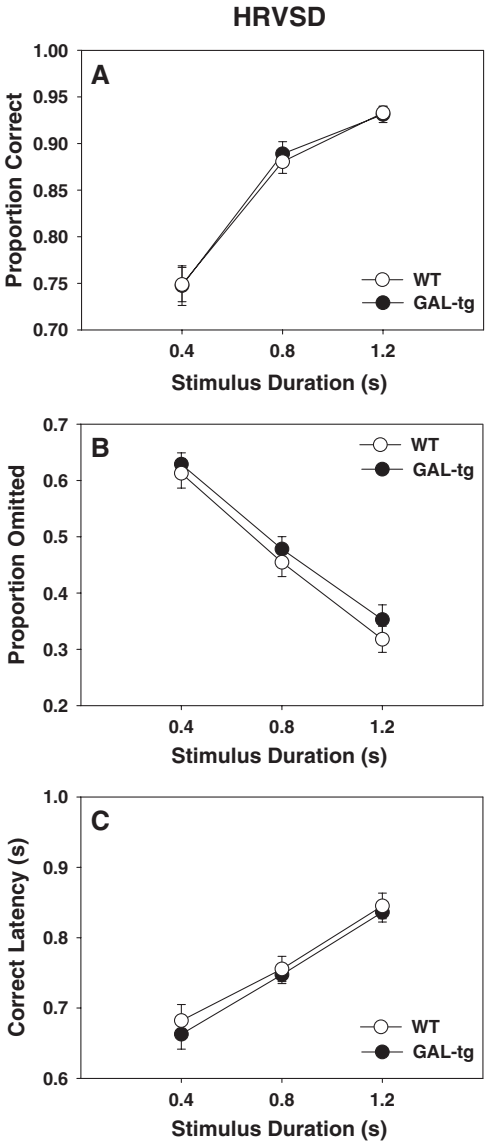

Fig. 3. Performance in the 5-choice serial reaction time task under conditions of variable stimulus duration with high, variable event rate (HRVSD). Accuracy (A), omission rate (B), and correct latency (C) are shown at each stimulus duration collapsed across 4 weeks of testing. There was no effect of genotype on these measures.

Table 1), and anticipatory responses ($F_{(2,\ 28)}=15.45$, $p<0.001$; Table 1) significantly decreased with increasing stimulus duration. The GAL-tg mice were not differentially affected by this manipulation, as there was no effect of genotype on any performance measure.

3.5. Tone distracter

Use of additional stimuli to increase the attentional load placed on the animal has yielded impairments of performance in previous studies of sustained attention (Humby et al., 1999; McGaughy and Sarter, 1995). The mice were subjected to a single session wherein an auditory distracter (2.9 kHz, 75 dB) was presented simultaneously with the stimulus light in the context of the high, variable event rate and variable stimulus duration. As observed in previous challenges, accuracy ($F_{(2,\ 28)}=11.21$, $p<0.001$; data not shown) significantly increased, and omissions ($F_{(2,\ 28)}=49.23$, $p<0.001$; data not shown) significantly decreased with increasing stimulus duration. Correct latency, dipper latency, anticipatory responses, and perseverative responses did not vary with stimulus duration in this challenge (data not shown). The GAL-tg and WT mice did not differ on any performance measure from the tone distracter session. When compared with baseline data, addition of a tone distracter only affected correct response latency ($F_{(1,\ 27)}=22.29$, $p<0.0001$), indicating that while the tone was perceived by the mice and did interfere with their performance, it did not differentially affect the animals by genotype.

3.6. No house light (NHL)

Throughout training and testing, incorrect responses and omissions resulted in a 5 s timeout that was signaled by illumination of the house light. To address how this explicit demarcation of the timeout period might contribute to performance, the mice were challenged by leaving the house light extinguished during timeouts in the context of high, variable event rate and variable stimulus duration. The consistent pattern of significantly increasing accuracy ($F_{(2,\ 28)}=85.13$, $p<0.001$), correct response latency ($F_{(2,\ 28)}=26.65$, $p<0.001$) and perseverative responses ($F_{(2,\ 28)}=20.56$, $p<0.001$) with increasing stimulus duration was maintained under this condition (data not shown). Omission rate ($F_{(2,\ 28)}=91.35$, $p<0.001$) significantly decreased as stimulus duration increased (data not

Table 1
Indices of inhibitory control and motivation in the 5-choice serial reaction time task under conditions of a high variable event rate and variable stimulus duration, no house light, and constant house light

High, variable event rate and variable stimulus duration				
	Stimulus duration (s)	Anticipatory responses (per trial)	Perseverative responses (per trial)	Dipper latency (s)
WT	0.4	0.16±0.02	0.06±0.01	1.12±0.06
	0.8	0.13±0.02	0.10±0.01	1.09±0.07
	1.2	0.11±0.02	0.13±0.02	1.08±0.06
GAL-tg	0.4	0.16±0.02	0.08±0.01	1.10±0.03
	0.8	0.12±0.01	0.11±0.02	1.05±0.02
	1.2	0.12±0.02	0.13±0.02	1.07±0.02
No house light				
WT	0.4	0.20±0.04	0.08±0.02	1.11±0.06
	0.8	0.19±0.05	0.13±0.02	1.11±0.07
	1.2	0.18±0.04	0.15±0.03	1.10±0.07
GAL-tg	0.4	0.26±0.06	0.09±0.01	1.13±0.03
	0.8	0.23±0.06	0.14±0.02	1.15±0.04
	1.2	0.19±0.03	0.17±0.02	1.15±0.03
Constant house light				
WT	0.4	2.25±0.30	0.14±0.02	1.81±0.17
	0.8	2.05±0.25	0.18±0.03	1.59±0.07
	1.2	2.18±0.33	0.20±0.04	1.64±0.13
GAL-tg	0.4	1.69±0.25	0.13±0.02	1.89±0.20
	0.8	1.62±0.17	0.16±0.02	1.68±0.09
	1.2	1.56±0.13	0.20±0.02	1.86±0.15

There was no effect of genotype on any measure.

shown). Removal of the overt indication of a timeout had a similar effect on both GAL-tg and WT mice.

Analyses comparing performance for the sessions wherein the timeout period was not overtly indicated versus those sessions in which the house light marker was utilized suggested that the mice employed the house light offset as a cue for imminent stimulus presentation (data not shown). Removal of demarcation of the timeout by the house light resulted in a decrease in accuracy ($F_{(1, 28)}=37.60$, $p<0.0001$), a decrease in omissions ($F_{(1, 28)}=7.62$, $p=0.01$), an increase in anticipatory responses ($F_{(1, 28)}=7.51$, $p=0.01$), and an increase in correct latency ($F_{(1, 28)}=7.88$, $p=0.01$). All of these effects were similar in WT and GAL-tg mice. This pattern of changes in performance suggests that the mice respond to the absence of the demarcation of the timeout by increasing responses during the ITI (anticipatory responses). Some of these responses occur, by chance, after stimulus onset which leads to decreased accuracy and omissions. Additionally, the increase in correct response latency for the timeout-no house light condition reflects probable use of the house light offset as a cue for stimulus onset.

3.7. Constant house light (CHL)

Reduction of stimulus discriminability was the final manipulation executed to challenge the attentional capacities of the mice. With the house light illuminated throughout the session, it was anticipated that performance would be impaired relative to data derived from the previous environment, which allowed for higher signal contrast. In general, accuracy (Fig. 4A) was much lower under conditions of constant house light, high asynchronous event rate, and dynamic signal durations than in the any of the other challenges (~40–50% vs. ~85–95%); however, there was no effect of genotype on accuracy. As in other challenges, signal-length dependent performance was maintained: accuracy ($F_{(2, 28)}=31.93$, $p<0.001$; Fig. 4A) and perseverative responses ($F_{(2, 28)}=6.90$, $p=0.002$; Table 1) significantly increased and correct response latencies ($F_{(2, 28)}=3.74$, $p=0.03$; Fig. 4C) significantly decreased as stimulus duration increased.

As expected, omission rate ($F_{(2, 28)}=30.61$, $p<0.001$; Fig. 4B) significantly decreased as stimulus duration increased. Interestingly, there was a significant interaction between stimulus duration and genotype ($F_{(2, 56)}=3.31$, $p=0.04$). Post hoc analysis determined that this interaction was due to the GAL-tg mice committing significantly more omissions than WT mice at 0.8 and 1.2 s but not at 0.4 s (GAL-tg vs. WT: 0.4 s, $p=0.57$; 0.8 s, $p=0.046$; 1.2 s, $p=0.03$).

3.8. No reinforcement

A general observation in the various manipulations of the 5-choice task was that the responsiveness of the mice decreased with session length. The most reliable measure of this phenomenon was the increase in omission rate that occurred with trial block. To explore whether this increase in omissions was due to satiety mechanisms, a session was performed under baseline conditions without reinforcement present. Fig. 5A shows the omission data from this session compared to baseline data with reinforcement present (collapsed from two sessions that preceded the no reward session). As expected, omissions significantly increased with trial block ($F_{(5, 28)}=41.236$, $p<0.0001$). There was no main effect of genotype or session type, but there was a significant three way interaction between block, session type, and genotype ($F_{(5, 140)}=2.66$, $p=0.03$). Post hoc analysis determined that this three-way interaction was due to the GAL-tg mice omitting significantly more in block 3 under the no reward condition than WT mice ($p=0.007$). Overall, the data show that omission rate increases with block regardless of whether food reinforcement is present.

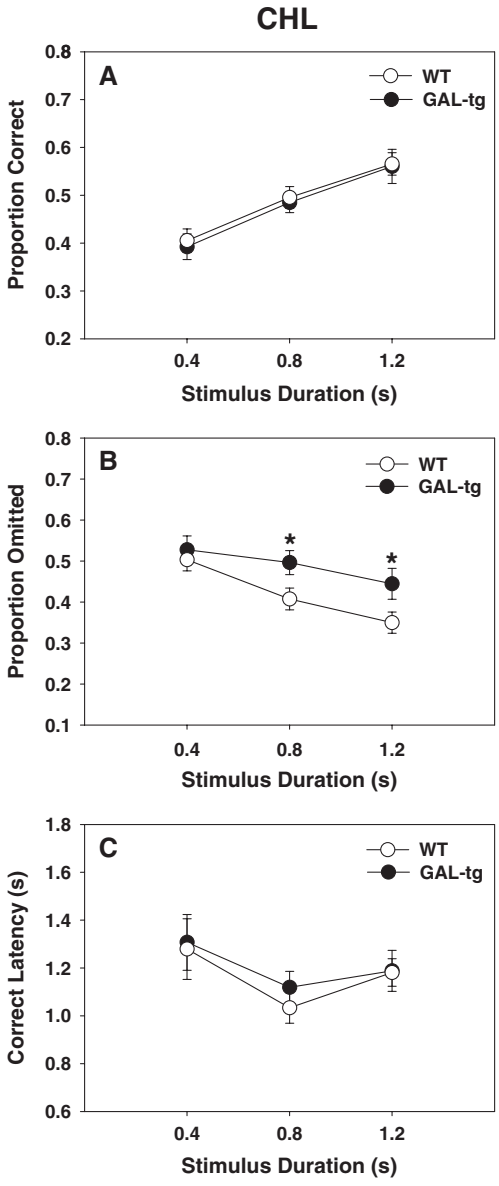

Fig. 4. Performance in the 5-choice serial reaction time task under conditions of constant house light (CHL). Accuracy (A), omissions (B), and correct latency (C) are shown as a function of stimulus duration collapsed across five once-daily sessions (54 trials per session). Under conditions of constant house light, GAL-tg mice omitted significantly more trials than the WT mice at stimulus durations of 0.8 and 1.2 s (*, $p<0.05$).

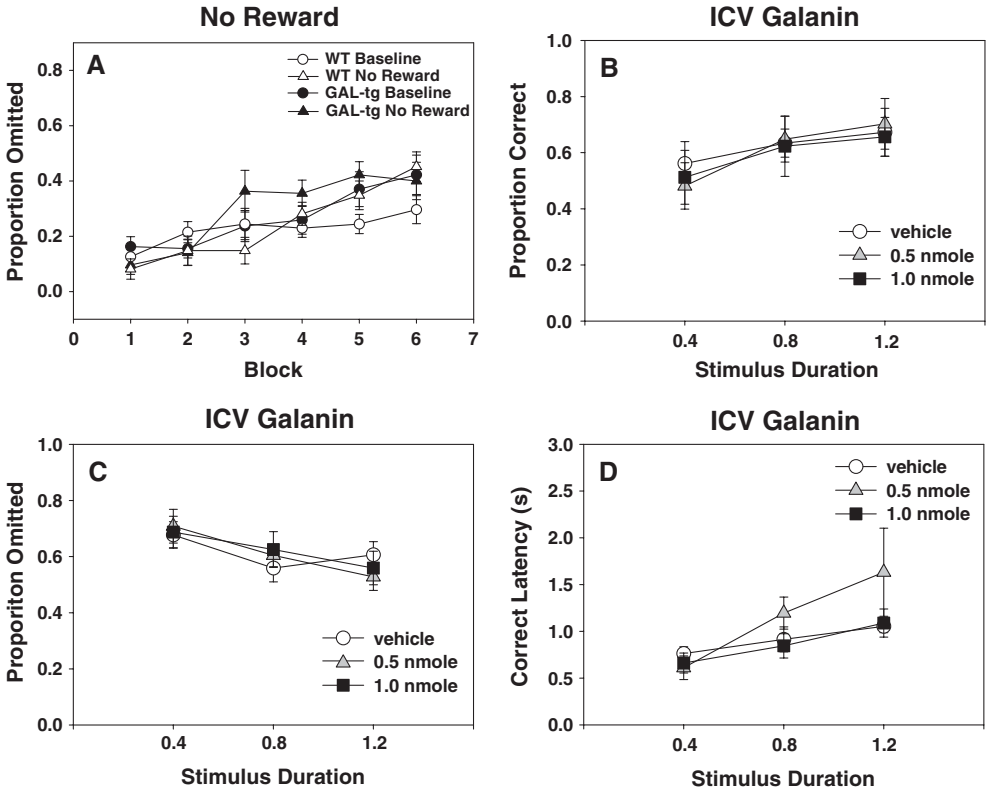

Fig. 5. (A) Performance of WT and GAL-tg mice under baseline conditions with and without reinforcement. The general pattern of an increase in omissions with trial block was not changed by the absence of reinforcement. (B–D) Performance of WT mice in the 5-choice serial reaction time task after intraventricular injection of various doses of vehicle, 0.5 nmol of galanin, or 1.0 nmol of galanin. Accuracy (B), omissions (C), and latencies on correct responses (D) are shown as a function of stimulus duration. There was no significant effect of genotype on these measures.

3.9. Intraventricular galanin

To test the ability of exogenous galanin to affect performance in the 5-choice serial reaction time task, each WT mouse received a permanent cannula targeting the left lateral ventricle. The cannulated mice were subsequently tested in the task after intraventricular injection of vehicle, 0.5 nmol, or 1 nmol of galanin under conditions of high, variable event rate and variable stimulus duration. As seen in other challenges, accuracy ($F_{(2,\ 14)}$=9.96, p=0.002; Fig. 5B) and correct latency ($F_{(2,\ 16)}$=4.37, p=0.04; Fig. 5D) significantly increased with increasing stimulus duration while omission rate ($F_{(2,\ 14)}$= 14.10, p<0.001; Fig. 5C) significantly decreased with increasing stimulus duration. Perseverative responses (Table 2) were unaffected by stimulus duration, but there was a main effect of galanin injection on this measure ($F_{(2,\ 16)}$=4.73, p=0.03). Post hoc analysis determined that the mice made significantly more perseverative responses after injection of 1 nmol galanin than after injection of vehicle (p=0.04) or 0.5 nmol of galanin (p=0.03).

Comparison of the accuracy and omission data from the intraventricular injection days with that of the other challenges reveals generally lower accuracy and higher omissions after injection. To examine whether this difference was a result of the injection procedure, data from the intervening non-injection days (data not shown) was compared to that of the injection days. There was not a significant effect of injection on choice accuracy. There was a significant effect of injection on omissions ($F_{(1,\ 7)}$=7.10, p=0.03), but this effect was due to omissions being *higher* in the non-injection sessions. Thus, these data indicate that the decreased accuracy and increased omissions relative to other challenges cannot be ascribed specifically to the injection procedure.

Table 2
Indices of inhibitory control and motivation in the 5-choice serial reaction time task in WT mice after intraventricular injection of vehicle, 0.5 nmol of galanin, or 1.0 nmol of galanin

Cannulated mice — variable stimulus duration				
	Stimulus duration (s)	Anticipatory responses (per trial)	Perseverative responses (per trial)	Dipper latency (s)
Vehicle	0.4	0.19±0.05	0.04±0.02	2.36±0.30
	0.8	0.21±0.03	0.06±0.02	2.34±0.37
	1.2	0.18±0.05	0.06±0.01	2.66±0.43
0.5 nmol galanin	0.4	0.16±0.07	0.02±0.01	1.60±0.30
	0.8	0.27±0.07	0.03±0.01	2.15±0.25
	1.2	0.26±0.09	0.04±0.01	1.94±0.20
1.0 nmol galanin	0.4	0.32±0.14	0.14±0.05*	2.00±0.34
	0.8	0.18±0.04	0.11±0.04*	2.21±0.42
	1.2	0.23±0.05	0.12±0.03*	2.00±0.27

Perseverative responses significantly increased after 1.0 nmol of galanin (*, p<0.05).

4. Discussion

The principal finding of the present study is that overexpression of the inhibitory neuropeptide galanin in the adrenergic neurons of the mouse does not impair attention as assessed by the 5CSRTT. In task acquisition, the only performance measure affected by galanin overexpression was omission rate, and this effect occurred only for the stimulus duration of 2 s. Moreover, in a number of attentional challenges designed to increase the demands on sustained attention, GAL-tg mice performed as well as their WT littermate controls. One exception to this pattern occurred under conditions of constant house light in which GAL-tg mice had higher omission rates than WT mice. While it is tempting to associate this disparity with attentional functions (i.e., the GAL-tg mice adopted a more conservative response strategy under conditions of high attentional load), the lack of any other genotype differences in challenges that are documented to tax attentional processes (McGaughy et al., 2002, 1996) casts doubt on the importance of this single behavioral difference.

In the current study, attentional challenges included increasing the visual stimulus event rate in conjunction with introducing event asynchrony, varying the signal duration, presenting a distracter stimulus of a different sensory modality, removing explicit demarcation of the timeout period, and decreasing signal discriminability. Importantly, and in contrast to some previous studies (Dalley et al., 2002; Humby et al., 1999), the mice were tested in the context of trials that were not self-paced in order to satisfy the conceptual constraint of temporal unpredictability in a sustained attention task (Parasumaran et al., 1987). Finally, in an attempt to map task parameters in accordance with theoretical considerations for sustained attention functions, animals were tested when several of these challenges were performed concurrently. Like rats (Dalley et al., 2002), our mice were able to reach performance criteria, effectively discriminating signal events under conditions of high (as well as low) event rate with reasonable levels of accuracy and low levels of impulsive responding. Similar to experiments involving rats (Dalley et al., 2002), as well as other work involving mice (Humby et al., 1999), the WT and GAL-tg mice were also able to perform accurately in the presence of a tone distracter. In each instance, and complementary to findings from rat studies (Dalley et al., 2002; McGaughy et al., 2002), the mice exhibited signal-length dependent performance, with greatest accuracy for the longest signal duration. As anticipated, the session wherein the house light was illuminated throughout the session, in conjunction with high, variable event rate and dynamic signal durations, was the most demanding. However, as with each of the prior manipulations, this parameter change failed to elicit a significant difference in accuracy levels between the GAL-tg and WT groups.

In the mice used in the present study, the overexpression of galanin is driven by the DBH promoter, which specifies the overexpression to noradrenaline-synthesizing neurons. For this reason, a possible explanation for the lack of effect of galanin-overexpression on 5CSRTT performance is that the noradrenergic pathways in which galanin is overexpressed are not involved in 5CSRTT performance. This explanation is unlikely for a number of reasons. First, converging data have shown that the noradrenergic system is involved in various aspects of 5CSRTT performance (Robbins, 2002). Secondly, the corticopetal, cholinergic neurons of the nucleus basalis, which are clearly involved in attentional function, receive significant noradrenergic input from the locus coeruleus (Hajszán and Zaborszky, 2002; Zaborszky and Cullinan, 1996) and express adrenoreceptors (Zaborszky et al., 2004). Thirdly, galanin inhibits cortical acetylcholine release (Wang et al., 1999), and GAL-tg mice have significantly increased levels of galanin mRNA and galanin peptide (10×) in the frontal cortex (He et al., 2005; Wrenn et al., 2002). With these facts in mind, the overexpression of galanin in noradrenergic neurons seems particularly well-placed to inhibit both the function of the noradrenergic system and the cortically projecting cholinergic system.

It must be noted, however, that previously documented effects of galanin on cognition are mostly derived from hippocampal-related tasks. For instance, both galanin overexpression and intraventricular galanin injection impair trace fear conditioning, which is hippocampal-dependent, but galanin has no effect on standard delay cued fear conditioning, which does not require an intact hippocampus (Kinney et al., 2002). Further, in an operant delayed non-matching to position task, intrahippocampal galanin disrupted performance, but galanin injections into the amygdala, nucleus basalis, entorhinal cortex, or prefrontal cortex had no effect (Robinson and Crawley, 1994). Thus, the hippocampus seems to be a major neuroanatomical substrate for the deleterious effects of galanin on learning and memory. Interestingly, a great deal of evidence has shown that the hippocampus is not critical to 5CSRTT performance but rather that 5CSRTT performance is regulated by anterior cortical regions (Hahn et al., 2003; Kirkby and Higgins, 1998; Lehman et al., 2003; Muir et al., 1996a,b). In sum, the current data show that despite the fact that galanin is significantly overexpressed in cortical areas in GAL-tg mice (Wrenn et al., 2002), and the fact that anterior cortical regions are critical for 5CSRTT performance (Muir et al., 1996b), attentional function remains largely intact in these GAL-tg mice.

The well described inhibitory effect of galanin on central cholinergic function (Consolo et al., 1991; Dutar et al., 1989; Fisone et al., 1991, 1987; McDonald and Crawley, 1997; Palazzi et al., 1991; Robinson et al., 1996) was the basis for our hypothesis that galanin overexpression would impair attention. The general lack of effects associated with galanin overexpression on performance in the 5CSRTT task, especially in terms of accuracy, contrasts with the effects of other manipulations of the cholinergic system on attentional processes (see Introduction). Thus the lack of attentional dysfunction in GAL-tg mice may reflect marginal effects of galanin overexpression on cortical release of acetylcholine. As mentioned briefly above, galanin inhibited acetylcholine release in rat cortical slices and synaptosome preparations (Wang et al., 1999). While GAL-tg mice show decreased acetylcholine release in the ventral hippocampus as determined by microdialysis (Laplante

et al., 2004), it remains unknown whether GAL-tg mice show similar reductions of acetylcholine release in cortical areas. Such data would shed light on the current behavioral results.

An important aspect of the present study is that we corroborated the negative findings in the GAL-tg mice by examining the effect of intraventricular galanin on 5CSRTT performance in WT mice. In agreement with the findings from the GAL-tg mice, microinjected galanin had no effect on measures of attentional function. This finding is important because it argues against the interpretation that life-long, chronic overexpression of galanin is counteracted by unknown compensatory mechanisms that serve to support normal attentional function. If such a compensation argument was correct, acute intraventricular galanin would be expected to impair 5CSRTT performance in WT mice in which compensatory mechanisms have not been recruited. While microinjected galanin had no effect on measures of attentional function, the highest dose of 1.0 nmol significantly increased perseverative responses, a measure akin to compulsive behavior (Chudasama et al., 2003). This finding is consistent with the observation in rats that injection of galanin into the ventral hippocampus or amygdala increased response perseveration in an operant delayed non-matching to position task (McDonald and Crawley, 1996; Robinson and Crawley, 1994). A more comprehensive description of the conditions under which galanin may influence inhibitory control will require further study and replication.

An interesting aspect of the data from the cannulated mice was the decrease in choice accuracy and increase in omissions relative to their performance on the various challenges. This change in performance was also seen in the intervening non-injection days, ruling out the injection procedure as the cause. Whether this effect was due to the surgical procedure, stress related to the presence of the cannula, or simply increased difficulty in performing the 5CSRTT with a cannula present cannot be determined from the present data. Further, we cannot rule out that these changes in accuracy and omissions obscured a smaller galanin effect.

One consistent performance characteristic displayed by the mice in the current study was the tendency of responsiveness to decrease with time on task. This tendency was most consistently manifest as an increase in omissions with successive trial block. One possible cause of such a pattern of behavior is within-session satiation to the liquid food reward. This explanation seems unlikely given the very low volume (10 μl) of reinforcement used and the maintenance of this pattern in sessions in which there was no reinforcement present. However, mechanisms of motivation separate from those relevant to satiation cannot be entirely ruled out because dipper latency often increased along with omission rate.

The behavioral index of omissions raises an important issue concerning the viability of sustained attention paradigms for murine applications because the overall omission rate was substantively higher among the mice than has typically been documented in rats. This high omission rate forced the use of much shorter sessions compared to what is usually used in the rat (54 trials vs. 100). Although the available literature is relatively nascent, previous studies using the mouse in the 5CSRTT have also used a low number of trials per session and report omission rates higher than what is normally seen in control rats (Greco et al., 2005; Humby et al., 1999; Steckler et al., 2000; van Gaalen et al., 2003). The necessarily limited number of trials per session, associated with apparently different motivation processes among mice (or at least mice of this strain) hinders interpretation of the 5CSRTT as a sustained attention task in this instance. More specifically, the omission rate precluded the use of enough trials per session to have sufficient time on task to observe a meaningful vigilance decrement (Dalley et al., 2004). Additionally, while the mice in the present study exhibited both the capacity for signal discrimination, as well as signal-length dependent performance, the signal durations tested were also longer than those utilized in some rat studies (i.e., 500, 250 and 125 ms, McGaughy et al., 2002). Clearly, there are important differences in how mice and rats perform this task, and these differences may reflect species differences in attentional function. The delineation of the parameters governing these differences will be a necessary area of study as various attentional paradigms continue to be incorporated in murine-based models (Mohler et al., 2001). Nevertheless, the successful performance of C57BL/6J mice on challenging versions of the 5CSRTT in the present experiments indicates that this task can be effectively applied to the phenotyping of mutant mouse lines.

In summary, our experiments revealed that excess galanin, derived from either overexpression of endogenous galanin or acute intraventricular administration of exogenous galanin, has only very minor effects on performance in the 5CSRTT. Importantly, these findings suggest that the cognitive effects of galanin are reasonably interpreted as learning and memory deficits per se rather than as a consequence of attentional dysfunction. Further study using paradigms that assess other operationally defined domains of attention, such as divided attention, will be necessary to confirm this interpretation.

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