Original Article

Effects of Cholestasis on Learning and Locomotor Activity in Bile Duct Ligated Rats

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Abstract

Background: **Cognitive functions are impaired in patients with liver disease. Bile duct ligation causes cholestasis that impairs liver function. This study investigated the impact of cholestasis progression on the acquisition and retention times in the passive avoidance test and on the locomotor activity of rats.**

Methods: **Cholestasis was induced in male Wistar rats by ligating the main bile duct. Locomotor activity, learning and memory were assessed by the passive avoidance learning test at day 7, day 14, and day 21 post-bile duct ligation. The serum levels of bilirubin, alanine aminotransferase, and alkaline phosphatase were measured.**

Results: **The results showed that acquisition time and locomotor activity were not affected at day 7 and day 14, but they were significantly ($P < 0.05$) impaired at day 21 post-bile duct ligation compared with the results for the control group. Additionally, memory was significantly impaired on day 7 ($P < 0.01$), day 14, and day 21 ($P < 0.001$) compared with the control groups. The levels of total bilirubin, direct bilirubin, indirect bilirubin, alanine aminotransferase, and alkaline phosphatase were significantly higher at day 7, day 14, and day 21 post-bile duct ligation compared with the levels in the sham group.**

Conclusion: **Based on these findings, both liver and memory function were affected in the early stage of cholestasis (7 days after bile duct ligation), while learning and locomotor activity were impaired at 21 days after bile duct ligation following the progression of cholestasis.**

Keywords: *cholestasis, passive avoidance, locomotor activity, BDL, rat*

Introduction

Normal brain function requires interactions between the liver and brain. The liver plays an important role by providing nutrients for the brain and removing toxic substances, such as neurotoxins, from the brain. Therefore, liver disease and subsequent liver dysfunction can cause brain damage and impair brain function (1).

Cholestasis is a frequent, prominent and severe manifestation of many liver diseases (2) in both humans and animals (3). It results from structural and functional impairments of the hepatobiliary system, (4) including a failure of bile secretion in hepatocytes and ductular cells as well as the impairment of bile flow and the accumulation of bile salts in the body. Bile duct ligation (BDL) is a well-known model of cholestatic liver disease that causes jaundice and liver dysfunction (5–7).

Liver disease is associated with cardiovascular complications (8), acute renal failure (9), and systemic inflammatory responses (10). In addition, it is known as one factor that can affect brain function (11). Cognitive impairment is one of the symptoms of liver disease described in both patients (12,13) and animal models (14). For example, children with biliary atresia have deficits in their performance intelligence quotient, learning and memory, and visuospatial functions (15). Additionally, some studies have shown that in adults with liver disease, visuospatial function correlates with liver function (16,17).

Hepatic encephalopathy (HE) is a neuropsychiatric metabolic syndrome and a major complication of both acute and chronic liver disease (18). Hyperammonemia is considered to be one of the main factors responsible for HE (19). Ammonia neurotoxicity has been shown to be associated with a number of pathophysiological, biochemical, and molecular changes in the brain, such as cognitive, psychiatric, and motor dysfunctions, which consequentially lead to cerebral dysfunction (20).

Many investigators have reported that the hippocampus is critical for learning and memory mechanisms in the brain (21,22). Moreno et al. (23) reported decreased neuronal synaptic activity in the hippocampal cortical areas 3 (CA3) and cortical area 1 (CA1), the dentate gyrus (GD) and the inferotemporal cortex (INF) in cholestatic rats with hepatic encephalopathy. Blei et al. (24) also found neurochemical alterations such as increases in serotonin, dopamine turnover, and ammonium levels as well as decreases in glutamate levels in several brain areas including the hippocampus, hypothalamus, and striatum. Cholestatic liver disease is also associated with clinical and experimental findings consistent with increased opioidergic neuromodulation (25–27). The mechanism behind the effect of cholestasis on the opioid system is unknown, but it has been proposed that it may increase the availability of endogenous opioid agonist ligands that bind to opioid receptors in BDL rats (28).

In some studies, it has been found that compared with jaundiced-free rats, cholestatic rats have poorer performance in passive avoidance tasks (7,29) such as the Morris water maze task and in motor coordination (5,14). Previous studies have also shown that memory was impaired in mice 24 days after BDL (7).

Nevertheless, there is not sufficient information describing the time course of the impact of cholestasis on cognitive performance, such as learning and memory, and locomotor activity. In the present study, we examined the cognitive function of rats with obstructive jaundice using the passive avoidance learning test, a standard test to assess learning and memory function in rodents (30). This study was designed to evaluate the effects of the progression of cholestasis in bile duct ligated rats (7, 14, or 21 days after bile duct ligation) on the acquisition (pre-shock latency) and retention time (24 hours post-shock) in the passive avoidance test and on locomotor activity.

Materials and Methods

Animals

Thirty male Wistar rats (220–250 g) were obtained from Jondishapour Institute (Ahwaz, Iran). The animals were kept in animal cages and provided with food and water ad libitum. They were housed in a temperature controlled environment 22 °C (SD2) with a 12:12 hours light–dark cycle (07:00–19:00). The animals were allowed to adapt to the laboratory conditions for at least one week before surgery. Each rat was handled for approximately 3 minutes each day prior to behavioral testing. All experiments were performed between 9:00 hours and 12:00 hours, and each rat was tested only once. The Animal Research Ethic Committee at Isfahan University approved the study, and all experiments were conducted in accordance with the international guiding principles for biomedical research involving animals, which were revised in 1985.

Bile duct ligation surgery and induced cholestasis

There were five experimental groups and six animals in each group:

1. Group 1: Control group (non-operated)
2. Group 2: Sham group (abdominal surgery was performed without BDL)
3. Group 3: BDL7 group (experiments were performed 7 days after bile duct ligation, and the rats were sacrificed 8 days after BDL)
4. Group 4: BDL14 group (experiments were performed 14 days after BDL, and the rats were sacrificed 15 days after BDL)
5. Group 5: BDL21 group (experiments were performed 21 days after BDL, and the rats were sacrificed 22 days after BDL)

Laparotomy was performed under general anesthesia induced by the injection of chloral hydrate (400 mg/kg, ip). The sham group was

subjected to laparotomy as well as bile duct identification and manipulation, but ligation or resection was not performed (with the aim of measuring possible stress induced by surgery). In the bile duct ligation groups, the main bile duct was first ligated using two ligatures approximately 0.5 cm apart and then transected at the midpoint between the two ligatures (31). In the immediate post-operative period, each animal was placed in a cage by itself to prevent wound dehiscence and was moved to its original cage 4 hours after the surgery (32). Post-operative analgesia was achieved with subcutaneous injection of 0.05 mg/kg rat buprenorphine (33). Passive avoidance tests, locomotor activity analysis and biochemical analysis were performed for all experimental groups, and the results from the sham operated, BDL 7, BDL 14, and BDL 21 groups were compared with those of the control group.

Memory testing and apparatus

The training apparatus had two compartments consisting of a small chamber (25 × 25 × 20 cm) and a large dark compartment (25 × 25 × 20 cm). The compartments were separated by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. At the beginning of the experiment, each rat was placed in the apparatus for 5 minutes to habituate. On the second day, an acquisition trial was performed, and the rats were placed individually in the illuminated chamber. After a habituation period (1 minute), the guillotine door was lifted (the pre-shock delay time to enter into the dark chamber was considered the initial latency or acquisition time). After the rat had entered the dark chamber, the door was lowered, and an inescapable, scrambled, single electric shock (0.2 mA, 50 Hz) was delivered for 3 seconds. In the probe trial (24 hours after receiving a foot shock), the time interval from the placement of the rat into the illuminated chamber until its entry into the dark chamber was measured (the post-shock delay time to enter into the dark chamber 24 hours after receiving a foot shock was considered the retention time) (34,35).

Locomotors activity

A straightaway open field apparatus was used for locomotor activity assessment. After the behavioral test, the animals from all of the experimental groups were put in a transparent box (25 × 25 × 36 cm) over a white floor that was divided into four squares. Blue lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The movement of each rat as indicated by the crossing of gridlines was determined in both sham-operated and BDR-rats. Initially the rats were put in the apparatus for 5 minutes to acclimate to the environment, and the movement of each rat was recorded for 5 minutes following the acclimation period. For data analysis, the floor was divided into a grid of four squares (5 × 5 cm) and locomotor activity was measured by counting the total number of squares crossed during the testing session (36,37).

Biochemical analysis

Rats were anesthetised with ether for sacrifice, and samples of their blood (3–4 mL) were collected. The whole blood was centrifuged at 3000 rpm for 10 minutes to separate the plasma and was then stored at –70 °C. Plasma bilirubin (total bilirubin, direct and indirect bilirubin), alkaline phosphatase, and alanine aminotransferase (ALT) levels were measured with colorimetric methods using commercially available kits (Zist-Shimi Co, Tehran, Iran) and a spectrophotometer (38,39).

Statistical analysis

One way ANOVAs and post-hoc tests (Tukey test) were used for data analysis. Differences between the experimental groups were deemed significant at $P < 0.05$ for each time point. The results are presented as the mean (standard deviation).

Results

Induction of cholestasis

One day after bile duct ligation, the animals showed signs of cholestasis (jaundice, dark urine, and steatorrhea), which were assessed qualitatively and quantitatively (Table 1). BDL rats showed biochemical evidence of cholestasis with significant elevations in serum bilirubin and alanine amino transaminase levels.

Passive avoidance learning test

The latencies were measured at the pre-foot shock stage (acquisition time) and 24 hours after the foot shock (retention time) in all experimental groups. A lower latency (a shorter time before entering the dark chamber after receiving a foot shock) indicates that memory function was impaired. One way ANOVA and post-hoc Turkey tests revealed that there was no significant difference between control and sham groups; thus, surgery did not affect the pre-shock latencies in the experimental groups (Figure 1a). There was no significant difference between the

control group and the two other groups (BDL 7 and BDL 14) with respect to pre-shock latency as well. Therefore, in this experiment, acquisition time was not affected during the early stages of cholestasis. The results indicated that pre-shock latency was significantly (P = 0.036) longer in the BDL 21 group than in the control group (Figure 1b). Our data showed that the acquisition time was altered after 21 days in cholestatic rats.

Our results showed that there was no significant difference between control and sham groups with respect to the post-shock latency. Thus, surgery did not affect latency or memory function in the experimental groups (Figure 2a). In contrast, there was a significant (P = 0.04) difference between the control and BDL7 groups with respect to post shock latency. Therefore, short-term memory was affected in the early stage of cholestasis. Additionally, the post-shock latencies to entering the dark chamber in the BDL 14 and BDL 21 groups were significantly shorter than that of the control group (P = 0.009, P = 0.002; respectively) (Figure 2b). Thus, short-term memory deteriorated with cholestasis progression.

Locomotor activity

Our data analysis revealed that there was no significant difference between the control and sham group and that surgery did not affect the locomotor activity of the experimental groups (Figure 3a). Additionally, there was no significant difference in locomotor activity between the control group and the BDL 7 or BDL 14 group. However, there was a significant difference between the control and BDL 21 groups (P = 0. 028), indicating that the BDL 21 rats were less active than the normal rats (Figure 3b).

Discussion

The effect of cholestasis on learning and memory formation

The main finding of this study was that learning and memory retrieval, as measured in the passive avoidance test, were impaired with the progression of cholestasis at 7, 14, and 21 days after BDL (Figure 1,2). In the passive avoidance test, the animals learned to avoid entering the dark chamber after receiving an electrical foot shock (34,35). Some articles reported that BDL causes biliary cirrhosis after 3–4 weeks, and this condition occurs in association with fibrosis, portal hypertension, portal-systemic shunting, and immune system dysfunction (6,40,41). Mild cognitive impairment was found in patients with liver cirrhosis (42). Furthermore, patients with liver disease and signs of hyperamonia may

Table 1: Liver biochemistries from BDL7 (8 days after laparotomy), BDL14 (15 days after laparotomy), BDL21 (22 days after laparotomy) and sham operated (22 days after laparotomy) groups

	Sham[a]	BDL7[a]	BDL14[a]	BDL21[a]
Alanin Trans Aminase (IU/L)				
F statics (df); (3,20) = 49.75	187.17 (27.27)	732.83 (119.54) [b]	714.33 (119.54) [b]	673 (121.10) [b]
P value = 0.000 [c]		P value = 0.000	P value = 0.000	P value = 0.000
Alkalin Phosphatase (IU/L)				
F statics (df); (3,20) = 6.30	467.33 (96.17)	650.83 (92.31) [b]	661.83 (101.37) [b]	670 (88.54) [b]
P value = 0.0005 [c]		P value = 0.001	P value = 0.003	P value = 0.007
Total Bilirubin (mg/dl)				
F statics (df); (3,20) = 17.95	0.483 (0.08)	5.986 (1.84) [b]	6.63 (1.88) [b]	6.03 (2.03) [b]
P value = 0.0005 [c]		P value = 0.005	P value = 0.001	P value = 0.004
Direct Bilirubin (mg/dl)				
F statics (df); (3,20) = 45.90	0.19 (0.023)	5.36 (1.06) [b]	5.33 (1.23) [b]	5.88 (1.04) [b]
P value = 0.0005 [c]		P value = 0.004	P value = 0.005	P value = 0.001
In direct Bilirubin (mg/dl)				
F statics (df); (3,20) = 40.61	0.67 (0.09)	2.45 (0.36) [b]	3.31 (0.70) [b]	3.76 (0.68) [b]
P value = 0.0005 [c]		P value = 0.002	P value = 0.001	P value = 0.001

[a] Mean (SD), [b] with respect to the sham group, [c] analysis of variance (ANOVA). There were six rats per group. Abbreviation: bile duct ligation = BDL.

also show impairments in attention, memory, and cognitive function and alterations in motor function including psychomotor slowing, bradykinesia, and hypokinesia (7).

In support of our data, some studies have found incomplete passive avoidance test results (7,26,43), such as an impaired spatial memory in the Morris water maze task (12) and impairments in the ability to discriminate novel objects after BDL in rodents (44). Moreover, cases of deficits in attention, visual perceptions and working memory have been reported in patients with liver disease and hyperamonia (45,46).

BDL is a model of chronic liver injury. It has been shown that both acute and chronic liver failure induce cholestasis and hepatic encephalopathy, which affect brain function (14,36,47). The liver impairs cognitive function through an unknown molecular mechanism. Some studies have suggested that hyperamonia

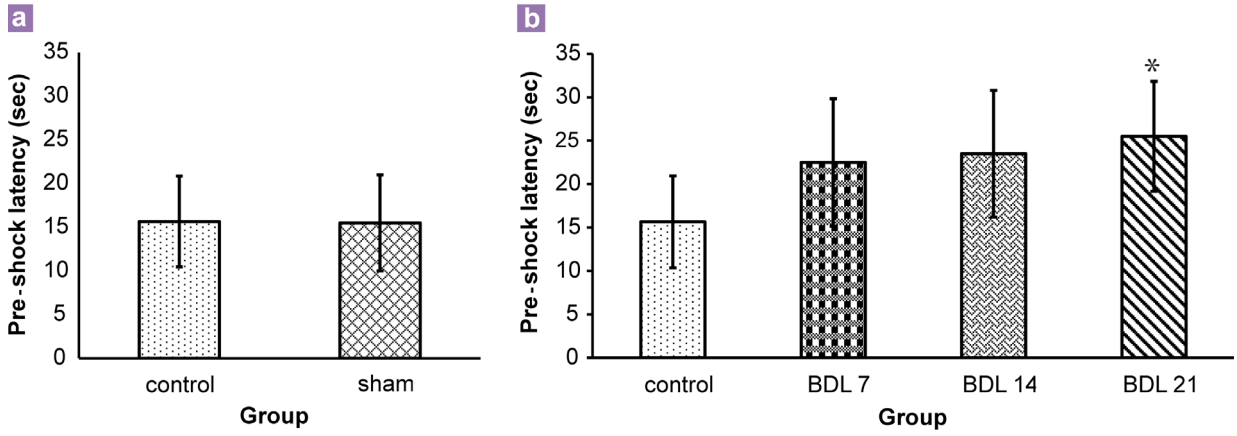

Figure 1: Comparison of the latency period for each rat to enter the dark chamber before receiving a foot shock (acquisition time). Each bar represents the mean (SD). One way ANOVA. (a) There were no significant differences between the control and sham groups. (b) Differences between the control group and the BDL 7 or BDL 14 groups were not significant. There were significant differences between the control and BDL 21 groups (*P = 0.036). Each group consisted of six rats.

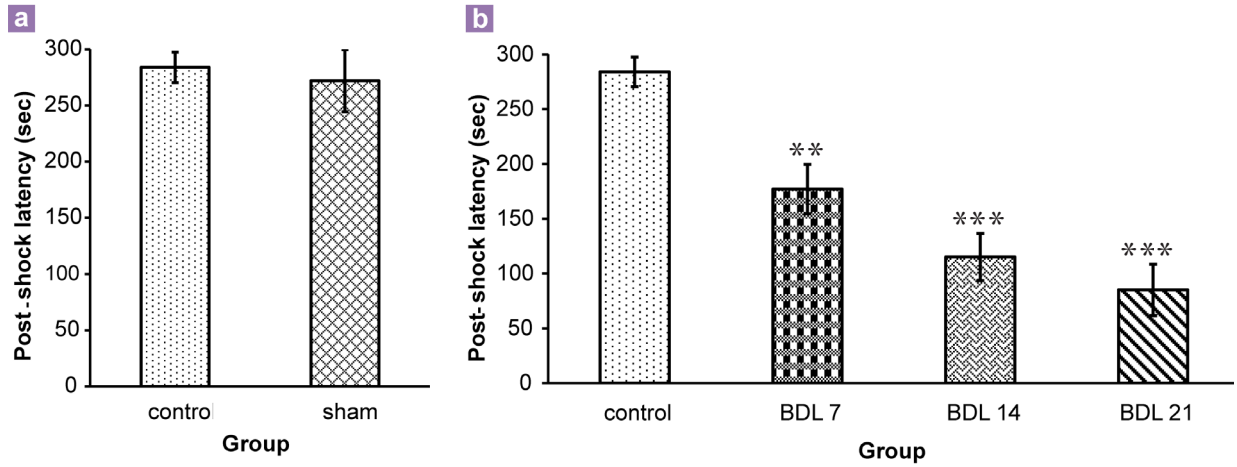

Figure 2: Comparison of the latency period for each rat to enter the dark chamber 24 hours after receiving a foot shock (the retention time). Each bar represents the mean (SD). One way ANOVA. (a) There were no significant differences between the control group and the sham group. (b) The retention time was significantly decreased in the BDL7 (**P = 0.04), BDL14 (***P = 0.009) and BDL21 (***P = 0.002) group compared with the control group. Each group consisted of six rats.

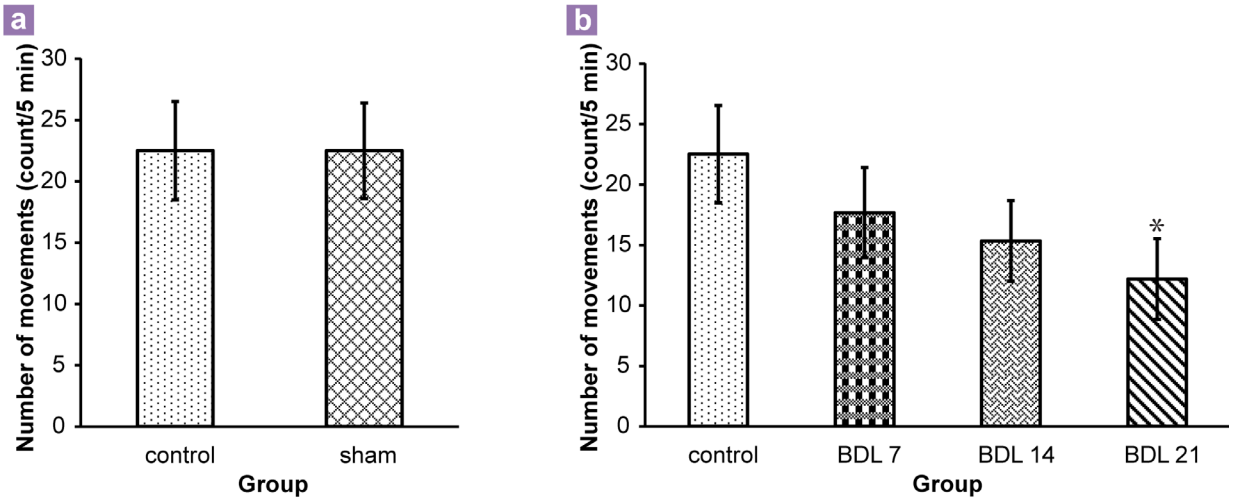

Figure 3: The effects of cholestasis on locomotor activity. Each bar represents the mean (SD). One-way ANOVA. (a) There were no significant differences between the control group and the sham group. (b) Differences between the control group and the BDL 7 or BDL 14 groups were not significant. There were significant differences in locomotor activity between the control and BDL 21 groups (*P = 0.028). Each group consisted of six rats.

is one of the main factors responsible for neurological alterations (48). Moreover, some mechanisms of induced amnesia that involve the glutamatergic system, such as changes in brain NO, oxidative stress, disruption of calcium homeostasis, membrane damage, and cell death (5,49,50), that also have detrimental effects on several key enzymes involved in glutamate and glucose transport have been suggested (51). All of the biological consequences mentioned above may cause cognitive deficits that are a result of induced amnesia in BDL rats. However, the mechanisms of amnesia induced by cholestasis in BDL rats have not been fully elaborated.

The effect of cholestasis on locomotor activity

The behavioral data showed that locomotor activity was not altered 7 or 14 days after BDL. Although it trended toward a decrease, this reduction was not significant. It was shown that cholestatic rats exhibited markedly less locomotor activity in the BDL 21 group compared with normal rats (Figure 3). Previous studies have shown reduced locomotor performance in the forced swim test, treadmill running and open field test 5 days after BDL in cholestatic rats (52,53). Additionally, locomotor activity in the open field test decreased after 4 weeks in BDL mice (11) and after 6 weeks (18) in BDL rats. In these studies, locomotor activity was assessed during the night (active) and day (inactive) periods (18). It seemed that locomotor activity disturbances began in the early stage of cholestasis in rats. In our study, rats showed disturbances in locomotor activity after 21 days of cholestasis. These conflicting observations might result from different methodologies to evaluate locomotor activity during prolonged periods (night and day) and from the fact that during the swimming and treadmill running, the animals had to expend energy to perform these two stressful physical activities. Therefore, because the animals were tired, they performed poorly in the test condition. In some studies, it has been proposed that fatigue is the reason for locomotor activity disturbances in cholestatic rats. It has been previously shown that the mechanisms involved in the fatigue that accompanies cholestasis may occur as a result of changes in the central nervous system (54,55). Among the neurotransmitter systems, the serotonergic and noradrenaline pathways are both implicated in fatigue states (56). It has been shown that these systems are intimately involved in the control of central corticotropine releasing hormone (CRH) release (56,57). Both the serotonergic system and the opioidergic system has been proven to be involved in cholestasis (58–60).

One of the main symptoms observed in chronic liver diseases, such as HE, is motor deficits including rigidity, asterixis (flapping tremor) and poor muscular coordination (61,62). Terzioglu et al. (63) reported increased 5-HT and dopamine

turnover in the hippocampus, hypothalamus, and in the striatum in BDL rats suggesting the involvement of hippocampus and hypothalamus in central fatigue (64,65).

These results showed an acquisition time impairment after 21 days. Moreover, locomotor activity in these animals decreased after 21 days. It seems that our results regarding memory impairment may be induced in part by disturbances in locomotor activity. Hence, to elucidate the mechanisms underlying the locomotor activity, learning, and memory impairments, it is necessary to further investigate individual brain areas such as the hippocampus, hypothalamus, striatum and their related neurotransmitters systems.

Studies have shown an activation of pro-inflammatory cytokines in BDL animals that did not have obvious signs of infection, fever, or signs of sepsis (10). Correspondingly, in humans with liver disease (62), the activation of inflammatory mediators is associated with a greater behavioral impairment.

Conclusion

In summary, the results revealed that cholestasis led to learning and memory, locomotor activity and liver function impairments and that cognition deteriorated with cholestasis progression.

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Conflict of Interest

None.

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Authors' Contributions

Conception and design, obtaining of funding collection and assembly of data: NH
Analysis and interpretation of the data: NH, MR
Drafting of the article: NH, HA, MN
Critical revision of the article for the important intellectual content: NH, MN
Final approval of the article: HA, MN, MR, MZ
Statistical expertise and administrative, technical or logistic support: MZ

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Reference

1. Blendis L. Hepatic encephalopathy forward to the past. *Gastroenterology*. 2006;**130(7)**:2239–2240. doi: 10.1053/j.gastro.2006.01.087.

2. Garcia-Moreno L, Conejo N, Gonzalez-Pardo H, Aller M, Nava M, Arias J, et al. Evaluation of two experimental models of hepatic encephalopathy in rats. *Braz J Med Biol Res*. 2005;**38(1)**:127–132. doi: 10.1590/S0100-879X2005000100019.

3. Butterworth RF, Norenberg MD, Felipo V, Ferenci P, Albrecht J, Blei AT. Experimental models of hepatic encephalopathy: ISHEN guidelines. *Liver Int*. 2009;**29(6)**:783–788. doi: 10.1111/j.1478-3231.2009.02034.x.

4. Roma M, Crocenzi F. Hepatocellular transport in acquired cholestasis: new insights into functional, regulatory and therapeutic aspects. *Clin Sci*. 2008;**114(9)**:567–588. doi: 10.1042/CS20070227.

5. Sheen JM, Huang LT, Hsieh CS, Chen CC, Wang JY, Tain YL. Bile duct ligation in developing rats: temporal progression of liver, kidney, and brain damage. *J Pediatr Surg*. 2010;**45(8)**:1650–1658. doi: 10.1016/j.jpedsurg.2009.12.019.

6. Aksu B, Umit H, Kanter M, Guzel A, Aktas C, Civelek S, et al. Effects of methylene blue in reducing cholestatic oxidative stress and hepatic damage after bile-duct ligation in rats. *Acta histochem*. 2010;**112(3)**:259–269. doi: 10.1016/j.acthis.2008.12.002.

7. Huang LT, Chen CC, Sheen JM, Chen YJ, Hsieh CS, Tain YL. The interaction between high ammonia diet and bile duct ligation in developing rats: assessment by spatial memory and asymmetric dimethylarginine. *Int J Dev Neurosc*. 2010;**28(2)**:169–174. doi: 10.1016/j.ijdevneu.2009.11.006.

8. Garcia-Ayllon M, Silveyra MX, Candela A, Compan A, Claria J, Jover R, et al. Changes in liver and plasma acetylcholinesterase in rats with cirrhosis induced by bile duct ligation. *Hepatology*. 2006;**43(3)**:444–453. doi: 10.1002/hep.21071.

9. Zarrindast MR, Hoseindoost S, Nasehi M. Possible interaction between opioidergic and cholinergic systems of CA1 in cholestasis-induced amnesia in mice. *Behav Brain Res*. 2012;**228(1)**:116–124. doi: 10.1016/j.bbr.2011.11.039.

10. Hajrasouliha AR, Tavakoli S, Jabehdar-Maralani P, Ebrahimi F, Shafaroodi H, Mirkhani SH, et al. Cholestatic liver disease modulates susceptibility to ischemia/reperfusion-induced arrhythmia, but not necrosis and hemodynamic instability: the role of endogenous opioid peptides. *J Hepatol*. 2005;**43(3)**:491–498. doi: 10.1016/j.jhep.2005.02.043.

11. Leung N, Croatt AJ, Haggard JJ, Grande JP, Nath KA. Acute cholestatic liver disease protects against glycerol-induced acute renal failure in the rat. *Kidney Int*. 2001;**60(3)**:1047–1057. doi: 10.1046/j.1523-1755. 2001.0600031047.x.

12. Swain MG. Cytokines and neuroendocrine dysregulation in obstructive cholestasis: pathophysiological implications. *J Hepatol*. 2001;**35(3)**:416–418. doi: 10.1016/S0168-8278(01)00174-X.

13. Magen I, Avraham Y, Ackerman Z, Vorobiev L, Mechoulam R, Berry EM. Cannabidiol ameliorates cognitive and motor impairments in mice with bile duct ligation. *J Hepatol*. 2009;**51(3)**:528–534. doi: 10.1016/j.jhep.2009.04.021.

14. Forton DM, Thomas HC, Murphy CA, Allsop JM, Foster GR, Main J, et al. Hepatitis C and cognitive impairment in a cohort of patients with mild liver disease. *Hepatology*. 2002;**35(2)**:433–439. doi: 10.1053/jhep.2002.30688.

15. Monfort P, Munoz MD, ElAyadi A, Kosenko E, Felipo V. Effects of hyperammonemia and liver failure on glutamatergic neurotransmission. *Metab Brain Dis*. 2002;**17(4):**237–250. doi: 10.1023/A:1021993431443.

16. Huang LT, Tiao MM, Tain YL, Chen CC, Hsieh CS. Melatonin ameliorates bile duct ligation-induced systemic oxidative stress and spatial memory deficits in developing rats. *Pediatr Res*. 2009;**65(2)**:176–180. doi: 10.1023/A:1021993431443.

17. Ryan C, Than T, Blumgart L. Choledochoduodenostomy in the rat with obstructive jaundice. *J Surg Res*. 1977;**23(5)**:321–331. doi: 1016/0022-4804(77)90069-5.

18. Amodio P, Del Piccolo F, Marchetti P, Angeli P, Lemmolo R, Caregaro L, et al. Clinical features and survivial of cirrhotic patients with subclinical cognitive alterations detected by the number connection test and computerized psychometric tests. *Hepatology*. 1999;**29(6)**:1662–1667. doi: 10.1002/hep.510290619.

19. Morris R, Schenk F, Tweedie F, Jarrard L. Ibotenate lesions of hippocampus and/or subiculum: dissociating components of allocentric spatial learning. *Eur J Neurosci*. 1990;**2(12)**:1016–1028. doi: 10.1111/j.1460-9568.1990.tb00014.x.

20. Bosoi CR, Parent-Robitaille C, Anderson K, Tremblay M, Rose CF. AST-120 (spherical carbon adsorbent) lowers ammonia levels and attenuates brain edema in bile duct ligated rats. *Hepatology*. 2011;**53(6)**:1995–2002. doi: 10.1002/hep.24273.

21. Rodrigo R, Cauli O, Gomez–Pinedo U, Agusti A, Hernandez–Rabaza V, Garcia–Verdugo JM, et al. Hyperammonemia induces neuroinflammation that contributes to cognitive impairment in rats with hepatic encephalopathy. *Gastroenterology*. 2010;**139(2)**:675–684. doi: 10.1053/j.gastro.2010.03.040.

22. Bosoi CR, Rose CF. Identifying the direct effects of ammonia on the brain. *Metab Brain Dis*. 2009;**24(1)**:95–102. doi: 10.1007/s11011-008-9112-7.

23. Khakpai F, Nasehi M, Haeri-Rohani A, Eidi A, Zarrindast MR. Scopolamine induced memory impairment; possible involvement of NMDA receptor mechanisms of dorsal hippocampus and/or septum. *Behav Brain Res*. 2012;**231(1)**:1–10. doi: 10.1016/j.bbr.2012.02.049.

24. McClelland JL, McNaughton BL, O'Reilly RC. Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev*. 1995;**102(3)**:419–457. doi: 10.1037/0033-295X.102.3.419.

25. Garcıia-Moreno LM, Angeles Aller M, Conejo NM, Gomez MA, Martıin FR, Arias J, et al. Brain Ag-NOR activity in cholestatic rats with hepatic encephalopathy. *Hepatol Res*. 2002;**24(3)**:275–281. doi: 10.1590/S0100-879X2005000100019.

26. Butterworth RF, Norenberg MD, Felipo V, Ferenci P, Albrecht J, Blei AT. Experimental models of hepatic encephalopathy: ISHEN guidelines. *Liver Int*. 2009;**29(6)**:783–788. doi: 10.1111/j.1478-3231.2009.02034.x.

27. Bergasa NV, Liau S, Homel P, Ghali V. Hepatic Met–enkephalin immunoreactivity is enhanced in primary biliary cirrhosis. *Liver*. 2002;**22(2)**:107–113. doi: 10.1034/j.1600-0676.2002.01458.x.

28. Nasehi M, Piri M, Abbolhasani K, Zarrindast MR. Involvement of opioidergic and nitrergic systems in memory acquisition and exploratory behaviors in cholestatic mice. *Behav Pharmacol*. 2013;**24(3)**:180–194. doi: 10.1016/j.physbeh.2012.10.006.

29. Eslimi D, Oryan S, Nasehi M, Zarrindast MR. Effects of opioidergic systems upon anxiolytic-like behaviors induced in cholestatic rats. *Eur J Pharmacol*. 2011;**670(1)**:180–185. doi: 10.1016/j.ejphar.2011.08.024.

30. Bergasa NV, Vergalla J, Swain MG, Jones EA. Hepatic concentrations of proenkephalin - derived opioids are increased in a rat model of cholestasis. *Liver*. 1996;**16(5)**:298–302. doi: 10.1111/j.1600-0676.1996.tb00749.x.

31. Nasehi M, Piri M, Abdollahian M, Zarrindast MR. Involvement of nitrergic system of CA1in harmane induced learning and memory deficits. *Physiol Behav*. 2013;**109**:23–32. doi: 10.1016/j.physbeh.2012.10.006.

32. Garcia-Moreno L, Conejo N, González-Pardo H, Aller M, Nava M, Arias J, et al. Evaluation of two experimental models of hepatic encephalopathy in rats. *Braz J Med Biol Res*. 2005;**38(1)**:127–132. doi: 10.1590/S0100-879X2005000100019.

33. Cahill L, Weinberger NM, Roozendaal B, McGaugh JL. Is the amygdala a locus of "conditioned fear"? Some questions and caveats. *Neuron*. 1999;**23(2)**:227–2228. doi: 10.1016/S0896-6273(00)80774-6.

34. Rastegar H, Homayoun H, Afifi M, Rezayat M, Dehpour AR. Modulation of cholestasis-induced antinociception by CCK receptor agonists and antagonists. *Eur Neuropsychopharmacol*. 2002;**12(2)**:111–118. doi: 10.1016/S0924-977X(02)00008-1.

35. Jablonski P, Howden B, Baxter K. Influence of buprenorphine analgesia on post-operative recovery in two strains of rats. *Lab Anim*. 2001;**35(3)**:213–222. doi: 10.1258/0023677011911651.

36. Sandi C, Rose SP, Patterson TA. Unilateral hippocampal lesions prevent recall of a passive avoidance task in day-old chicks. *Neurosci Lett*. 1992;**141**:255–258. doi: 10.1016/0304-3940(92)90907-O.

37. Lorenzini CA, Baldi E, Bucherelli C, Sacchetti B, Tassoni G. Role of dorsal hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response: a tetrodotoxin functional inactivation study. *Brain Res*. 1996;**730(1–2)**:32–39. doi: 10.1016/0006-8993(96)00427-1.

38. Jover R, Rodrigo R, Felipo V, Insausti R, Saez-Valero J, Garcia-Ayllon M, et al. Brain edema and inflammatory activation in bile duct ligated rats with diet-induced hyperammonemia: A model of hepatic encephalopathy in cirrhosis. *Hepatology*. 2006;**43(6)**:1257–1266. doi: 10.1002/hep.21180.

39. Walsh RN, Cummins RA. The open-field test: A critical review. *Psychol Bull*. 1976;**83(3)**:482–504. doi: 10.1037/0033–2909.83.3.482.

40. Mani AR, Nahavandi A, Moosavi M, Safarinejad R, Dehpour AR. Dual nitric oxide mechanisms of cholestasis-induced bradycardia in the rat. *Clin Exp Pharmacol Physiol*. 2002;**29(10)**:905–908. doi: 10.1046/j.1440-1681.2002.03748.x.

41. Borhani AA, Houshmand G, Samini M, Namiranian K, Hajrasouliha AR, Tavakoli S, et al. α 2-Adrenoceptor subsensitivity in mesenteric vascular bed of cholestatic rats: The role of nitric oxide and endogenous opioids. *Eur J Pharmacol*. 2005;**514(2–3)**:183–189. doi: 10.1016/j.ejphar.2005.03.015.

42. Gieling RG, Burt AD, Mann DA. Fibrosis and cirrhosis reversibility–molecular mechanisms. *Clin Liver Dis*. 2008;**12(4)**:915–937. doi: 10.1016/j.cld.2008.07.001.

43. Michalak A, Knecht K, Butterworth RF. Hepatic encephalopathy in acute liver failure: role of the glutamate system. *Adv Exp Med Biol*. 1997;**420**:35–43. doi: 10.1007/978-1-4615-5945-0-2.

44. Arria AM, Tarter RE, Kabene M, Laird S, Moss H, Thiel DV. The role of cirrhosis in memory functioning of alcoholics. *Alcohol Clin Exp Res*. 1991;**15(6)**:932–937. doi: 10.1111/j.1530-0277.1991.tb05192.x.

45. Jover R, Madaria E, Felipo V, Rodrigo R, Candela A, Compan A. Animal models in the study of episodic hepatic encephalopathy in cirrhosis. *Metab Brain Dis*. 2005;**20(4)**:399–408. doi: 10.1007/s11011-005-7925-1.

46. Aronson DC, Chamuleau RA, Frederiks WM, Gooszen HG, Heijmans HS, James J. Reversibility of cholestatic changes following experimental common bile duct obstruction: fact or fantasy? *J Hepatol*. 1993;**18(1)**:85–95. doi: 10.1016/S0168-8278(05)80014-5.

47. Lozeva V, Montgomery JA, Tuomisto L, Rocheleau B, Pannunzio M, Huet PM, et al. Increased brain serotonin turnover correlates with the degree of shunting and hyperammonemia in rats following variable portal vein stenosis. *J Hepatol*. 2004;**40(5)**:742–748. doi: 10.1016/j.jhep.2004.01.003.

48. Weissenborn K, Heidenreich S, Giewekemeyer K, Ruckert N, Hecker H. Memory function in early hepatic encephalopathy. *J Hepatol*. 2003;**39(3)**:320–325. doi: 10.1016/S0168-8278(03)00295-2.

49. Cauli O, López-Larrubia P, Rodrigues TB, Cerdán S, Felipo V. Magnetic resonance analysis of the effects of acute ammonia intoxication on rat brain. Role of NMDA receptors. *J Neurochem*. 2007;**103(4)**:1334–1343. doi: 10.1111/j.1471-4159.2007.04878.x.

50. Pantiga C, Rodrigo LR, Cuesta M, Lopez L, Arias JL. Cognitive deficits in patients with hepatic cirrhosis and in liver transplant recipients. *J Neuropsychiatry Clin Neurosci*. 2003;**15(1)**:84–89. doi: 10.1176/appi.neuropsych.15.1.84.

51. Stewart SM, Kennard BD, Waller DA, Fixler D. Cognitive function in children who receive organ transplantation. *Health Psychol*. 1994;**13**:3. doi: 10.1037/0278–6133.13.1.3.

52. Parfenova H, Basuroy S, Bhattacharya S, Tcheranova D, Qu Y, Regan RF, et al. Glutamate induces oxidative stress and apoptosis in cerebral vascular endothelial cells: contributions of HO-1 and HO-2 to cytoprotection. *Am J Physiol Cell Physiol*. 2006;**290(5)**:C1399–1410. doi: 10.1152/ajpcell.00386.2005.

53. Jover R, Compañy L, Gutiérrez A, Lorente M, Zapater P, Poveda MJ, et al. Clinical significance of extrapyramidal signs in patients with cirrhosis. *J Hepatol*. 2005;**42(2)**:659–665. doi: 10.1016/j.jhep.2004.12.030.

54. Magistretti PJ. Role of glutamate in neuron-glia metabolic coupling. *Am J Clin Nutr*. 2009;**90(3)**:S875–S880. doi: 10.3945/ajcn.2009.27462CC.

55. Swain MG, Maric M. Improvement in cholestasis-associated fatigue with a serotonin receptor agonist using a novel rat model of fatigue assessment. *Hepatology*. 1997;**25(2)**:291-294. doi: 10.1002/hep.510250206.

56. Soares DD, Coimbra CC, Marubayashi U. Tryptophan-induced central fatigue in exercising rats is related to serotonin content in preoptic area. *Neurosci Lett*. 2007;**415(3)**:274–278. doi: 10.1016/j.neulet.2007.01.035.

57. Celik T, Goren MZ, Cınar K, Gurdal H, Onder FO, Tan A, et al. Fatigue of cholestasis and the serotoninergic neurotransmitter system in the rat. *Hepatology*. 2005;**41(4)**:731–737. doi: 10.1002/hep.20617.

58. Burak KW, Le T, Swain MG. Increased midbrain 5-HT- 1A receptor number and responsiveness in cholestatic rats. *Brain Res*. 2001;**892(2)**:376–379. doi: 10.1016/S0006-8993(00)03058-4.

59. Bearn J, Wessely S. Neurobiological aspects of the chronic fatigue syndrome. *Eur J Clin Invest*. 1994;**24(2)**:79–90. doi: 10.1111/j.1365-2362.1994.tb00971.x.

60. Sullivan Hanley N, Van de Kar L. Serotonin and the neuroendocrine regulation of the hypothalamic–pituitary–adrenal axis in health and disease. *Vitam Horm*. 2003;**66**:189–255. doi: 10.1016/S0083-6729(03)01006-9.

61. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. *N Engl J Med*. 2005;**353(12)**:1261–1273. doi: 10.1056/NEJMra043898.

62. Jones E, Neuberger J, Bergasa N. Opiate antagonist therapy for the pruritus of cholestasis: the avoidance of opioid withdrawal-like reactions. *QJM*. 2002;**95(8)**:547–552. doi: 10.1093/qjmed/95.8.547.

63. Terzioglu B, Aypak C, Yananlı HR, Kucukibrahimoglu E, Yurdaydın C, Goren MZ. 5-hydroxytryptamine release in the anterior hypothalamic and the hippocampal areas of cholestatic rats. *Life Sci*. 2006;**78(10)**:1078–1083. doi: 10.1016/j.lfs.2005.06.025.

64. Montes S, Alcaraz-Zubeldia M, Muriel P, Rı´os C. Striatal manganese accumulation induces changes in dopamine metabolism in the cirrhotic rat. *Brain Res*. 2001;**891(1)**:123–129. doi: 10.1016/S0006-8993(00)03208-X.

65. Zietz B, Wengler I, Messmann H, Lock G, Schölmerich J, Straub RH. Early shifts of adrenal steroid synthesis before and after relief of short-term cholestasis. *J Hepatol*. 2001;**35(3)**:329–337. doi: 10.1016/S0168-8278(01)00152-0.