Hypoxia-ischemia induced neurological dysfunction and brain injury in the neonatal rat

Lir-Wan Fan a, Shuying Lin a, Yi Pang a, Manping Lei b, Feng Zhang b, Philip G. Rhodes a, Zhengwei Cai a, *

a Department of Pediatrics, Division of Newborn Medicine, University of Mississippi Medical Center, Jackson, MS 39216, USA
b Department of Surgery, University of Mississippi Medical Center, Jackson, MS 39216, USA

Received 7 April 2005; received in revised form 20 June 2005; accepted 21 June 2005

Available online 2 September 2005

Abstract

Bilateral carotid artery occlusion (BCAO) followed by exposure to a hypoxic condition (8% oxygen for 10 or 15 min) was performed in postnatal day 4 SD rats. Brain injury and myelination changes were examined on postnatal day 21 (P21) and tests for neurobehavioral toxicity were performed from P3 to P21. BCAO followed by 10 or 15 min hypoxic insult resulted in mild and severe, respectively, brain injury, reduction in mature oligodendrocytes and tyrosine hydroxylase positive neurons and impaired myelination as indicated by decreased myelin basic protein immunostaining in the P21 rat brain. Hypoxia-ischemia also affected physical development (body weight gain and eye opening) and neurobehavioral performance, such as righting reflex, wire hanging maneuver, cliff avoidance, locomotor activity, gait analysis, responses in the elevated plus-maze and passive avoidance. BCAO followed by 15 min of hypoxia caused more severely impaired neurobehavioral performance as compared with BCAO followed by 10 min of hypoxia in the rat. The overall results demonstrate that hypoxia-ischemia-induced brain injury not only persists, but also is linked with neurobehavioral deficits in juvenile rats. The present data also indicate that the degree of brain injury and the deficits of neurobehavioral performance in the rat are dependent on the hypoxic-ischemic condition, i.e., the exposure time to hypoxia.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Hypoxia-ischemia; Brain injury; Hypomyelination; Tyrosine hydroxylase; Neurobehavioral performance

1. Introduction

Periventricular leukomalacia (PVL), an important form of white matter disease in very premature infants, is closely associated with severe neurological disorders, such as cerebral palsy, mental retardation and visual impairment [36]. Hypoxia-ischemia (HI) has been considered as a major cause for the occurrence of PVL resulting in acute mortality and chronically disturbed central nervous system functioning, including behavioral alterations, motor disturbances and learning disabilities [12,36]. In our previous studies, we found that bilateral carotid artery occlusion (BCAO) followed by exposure to hypoxia (8% oxygen for 20 min) in postnatal day 4 (P4) rats caused severe white matter damage in the P9 rat brain (5 days after the HI insult) [19]. The lesions included rarefaction, necrosis and cavity formation in the corpus callosum, external and internal capsule areas and hypomyelination. These white matter changes resembled injuries found in newborn infants with PVL.

PVL is a major form of brain injury in preterm infants and this injury can have long-term effects on physical, motor, sensory, cognitive and social development [11,29]. Our previous studies have investigated the biochemical and pathological changes induced by HI in the neonatal rat brain [8,19]. It is essential to know whether the HI insult in our model also has long-term effects on neurobehavioral performance in the developing rat. The severity of HI-induced brain injury in the neonatal rat model has been shown to closely link with the duration of hypoxia [28]. Therefore, it is also important to...
know whether alterations in neurobehavioral performance are dependent on the HI condition (i.e., the duration of hypoxia).

The present study was designed to examine the physical development (body weight and eye opening) and neurobehavioral alterations, including sensorimotor and locomotor functions, learning and memory, in juvenile rats after BCAO followed by hypoxia for 10 or 15 min.

2.2. Animals

Timed pregnant Sprague–Dawley (SD) rat arrived in the laboratory on day 19 of gestation. Animals were maintained in an animal room on a 12-h light/12-h dark cycle and at constant temperature (22 ± 2 °C). After birth, the litter size was adjusted to twelve pups per litter to minimize the effect of litter size on body weight and brain size. All procedures for animal care were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center.

2.3. Surgery procedures and animal treatment

The surgery procedures were performed as described by Cai et al. [8] and Liu et al. [19] with modification. Late oligodendrocyte (OL) progenitor cells, which have been proposed to be the major target in cerebral white matter injury in human infants [4], are the predominant OL lineage stage in the rat cerebral hemispheres between P2 and P7 [4]. For ease of operation, we performed the operation in P4 SD rats. Pups were lightly anesthetized with isoflurane (4% induction, 1.5% maintenance). A skin incision was made in the neck surface and the common carotid arteries were exposed. Bilateral common carotid arteries were separated from the vagal nerves and BCAO was performed with 8-0 nylon sutures under a surgical microscope. The wound was sutured and the animals were then placed on a warm heating pad (34–35 °C) for recovery from anesthesia. Sham-operation (surgery without BCAO) was conducted in the littersmate. Naive and sham-operated rats were considered as the controls. All animals survived the operation.

After BCAO-operated animals were further divided into two groups: one exposed to hypoxia for 10 min and another for 15 min in a chamber maintained at 37 °C and provided with a humidified gas mixture flow (8% oxygen–92% nitrogen). The duration of hypoxic exposure after BCAO treatment was chosen based on the results reported previously, which resulted in preferential white matter injury [19]. Sham-operated rats were also exposed to hypoxia for 15 min. Since neonatal rats exposed to hypoxia alone do not show any brain injury [28], the current study did not include a group with only the hypoxia exposure. Each dam had the same litter size (12 pups) and equal numbers of rat pups (3 pups) from each treatment group were included in a litter. There were nine pups in each group. Seventeen days after the operation (P21), rat pups were sacrificed by transcardiac perfusion with normal saline followed by 4% paraformaldehyde for brain section preparation.

2.4. Behavioral testing

The developmental test battery used was based on the tests for neurobehavioral toxicity [2,12,37]. These tests were performed for all rat pups from P3 to P21. Body weights of rat pups were recorded daily.

2.4.1. Righting reflex

This test is believed to be a reflection of subcortical maturation [3,12]. Pups were placed on their back, and the time required to turn over on all four feet and touch the platform was measured [3].

2.4.2. Wire hanging maneuver

This maneuver tests neuromuscular and locomotor development [2,12]. Pups suspended by their forelimbs from a horizontal rod (5 mm × 5 mm area, 35 cm long, between two poles 50 cm high) tend to support themselves with their hind limbs, preventing them from falling and aiding in progression along the rod. Suspension latencies were recorded.

2.4.3. Cliff avoidance test

This test was used to assess the integration of exteroceptive input (vibrissae) and locomotor output [2,12,32]. Pups placed on the edge of a platform (20 cm × 35 cm × 20 cm) with forepaws and chest extending over the edge tend to move away by backing up or turning to the side. Avoidance was scored by reflex latency between being placed on the edge and turning the body sideways with forepaws or turning the head away. If the pup did not make any response within 60 s or fell off from the platform, it was recorded as 60 s [32].

2.4.4. Locomotor activity

This test measures the activity and habitation response of animals on placement in a novel environment [12,35]. Locomotor...
activity was measured by using the Video Tracking System SMART-2000 (San Diego Instruments Inc., San Diego, CA). Pups were placed in the activity chambers in a quiet room with dimmed light. The total distance traveled by the animal was recorded during a 10-min testing period [35].

2.4.5. Passive avoidance
Passive avoidance gives information about learning and memory capabilities as well as maturation of the inhibitory process [12]. The passive avoidance procedure consists of two sessions. In the first session (P20), rats were trained in a step-down type of passive avoidance apparatus. The experimental chamber (30 cm × 30 cm × 40 cm) was made of plexiglass. The floor of the chamber was made of parallel 2-mm-caliber stainless steel rods spaced 1 cm apart from each other and connected with an electric shock generator. The safe part was a piece of wood board (8 cm × 25 cm × 2.5 cm) placed at a corner of the chamber above the metal rods. Each animal was placed initially on the safe platform. When the rat stepped down onto the floor, it received a foot shock (1 s, 0.5 mA). Although the rats repeatedly stepped up and down, they eventually remained on the board. The number of shocks required to retain an individual animal on the board for 2 min was recorded as a measure of acquisition of passive avoidance. The second session was carried out 24 h after the first session (P21). The rat was placed on the safe board and steel rods were not connected with the electric shock generator. The retention latency, i.e., the time elapsed before the rat stepped down to the grid floor, was recorded as a measure of the retention of passive avoidance. If the rat did not step down to the grid floor within 2 min, a ceiling score of 2 min was assigned.

2.4.6. Gait analysis
This test was used to assess integrity of the cerebellum [12]. On P19, the hind paws of each rat were smeared with ink. The animal was allowed to walk up a runway (80 cm × 10 cm) covered with white paper and with a darkened part at the end. For motivational purposes, a white light (60 W) was placed at the beginning of the ramp. Six variables from footprint pattern were measured as follows: (1) foot length, (2) spreading toes 1–5, (3) spreading toes 2–4, (4) stride length, (5) stride width and (6) step angle.

2.4.7. Elevated plus-maze test
The elevated plus-maze test is used to assess anxiolytic behavior [1,30,31]. The procedure is based on rodents’ natural tendency to avoid open space and it does not contain any experimenter-controlled aversive element. Nevertheless, exposure to it is stressful for the subjects [1]. The plus-maze consists of two open arms (30 cm × 5 cm × 10 cm) and two enclosed arms (30 cm × 5 cm × 10 cm) emanating from a common central platform (5 cm × 5 cm) to form a plus shape. The entire apparatus was elevated to a height of 40 cm above the floor. A video camera and illumination-lamps were mounted at the ceiling. The anxiety-related behaviors for each animal were recorded for a period of 5 min by a VCR-recording system on P19. At the beginning of the test, the rat was placed in the central platform with its head facing an open arm. The parameters recorded were the numbers of open arm or enclosed arm entries (arm entry defined as all four paws into an arm), and the total time each animal spent in various sections of the maze (open arms, center and enclosed arms) [30]. The results were expressed as the percentage of open arm or enclosed arm entries (open arm or enclosed arm entries divided by the sum of open arm and enclosed arm entries) and the percentage of the time spent in open arms or enclosed arms (time spent in open arms or enclosed arms divided by the sum of time spent in either arm).

2.5. Immunohistochemistry studies
Brain injury was estimated based on the results of Hematoxylin and Eosin (H&E) staining and immunohistochemistry in consecutive frozen sections at a thickness of 10 μm prepared from the P21 rat brain (17 days after the HI insult). Increasing evidence indicates that not only white matter injury, but also neuronal and axonal injury is involved in PVL [5,15]. Therefore, in addition to the injury to oligodendrocytes and myelination, we also examine axonal injury with MAP1 antibody and dopamine neuron injury with TH antibody. For immunohistochemistry, the final concentrations of the primary antibodies were diluted as follows: APC (1:20), MBP (1:100), TH (1:20 000) and MAP1 (1:200). Mature oligodendrocytes were identified with APC which immunostains oligodendrocyte cell bodies primarily observed in the corpus callosum, the subcortical white matter tract and the internal capsule areas without labeling O1 processes or astrocytes [9,21]. MAP1 has selective staining of neurons and stronger staining of axons and dendrites. After incubation with biotinylated second antibodies for 1 h at room temperature (RT), brain sections were further incubated with Cy3-conjugated avidin or the avidin–horseradish peroxidase system (ABC kit from Vector Laboratories, Burlingame, CA) for an additional 1 h in dark at RT. In immunofluorescence staining, DAPI (100 ng/ml) was simultaneously used to identify nuclei in the final visualization. Sections incubated in the absence of primary antibody were used as negative controls.

2.6. Quantification of immunostaining data and statistics
To compare the cell number among the treatment groups, positively stained cells were counted. Our previous studies indicate that this neonatal rat model of HI produces preferential white matter injury primarily in the forebrain [8,19]. APC+ cell counting was performed in the forebrain sections at the bregma level. TH+ cell counting was performed in the mid-brain sections at a level 1/3 rostral from the lambda to the bregma. A digital microscopic image for each brain section was captured at the circular area where the APC+ cells were most abundant or at the SN where the TH+ cells were most abundant. Three brain sections at the same brain level were counted bilaterally in each animal. The mean thickness of the corpus callosum (as observed by H&E staining) has been suggested to be related to neuropsychological outcome [23]. The thickness of the corpus callosum at the bregma level in each rat brain was determined at the center of the corpus callosum with a NIH Image software. Perinatal asphyxia has been reported to reduce the thickness of corpus callosum MBP staining [17]. We found in our preliminary study that HI resulted in a thinner MBP staining at the subcortical white matter tract. To quantitatively examine effects of HI on the thickness of the subcortical MBP staining at the bregma level, the mean thickness of the subcortical MBP staining under the forebrain area of the cortex in a microscopic image was determined by the NIH Image software. The thickness of MBP staining was measured at the beginning, the middle and the end of the area (see Fig. 6 for the position) and the average of the three measures were calculated bilaterally for a single brain.
Cell number or thickness of the corpus callosum and the MBP staining were taken from the average of three sections for each brain. The results were presented as the mean ± S.E.M. cells per mm² or the mean ± S.E.M. μm or mm of nine animals. The immunostaining and the neurobehavioral performance data were analyzed by one-way ANOVA followed by Student-Newman-Keuls test. Results with a P < 0.05 were considered statistically significant.

3. Results

No apparent differences in neurobehavioral performances were observed between the naïve and the sham group. Therefore, we used data from the naïve group as the control.

3.1. Hypoxia-ischemia induced neurobehavioral performance deficits in rats

3.1.1. Physical development

Compared with the naïve group, HI insult on P4 resulted in a significantly lower body weight from P5 to P21 (Fig. 1A). The degree of retarded somatic development was dependent on the HI condition. Compared with the naïve group on P21, the body weight for the BCAO following 10-min hypoxia (HI-10 min) and 15-min hypoxia (HI-15 min) groups were 10% and 22% lower, respectively. HI treatment also delayed the date of eye opening. The date of eye opening for the naïve and sham groups was P15.3 ± 0.1 and P15.2 ± 0.1, respectively. The date of eye opening for HI-10 min and HI-15 min groups was P16.2 ± 0.2 and P17.3 ± 0.3, respectively.

3.1.2. Righting reflex

As shown in Fig. 1B, the HI-10 min and HI-15 min groups exhibited significantly longer mean latency times as compared to the naïve and the sham groups from P5 to P13 and P15, respectively. Recovery from this impairment occurred on P14 and P16.

3.1.3. Wire hanging maneuver

The wire hanging ability of the rat increased with age. The mean latency time of the HI-10 min and HI-15 min groups were significantly less than that of the naïve group from P6 to P13 and P16, respectively (Fig. 2A).
3.1.4. Cliff avoidance test

All pups from the naïve and the sham groups succeeded in the cliff avoidance test by P11 and the avoidance latency decreased with age (Fig. 2B). It was not until P13 and P14, respectively, that pups from the HI-10 min and HI-15 min groups had cliff avoidance responses. The avoidance response latency in the HI-10 min group reached the level of the naïve group by P17 and that in the HI-15 min group reached the level of the naïve group by P18.

3.1.5. Locomotor activity

Locomotor activity, as measured by the total crossing distance of an individual rat during a 10-min period, increased with age until P15 in all groups (Fig. 3A). The HI-10 min treatment resulted in hyperactivity from P13 to P17 and the HI-15 min treatment resulted in hyperactivity from P12 to P18 as compared to the naïve group.

3.1.6. Passive avoidance

The number of electric foot shocks needed to retain the rat on the safe board was significantly increased in the HI group at P20 (Fig. 3B). HI also reduced the retention latency to step down from the board the next day (P21) as compared to the control group. The degree of impairment in learning and memory was dependent on the HI condition.

3.1.7. Gait analysis

The performances of all groups in gait analysis at P19 are summarized in Table 1. HI administration resulted in increased foot length, stride width and step angle. HI treatment decreased total spreading (spreading toes 1–5), intermediary toes (spreading toes 2–4) and stride length. The degree of impairment in the gait performance was dependent on the HI condition.

3.1.8. Elevated plus-maze test

As shown in Fig. 4, a higher proportion of open arm entries was observed in the HI groups without increases in enclosed arm entries (referring to the actual number of entries, data not shown) as compared with the naïve group at P19. HI treatment also increased the proportion of time spent in the open arms while decreased the proportion of time spent in the enclosed arms. The effect of HI on the plus-maze performance (less anxiolytic behavior) in the HI-15 min group was more prominent than that in the HI-10 min group.

3.2. Hypoxia-ischemia induced brain injury in the rat brain

Brain injury was estimated based on the results of H&E staining and immunohistochemistry in consecutive frozen sections. Hypoxia-ischemia induced deficits in gait analysis performed 15 days (P19) after the hypoxic-ischemic insult in the rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Naïve</th>
<th>Sham</th>
<th>Hypoxia-ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot length</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total spreading (1–5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate toes (2–4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stride length</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step angle (°)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.M. cm of nine animals. Data were analyzed by one-way ANOVA followed by Student–Newman–Keuls test. *P < 0.05 compared with the naïve group.

Fig. 3. Hypoxia-ischemia-induced increases in locomotor activity, as determined by the open field test in rats (A), and learning and memory deficits, as determined by the passive avoidance test, 16 (P20) and 17 (P21) days after the hypoxia-ischemia treatment (B). In (B), the number of electric foot shocks needed to retain the rat on the safe board was recorded as an index of acquisition of passive avoidance and the retention latency was determined the next day as an index of retention of passive avoidance. The results are expressed as the mean ± S.E.M. of nine animals in each group. *P < 0.05 compared with the naïve group. #P < 0.05 compared with the HI-10 min group.
Fig. 4. Hypoxia-ischemia-induced less anxiolytic-like behavior, as determined by the elevated plus-maze test, 15 days (P19) after the hypoxia-ischemia treatment. The results are shown as the percentage of open arm or enclosed arm entries (open arm or enclosed arm entries divided by the sum of open arm and enclosed arm entries) and the percentage of time spent in open arms or enclosed arms (time spent in open arms or enclosed arms divided by the sum of time spent in either arm). Data are expressed as the mean ± S.E.M. of nine animals in each group. *P < 0.05 compared with the naive group. #P < 0.05 compared with the HI-10 min group.

As we reported previously [19], HI insult caused white matter rarefaction or severe necrosis at the corpus callosum area (data not shown) in 1 out of 6 rat brains from the HI-10 min group and 4 out of 6 P21 rat brains from the HI-15 min group. Although the injury was largely in the white matter, necrotic neuronal cells in the cortex and compressed hippocampus were also observed of the severely injured HI-15 min rat brain. HI treatment significantly decreased the thickness of the corpus callosum at the bregma level (the mean thickness (μm): naïve, 239.7 ± 6.9; sham, 242.6 ± 10.0; HI-10 min, 192.8 ± 7.4; HI-15 min, 108.3 ± 9.3 *; #P < 0.05 compared with the naive group; *P < 0.05 compared with the HI-10 min group).

3.3. Hypoxia-ischemia induced mature oligodendrocyte loss in the rat brain

Mature OLs were identified with APC which immunostains only the cell body of OLs. APC positive staining was primarily observed in the corpus callosum and the subcortical white matter tract at the bregma level and in the internal capsule areas (Fig. 5). In the naïve and the sham P21 rat brain, APC positive cells at the bregma level were 1465.3 ± 62.6 and 1476.0 ± 79.0 cells/mm², respectively (Fig. 5A and B). The APC positive cells in the HI-10 min group were reduced (HI-10 min, 1228.0 ± 57.7) (Fig. 5C). HI-15 min treatment further significantly reduced the number of APC positive cells (962.7 ± 74.9 *; #P < 0.05 compared with the naive group; *P < 0.05 compared with the HI-10 min group) in the P21 rat brain (Fig. 5D).

3.4. Hypoxia-ischemia induced hypomyelination in the rat brain

MBP positive staining was clearly detected in the P21 sham rat brain. MBP positive staining was primarily observed

Fig. 5. Representative photomicrographs of APC staining at the bregma level of the P21 rat brain. Hypoxia-ischemia treatments were performed as described in the method. (A and B) APC positive staining was detected in the P21 naïve and sham rat brains, respectively. (C and D) the HI treatment resulted in loss of APC positive staining in the P21 HI-10 min and HI-15 min rat brain. Scale bar is shown in (A).
in the corpus callosum and the subcortical white matter tract at the bregma level and in the internal capsule areas. HI resulted in severe impairment of MBP staining at the subcortical white matter tract (Fig. 6). In four out of six HI-15 min rat brain, MBP staining in the subcortical white matter under the forelimb area of the cortex was completely lost (Fig. 6D). The mean thickness of the subcortical white matter MBP staining under the forelimb area of the cortex was measured for comparison. The HI-10 min and HI-15 min treatments significantly reduced the thickness of the MBP positive staining (138.3 ± 3.0 and 84.4 ± 13.8 μm) compared with the naive group (176.0 ± 3.9 μm) and the sham group (182.0 ± 4.9 μm) (P < 0.05).

3.5. Hypoxia-ischemia induced tyrosine hydroxylase neuron loss in the rat brain

Positive staining of TH was used to detect dopamine neurons in the SN. All TH positive cell in the mid-brain sections at a level 1/3 rostral from the lambda to the bregma were counted bilaterally in three sections for each animal. In the naive and the sham P21 rat brain, TH positive cells were more predominant in the lateral regions of the SN (61.3 ± 2.9 cells/mm², 63.9 ± 1.3 cells/mm²) (Fig. 7A and B). HI significantly reduced the number of TH positive cells (HI-10 min, 39.4 ± 1.0*; HI-15 min, 22.0 ± 2.4* cells/mm², *P < 0.05 compared with the naive group; *P < 0.05 compared with the HI-10 min group) in the P21 rat brain (Fig. 7C and D).

3.6. Hypoxia-ischemia induced axonal and dendritic damages in the rat brain

While neurons in the cerebral cortex, hippocampus and cerebellum had very weak immunoreactivity for MAP1, neuronal axons and dendrites were detected by strong MAP1 immunostaining. In the naive and the sham P21 rat brain, the length of axons in the hippocampal CA1 region neurons was around 40–70 μm (Fig. 8A and B). As shown in Fig. 8C and D, the HI-10 min and HI-15 min treatments induced damage to axons and dendrites in the hippocampal neurons, as indicated by the shorter length of the MAP1 immunostained axons (around 10 and 30 μm) as compared with the naive group and the sham group.

4. Discussion

The major finding of the present study is that neonatal HI results in not only persistent white matter as well as neuronal and axonal injury, but also impaired neurobehavioral performance in juvenile rats. Both the brain injury and impairment of behavioral performance were dependent on the duration of the hypoxic exposure.
Fig. 7. Representative photomicrographs of TH staining in the substantia nigra of P21 rat brain. Hypoxia-ischemia treatments were performed as described in the method. (A and B) TH positive staining was detected in the P21 naive and sham rat brains, respectively. (C and D) HI treatment resulted in loss of TH positive staining in the P21 HI-10 min and HI-15 min rat brain. Scale bar is shown in (A).

Fig. 8. Representative photomicrographs of MAP1 staining in the hippocampal CA1 region of P21 rat brain. Hypoxia-ischemia treatments were performed as described in the method. (A and B) MAP1 positive staining was detected in the P21 naive and sham rat brains, respectively. (C and D) HI treatment resulted in axonal and dendritic impairment in the P21 HI-10 min and HI-15 min rat brain. Scale bar is shown in (C).
Results form the present study show that HI insult retards somatic development as indicated by the lower body weight and delayed eye opening in the HI rats. Similar results have been reported in other studies for lower daily weight gain [6,20] and delayed eye opening following neonatal HI in the rat [20,24]. Obta et al. [24] reported that BCAO caused reduction in cerebral blood flow and resulted in shrinkage of the optic nerves in rats. Delayed eye opening has been reported to accompany with delayed brain development [27]. On the other hand, early eyelid opening has been found to promote the development of perforant path synaptic strength in the hippocampus of juvenile rats [10]. These findings suggest that visual input may act as an extrinsic factor that drives hippocampal development and the emergence of hippocampal-dependent behavior. The present study shows that HI insult in the neonatal rats delays the appearance of some reflexes, such as righting reflex and cliff avoidance, in the assessment of sensorimotor deficiencies in early postnatal days in rats. Ten et al. [34] reported that such HI-induced deficits in sensorimotor activity are likely caused by cortical damage in the brain. HI insult also resulted in short-term deficits in motor coordination and locomotion tasks, such as wire hanging maneuver and locomotor activity, respectively. These short-term sensorimotor and locomotor deficiencies are HI condition-related and age-related changes in the rat and these impairments appeared to be recoverable. Since performance on some neurobehavioral tests, such as righting and wing hanging may also reflect strength of the rat pup in addition to neurologic integrity, the lower body weight in the HI group may also contribute to the poor outcomes in these tests. Further studies are needed to clearly distinguish the contribution of neurologic factors versus growth on the behavioral outcome measurement.

The long-term deficiencies caused by HI insult were also observed in neuromuscular and locomotor development, anxiety response and learning and memory performance, as demonstrated in the gait analysis, the elevated plus-maze and passive avoidance tests, respectively. The passive avoidance test gives information about learning and memory capabilities as well as the learned inhibition of a natural response. Cerebral injury generally induces a reduction in inhibition [12]. In the current study, there was no significant difference in locomotor activity among groups from P19 to P21 (Fig. 3A). Therefore, the increased electric shocks required for avoidance acquisition is unlikely due to the difference in locomotor activity. HI also induced spatial memory impairments in the rat, as indicated by the decreased retention latency to step down from the board the next day, as compared to the naive group. The HI-induced cognitive dysfunction has also been reported in other studies [14,33,34]. In the present elevated plus-maze test, a higher proportion of open arm entries and the time spent in the open arms was observed in the HI groups as compared with the naive group at P19. This is indicative of an effect independent of possible actions due to altered locomotor activity [1]. These results also indicate that HI may result in less anxious-like behavior. Disturbances of the hypothalamic–pituitary–adrenal (HPA) axis has been linked with alterations in anxiety responses in the elevated plus-maze test [18,22]. Whether these alterations observed in the current study are linked with disturbances of the HPA axis needs further investigation.

In the open field test, all groups showed an increase in crossing distance from P3 to P15 and then a decrease (Fig. 3). One possible reason for the significantly decreased activity of all animals in a novel environment after P15 may be the result of eye opening [12,24,38]. In the present study, however, the mean eye opening day varied among groups, while the peak activity appeared on the same day in all groups. This is an indication that besides eye opening, other mechanisms may also be involved in performance in the open field test. Development of locomotor activity in the rat has also been correlated with the maturation of neurotransmitter systems mediating inhibition and excitation [12]. Shen et al. [32] reported that the hippocampal injury caused hyperactivity in an open field test. In the present study, the hippocampal injury in addition to the white matter injury was also observed in some H&E stained brain sections from the HI-15 min group. The MAP1 immunostaining also indicated that HI-10 min and HI-15 min treatments induced damages in axons and dendrites of the hippocampal neurons. These observations may partially explain the hyperactivity found in the HI-15 min group. Furthermore, the nigrostriatal dopaminergic system plays a major role in motor control [7]. An increased locomotor activity in the rats following intranigral injection of lipopolysaccharide has been linked with the decreased dopamine level in the injured SN and the striatum [13]. Long-term prenatal hypoxia (10% O2) from the 5th to 20th day of gestation in the rat has been indicated to reduce tyrosine hydroxylase activity in the motor cortex and hippocampus, but not in striatum of offspring at P21 [26]. On et al. [25] and Burke et al. [7] indicated that neonatal HI (unilateral carotid ligation followed by 3–4 h exposure to 8% O2) on P7 rats resulted in a decrease in TH positive neurons in the SN in neonatals (P9) and adult rats (P56–P84), respectively. Data from the present results show that both the reduction in the number of TH positive neurons in the SN and the extent of hyperactivity in the juvenile rat (P21) following HI were hypoxia duration-dependent. Therefore, the altered TH activity in the SN of the juvenile rat in the current study may be part of the neural mechanisms contributing to the motor behavioral impairments induced by neonatal HI. In addition, the increased locomotor activity in the HI groups indicates that these animals may have a delay of accommodation to this experience with repeated exposure to the field. This might reflect impaired memory and/or alteration of dopaminergic system. The disappearance of hyperactivity in the HI groups after P18 indicates factors other than the dopaminergic system are also involved in the regulation of locomotor activity in the rat, but the altered dopaminergic system may still have effects on the locomotor behavior. One good example for such a scenario is that apomorphine, a dopaminergic drug, induced locomotor behavioral differences between the normal and the...
HI animals in P21 rats, even though HI-induced locomotor hyperactivity was not observed on P21 [16]. Although results from the present study are not sufficient to link the specific neurobehavioral alteration with injuries in specific brain regions, our data demonstrate that the altered neurobehaviors might be associated with both white matter injury and neuronal and axonal injury. Our results also indicate that both the extent of brain injury and the extent of impairment in neurobehavioral performance induced by HI in the rat were dependent on the HI condition, i.e., the duration of hypoxic exposure. It is likely that for a pharmacological treatment of HI-induced brain injury, it is important to assess not only the histopathological evidence, but also the improvement of neurobehavioral performance.

Acknowledgement

This work is supported by HD 35496.

References


