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The *N*-methyl-D-aspartate receptor modulator GLYX-13 enhances learning and memory, in young adult and learning impaired aging rats

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Abstract

NMDA receptor (NMDAR) activity has been strongly implicated in both *in vitro* and *in vivo* learning models and the decline in cognitive function associated with aging and is linked to a decrease in NMDAR functional expression. GLYX-13 is a tetrapeptide (Thr-Pro-Pro-Thr) which acts as a NMDAR receptor partial agonist at the glycine site. GLYX-13 was administered to young adult (3 months old) and aged (27–32 months old) Fischer 344 X Brown Norway F1 rats (FBNF1), and behavioral learning tested in trace eye blink conditioning (tEBC), a movable platform version of the Morris water maze (MWM), and alternating t-maze tasks. GLYX-13 (1 mg/kg, i.v.) enhanced learning in both young adult and aging animals for MWM and alternating t-maze, and increased tEBC in aging rats. We previously showed optimal enhancement of tEBC in young adult rats given GLYX-13 at the same dose. Of these learning tasks, the MWM showed the most robust age related deficit in learning. In the MWM, GLYX-13 enhancement of learning was greater in the old compared to the young adult animals. Examination of the induction of long-term potentiation (LTP) and depression (LTD) at Schaffer collateral-CA1 synapses in hippocampal slices showed that aged rats showed marked, selective impairment in the magnitude of LTP evoked by a sub-maximal tetanus, and that GLYX-13 significantly enhanced the magnitude of LTP in slices from both young adult and aged rats without affecting LTD. These data, combined with the observation that the GLYX-13 enhancement of learning was greater in old than in young adult animals, suggest that GLYX-13 may be a promising treatment for deficits in cognitive function associated with aging.

Keywords: Learning; Memory; Aging; Rat; Hippocampus; Eye blink conditioning; Morris water maze; T-maze; GLYX-13

1. Introduction

The glutamate *N*-methyl-D-aspartate receptor (NMDAR) plays a critical role in learning and memory. The NMDAR is critical for the induction of both long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength, forms of activity-dependent synaptic plasticity believed to be associated with learning and memory formation (Martin et al., 2000; Rison and Stanton, 1996; Stanton, 1996). NMDAR activation has been suggested to be critical for the acquisition of hippocampal-dependent learning tasks such as trace eye blink conditioning (tEBC; Weiss et al., 1999a,b), because NMDAR antagonists MK-801 and PCP prevent the acquisition of tEBC (Thompson and Disterhoft, 1997a) and the NMDAR glycine site partial agonist D-cycloserine (DCS) facilitates acquisition of tEBC (Thompson and Disterhoft,

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1997b). Mice with point mutations in the NMDAR glycine binding site that greatly reduce NMDAR function show severe deficits in learning in the MWM, and learning is rescued by administration of the NMDAR glycine site partial agonist D-serine (Ballard et al., 2002; Kew et al., 2000).

There are age related decreases in NMDAR protein expression and function in humans and other animals. Receptor binding studies have shown an age related decrease in NMDAR binding in the frontal cortex and hippocampus of mice (Magnusson and Cotman, 1993), rats (Tamaru et al., 1991), rhesus monkeys, (Wenk et al., 1991), and humans (Piggott et al., 1992). In addition, hippocampal protein levels of the NMDAR obligatory NR1 subunit that contains the glycine binding sites (Lynch and Guttmann, 2001), decrease as a consequence of aging in mice (Magnusson et al., 2002), rats (Eckles-Smith et al., 2000), and rhesus monkeys (Gazzaley et al., 1996).

NMDAR alterations have also been reported in Alzheimer's disease (AD). Levels of NMDAR receptor binding (Jansen et al., 1990; Ułas et al., 1992), NR1 protein expression (Hynd et al., 2004; Mishizen-Eberz et al., 2004) and gene expression (Mishizen-Eberz et al., 2004) are all decreased in the cortex and hippocampus of AD patients compared to aged-matched controls.

Cognitive deficits in aging correlate with NMDAR deficits. Protein levels of NR1 (Adams et al., 2001) and NMDAR binding (Davis et al., 1993) in rats both correlate positively with learning acquisition rates in the MWM. Moreover, old rats are more sensitive than young adult rats to the memory impairing effect of the NMDA open channel blocker MK-801 (Ingram et al., 1992).

GLYX-13 is a recently developed tetrapeptide (Thr-Pro-Pro-Thr) that acts as a NMDAR receptor partial agonist at the glycine site with therapeutic potential as a cognitive enhancer (Moskal et al., 2005). GLYX-13 readily crosses the blood brain barrier and has been shown to increase both Schaffer collateral-CA1 LTP *in vitro,* and learning and memory in 3-month-old rats in a trace eye blink conditioning paradigm (Moskal et al., 2005). GLYX-13 administration in concert with a learning task has also been shown to elevate gene expression of hippocampal NR1 subunit in 3-month-old rats (Moskal et al., 2007).

In the present study, we examined the effect of GLYX-13 on learning and memory in the MWM, and t-maze tasks in young adult (3 months old) and aged F1 hybrid Fischer 344 X Brown Norway rats (FBNF1). We also examined the effect of GLYX-13 on tEBC in aging rats in order to compare their performance to that of young adult rats reported previously (Moskal et al., 2005).

Twenty-seven months of age was chosen because a study examining four different ages of adult rats (from 6 to 35 months), found that half of the 27-month-old group exhibited impairment of the trace eye blink conditioning task (Knuttinen et al., 2001). Since GLYX-13 increases learning and memory in hippocampally dependent tasks (Moskal et al., 2005), and enhances hippocampal LTP (Zhang et al., 2008), we also tested the hypothesis that the effects of GLYX-13 on both learning and synaptic plasticity might be greater in aged animals. Thus, we examined the effects of GLYX-13 on long-term potentiation (LTP) and depression (LTD) at Schaffer collateral-CA1 synapses in hippocampal slices from young adult (2-month-old) and aged (24-month-old) Fischer 344 rats.

2. Methods

2.1. Animals and housing

Male F1 hybrid Fischer 344 X Brown Norway (FBNF1) specific pathogen-free rats were used in these studies. Two groups of FBNF1 were used; young adult (3 months of age) and old (27–32 months of age). Animals were purchased from the National Institute of Aging Colony Maintained at Harlan (USA). By 32 months of age these animals have approximately a 50% mortality (Turturro et al., 1999). Animals were equipped with subdural femoral vascular access ports (Access Technologies, Chicago). Patency was maintained by flushing with 0.15 ml 0.9% saline followed by 0.15 ml heparinized glycerol lock flush solution to clear the dead space in the catheter every third day. Animals were housed in opaque polycarbonate cages with wood shaving bedding and were maintained on a 14:10 light:dark cycle with lights on at 6am and off at 8pm in the Northwestern University AAALAC-approved animal facility. Measures were taken in these studies to ensure that old rats were healthy during behavioral assessments, including daily weights and gross assessment of overall health. Animals were given access to food and water *ad libitum* throughout the studies unless otherwise noted. All experiments were approved by the Northwestern University Animal Care and Use Committee, or the Animal Care and Use Committee of New York Medical College, in compliance with National Institutes of Health guidelines.

2.2. Trace eye blink conditioning

Methods were identical to Weiss et al. (1999a,b). Animals were anesthetized with either a combination of xylazine (13 mg/kg, i.p.) and ketamine (87 mg/kg, i.p.), or isoflurane (to effect) and placed in a stereotaxic device. An incision was made on the top of the skull allowing for retraction of the periosteum. A total of six bilateral holes were drilled into the skull for insertion of stainless steel screws. A strip connector with two Teflon coated stainless steel wires and an uninsulated wire for an animal ground was then placed on the skull. EMG activity was recorded from the orbicularis oculi muscle via the recording wires, which were inserted underneath the skin until they penetrated the upper eyelid of the right eye. A tether holding a connector for relaying EMG activity and a tube for airpuff delivery was attached to the strip connector. Dental cement was then placed around

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the connector and over the screws until the connector was firmly in place. Following surgery, animals were placed on a heating pad and given buprenex (0.5 mg/kg, s.c.) for possible discomfort due to surgery. Animals were given a minimum of 5 days to recover before beginning the behavioral training.

Aged rats received vehicle ($n = 15$) or 1.0 mg/kg ($N = 10$) GLYX-13 dissolved in 0.9% saline 10 min prior to habituation and each daily training session. All injections were given in a volume of 1 ml/kg. This dose of GLYX-13 was chosen because it produced the optimal enhancement in learning in both young adult and old animals in a previous dose response study using tEBC (Moskal et al., 2005; Weiss, unpublished observation). After injection of either drug or vehicle, 0.15 ml 0.9% saline was injected to flush the catheter, followed by 0.15 ml heparinized glycerol to clear the dead space. Animals were then subjected to the trace conditioning paradigm as described below.

A habituation session preceded testing and was of equal duration to the training sessions. No stimuli were presented during the habituation session. During tEBC, rats received 10 sessions (1 session/day) of 30 paired presentations per session of an auditory conditioned stimulus (CS, 250 ms, 8 kHz, 85 dB, 5 ms rise/fall) and, following a 250 ms trace interval, an air puff unconditioned stimulus (US, 100 ms, 4.5 psi corneal airpuff), with an inter-trial interval (ITI) of 20–40 s (30 s average). Data were collected and analyzed as described previously (Weiss et al., 1999a,b). Statistical analyses were performed using one-way analysis of variance (ANOVA) and Fisher's PLSD post hoc test where appropriate.

2.3. Morris water maze

Testing was performed using methods of Kuo et al. (2006). Old or young adult animals were pre-treated 10 min before the start of each testing day with i.v. injections of 1.0 mg/kg GLYX-13 (old $n = 7$, young adult $n = 12$) or saline vehicle (old $n = 9$, young adult $n = 10$). Prior to the first training session, cue learning was measured without drug or vehicle injection. A black platform extending 2 cm above water level was used for these trials, to contrast with the white opaque tank water. For each trial, the platform was randomly moved, and rats were placed in the tank at different start positions. Rats received four cue trials. Cue learning served as a control procedure for place learning to ensure that the rats did not suffer from sensory, motor, or motivational deficits. All rats were able to find the visible platform within 60 s, did not show overt sensory/motor deficits, and were randomly assigned to drug or vehicle groups for subsequent training session. Rats were tested for place learning in the MWM on subsequent sessions; each session consisted of four trials/session, with an inter-trial interval of 60 s. Prior to the first training trial, rats were placed on the platform and allowed to remain there for 30 s to familiarize themselves with its location. The platform was located in a novel location each test day, remaining constant over trials within a daily session. A matching to sample version of the MWM task was employed for this study because of the increased difficulty of this task over a standard reference memory MWM task. On each trial, rats were placed in the water, with their heads facing toward the tank wall, at one of four equally spaced positions around the tank (north, south, east, west). Rats started from a different position each trial, and were allowed to swim until reaching the platform (10 cm square, submerged ~3 cm below the top of the water) and climbing on top, or until 60 s had expired, at which time rats that did not find the platform were hand-guided to it. Rats remained on the platform for 15 s before being removed. Tank water was made opaque with a powdered, nontoxic white paint, and color was kept consistent throughout the training period. Daily readings were taken to verify that tank water was at $25 \pm 1\,^{\circ}C$ before animals were run. During all trials, rats' performance was recorded by a FC-82B video camera and wide-angle camera lens mounted above the center of the pool. The animal's location in the tank was digitized with a VP200 tracker and data collected by HVS Water for Windows software from HVS Image (United Kingdom). The tank (180 cm in diameter) was surrounded by curtains with detailed posters attached to them serving as extra-maze cues. The experimenter remained outside the curtains during trials and entered at the end of a trial to retrieve the rat. Placement of the extra-maze cues remained consistent from day to day. Place learning was assessed by analyzing the distance in meters the animal traversed to locate the platform averaged across 4 trials each test day. Statistical analyses were performed using analysis of variance (ANOVA) and Fisher's PLSD post hoc test where appropriate.

2.4. T-maze

T-maze testing was performed using methods similar to Monahan et al. (1989). Young adult and old rats were pre-treated 10 min before the start of testing with i.v. injections of either 1.0 mg/kg GLYX-13 or an equal volume of saline vehicle (all $ns = 7$ per group). In brief, the t-maze was constructed with arms (45 cm long × 10 cm wide × 10 cm high) made of black Plexiglas enclosing the maze. Two plastic bottle caps, lined with wire mesh, were secured to the end of each goal arm in which the food reward (Cheerios, ~100 mg/piece) was placed. Before the start of testing, animals were gradually deprived of food to approximately 85% of their free feeding weight. On three successive days before the start of testing, animals were habituated to the t-maze with food located throughout the maze. On the first day of testing, animals were rewarded for right arm choices and were trained to a criterion of 9 out of 10 consecutive correct choices. The next day, animals were rewarded for left arm choices, and were trained to a criterion of 9 out of 10 consecutive correct choices. Animals were tested in a round robin manner with an ITI of approximately 7 min. Total trials to criterion (trials to criterion day 1+ trials to

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criterion day 2) were used for statistical analysis. Statistical analyses were performed using analysis of variance (ANOVA) and Fisher's PLSD post hoc test where appropriate.

2.5. Hippocampal slice preparation

Fischer 344 rats (70–80 days or 24 months old; Harlan Labs) were used in these studies. At 24 months of age, male Fischer 344 rats show, on average, a 40% mortality rate (Turturro et al., 1999), and marked age related learning and memory deficits in the Morris water maze (Frick et al., 1995), both of which are similar to 27-month-old FBNF1 rats (Present study; Turturro et al., 1999; Knuttinen et al., 2001). Animals were deeply anesthetized with isoflurane and decapitated. The brain was removed rapidly, submerged in ice-cold artificial cerebrospinal fluid (ACSF, 2–4 °C), which contained (in mM): 124 NaCl, 4 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose; at pH 7.4, gassed continuously with 95% O_2/5% CO_2. The brain was hemisected, the frontal lobes cut off, and individual hemispheres glued using cyanoacrylate adhesive onto a stage immersed in ice-cold ACSF gassed continuously with 95% O_2/5% CO_2 during slicing. 300 μm thick coronal slices were cut using a Vibratome (DSK DTK-1000), and transferred to an interface holding chamber for incubation at room temperature for a minimum of one hr before commencing recording.

2.6. Extracellular recordings

Slices were transferred to a Haas-style interface recording chamber and continuously perfused at 3 ml/min with oxygenated ACSF at 32 ± 0.5 °C. Low resistance recording electrodes were made from thin-walled borosilicate glass (1–2 MΩ after filling with ACSF) and inserted into the apical dendritic region of the Schaffer collateral termination field in stratum radiatum of the CA1 region to record field excitatory postsynaptic potentials (fEPSPs). A bipolar stainless steel stimulating electrode (FHC Co.) was placed on Schaffer collateral-commissural fibers in CA3 stratum radiatum, and constant current stimulus intensity adjusted to evoke approximately half-maximal fEPSPs once each 30 s (50–100 pA; 100 μs duration). fEPSP slope was measured by linear interpolation from 20 to 80% of maximum negative deflection, and slopes confirmed to be stable to within ± 10% for at least 15 min before commencing an experiment. Bath application of GLYX-13 (1 μM) vs. no bath application control was made before LTP or LTD inducing stimulation. The concentration of GLYX-13 used in this study (1 μM) was previously shown to produce optimal enhancement of LTP (Zhang et al., 2008). LTP was induced by stimulation of Schaffer collateral axons with four high frequency theta burst stimulus trains of 10 × 100 Hz/5 pulse bursts each, applied at an interburst interval of 200 ms. Each train was 2 s in duration, and trains were applied 15 s apart. LTD was induced by a single low frequency train of 2 Hz stimuli lasting 10 min, for a total of 1200 stimuli. Signals were recorded using a Multiclamp 700B amplifier and digitized with a Digidata 1322 (Axon Instruments, USA). Data were analyzed using pClamp software (version 9, Axon Instruments) on an IBM-compatible personal computer.

All external recording pipette solutions were made with deionized distilled water (resistance > 18 MΩ cm^{-2}; Milli-Q system). The chemicals for making extra- and intracellular solutions were purchased from Sigma (USA) and Fluka (USA). Electrophysiological data were analyzed initially with Clampfit (v9) (Axon Instruments, USA), and further processed and presented with Origin 6.1 (Microcal Software, USA) and CorelDraw 10.0 (Corel, Canada) programs. Statistical analyses were performed with SPSS (v11, USA). Statistical data are presented as mean ± SEM.

3. Results

3.1. Trace eye blink conditioning

As illustrated in Fig. 1, GLYX-13 (1.0 mg/kg, i.v.) enhanced tEBC learning in the aged rats, indexed by percent adaptive CRs across the 10 test days compared to vehicle treated animals (main effect of drug – ($F(1,23) = 14.4$, $P < .001$). In both vehicle-treated and GLYX-13-treated 27–32 months old FBNF1 rats, performance improved across test days ($F(9,23) = 16.0$, $P < .0001$). GLYX-13-treated animals required 90% fewer trials to learn the task (8 out of 10 consecutive trials with an adaptive CRs) compared to vehicle treated animals ($F(1,23) = 7.9$, $P < .01$). The mean ± SEM trials to criterion for the GLYX-13-treated rats was 11.2 ± 2.7, compared to 100.8 ± 25.7 for vehicle-treated rats. Fig. 1B is presented to show that although the percent of trials with CRs was relatively large for the first day of training, there was still a learning curve across blocks of trials for that session. GLYX-13 enhancement in tEBC in 3-month-old FBNF1 rats has been previously reported (Moskal et al., 2005).

3.2. Morris water maze

GLYX-13 (1.0 mg/kg, i.v.; Fig. 2) also enhanced learning and memory in the MWM, as indexed by mean path length to find the hidden submerged platform (main effect of Drug–($F(1,34) = 28.7$, $P < .0001$). Fisher PLSD post hoc test revealed that GLYX-13 significantly decreased mean path length compared to vehicle injected animals in both young adult ($P < .005$) and aged ($P < .005$) rats. A significant Drug × Age interaction ($F(1,34) = 6.0$, $P < .05$) indicated that GLYX-13 was more potent in increasing learning in old rats compared to young adult rats. This interaction likely occurred because the young rats were already performing so well that no further improvement was possible, i.e. there is a floor effect preventing further enhancement by the young rats. In addition, GLYX-13 treated old animals showed a greater reduction in mean path length average

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across test days (mean ± SEM; 2.2 ± 0.3 M) compared to vehicle treated old animals then young adult GLYX-13 treated animals (1.1 ± 0.2 M) compared to young adult vehicle controls ($F(1,17) = 12.1$, $P < .005$). Aged rats showed a significant impairment in MWM learning acquisition, as indexed by mean path length across the 7 test days compared to young adult rats ($F(1,34) = 122.7$, $P < .0001$). Old vehicle treated animals failed to learn the task at all across the seven test days (one way ANOVA – $F(6,56) = 1.3$, $P > .05$), whereas GLYX-13 treated animals were able to learn the task, demonstrated by a significantly reduced path length (one-way ANOVA – $F(6,42) = 4.4$, $P < .005$).

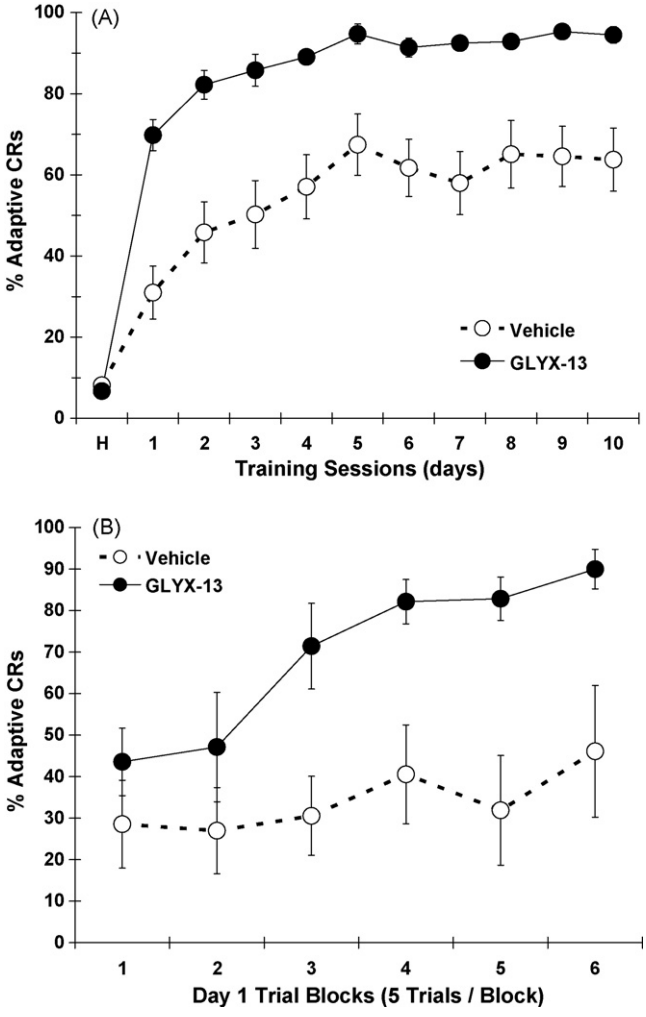

Fig. 1. GLYX-13 enhanced learning in the tEBC paradigm in old rats. (A) mean ± SEM percent adaptive conditioned responses (CRs) during trace eye blink conditioning in old (27–32 month) rats. GLYX-13 (1.0 mg/kg IV) or vehicle was administered 10 min before the start of habituation or testing. This enhancement persisted throughout the entire experiment. GLYX-13 produced a similar enhancement of learning in young adult rats (Moskal et al., 2005). (B) The learning enhancement developed slowly during the first day of training. Old animals treated with GLYX-13 showed a significant increase in learning (as measured by adaptive CRs) compared to controls on blocks 3–6 of the first day of training ($P < .05$; Fisher's PLSD planned comparison), but not during the first two blocks of training.

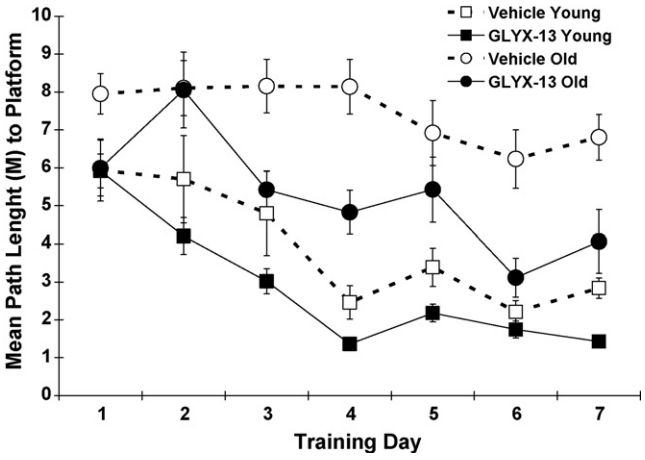

Fig. 2. GLYX-13 enhanced learning in the Morris water maze in both young and old rats. Data are presented as the mean ± SEM path length to the hidden platform for each of the 7 test days in the movable platform version of this task. Young adult (3 months) or old (27–32 months) rats were given IV injections of GLYX-13 (1.0 mg/kg, i.v.) or vehicle 10 min before the start of testing. GLYX-13 enhanced learning and memory in both the young adult and old animals. GLYX-13 facilitated learning and memory to a greater extent in the old animals as indexed by both an Age X Drug interaction ($P < .05$), and a greater reduction in mean path length in drug vs. vehicle treated animals ($P < .005$). This may be due to a "floor effect" for the young animals (see Section 3 for more details).

3.3. T-maze

GLYX-13 at 1.0 mg/kg, i.v. compared to vehicle enhanced learning as indexed by a decrease in trials to criterion compared to vehicle treated animals on both the non-alternating t-maze on test day 1 (Fig. 3; main effect of Drug – $F(1,24) = 23.7$, $P < .0001$), and the alternating t-maze on test day 2 (Fig. 3; main effect of Drug – $F(1,24) = 8.3$, $P < .01$). There was no significant main effect for age or age by drug interaction for either the non-alternating or alternating tasks (all Ps > .05).

3.4. Long-term potentiation and long-term depression at the Schaffer collateral-CA1 synapses

Fig. 4 shows the effects of GLYX-13 on long-term activity-dependent plasticity at Schaffer collateral synapses in hippocampal slices from young adult (2-month-old) Fischer 344 rats. Bath application of GLYX-13 (1 μM) prior to application of theta burst stimulation to Schaffer collateral axons produced a 40% increase in the amplitude of LTP evoked in slices from 2-month-old Fischer 344 rats (Fig. 4A), while the same concentration of GLYX-13 did not significantly alter the magnitude of LTD elicited by a low-frequency stimulus train (Fig. 4B; 2 Hz/10 min). GLYX-13 was significantly more potent in enhancing the magnitude of LTP at Schaffer collateral-CA1 synapses in slices from aged 24-month-old Fischer 344 rats, where it produced an 85% enhancement of LTP ($P < 0.05$, one-way ANOVA; Fig. 5A). The facilitation of LTP by GLYX-13

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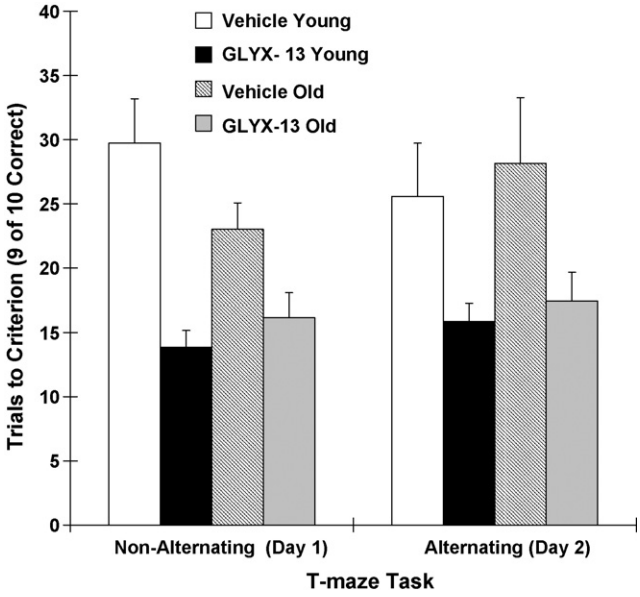

Fig. 3. GLYX-13 enhanced learning in the t-maze task in both young and old rats. Data are presented as the mean ± SEM trials to criterion (9 out of 10 consecutive correct choices on each testing day). Food deprived rats were rewarded for left arm choices on day 1 and right arm choices on day 2. Ten minutes before the start of each testing day, animals were given i.v. injections of 1.0 mg/kg of GLYX-13 or vehicle. GLYX-13 significantly facilitated learning in both young adult and aging rats in both the non-alternating and alternating t-maze ($P < .05$).

remained selective, since LTD was not altered in aged rats (Fig. 5B).

4. Discussion

GLYX-13 enhanced learning in all three hippocampus-dependent learning tasks (tEBC, MWM, T-Maze) in both young adult and age impaired old animals. In the MWM in which a clear age related deficit in learning was seen, GLYX-13 enhancement of learning was greater in old compared to young adult rats. These data corroborate and extend our previous report that GLYX-13 enhanced hippocampally dependent tEBC *in vivo* in young adult animals (Moskal et al., 2005). The previous tEBC study suggested that the effects of GLX-13 were acting upon the forebrain, since rats trained with delay conditioning learned at a similar rate and to a similar level whether they were given GLYX-13 or vehicle. Those results also suggested that the effects of GLYX-13 were associative in nature since young adult rats given explicitly unpaired presentations of tones and corneal air puffs did not exhibit pseudoconditioning. Moreover, the amplitude of the unconditioned response was similar in both the GLYX-13 and vehicle treated groups. The learning enhancement in the aging rats is likely also associative in nature, especially since there was no effect of GLYX-13 on the peak amplitude of the UR (data not shown). However, pseudoconditioning controls were not run to explicitly rule out this possibility.

In contrast to GLYX-13, the facilitation of tEBC in aged animals by DCS produced a smaller enhancement in learning as measured by trials to criterion (~50% reduction with DCS, compared to an 84% reduction in trials to criterion with GLYX-13; Thompson and Disterhoft, 1997b). In addition, twice the dose of DCS was necessary to elicit optimal facilitation in learning (Thompson and Disterhoft, 1997b).

In the present study, we did not find an age related deficit in performance in the t-maze task. However, GLYX-13 significantly enhanced performance on both the acquisition and reversal test days in the t-maze task for both young adult and old animals. Aggleton et al. (1986) have reported that lesions to the hippocampus disrupt alternating t-maze learning but not non-alternating t-maze learning. GLYX-13 enhanced learning in both the alternating and non-alternating component of the t-maze paradigm used in these studies. Therefore, GLYX-13 may facilitate hippocampus-dependent as well as hippocampus independent forms of learning.

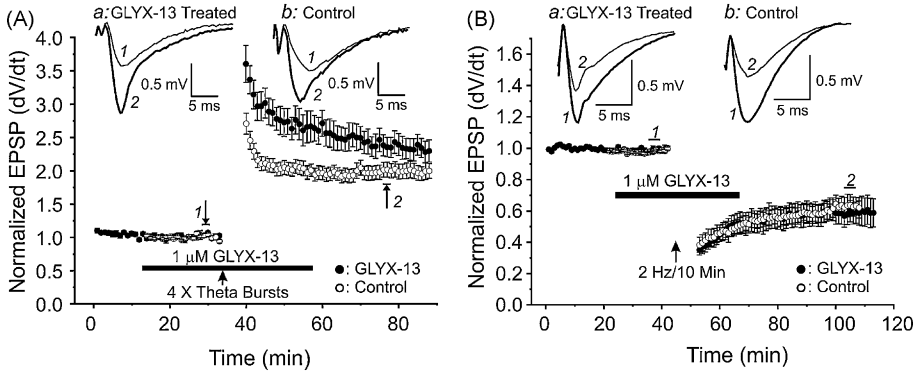

Fig. 4. 1 μM GLYX-13 enhances magnitude of long-term potentiation (LTP), without affecting long-term depression (LTD), in slices from young adult (2-month-old) Fischer 344 rats. (A) Time course of LTP induced by four high frequency theta burst stimulus trains (10 × 100 Hz 5 pulse bursts 200 ms inter-burst interval; arrow) at Schaffer collateral-CA1 synapses in slices pre-treated with GLYX-13 (1 μM; solid bar; filled circles; $n = 12$), compared to control, untreated slices (open circles; $n = 12$). (Insets: sample field e.p.s.p.s before (1) and after (2) induction of LTP in a GLYX-13 treated (a) and control (b) slices. (B) Time course of LTD induced by a low-frequency stimulus train (2 Hz/10 min; arrow) at Schaffer collateral-CA1 synapses in slices pre-treated with GLYX-13 (1 μM; solid bar; filled circles; $n = 9$), compared to control, untreated slices (open circles; $n = 10$). (Insets: sample field e.p.s.p.s before (1) and after (2) induction of LTD in a GLYX-13 treated (a) and control (b) slices.

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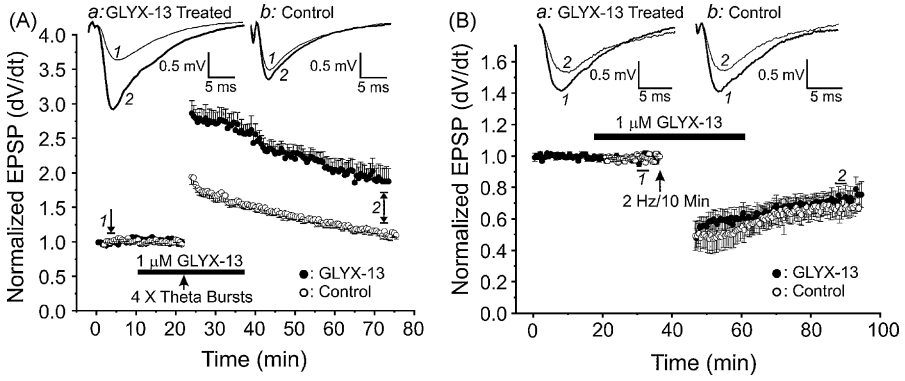

Fig. 5. GLYX-13 produces a greater enhancement in Schaffer collateral-CA1 LTP, without altering LTD, in slices from aged (24-month-old) Fischer 344 rats. (A) Time course of LTP induced by four high frequency theta burst stimulus trains (10 × 100 Hz 5 pulse bursts 200 ms inter-burst interval; arrow) at Schaffer collateral-CA1 synapses in slices pre-treated with GLYX-13 (1 μM; solid bar; filled circles; $n = 12$), compared to control, untreated slices (open circles; $n = 12$). (Insets: sample field e.p.s.p.s before (1) and after (2) induction of LTP in a GLYX-13 treated (a) and control (b) slices. (B) Time course of LTD induced by a low-frequency stimulus train (2 Hz/10 min; arrow) at Schaffer collateral-CA1 synapses in slices pre-treated with GLYX-13 (1 μM; solid bar; filled circles; $n = 9$), compared to control, untreated slices (open circles; $n = 10$). (Insets: sample field e.p.s.p.s before (1) and after (2) induction of LTD in a GLYX-13 treated (a) and control (b) slice). GLYX-13 enhanced LTP to a greater extent in young adult as compared to old rats as indexed by an Age X Drug interaction ($P < .05$).

The version of the t-maze task used may not have been cognitively demanding enough to capture age related deficits. In contrast to the MWM, in which a marked age related deficit was observed, in the t-maze task the animals only had to learn which of two arms were rewarded each day for just two test days. Whereas in the MWM task, rats had to locate a hidden platform whose location changed each day across 7 test days. It is possible that by increasing the difficulty of the task by using an alternating t-maze protocol, or by increasing the inter-trial interval, an age related cognitive deficit might be uncovered. Also, the failure to see an age related deficit in the t-maze task could have been associated with the age chosen for testing (27 months). This age was chosen based on a study examining four different ages of adult rats of the same strain (from 6 to 35 months), where half of the 27-month group (old) exhibited impairment on the trace eye blink conditioning task (Knuttinen et al., 2001). It is quite possible that even older animals would have shown more impairment on the t-maze task.

GLYX-13 enhances learning and memory in hippocampal-dependent tasks by facilitating NMDAR activity through partial agonist activity at the glycine site (Moskal et al., 2005). NMDAR binding and protein levels are both decreased in aged hippocampus of laboratory animals and humans (reviewed in Magnusson, 1998). Furthermore, aged rats that show decreased NMDAR binding in the hippocampus are more sensitive to the NMDAR antagonist MK-801 than young adult rats (Ingram et al., 1992). The greater enhancement in MWM learning seen in GLYX-13 treated old rats compared to young adult rats may be due to a greater net GLYX-13 mediated facilitation of down regulated NMDAR in old animals. GLYX-13 may facilitate learning in old rats to a greater extent then young adult rats due to greater functional NMDAR expression generating a ceiling effect in young adult rats.

Another hypothesis is that GLYX-13 may enhance learning to a greater extent in the MWM in old compared to young adult rats because it corrects an age-related deficit in D-serine biosynthesis and or release, since D-serine is now considered to be the endogenous co-agonist of the NMDAR glycine site (Miller, 2004; Mothet et al., 2006). GLYX-13, acting as an agonist at the glycine site, may therefore act as a replacement therapy for age-related deficits in D-serine to promote learning and memory in aging.

In aged rats, some studies have found significantly less LTP (Shankar et al., 1998; Mori et al., 2000; Reis et al., 2005), while others have reported no differences (Kumar et al., 2007). Similarly, studies on LTD, all of which used a 1 Hz stimulus frequency rather than our 2 Hz frequency, reported greater LTD (Norris et al., 1996; Foster and Kumar, 2007), while others found no changes (Norris et al., 1998; Kumar et al., 2007). Here, we confirmed that control LTP in aged Fisher 344 rat slices was markedly impaired, but found that aging produced only a slight decrease in the amplitude of LTD. Species, synapse, and stimulus frequency differences could all contribute to this variability.

The selectivity of GLYX-13 for enhancing LTP over LTD suggests that the NMDARs involved in induction of these two forms of plasticity, and the effects of GLYX-13 on glycine sites in differing NMDAR subunits, are likely to be different.

In summary, GLYX-13 enhanced learning and memory in 3 different hippocampal-dependent learning tasks in both young adult and learning impaired aging rats. GLYX-13 also facilitated the formation of LTP and to a greater extent in aged compared to young adult animals. GLYX-13 had no effect on the production of LTD in either young adult or aged animals. These data suggest that GLYX-13 may be a promising drug treatment for deficits in learning and cognition associated with human aging, and in neurodegenerative diseases involving cognitive dysfunction.

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Disclosure statements

1.

(a) Joseph Moskal is the inventor of the Glyxin family of neuropeptides of which GLYX-13 is one. He is a co-founder and stock holder in Naurex, Inc. Naurex, Inc. owns the Intellectual Property for GLYX-13. There are no financial, personal or other relationships with other people or organizations that could result in an actual or perceived conflict of interest by any other authors of this manuscript. That is there were no consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, grants or other funding to any of the coauthors. Coauthors Disterhoft and Stanton were paid a fee as consultants for overseeing some of the experiments, data analysis and interpretation as described in the NIH grant used to fund these studies.

(b) There are no Institutional contracts relating to this research or any other organization that could stand to gain financially now or in the future.

(c) There are no agreements of any authors or their institutions that could be seen as involving a financial interest in this work.

2.

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3.

The data contained in the manuscript being submitted have not been previously published, have not been submitted elsewhere and will not be submitted elsewhere while under consideration at Neurobiology of Aging.

4.

Statements verifying that appropriate approval and procedures were used concerning human subjects and animals are included in the manuscript.

5.

I verify that all authors have reviewed the contents of the manuscript being submitted, approved of its contents and validated the accuracy of the data.

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