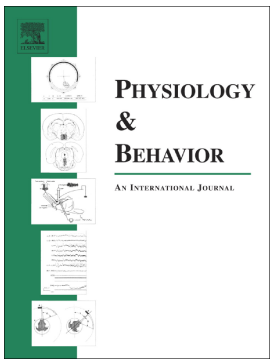

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Evaluation of prepulse inhibition and memory impairments at early stage of cirrhosis may be considered as a diagnostic index for minimal hepatic encephalopathy

Iraj Aghaei[1, 2], Seyed Soheil Saeedi Saravi[3, 4, 5], Masoumeh Nozari[2], Samaneh Ghotbi Ravandi[2], Afshin Dalili[6], Mohammad Shabani[2*], Ahmad Reza Dehpour[3, 4 *]

[1] Social Determinants of Health Research Center, Gilan University of Medical Sciences, Rasht, Iran

[2] Neuroscience Research Center, Neuropharmacology Institute, Kerman University of Medical Sciences, Kerman, Iran

[3] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[4] Department of Toxicology-Pharmacology, Faculty of Pharmacy, Gilan University of Medical Sciences, Rasht, Iran

[5] Experimental Medicine Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[6] Razi Hospital, Gilan University of Medical Sciences, Rasht, Iran

* These two authors contributed equally to this paper as corresponding author: Dr Mohammad Shabani (PhD), Neuroscience Research Center, Neuropharmacology institute, Kerman University of Medical Sciences, Kerman, Iran. P.O. Box: 76198-13159; Tel: +98 913 397 8116; Fax: +98-343-226-4198; E-mail: shabanimoh@yahoo.com, shabani@kmu.ac.ir (M. Shabani).

Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box: 13145-784, Tehran, Iran. Fax: +98 21 6640 2569. E-mail address: dehpour@yahoo.com, dehpourar@tums.ac.ir (A.R. Dehpour).

Abstract

Minimal hepatic encephalopathy (MHE), which represents the early stage of this condition, is not clinically apparent and is prevalent in up to 80% of patients. The poor outcomes of MHE encouraged us to identify more simple methods for early diagnosis of MHE. To this purpose, we evaluated the contemporary manifestations of motor, cognitive and sensorimotor gaiting deficits following bile duct-ligation (BDL). Male Wistar rats were undergone BDL to induce cirrhosis and locomotor, spatial learning and memory and sensorimotor gating were assessed 2, 3, and 4 weeks after the operation by Rota rod, Morris water-maze and Prepulse inhibition (PPI) tests. PPI was examined 6 weeks after BDL until appearance of hepatic encephalopathy. Results showed that although PPI was significantly enhanced in the 6-week BDL animals, locomotor activity reduced in 4-week BDL rats compared to the BDL rats after a 2-week period. The total distance travelled and swimming time to reach the platform increased in the 4-week BDL rats and, in contrast, the percentage of time spent and space travelled in correct quadrant decreased. Moreover, memory index decreased in the 3-week BDL group compared to sham-operated group. It was observed an increase in global PPI in 3- and 4-week BDL animals in comparison with either 2-week BDL or sham-operated rats. Consequently, it is indicated that BDL animals manifest spatial learning and memory deficits and PPI disruption in early stage of HE and evaluation of these factors can be considered as indices for simple and early diagnosis of MHE.

Keywords: Bile duct-ligation, Minimal hepatic encephalopathy, Prepulse inhibition, Cognition

Abbreviations: MHE, Minimal hepatic encephalopathy; BDL, Bile duct-ligation; PPI, Prepulse inhibition; MWM, Morris water-maze.

Running title: PPI impairment as a diagnostic index of MHE

1. Introduction

Hepatic cirrhosis, one of the most common health threatening diseases with high rate of mortality, affects the quality of life in the patients at different stages (Butterworth 2011). This condition may resulted in hepatic encephalopathy

(HE), one of the neurologic complications associated with cirrhosis, which is prevalent in a considerable percentage of population at different age groups(Butterworth 2011, Felipo 2013). This phenomenon is a progressing condition, which finally may lead to coma anddeath (Butterworth 2002). HE is classified in three forms: (1) minimal hepatic encephalopathy (MHE) in early stage, (2) episodic hepatic encephalopathy, and (3) persistent hepatic encephalopathy in progressive stage or overt HE (OHE) (Ferenci et al. 2002). Although the distinct prevalence of OHE in cirrhosis ranges from 30% to 40% (Murray and Carithers 2005), the exact prevalence of MHE is not well found. Despite of the difficult diagnosis of MHE (Masson et al. 2008), studies have reported 60% to 80% prevalence in the patients with cirrhosis (Ortiz et al. 2005). Progression of HE from minimal to overt condition in cirrhotic patients is 3.7 times more likely than in patients without a diagnosis of MHE (Romero-Gómez et al. 2001). Regarding to poor outcomes of OHE, the initiative stage of MHE is severely important to diagnose and treat the patients at early stages of hepatic encephalopathy (Bustamante et al. 1999, Kircheis et al. 2009).

In subclinical hepatic encephalopathy, represented as MHE, the apparent lack of manifestations causes a great diagnostic challenge, but a thorough history may detect some aiding factors such as poor social interaction, personality changes, poor performance at work, and recent traffic violations or motor vehicle accidents. Neurological deficits associated with subclinical form of MHE include the domains of attention, working memory, visuo-spatial ability and fine motor skills (Amodio et al. 2004, Wakim-Fleming 2011). These deficits are not reliably detected via standard clinical evaluation, but neuropsychiatric and neurophysiologic tests can be used for early detection. However, psychometric tests are time-consuming, liable to observational bias, difficult to be standardized based on age and education, and subject to a “practice effect”, improvement with repeated testing (Ortiz et al. 2005). Furthermore, critical flicker frequency test demonstrates a high specificity, but moderate sensitivity (Torlot et al. 2013). Therefore, early diagnosis results in a decreased risk of injury, and earlier and more successful treatment of cirrhotic patients. HE is a potentially fatal complication of liver disease encompassing a spectrum of cognitive impairment that can be semi-quantitatively scaled (0 to 4) by the West Haven Criteria (WHC) (Amodio et al. 2004; Blei et al. 2001). Regarding to the poor performance of the patients at the bottom end of this scale, with apparently unimpaired verbal and motor ability, in psychometric tests (Weissenborn et al. 2001) and lack of obvious and validated methods to assess MHE, finding easier and more prompt evaluation methods s are needed. While several

tests for diagnosis of MHE have been developed, they are needed to validate in large trials (Dhiman et al. 2010, Wakim-Fleming 2011) Therefore, novel simple investigations are necessary to innovate.

Bile duct-ligation (BDL) has been introduced as a reliable animal model of hepatic cirrhosis (Butterworth et al. 2009). Several studies have shown impairments in motor functions (Magen et al. 2009) short-term memory (Leke et al. 2013), spatial learning and memory (Aghaei et al. 2014) and passive avoidance learning (Aguilar et al. 2000) in the BDL-induced cirrhotic animals, similar to that observed in clinical conditions. Although the pathophysiology of HE in cirrhotic rats by bile duct ligation is not clearly understood, it is assumed that this condition occurs due to alterations in the neuromodulators and neurotransmitters in the central nervous system, including gamma amino butyric acid (GABA), glutamate, serotonin, endogenous opioids and nitric oxide (Butterworth 2001; Butterworth et al. 2009; Albrecht and Jones 1999).

Prepulse inhibition measurement, a method for analysis of sensorimotor gating, is considered as a protective procedure that impedes the processing of a stimulus (Braff and Geyer 1990). Healthy function of this mechanism is crucial for normal cognition, because an individual can focus its attention on the most salient stimuli in the environment (Kohl et al. 2013). Recently, Shadwick and sun (2014) have shown that prepulse inhibition and acoustic startle reflex can be useful to diagnose impaired sensorimotor gating in patients with tinnitus (Shadwick and sun, 2014). The pharmacology and circuitry of this reflex is virtually identical in rodents and humans (Caeser et al. 1989, Braff et al 2001). The physiological basis of PPI seems to be based on modulatory role of several neurotransmitters such as GABA, glutamate, dopamine and acetylcholine, which regulate the magnitude of startle response and its inhibition (Larrauri and Schmajuk 2006). In HE, there are abnormal release, degradation and reuptake of the neurotransmitters (Braissant et al. 2013), as well as, receptor affinity (Cabrera-Pastor et al. 2012).

A disruption in attention and cognitive functions has been observed in patients and experimental animals with HE (Wakim-Fleming 2011). The prepulse inhibition and cognitive deficits may be linked to impairment of sensorimotor gating. Furthermore, diagnosis of MHE is difficult, due to subclinical nature of the disease. Therefore, the present study examined the hypothesis that an impaired sensorimotor gating is occurred following bile duct-ligation. Moreover, the time course manifestation of impairment of motor, cognitive and sensorimotor gating in BDL rats

were examined for MHE diagnosis. The current study is designed to present a simple method for early diagnosis of MHE using alterations in behavioral indices.

2. Materials and methods

2.1. Animals

Sixty male Wistar rats (2 months old) weighing 180-220 g were used in the current study. All the procedures were carried out in agreement with the guidelines for *use and care of laboratory animals* (Kerman University of Medical Sciences, Ethics code: KNRC/92/6) and in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Animals were kept under standard conditions (12-h light/dark cycle, environment temperature at 21±2 ˚C) with free access to food and water except for the brief time of surgery, drug administration and the behavioral tests. The rats were randomly allocated into two main experimental groups: group 1, divided in two sub-groups: sham and BDL (n=10 per sub-group) for PPI assays in a pilot study; group 2, divided in four sham (3 weeks after surgery) and BDL sub-groups (n=10 per sub-group) for behavioral (rotarod and MWM) and PPI assays, 2, 3 and 4 weeks following BDL.

2.2. BDL operation

Animals were generally anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine(20 mg/kg). An incision was made in the midline of the abdominal wall; and after cutting fascia and the muscles, the common bile duct was ligated with a 4–0 non-absorbable silk suture at two specific sites (easposterior to the hilum of liver and anterior to pancreas). Then, the abdominal wall incision was sutured in two layers and all animals were administered to 1 mL of sterile 0.9% normal saline (Aghaei et al. 2015, Javadi-Paydar et al. 2013). In the sham-operated group, only surgical stress was occurred by immediately retraction and replacement of the bile duct.

2.3. Rotarod

The animals in all examine groups were placed on an accelerating rotating drum (Hugo Sachs Elektronic, Germany). The speed of rotarod was started from 10rpm to maximum speed of 60 rpm over a 5-min period. The total time the rats maintained its balance on the rod was recorded. Each rat was repeatedly undergone three trials with an interval of 15 min between attempts (Shabani et al. 2012, Nozari et al. 2014).

2.4. Morris water-maze (MWM)

The spatial learning and memory was evaluated in the MWM test. The test was performed as previously described (Frick et al. 2000; Shabani et al. 2012). Briefly, the test chamber was a circular pool (140 cm diameter, 45 cm depth), surrounded by extra-pool visual cues. We used a black pool and we conducted the MWM task in a dimly lit room. An invisible escape platform (15 cm wide, 35 cm height) was submerged 1.5 cm below the water surface in the center of the quadrant NE (target quadrant). In a single day training protocol of MWM task, rats were fully trained in approximately 1.5 h which is sufficient for evaluating the effects of long-lasting treatments. In fact, the classic version of MWM task (5 days training protocol) is not applicable for evaluation of early stage of cirrhosis. Since time period is considered as an important factor in manifestations of HE, we tried to assess the contemporary motor, cognitive and prepulse inhibition impairments at 2, 3 and 4 weeks after BDL. On each trial, animals were randomly released into the water from one of the four quadrants of the maze with its face toward the wall of the quadrant where it was released. Each rat had 4 different starting points. During acquisition trials, the position of the platform was constant and rats were allowed to swim to the hidden escape platform in 60 s (maximum time). The pool was filled with water containing non-toxic white paint and temperature was maintained at 21–23 °C. The rats' behavioral preferences were recorded and video analyzed using a set of EthoVision XT6 (Noldus Information Technology, Leesburg, VA). The following parameters were recorded for each rat: total distance and time spent to reach the platform in three consecutive trials, number of crosses in the correct quadrant in the retention phase, percentage of time and distance travelled in the correct quadrant. At the training phase, each rat was undergone three blocks of trials with an interval of 30 min between attempts and four comprising trials with an interval of 30 s between the trials. The animals were placed at the starting position (one of the quadrants adjacent (A) to the target maze). Then, starting positions were regularly clock-wise changed for the other three trials in each block (four trials/

block). Each rat was given 60-s opportunity to arrive on the platform and if they could not, the trial has been elapsed. At the acquisition phase, there was three blocks which were separated by 30-min resting period between each block. In a single training protocol, each rat accomplished three blocks (Each block consisted of four successive trials with four different releasing points) which were separated by a 30-min resting period. After the animal found the platform, it was allowed to remain there for 20–30 seconds and then was located in animal cage until 20–30 seconds before start of the next trial. Therefore, there were two 30-min resting periods between three blocks and three 1-min (20–30 seconds on platform and 20–30 seconds in its cage) resting periods between four trials in each block phase (consisted of three blocks, which was separated by 30-min resting period between each block). Two hours after the last training trial, a single probe trial was again performed in which the platform was removed from the pool and the number of crosses from the correct quadrant and the total time spent in target quadrant were recorded.

One day before the performance of behavioral tests, the animals were put on MWM tank and permitted to swim for 30 second adaptations while platform was removed from tank to prevent the rats to find platform before main tests. Animals were, at first, habituated to the pool by allotting 30 seconds free swim. However, following the probe trial, 5 rats in each group completed a visible platform test to determine any possibility of BDL interference with sensory and motor coordination or motivation (Buccafusco 2009; Hajali et al. 2012; Hajali et al. 2015). In this test, the animals' ability to escape to a visible platform (platform was raised 2 cm above the water surface and became visible with aluminum foil) was evaluated.

2.5. Prepulse inhibition

2.5.1. Apparatus

Auditory startle reflex amplitude and PPI were measured using Med Associates Startle Reflex System (St. Albans, VT, USA). The Apparatus includes a response platform (piezoelectric accelerometer), placed in the roof of sound attenuating chamber, a loudspeaker, placed within the chamber midway on the long axis of the platform and a plexiglas cylinder, mounted on the platform. Vibrations of the Plexiglas enclosure caused by the whole-body startle

response of the animal were converted into analog signals by a piezoelectric unit attached to the platform. The signals were digitized and stored by a computer as a sinusoidal motion pattern. Ultimately, startle amplitudes were taken from maximum peak to minimum peak ratio of the sinusoidal response (Pisansky et al. 2013).

2.5.2. Behavioral procedure

The test sessions include: a 5-min acclimatization on period with a 68 dB background noise, which initiates when the animals were placed in the chambers; 14 pulse alone trials (120 dB broad-band noise burst for 40 ms period); 30 prepulse + pulse trials at three different 20 ms broad-band intensities of 71, 74 and 80 dB (3, 6 and 12 dB over the 68 dB background noise), followed by a startle pulse (120 dB for 100 ms); and 8 no-stimulus null trials with only a 68 dB background noise. All trials were presented in a pseudo-random order and separated by an average of 22 s (15–30 s) inter-trial interval. Four 120 dB pulse trials were presented at the beginning and end of the test sessions (for all 60 trials), but were not analyzed for calculation of the PPI values.

The effect of prepulses on the startle responses of the animals was determined as percentage of prepulse inhibition (Powell et al. 2003):

%PPI = (1- [startle amplitude on prepulse + pulse trial / mean startle amplitude on pulse alone trials]) × 100

Global PPI = average of %PPI following three prepulse intensities for each animal (Ces et al. 2012).

2.6. Timing of the behavioral tests

Rotarod and Morris water-maze assays were performed at 13, 20 and 27 days after BDL operation with 2 h interval among each assay and PPI test was conducted on the day after these behavioral tests. One day before the performance of behavioral tests, the animals were put on rotarod (constant speed of 2 rpm for 3 min) and MWM tank (30 seconds) for adaptations.

2.7. Statistical analysis

All data were expressed as mean ± SEM and analyzed using SPSS software (version 16. IBM, USA). The Rotarod, PPI and probe data were analyzed by one-way ANOVA. Repeated measures ANOVA was performed on a motor learning phase of rotarod, and the learning phase of the Morris water maze. Individual comparisons were performed by Tukey's test. A value of $P<0.05$ was considered statistically to be significant.

3. Results

3.1. The effect of hepatic cirrhosis on motor coordination and balance skills in rotarod

The effects of BDL-induced cirrhosis on the time on rotarod are shown in the Fig. 1. Hepatic encephalopathy significantly reduced the time spent on rotarod in the 4-week BDL group compared to either the sham-operated (average of 3 trials without interaction groups× trials; $F(3,36)= 8.518$; $P<0.001$) or 2-week BDL groups ($F(3,36)= 6.9$; $P<0.01$). It means that a decrement of latency to fall from rod was observed following BDL in the rats.

3.2. The effect of hepatic cirrhosis on spatial learning and memory in MWM

The results of the learning trials are depicted in Fig. 2. All treated, control and BDL rats progressively took less time to locate the hidden platform over the course of the 12 trials during the training period (block 3 and 2 compared to block1 for all groups , repeated measures ANOVAs; at least $p<0.05$). As shown in Fig. 2, repeated measures ANOVA statistical analysis demonstrated that the total distance travelled to reach the platform was increased in the 4-week BDL group in comparison with sham-operated animals at the first, second and third blocks (interaction of time × training; $F(6, 63) = 4.06$; $P<0.05$, $F(6, 63) = 5.76$; $P<0.01$ and$F(6, 63) = 9.68$; $P<0.001$, respectively)and with 2-week BDL group at the second and third blocks ($F(6, 63) = 4.9$; $P<0.05$, Fig. 2A). Moreover, an increase in the swimming time to reach the platform (Fig. 2B) was observed in the animals undergoing BDL for 4 weeks compared with the sham-operated animals in the first ($F(6, 63) = 3.8$; $P<0.05$), second ($F(6, 63) = 7.3$; $P<0.01$) and third blocks ($F(6, 63) = 9.6$; $P<0.001$). The results demonstrated that there was no significant difference in the swimming speed

between thefour studied groups in MWM learning trials ($F(6, 63) = 1.5$; $P>0.05$). On the other hand, percentage of time spent in the target quadrant and distance travelled in the correct quadrant was significantly decreased in the 4-week BDL group compared with either the sham-operated group ($F(6,63) = 6.38$; $P<0.01$ and $F(6,63) = 8.2$; $P<0.001$, respectively) or 2-week BDL group ($F(6, 63) = 4.2$; $P<0.05$, Fig. 2C). Percentage of distance travelled in the correct quadrant was significantly decreased in the animals undergoing BDL for 3-week in comparison with the undergone sham-operation ($F(3, 36) = 3.9$; $P<0.05$, Fig. 2D). The number of crosses and swimming speed showed no significant differences between all groups of the investigation ($F(3, 36) =0.02$; $P>0.05$; $F(3, 36) = 1.05$; $P>0.05$, Fig. 2E and 2F).

The swimming speed and latency to find the visible platform in the MWM test is presented in Table 1.

Table 1. Swimming speed and latency to find the visible platform in the MWM test

Groups	Swimming speed (cm/s)	Escape latency (s)
Sham (3 w) [a]	21.4 ± 2.6	13.6 ± 0.9
BDL (2 w) [b]	20.4 ± 3.8	14.5 ± 2.4
BDL (3 w) [c]	19.3 ± 3.6	17.7 ± 2.6
BDL (4 w) [d]	19.01 ± 2.8	15.9 ± 2.5

a: Sham-operated rats; 3-weeks after bile duct ligation.

b, c, d: BDL rats; 2, 3, and 4-weeks after bile duct ligation, respectively.

3.3. The effect of hepatic cirrhosis on prepulse inhibition

Analysis of startle response and prepulse inhibition using an unpaired t-Test revealed that there was no significant difference in startle response and PPI following the prepulse intensities of 3, 6 ($F(1, 18) = 2.4$; $P<0.05$) and 12 dB ($F(1, 18) = 4.02$; $P<0.01$) over the68 dB background noise. Otherwise, a significant PPI increment was observed in the BDL animals 6 weeks after the operation (Fig. 3A and 3B).

3.4. Manifestation of PPI impairment in the early stage of hepatic cirrhosis

According to the results, although no significant differences were observed in startle responses and prepulse inhibition following a 71 dB prepulse, PPI was significantly enhanced after prepulse intensities of 74 and 80 dB, as well as, global prepulses in the 4-week BDL group compared with either the sham-operated ($F(6, 63) = 3.17$; $P<0.05$) or 2-week BDL animals ($F(6, 63) = 3.08$; $P<0.05$). After 80 dB and global prepulses, a significant PPI improvement was shown in the rats undergone BDL for 3 weeks in comparison with the sham-operated group ($F(6, 63) = 4.7$;$P<0.01$ and $P<0.05$, respectively; Fig. 4).

4. Discussion

It has been reported that two weeks after BDL, an acute obstructive jaundice can be occurred and can progress to cirrhosis after 4 to 6 weeks (Antoine et al. 2005). Ultimately, these manifestations, that are called HE, will lead to motor, memory and cognitive dysfunctions (Schuppan and Afdhal 2008). Impairment of detoxification system can cause hyperamonemia, leading to abnormality of neurotransmitter release, degradation (Petersen 2003), affinity to receptors (Cabrera-Pastor et al. 2012) and, subsequently, neurological (Llansola et al. 2013) and neural circuit deficits (Chen et al. 2014). The current study has indicated that bile duct-ligation, a model of hepatic cirrhosis in rodents, caused PPI impairment 6 weeks after the operation and, at this time, the animals manifested a progression of hepatic encephalopathy, leading to motor and cognitive dysfunctions. Our results have demonstrated a PPI increment following prepulse intensities of 74 dB and 80 dB and global prepulses in the animals undergoing 4-week BDL compared to the 2-week BDL and sham-operated groups. The finding was in agreement with the consequences resulted from the animals after 3-week BDL. It confirmed the hypothesis that development of PPI impairment may be occurred at early stage of HE. However, attention and visual perception deficits have been reported in the patients with cirrhosis (Weissenborn et al. 2005), while it is necessary to further evaluate the prepulse inhibition.

The mechanism underlying startle responses is relatively understood in rodents. Patients with liver cirrhosis may develop hepatic encephalopathy which leads to a wide range of cognitive alterations. The mechanisms leading to

impaired function of the pathway are beginning to be clarified. A main contributor to this impairment is chronic hyperammonemia. Experimental studies provide convincing evidence that BDL cause hyperamounium (Jover-Cobos et al., 2014) and induce many different effects including profound changes in cognitive and especially motor function (Dhanda and Sandhir, 2015; Rodrigo et al., 2010). They exert most of their actions through modulation of GABAergic (Cauli et al., 2009) or Glutamatergic (Llansola et al., 2013) neurotransmission. Many studies have demonstrated that cirrhosis can cause an increase in glutamate release and a decrease of its reuptake (Monfort et al. 2009), which resulted in PPI improvement. Furthermore, alteration in acetyl cholinesterase (AChE) activity is reported to be associated with PPI levels in the rats undergoing BDL operation. In this regard, Maria-Saludet al. (2008) have indicated that increment of AChE activity in cerebral cortex of patients with hepatic coma is consistent with the results from BDL rats (García-Ayllón et al. 2008), which can lead to fast degradation of acetylcholine in synaptic clefts located in pedancolopototegmental fibers and ventrocaudal of pontine reticular nucleus (PnC), a decrease of its inhibitory effect on PnC and possible increase in PPI. However, abnormality of some other neurotransmitter systems can be linked to neurobehavioral deficits induced by liver cirrhosis. Study of reasons for the PPI increment was not the main aim of this study. Therefore, declaration of the role of possible mechanisms, as briefly discussed above, in this condition needs future investigations to be clarified.

On the other hand, a reduction in motor coordination and balance skills was observed in the animals undergone 4-week BDL in comparison with the 2-week BDL and sham-operated groups, which is confirmed by previous studies of 6-week BDL rats (Aghaei et al. 2014, Aghaei et al. 2015). Alterations in motor function and cognition were occurred in the animals 4 weeks after BDL, implicating the possible association with onset of minimal HE at early stage of cirrhosis.

Moreover, the total distance travelled and swimming time to reach the target zone of platform in Morris water-maze have increased in the 4-week BDL group compared with 2-week BDL animals, emphasizing impairment of spatial learning. In addition, percentages of time spent and space travelled in the correct quadrant of MWM pool decreased in the 4-week BDL group in comparison with the 2-week BDL animals, indicating impairment of spatial memory. In contrast, percentage of space travelled in the correct quadrant was decreased in the 3-week BDL group compared with the sham-operated animals. The observed cirrhosis-induced learning and memory dysfunctions at early stage of hepatic encephalopathy are in agreement with the previous studies of 6-week BDL rats (Aghaei et al. 2014).

Although the beginning time of deficits in spatial learning and memory has not been studied, Hossini et al. (2014) have reported an impairment of memory retrieval 7 and 21 days after foot shock stress in animals undergone BDL operation. Furthermore, Weissenborn at al. (2003) have demonstrated that patients with early HE have low memory task scores, which is linked to attention and visual perception deficits. Studies have demonstrated that BDL is associated with impairment of spatial learning and memory (Huang L-T et al. 2009), short-term memory (Leke R et al. 2013), and passive avoidance learning (Aguilar M et al. 2000, Hossini et al. 2014). To find the predisposing factors and possible underlying mechanisms of MHE, the reports have indicated to the involvement of neurotransmitter systems, oxidative stress and role of brain edema (Huang L-T et al. 2009 , Rodrigo R et al. 2010, Leke R et al. 2013,). Huang et al. (2009) have suggested that BDL-induced impairment of spatial learning and memory are mediated by oxidative stress pathway (Huang L-T et al. 2009). Javadi-Paydar et al. (2013) have demonstrated that oxidative stress is responsible for the impairment of spatial learning and memory in BDL rats (Javadi-Paydar et al. 2013). In addition, Magen et al. (2010) implied that BDL impairs memory and motor learning through involvement of different neurotransmitter pathways (Magen et al. 2010).

5. Conclusions

Difficult and delayed diagnosis and also monitoring of HE in cirrhotic patients are needed to explore simple neurological scales and neuropsychological tests. As a result, the present study attempts were to design simple sensitive neurobehavioral tests under time course manifestation via assessment of motor, cognitive and sensorimotor gating in BDL rats for early diagnosis of MHE. We have demonstrated that evaluation of prepulse inhibition may be highly simple and available neuropsychological test. According to our findings, development of PPI disruption are appeared at early stage of hepatic cirrhosis. Moreover, it is shown that BDL can lead to spatial memory and learning dysfunctions at early stage of hepatic encephalopathy. Therefore, we have presumed that evaluation of memory and learning functions can be considered as diagnostic factors of minimal HE.To highlight this fact, further studies are necessary to identify the possible mechanisms underlying PPI deficits in patients with HE.

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Conflict of interest

The authors declare no conflict of interest.

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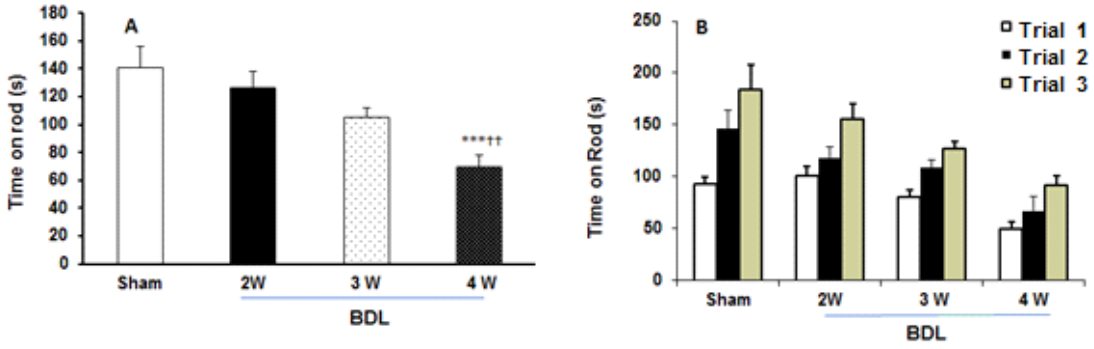
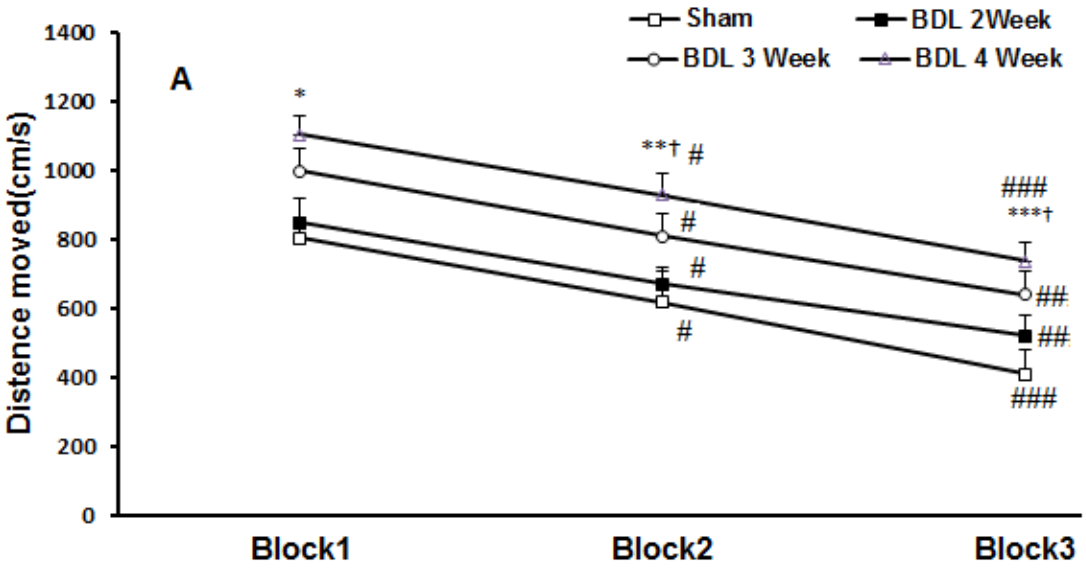
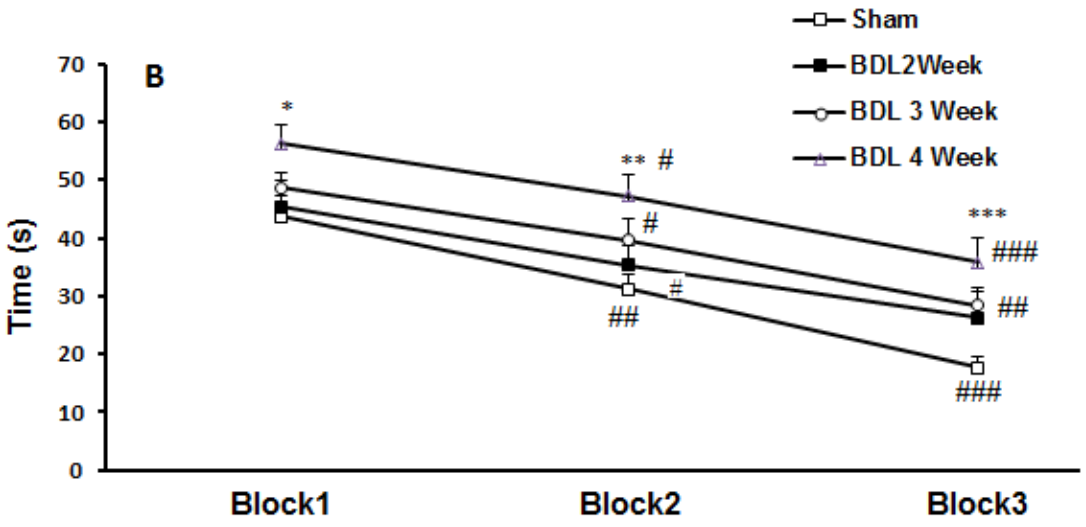
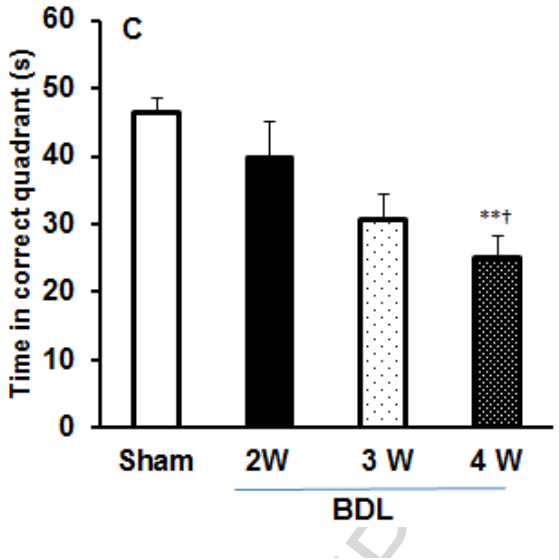


Fig. 1 The total time spent on the rotarod was significantly reduced after a 4-week bile duct-ligation in comparison with either a2-week BDL or sham-operation (A). No significant alteration in total time spent on the rotarod was observed in the three consecutive trials for each animal in the studied groups(B). ***P<0. 001compared with sham-operated ,and ††P<0.01 2-week BDL rats. All data are expressed as mean ± SEM.







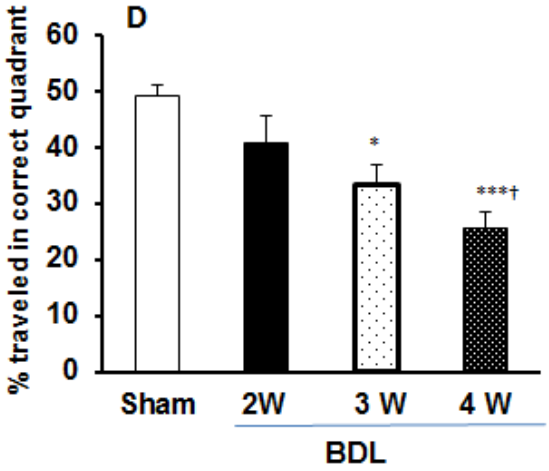
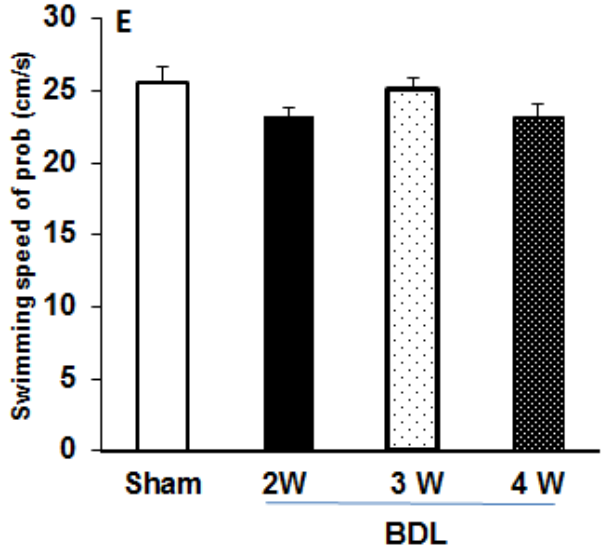


Fig. 2



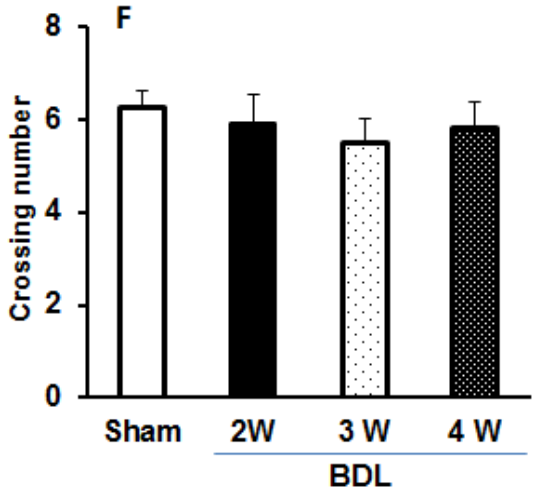


Fig. 2 A significant impairment of learning and memory functions were observed in the bile duct-ligatedrats after 3- and 4-week periods compared with either the sham-operated or 2-week BDL rats in the Morris water-maze test. Increased distance(A) and time(B) spent to reach the hidden platform were observed in the4-week BDL animals .Moreover ,percentage of swimming time in the correct quadrant was decreased in 3- and4-week BDL rats compared with the sham-operated and 2-week BDL groups(C). Percentage of totaltime spent in the correct quadrant was significantly decreased in the bile duct-ligated rats after a 4-week period compared with either the sham-operated or 2-week BDL rats (D). There was no significant alteration in swimming speed (E) and number of crosses(F) in BDL rats compared with other animals in the studied groups. *P<0.05, **P>0.01 and ***P<0.001 compared with the animals under sham-operation; †P<0.05 compared with the animals under bile duct-ligation after a 2-week period; the results of the learning trials are depicted in Fig. 2A and B. $^{\#}$P<0.05, $^{\#\#}$P<0.01 and$^{\#\#\#}$P<0.001 compared to block1 within each group of study. All data are expressed as mean ± SEM.

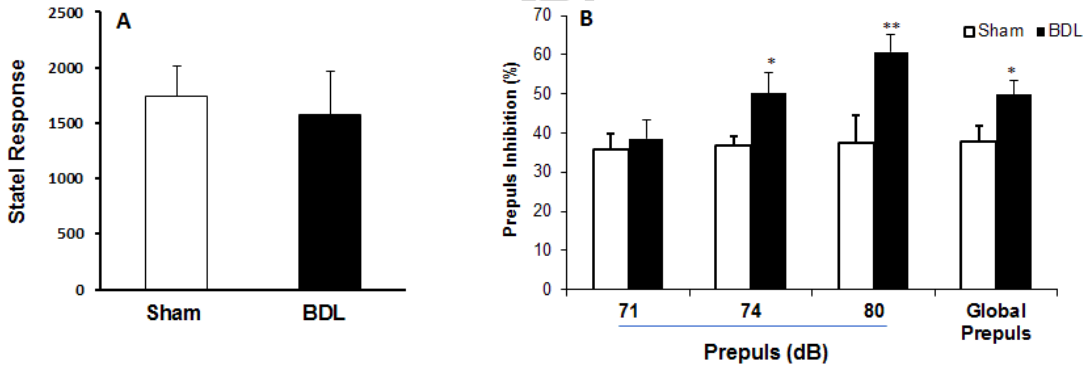


Fig. 3 Mean startle responses show no significant differences)A .(Prepulse inhibition)PPI (was increased in BDL rats 6weeks after the operation % .PPI (mean ± SEM) is shown in the sham-operated and BDL rats following three specific(71, 74and 80dB (and global prepulses)B* .(P>0.05 ,**P<0. 001 compared with the animals under sham-operation.

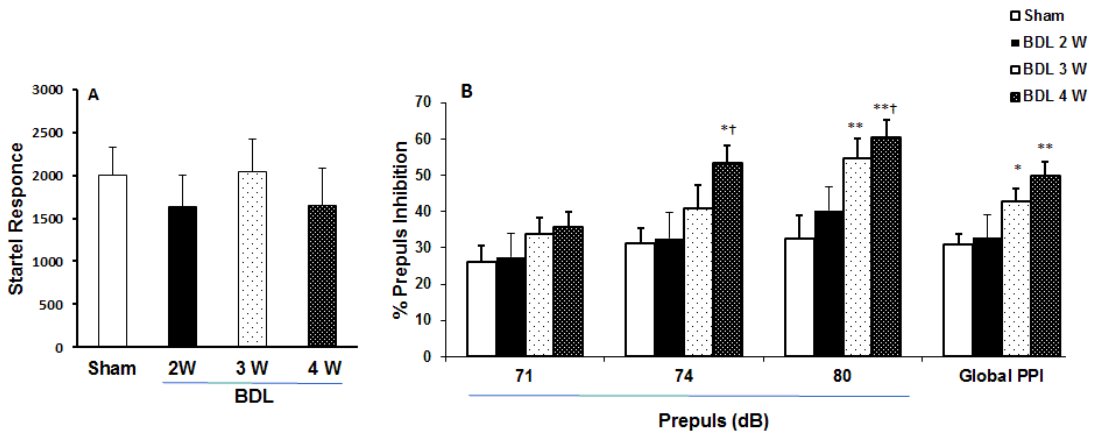


Fig. 4 Mean startle responses showed no significant differences (A). Prepulse inhibition (PPI) was increased in the 3- and 4-week BDL animals in comparison with the sham- operated and2-week BDL groups % .PPI)mean ± SEM (in the sham-operated and BDL rats after 2, 3and 4weeks are shown following three specific (71, 74 and 80 dB) and global prepulses (A). *P<0.05 and **P<0.01 compared with the animals under sham-operation; †P<0.05 compared with the animals under bile duct-ligation after a 2-week period.

Research highlights:

- Bile duct ligation (BDL) leads to animals show learning deficits in early stage of hepatic encephalopathy (HE)
- Spatial memory deficits are resulted from BDL in early stage of HE
- The animals under BDL operation demonstrate PPI disruption in early stage of HE
- Evaluation of spatial learning and memory deficits and PPI impairment can be considered as indices for simple and early diagnosis of minimal HE (MHE)