WITHDRAWN:
Effects of $GABA_A$ Receptors in Dorsal Hippocampus on Memory Retention of Cholestatic Rats

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Abstract

The molecular mechanisms that result in cognitive deficits following cholestasis are mainly unknown. As there are many $GABA_A$ receptors in the hippocampus CA1 region and the crucial role for GABA in modulating memory, we evaluated the effects of $GABA_A$ receptor agents in the CA1 of cholestatic rats on memory retention. The interaction between GABAergic and opioidergic systems in the CA1 on memory was also investigated. The effects of administration of $GABA_A$ receptor agonist and antagonist, muscimol (60, 120 and 240 ng/ side) and bicuculline (100, 200 and 400 ng/ side), into CA1 on memory retention were studies using passive avoidance learning (PAL) task in bile duct ligated (BDL) rats. Naloxone (250 ng/ side), the mu-opioid receptor antagonist, was also co-administered alone or with bicuculline (400 ng/ side) to indicate the interaction between opioidergic and GABAergic system. Cholestasis inhibited memory retrieval is shown by the decrease in the step-through latency (STLr). Administration of muscimol or bicuculline alone after training potentiated or attenuated respectively amnesia in BDL rats dose-dependently. Naloxone (250 ng /side) alone increased STLr in BDL-treated rats. Bicuculline (100 ng/side) alone antagonized the amnesic effect of muscimol (120 ng/side). Co-administration of bicuculline and naloxone or muscimol and naloxone caused a significant difference in STLr compared to only naloxone treated rats which show the interaction between two systems on memory retention in cholestssis. Bicuculline (100 ng/side) microinjection alone antagonized the amnesic effect of muscimol (120 ng/side). We indicated the contribution of intra-CA1 $GABA_A$ receptors on memory retention in cholestatic rats by the PAL test. Blockade of each $GABA_A$ or mu-opioid receptors alone could attenuate the amnesia in BDL rats. Furthermore, blockade of both $GABA_A$ or mu-opioid receptors reversed the memory deficit in BDL-treated rats, which shows the interaction between GABAergic and opioidergic systems on memory retention in this test.

Introduction

Cholestasis arises from structural and functional impairments of the hepatobiliary system (Roma and Crocenzi 2008) that finally leads to elevation of concentrations of biliary constituents in plasma, jaundice, and liver injury. Bile duct ligation (BDL) is an experimental model for cholestatic liver disorders that induces jaundice and liver dysfunction (Huang et al., 2010; Sheen et al., 2010). Neurocognitive deficits as the main complication of liver dysfunction have been indicated in both humans (Swain 2009) and animals (Magen et al., 2009).

The precise mechanism that results in cognitive deficits following cholestatic liver disease is mainly unknown and therefore, there is no effective treatment available (Bashiri et al., 2018). There are some reports indicate that dysfunctions in several neurotransmitter systems, including acetylcholine, opioids,gamma-aminobutyric acid, noradrenaline, serotonin, dopamine, and glutamate may be involved in the pathogenesis of behavioral and cognitive abnormalities caused by cholestatic liver disease (Agusti et al., 2016; Hosseini et al., 2013; Nasehi et al., 2013a; Palomero-Gallagher et al., 2009). For example, it has been shown that the endogenous opioids increase in the cholestatic liver disease, supporting the

involvement of the opioid system in its pathophysiology (Ebrahimkhani et al., 2006; Yurdaydin, 2001). Furthermore, it has been shown that GABAergic system dysfunction contributes to the pathogenesis of the cholestatic liver disease (Cauli et al., 2009).

$GABA_A$ receptors, as the major inhibitory receptors in the nervous system, play the main role in cognition (Chapouthier and Venault 2002; Reis et al., 2009; Torkaman-Boutorabi et al., 2013). They are found at high concentration in the hippocampus, which plays crucial roles in cognition processes including, acquisition, consolidation, and retention (Lorenzini et al., 1996). For example, application of GABAergic receptor agonists could impair memory, but the GABAergic receptor antagonists enhance memory processes in inhibitory avoidance tests (Castellano and McGaugh 1990; Rassouli et al., 2010; Sardari et al., 2014).

There is no integrative study about the effects of GABAergic system on cognition function in cholestatic liver disease. Therefore, we aimed to evaluate the involvement of $GABA_A$ receptors in the dorsal hippocampal on memory retention of cholestatic animals. In the first series of experiments, we microinjected a selective $GABA_A$ receptor agonist, muscimol, and the $GABA_A$ receptor antagonist, bicuculline, at different doses into CA1 of healthy and BDL rats. In another series of experiments, naloxone, the μ-opioid receptor antagonist, was microinjected alone or with bicuculline and muscimol into CA1 for detecting the possible interaction between the two opioidergic and GABAergic systems. Memory retention was evaluated by a passive avoidance learning (PAL) task in different animal groups.

Material And Methods

Animals Husbandry

Locally produced male Wistar rats (250-280 g) were used in this study. Animals were housed at about 26 ± 2°C temperature and a 12/12°h light/dark cycle. Rats had free access to the taped water and standard laboratory chow. Rats were handled following the protocols approved by the institutional ethics committee of Shaanxi Provincial People's Hospital. Furthermore, the present experimental

procedures were done in accordance with the guidelines for animal experimentation of the European Communities Council Directive (EU/63/2010) and reported according to the ARRIVE guidelines (Lilley et al., 2020).

Drugs

We used (+)-bicuculline (Sigma, St. Louis, USA), muscimol hydrochloride (Sigma, St Louis, MO) and naloxone hydrochloride (Tocris Cookson Inc., Bristol, UK). Naloxone and muscimol were dissolved in 0.9% saline. Bicuculline was dissolved in a drop of acetic acid glacial and then made up to 5 mL volume with 0.9% saline.

Bile duct ligation surgery

Laparotomy was done through the administration of sodium pentobarbital (50 mg/kg, *i.p.*) to cause general anesthesia. Surgery of BDL was performed by ligation of the main bile duct by two ligatures approximately 0.5 cm apart and transection at the midpoint between the two ligatures (Bergasa et al., 1994). To do postoperative care, rats were kept in separate cages to prevent wound dehiscence. About 4 h after surgery, rats were moved to their original cage (Rastegar et al., 2002). Operation-related mortality was less than 5%.

Cannula guide implantation

Animals were anaesthetized with xylazine/ketamine (5 and 50 mg/kg, *i.p.*, respectively). To do stereotaxic surgery, two 23 gauges guide cannulas were moved until the tips were above the CA1 (about 2 mm). The stereotaxic coordinates used for the CA1 were: AP: -2 mm from bregma, V: -1.5 mm from the skull surface and L: ±1.6 from the sagittal suture (Paxinos and Watson 1986). The cannulas were secured to anchor screws with dental acrylic. After one week of recovery, rats were used for the experiments.

Intracerebral drug microinjections

The injection cannula (30 gauge) were used for bilateral microinjection of drugs into the CA1. Then, injection cannula was attached to a Hamilton syringe (1μL) by a PE-10 connection tube. The volume of injection was 0.5 μL solution on each side (1 μL/rat) during a 60-s period. The needles were not removed until 1 min after injection for complete diffusion.

PAL and memory test (step-through test)

The test was performed for all experimental groups on 14 days after BDL surgery. The procedure and apparatus were approximately the same as the previous reports (Hasanein and Shahidi 2010; Hasanein and Teimuri Far 2015). The step-through passive avoidance apparatus included of a transparent plastic lighted chamber (20 cm×20 cm×30 cm) and a dark chamber in dark opaque plastic with the same size of light chamber. The floor of both compartments was made of stainless steel rods (spaced 1 cm apart). A shock generator provides electricity in the floor of dark chamber. An opaque guillotine door closed the opening between the two chambers.

Training

All the rats received two trials for habituating to the apparatus at first. To do these trials, the animals were placed in a lighted chamber facing away from the door and the guillotine door was raised 5 sec later. The rat prefers dark environment naturally. Once the animal enters the dark chamber, the door was closed and then after 30 sec the animal was moved to its home cage. The trial of habituation was repeated 30 min later and then followed by the first acquisition trial after 30 min. When the rat had placed all 4 paws in the dark chamber, step-through latency or STLa was recorded as the latency to enter the dark chamber. If rat waited more than 100 sec to enter the dark chamber, it was eliminated from the experiments. A mild electrical shock (0.5 mA) was applied (3 sec) after entering the animal to the dark chamber. The animal was moved from the dark chamber into its cage after 30 sec. Then the procedure was repeated after 2

min. Every time the animal reentered the dark and had placed all 4 paws in the dark chamber, it received a foot-shock. The training was terminated if the rat remained 120 sec in the light compartment.

Retention test

Twenty-four hours after the acquisition trial, the retention trial was done. First, the animal was placed in the lighted chamber and after 5 sec, the guillotine door was raised. We recorded step-through latency (STLr) up to 300 sec. If the animal did not enter the dark chamber in 300 sec, the retention trial was terminated.

Experimental design

The following experiments were performed on each rat group consisting of seven animals and each rat was used only once.

Experiment 1. Different doses of muscimol were microinjected into CA1 after training in BDL and non-BDL rats. Four groups of rats received intra-CA1 injections of saline or muscimol (60, 120 and 240 ng/side) immediately after training. In the retention trial, all of these rats were microinjected with saline, 5 min before the retention trial.

Experiment 2. Different doses of bicuculline were microinjected into CA1 after training in BDL and non-BDL rats. Four groups of rats were microinjected into CA1 with saline or bicuculline (100, 200 and 400 ng/side) immediately after training. On the retention trial, all of these rats were administered saline, 5 min before the retention trial.

Experiment 3. The effects of microinjection of naloxone alone or bicuculline plus naloxone after training on memory retention in BDL and non-BDL rats were investigated. Four groups of animals received intra-CA1 injections of saline, naloxone alone (250 ng/side), naloxone (250 ng/side) plus bicuculline (200 ng/side) and naloxone (250 ng/side) plus muscimol (120 ng/side) after training. On the retention trial, all of these rats received saline into CA1, 5 min before the retention trial.

Experiment 4. This experiment examined the effect of post-training microinjection of bicuculline with muscimol on memory retention in BDL rats. Four groups of BDL animals received intra-CA1 injections of saline, bicuculline (100 ng/side), muscimol (120 ng/side) and bicuculline (100 ng/side) with muscimol (120 ng/side) after training. On the test day, all of these animals received intra-CA1 injections of saline, 5 min before the test.

Statistical analysis

All data are expressed as mean ± S.E.M. The SPSS statistical software package (version 21.5; SPSS, Chicago, IL, USA) was used to do the analysis. One-way analysis of variance (ANOVA) with Tukey as the *post hoc* test was applied for showing differences among groups. Probability values less than 0.05 were considered significant. Two-way ANOVA identified a significant interaction between STLr of rats who

received different doses of drugs in BDL and non-BDL groups. *Post hoc* analysis (Tukey test) was applied following a significant F value, for assessing the specific inter-group comparisons.

Results

Effects of different doses of muscimol microinjected into CA1 on memory retention on the PAL test

Figure 1 indicates muscimol effects microinjected into CA1 at doses 60, 120 and 240 ng/side on STLr in different control and BDL rats. The results of two-way ANOVA showed a significant interaction between muscimol effects administered at different doses on STLr in BDL and non-BDL animals ($F (3, 48) = 43.61$, $P < 0.001$). More analysis revealed that intra-CA1 injection of muscimol (60, 120 and 240 ng/side) induced no significant change on STLr in non-BDL animals (P's > 0.05) (Fig. 1 left panel). However, post-training administration of muscimol (60 ng/side) decreased STLr in BDL rats (190.42 ± 2.8 sec) compared to saline-treated BDL rats (221.57 ± 6.28 sec) ($P < 0.001$) (Fig. 1 right panel). Furthermore, muscimol at 120 and 240 ng/side doses decreased STLr of BDL rats (161.14 ± 3.9 sec, 143.28 ± 5.18 sec, respectively) than saline-received BDL rats (221.57 ± 6.28 sec) ($P < 0.001$, $P < 0.001$, respectively) (Fig. 1, right panel). The effect of muscimol on STLr in BDL animals was dose-dependent, in this regard muscimol at the highest dose induced the most effect in reduction of STLr in BDL rats compared to muscimol 60 and 120 ng-treated BDL animals ($P < 0.001$, $P < 0.05$, respectively) (Fig. 1, right panel).

Effects of different doses of bicuculline microinjected into CA1 on memory retention in the PAL test

Muscimol effects microinjected into CA1 at doses 100, 200 and 400 ng/side on STLr in different control and BDL rats are indicated in Figure 2. Two-way ANOVA results showed a significant interaction between bicuculline effects administration at different doses on STLr in BDL and non-BDL animals ($F (3, 48) = 10.96$, $P < 0.001$). Tukey test revealed that post-training injection of bicuculline (100, 200 and 400 ng/side) in non-BDL rats could not alter STLr as shown in the left panel of Figure 2 (P's > 0.05). We found a significant difference in STLr of BDL rats, between post-training administered bicuculline (100 ng/side) (229.71± 3.63 sec) compared to vehicle-treated BDL group (201.71 ± 2.69 sec) ($P < 0.01$) (Fig. 2 right panel). Moreover, intra-CA1 injection of bicuclline 200 and 400 ng increased STLr (245 ± 4.1 sec, 262.85± 6.26 sec, respectively) than vehicle-received BDL rats (201.71 ± 2.69 sec) ($P < 0.001$, $P < 0.001$, respectively) (Fig. 2 right panel). Further analysis showed bicuculline increased STLr in BDL rats dose dependently, in this regard, we found a significant difference in STLr between bicuculline 100 ng and bicuculline 400 ng-treated BDL animals ($P < 0.001$). A significant difference in STLr between bicuculline 200 ng and bicuculline 400 ng-treated BDL animals ($P < 0.05$) were also found (right panel of Figure 2).

Effects of administration of bicuculline or muscimol with naloxone on memory retention in the PAL task

Figure 3 indicates the effects of naloxone alone and bicuculline or muscimol plus naloxone on memory retention in BDL and non-BDL rats. The results of two-way ANOVA showed a significant interaction between the effects of naloxone alone and bicuculline or muscimol plus naloxone on STLr in BDL and non-BDL rats ($F (3, 48) = 20.78$, $P < 0.001$). As shown in the left panel of figure 3, administration of

naloxone alone and bicuculline or muscimol plus naloxone could not change STLr of non-BDL rats (243.85 ± 1.86 sec, 246.85 ± 6.61 sec, 249.14 ± 5.47 sec, respectively) compared to saline-treated non-BDL animals (244.14 ± 4.66 sec) (P's > 0.05).

More analysis indicated that intra-CA1 injection of naloxone (250 ng/side) induced a significant increase in STLr in BDL rats (230.14 ± 0.49 sec) compared to saline-treated BDL group (210 ± 2.43 sec) ($P < 0.05$) (right panel, Fig. 3). However, we showed a significant difference in STLr in naloxone-treated BDL rats (226.14 ± 2.48 sec) compared to saline-treated non-BDL rats (244.14 ± 4.66 sec) ($P > 0.05$). Administration of both naloxone and bicuculline in BDL group reversed the amnesia induced by cholestasis in BDL group. In this regard, such treatment increased STLr of BDL rats (251.28 ± 3.84 sec) compared to saline-treated BDL animals (210 ± 2.43 sec) ($P < 0.001$). Furthermore, we could not find any significant change in STLr between co-administered naloxone and bicuculline BDL group (251.28 ± 3.84 sec) compared to saline-treated non-BDL animals (244.14 ± 4.66 sec) ($P > 0.05$). Further analysis indicated a significant difference in STLr between only naloxone treated BDL rats (230.14 ± 0.49 sec) and bicuculline plus naloxone treated BDL rats (251.28 ± 3.84 sec) ($P < 0.05$). On the other hand, microinjection of muscimol plus naloxone in BDL rats made a significant difference in STLr (194 ± 2.19 sec) compared to only naloxone treated BDL animals (230.14 ± 0.49 sec) ($P < 0.001$) as shown in the right panel of Figure 3.

Effects of administration of bicuculline to BDL rats on the response induced by administration of muscimol in the PAL task

Tukey post test revealed that microinjection of the minimum dose of bicuculline (100 ng/side) could not alter STLr of BDL rats compared to saline treated BDL animals as indicated in Figure 4 ($P > 0.05$). Administration of the medium dose of muscimol (120 ng/side) induces a hyperalgesic effect in BDL rats (156.85 ± 3.39 sec) compared to saline treated BDL group (225.42 ± 3.46 sec) ($P < 0.001$). However, co-administration of bicuculline (100 ng/side) with muscimol (120 ng/side) antagonized the hyperalgesic effect of muscimol (120 ng/side) by increasing STLr in bicuculline plus muscimol injected cholestatic animals (220.71 ± 2.92 sec) compared to only muscimol- treated cholestatic animals (156.85 ± 3.39 sec) ($P < 0.001$).

Discussion

The present work indicated the influences of $GABA_A$ receptor antagonist and agonist on memory retention in cholestasis, which is an animal model with increased tone of endogenous opioid system. We used PAL task which is a standard task for evaluating learning and memory based on the previous reports (Hasanein and Teimuri 2015; Nasehi et al., 2013a). During the retention trial of PAL task, the decrease in STLr indicates inhibitory effects on memory retention (Hasanein and Shahidi 2010; Torkaman-Boutorabi et al., 2013). Our results revealed that amnesia in the cholestatic animals was potentiated by intra-CA1 microinjection of muscimol. The involvement of $GABA_A$ receptor on memory retention in cholestatic model was confirmed by showing the effects of bicuculline as a selective $GABA_A$

receptor antagonist, which increased STLr in cholestatic animals. To exclude the hypomotility in inducing the observed effects, muscimol and bicuculiine doses in this study were selected based on the previous published report (Ebrahimi-Ghiri et al., 2018) and our own pilot studies. The drugs were administered immediately after training, therefore, the observed effects are attributed to consolidation of memory, which performs just after the training trial and lasts some time (Ghiasvand et al., 2011; McGaugh 1989). We have shown in a previous study bicuculline and muscimol effects in cholestatic animals on thermal pain modulation, which also suggests the pharmacological interactions between GABA and opioid systems (Hasanein and Parviz 2014).

In the current study, there was an impairment in memory retention of cholestatic animals in the PAL task, which is compatible with a previous study showing the negative effect of post-training microinjection of morphine on memory retention in PAL test (Hosseini et al., 2013). Furthermore, this report confirms previous behavioral studies about the inhibitory effect of morphine on learning and memory formation in the animal model of Y-maze (Ma et al., 2007). We have also shown that the amnesia induced in BDL rats was blocked by naloxone microinjection which verifies the role of endogenous opioids in the amnesia caused by cholestasis (Nasehi et al., 2013a, b). The influence of endogenous opioids in the amnesia caused by cholestasis in the current work was further confirmed by indicating the effect of a mu-opioid receptor antagonist, naloxone, on blocking memory deficits in BDL rats.

Different neurotransmitter systems could modulate learning and memory processes in cholestasis (Bashiri et al., 2018; Nasehi et al., 2013a, b; Zarrindast et al., 2012). For instance, glutaminergic system in the hippocampus influences on the cholestasis-induced memory deficits in male mice (Hosseini et al., 2013). $GABA_A$ receptors also have an important role on learning and memory processes (Chapouthier and Venault 2002; Reis et al., 2009; Torkaman-Boutorabi et al., 2013). We indicated that bicuculline and muscimol, at the doses which could not alter STLr in non-cholestatic animals, attenuate or potentiate respectively cholestasis-induced amnesia. This suggests that $GABA_A$ receptors have a tonic activity in controlling memory retention of cholestatic rats. For supporting this hypothesis, it has been shown that there is a tonic activity of GABA in cholestatic animals (Cauli et al., 2009).

$GABA_A$ receptors have a high density in the CA1 (Ebrahimi-Ghiri et al., 2018). GABAergic and opioidergic systems have several interactions in behavioral responses of cholestatic animals (Cauli et al., 2009; Hasanein and Parviz 2014). The interaction between $GABA_A$ and opioid receptors is an interesting topic that has been studied in behavioral models, including opioid analgesia and opioid tolerance (Mahmoudi and Zarrindast 2001; Rashvand et al., 2014). Mu-opioid receptors located in the hippocampus on the terminals of GABAergic neurons presynaptically and could decrease GABA release (Simmons and Chavkin 1996; Zarrindast et al., 2008). Therefore, muscimol facilitated the effect of endogenous opioids on reducing GABA release, which results in potentiating amnesic effect in BDL rats. On the other hand, bicuculline at each different dose in this work attenuated significantly memory deficit caused by cholestasis. It looks that blockade of $GABA_A$ receptors by bicuculline could prevent the effect of endogenous opioids in diminishing GABA release, leads to attenuation of amnesia in cholestatic animals.

Consistent with our findings it has been reported that muscimol and bicuculline could potentiate and attenuated respectively the amnesia caused by intra-CA1 microinjection of morphine in animals (Zarrindast et al., 2008).

The effects of bicuculline and muscimol on memory retention in non-BDL rats are in agreement with a previous study reported that microinjection of bicuculline and imuscimol into central amygdala, at similar doses with this work, could not alter memory retention in the PAL test (Rassouli et al., 2010). However, injection of muscimol intro amygdala has a negative influence on memory performance, the discrepancy is possibly due to rats strain used or the low doses used in the current study. We used muscimol at doses 60, 120 and 240 ng /side less than 1 µg/rat used in that report.

These experiments revealed that administration of naloxone alone reversed although not completely the amnesia induced by cholestasis in treated animals. However, there was still significant difference compared to saline-received healthy animals. This reconfirms the influence of opioid system is in pathophysiology of amnesia induced by cholestasis and could suggest opioid antagonists as a probable therapeutic strategy for treatment of cholestatic amnesia (Ebrahimkhani et al., 2006; Yurdaydin, 2001).

In the third series of the experiments, we showed that coadministration of bicuculline and naloxone could completely antagonize the amnesic effect of cholestasis in treated rats. Furthermore, there was not any significant difference in STLr than saline-received healthy animals. However, there was significant difference in STLr between naloxone and bicuculline received BDL rats and only naloxone received BDL animals. Furthermore, coadministration of muscimol and naloxone induced significant change in STLr than only naloxone administartion in BDL groups. These observations show the reciprocal interactions between GABAergic and opioidergic systems in the CA1 on retention memory in BDL rats and lend credence to previous studies on modulation of memory by the opioidergic and GABAergic systems (Dubrovina and Ilyutchenok 1995; Rassouli et al., 2010; Sharifi et al., 2017).

Our current experiments showed that microinjection of bicuculline (100 ng/side) that alone has no significant impact on STLr could antagonize the amnesic effect of muscimol (120 ng/side). Therefore, the observation that the amnesic effect of muscimol was antagonized by co-administration of the $GABA_A$ receptor antagonist, provides evidence that the effect was not a non-specific effect of the injection procedure.

Conclusions

These experiments showed the influence of intra-CA1 $GABA_A$ receptors in controlling memory retention in cholestatic animals by the PAL test. Our results indicate that decreasing and increasing GABAergic neurotransmission by bicuculline and muscimol respectively attenuates and potentiates amnesia caused by cholestasis. These effect were specific and could not be attributed to the effects of the injection procedure. Therefore, the present report reveals that the $GABA_A$ receptors have participated in the regulation of memory retention in cholestatic animals. Furthermore, we showed a possible interaction

between GABAergic and opioidergic systems in the CA1 of cholestatic rats on memory retention using PAL test by showing the effects of coadministartions of pharmacological opiodergic and GABAergic agents on STLr in BDL animals.

Declarations

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Compliance with Ethical Standards

Conflict of interest: The authors have no competing interests to declare that are relevant to the content of this article. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent to publish: Not applicable.

Consent to Participate: Not applicable.

Ethical approval: Ethical approval was waived by the local Ethics Committee of Shaanxi Provincial People's Hospital. Hakim Sabzevari University in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

Availability of data and material

All data generated or analyzed during this study are included in this published article. The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Authors' contributions

RC wrote the manuscript; DZ did the experiments; ST conceptualization and funding; NL was the leader of project and writing the manuscript.

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Figures

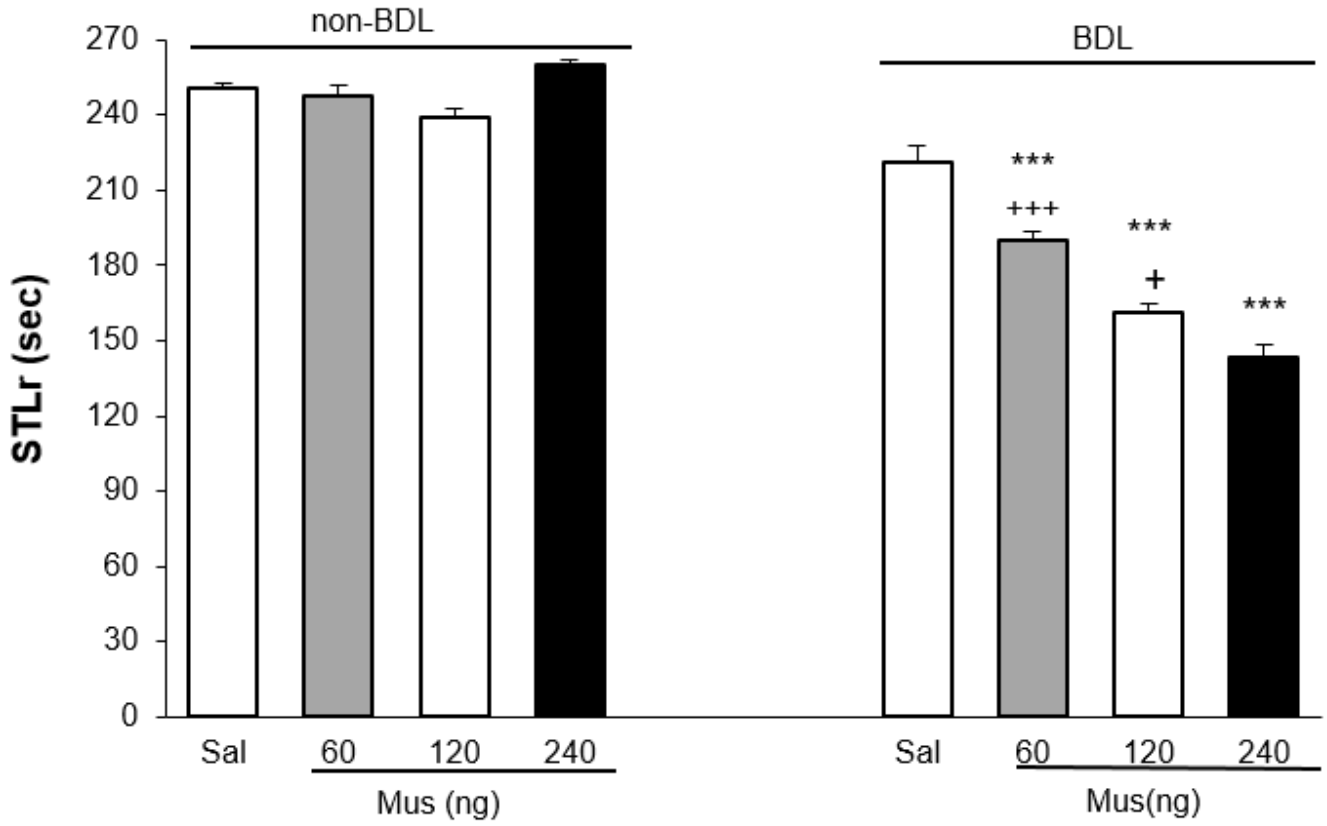

Figure 1

Effects of post training intra-CA1 administration of saline (Sal) or muscimol (Mus) (60, 120, and 240 ng/side) in pre-test saline-treated non-BDL and BDL groups on memory retention (N = 7). *** P < 0.001 compare to post training saline-treated BDL rats. + P < 0.05 and + + + P < 0.001 compare to post training Mus 240 ng -treated BDL aimals. Each bar and column represents mean ± S.E.M.

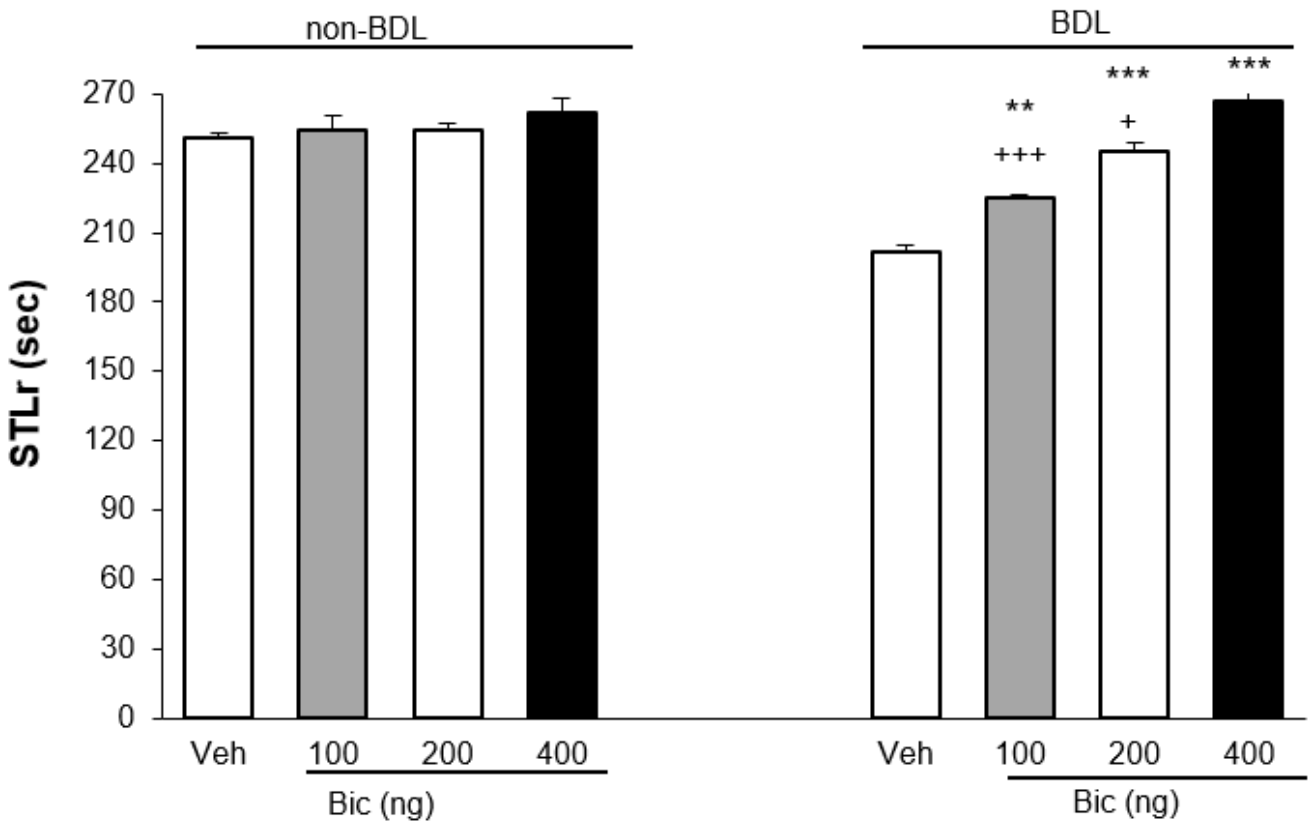

Figure 2

Effects of post training intra-CA1 administration of vehicle (Veh) or bicuculline (Bic) (100, 200, and 400 ng/side) in pre-test vehicle-treated non-BDL and BDL groups on memory retention (N = 7). ** P < 0.01 and *** P < 0.001 compare to post training saline-treated BDL animals. + P < 0.05 difference between Bic 200 and 400 ng -treated BDL rats. + + + P < 0.001 difference between Bic 100 and 400 ng -treated BDL rats. Each bar and column represents mean ± S.E.M.

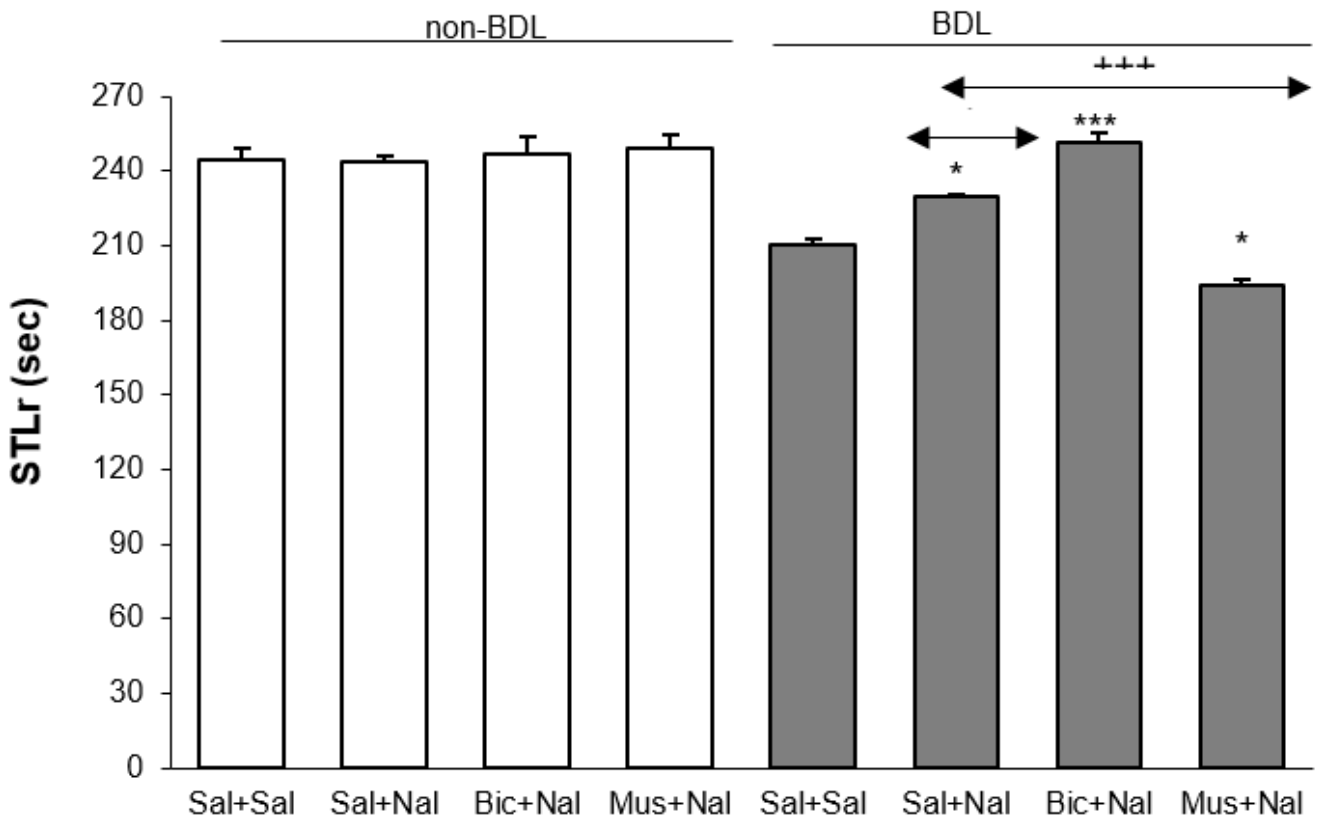

Figure 3

Effects of post training microinjection of bicuculline (Bic) (200 ng/side) or muscimol (Mus) (120 ng/side) with naloxone (Nal) (250 ng/side) into CA1 on retention memory in non-BDL and BDL groups (N = 7). * P < 0.05 and *** P < 0.001 compare to post training saline-treated BDL rats. + P < 0.05 and + + + P < 0.001 difference between BDL rats compared to Nal-treated -BDL rats. Each bar and column represents mean ± S.E.M.

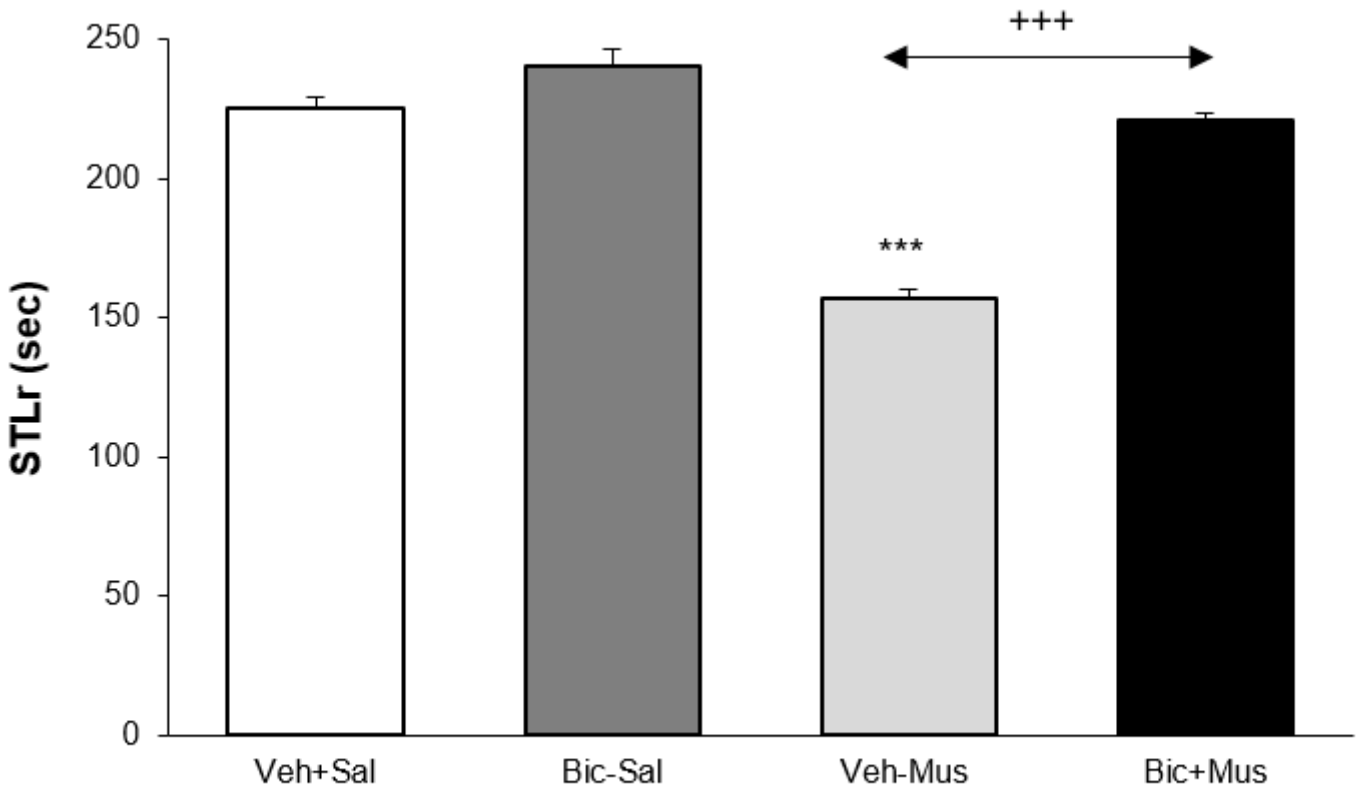

Figure 4

Effects of post training intra-CA1 microinjection of bicuculline (Bic) (100 ng/side) on the amnesia induced by muscimol (Mus) (120 ng/side) in BDL rats. (N = 7). *** P < 0.001 compare to saline-treated BDL rats. + + + P < 0.001 difference between Bic+Mus treated animals and only Mus-treated BDL rats. Each column and bar represents mean ± S.E.M.