ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Derleme *Review* DOI: 10.5281/zenodo.10894221

Examining the Effects of Oxygen Exposure on the Developing Brain Through Murine Models

Yenidoğan Rodent Modellerinde Hiperoksik Beyin Hasarının Değerlendirilmesi

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ABSTRACT

Hyperoxia is one of the key players contributing preterm brain injury. Researchers typically use rodent models to pinpoint the underlying pathologic alterations in hyperoxic brain damage. When evaluating the neurological effects of neonatal hyperoxic brain injury in an experimental model, choosing the appropriate assessment techniques is crucial. The goal of this article is to review the behavioral and learning tests that can be used to determine the impact of hyperoxia on the developing brain. Injuries to the nervous system can be recovered very quickly in newborn rodents. Thus, the timing of evaluation tests are very critical. A model that is appropriate for the brain's developmental processes and accurately simulates the damage in humans should be utilized in studies on neonatal hyperoxic brain injury, and the right test should be chosen at the appropriate time. In the first twenty days, physical and motor development tests, and subsequent evaluation of damaged brain structures are relevant. The open field and forced swim tests can be used to assess the animal's locomotor activity and depressive condition, while the watermaze, passive avoidance and new object recognition tests can be used to assess cognitive abilities. In laboratory mice and rats, physical development and motor reflex development tests can be started right after birth, while learning and memory tests can be done from 4 weeks at the earliest. Correlations between motor development, behavior, memory tests, and results of cellular/ molecular studies should be made and interpreted carefully.

Keywords: Brain injury, behavioral test, hyperoxia model, motor development tests, newborn rodent

ÖZ

Hiperoksi, preterm beyin hasarına katkıda bulunan önemli postnatal faktörlerden biridir. Hiperoksinin neden preterm beyin dokusunda yol açtığı olduğu patolojik süreçlerin aydınlatılabilmesi için deneysel kemirgen modelleri sıklıkla kullanılmaktadır. Bu derleme, yenidoğan hiperoksik beyin hasarının değerlendirmesinde, araştırmacıların davranış ve öğrenme testleri ile ilgili seçimlerine ışık tutmayı hedeflemektedir. Yenidoğan kemirgen modellerinde, hayvanların nörolojik hasarlarından hızla iyileşme konusunda yüksek yeteneğe sahip olduğu göz ardı edilmemeli ve değerlendirme testlerinin yapılma zamanı iyi belirlenmelidir. Beynin gelişimsel süreçlerine uygun, insanlardaki hasarı daha iyi yansıtacak hayvan modeli kullanılmalı, doğru değerlendirme testi seçilmeli ve seçilen testler doğru zamanda uygulanmalıdır. Yaşamın ilk yirmi gününde fiziksel ve motor gelişim testleri kullanılmalı, daha sonraki süreçte beyin olgunlaşmasına paralel olarak davranış ve bellek testleri ile değerlendirilme yapılmalıdır. Lokomotor aktivite ve depresyon varlığı açısından açık alan testi, bilişsel işlevlerin değerlendirilmesi için yeni obje tanıma, su labirenti ve pasif kaçınma testleri seçilebilir. Motor gelişim, davranış ve bellek testleri, hücresel ve moleküler değişiklikler ile korele edilerek yorumlanmalıdır.

Anahtar Kelimeler: Beyin hasarı, davranış testleri, hiperoksi modeli, motor gelişim testleri, yenidoğan rodent.

Geliş Tarihi/Received 24.04.2023
Kabul Tarihi/Accepted 17.01.2024
Yayın Tarihi/Publication Date 29.03.2024

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Cite this article: Yılmaz C., Engür D., Kumral A., & Yılmaz O. (2024). Examining the Effects of Oxygen Exposure on the Developing Brain Through Murine Models. *Journal of Laboratory Animal Science and Practices*, *4*(1), 15-25.

Introduction

Every year, around 15 million babies are delivered prematurely in the world (Chawanpaiboon et al., 2019). Although survival rates have improved significantly through scientific and technologic advances in perinatology and neonatology, these infants remain vulnerable to many complications in their future life due to immature organ systems at birth. One of the major organs that is adversely affected by preterm birth is the brain. Oxidative stress, arising from exposure to high oxygen levels (hyperoxia) is suggested to be one of the main contributing mechanisms causing brain injury in preterm babies (Reich et al., 2016). When compared to intrauterine life, preterm newborns are exposed to high oxygen levels after delivery, and the majority of them need supplementary oxygen for respiratory support. This situation results in the production of reactive oxygen species (ROS), which interferes with the critical processes in the developing brain(Saugstad et al. 2021). Although target saturation limits have been established and oxygen saturation is routinely monitored in intensive care units by pulse oximetry, premature newborns are still inevitably exposed to high levels of oxygen (Falsaperla et al., 2022). Despite the awareness of the possible detrimental effects of hyperoxia has grown dramatically over the past few decades, studies have demonstrated that preterm newborns frequently receive extra oxygen especially after hypoxic episodes brought on by apnea or bradycardia (van Zanten et al., 2014). Additionally, lung mechanics might vary, making it challenging to maintain a constant, stable target saturation range. ROS can be produced in excess even during brief periods of hyperoxia, which can cause oxidative stress and inflammation(Farrow et al., 2012). Furthermore, preterm newborns have an undeveloped antioxidant defense mechanism, making them particularly susceptible to hyperoxia. Both antioxidant enzymes and non-enzymatic antioxidant components have been reported to be lower in preterm infants than in term children (Perrone et al., 2017). Overall, this renders preterm newborns fundamentally unable to counterbalance a hyperoxic burden, leading to increased ROS generation that will surpass antioxidant capacity and might result in cellular damage. ROS can alter neural stem cell differentiation and interfere with developmental processes in the preterm brain (Micili et al.,2020). The most recent recommendations based on the available research suggest oxygen saturation objectives of 90-94% for optimum oxygenation of preterm infants during their stay in neonatal intensive care and designate levels above 95% as hyperoxia, which is linked to higher rates of mortality and morbidity (Andresen & Saugstad, 2020). In the study, when the effects of hyperoxia on gender were compared, it was shown that female infants were less harmed than male infants by the harmful effects of high oxygen concentration (Deulofeut et al. 2007).

Murine Models of Neonatal Oxygen Exposure

The newborn mouse and rat models are extensively used as experimental models for hyperoxic brain injury in the literature. In this review, the terms "rodent" and "sub-sublines to these two species" refer to the species of mouse and rat, respectively. For experimental models of hyperoxia, the developmental stage of the brain at birth in mouse and rat pups provides a substantial advantage in mimicking the preterm human brain.

The formation of the neural tube, which is the first stage in the development of the central nervous system in all vertebrates, is where the spinal cord and brain emerge from the ectoderm. Compared to human gestation, which lasts 266–280 days, mouse and rat gestation lasts 20–21 days. Neural tube formation occurs at 10-11th gestational days in rats and 9-9.5 th days in mice (DeSesso et al., 1999; Rice & Barone, 2000). When comparing the cerebral development of experimental animals and humans, it is important to take into account the timing of behaviors associated with growth that overlap with neurogenesis, synaptogenesis, gliogenesis, oligodendrocyte maturation, and developmentally regulated molecular and biochemical alterations. Rodents and humans both go through important stages of brain development in a roughly comparable order. Regional sensitivity and functional outcomes following brain lesions also clearly show similarities (Semple et al., 2013).

Mammalian brain development is a dynamic process that includes structural and functional maturation processes. In brain development, many processes such as neuronal cell proliferation and differentiation, migration, glial cell proliferation, axonal and dendritic growth, synaptogenesis and myelination of axons occur sequentially or simultaneously (Stiles & Jernigan, 2010). On the developing rodent brain, hyperoxia stimulates cellular degeneration, causes hypomyelination, and results in long-term cognitive damage. It also causes gene alterations linked to oxidative stress, inflammation, neurodegeneration, apoptosis, autophagy, and synaptic plasticity (Brehmer et al., 2012; Gerstner et al., 2008; Serdar et al., 2016). In recent studies, it has been associated with hyperactivity and coordination deficits and cognitive

deficits. The brain areas affected are the cortex, basal ganglia, hypothalamus, striatum, hippocampus, and white matter structures.

Treatment of immature rats with high doses of oxygen from birth in the first five days of life results in a significant increase in apoptotic cells and a decrease in brain weight. Oxygen sensitivity varies depending on age. Resistance to the harmful effects of hyperoxia has been observed in rodents starting from postnatal PN14 (Felderhoff-Mueser et al., 2004). There are differences in oxygen exposure time and oxygen concentration practices in rodent neonatal hyperoxic brain injury model protocols. In studies reported in the literature, oxygen concentrations vary between 40%, 70% and 95%, and the induction period varies between 2-7 days. Since the first seven days after normal birth in rodents correspond to the immature lungs of premature babies in humans, hyperoxia administration should be completed during this period (Giusto et al. 2021). There is also gender dimension of oxygen exposure and ROS susceptibility. Aside from evident morphological and physiological variations between the male and female brain, recent evidence suggests sex dependant alterations in fundamental cellular and molecular process including apoptosis and cell death. Sexual dimorphism has also been shown in cytosolic and mitochondrial pathways. Differences in mitochondrial related pathways in male and female cells may explain the differing susceptibility to mitochondrial dysfunction following oxygen exposure, with female cells displaying stronger resilience to insults. Researchers should carefully evaluate and analyse their results with regards to the sex of the animals (Di Florio et al., 2020).

A closed cabin system with an oxygen supplying intake is employed for rodent hyperoxia applications, where the oxygen content is continuously measured (Figure 1). The pups are placed in this cabin with their mothers in their own cages.

Sodaline is used to keep the humidity that will occur during hyperoxia treatments below 80%, and the pellet feeds are changed once in 24 hours to prevent moisture (Kwak et al., 2006).

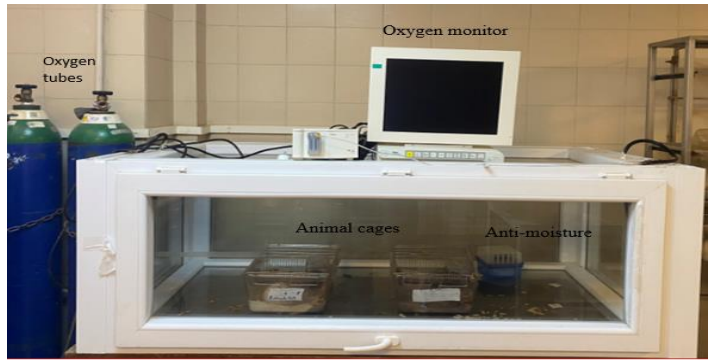

Figure 1. Mouse and Rat Neonatal Hyperoxia Cabinet

Şekil 1. Fare ve Sıçan Yenidoğan Hiperoksi Kabini

Evaluation in Hyperoxic Brain Injury Models

Following oxygen exposure, neonatal rodents suffer global damage to their developing brains. Because of this, it would be more suitable to conduct an early functional examination while taking the animal's physical growth into account. Using motor development tests during the first 20 days and behavioral and learning memory tests later on, in accordance with the maturation of the brain, may be a more accurate strategy (Eltokhi et al., 2020). In the rodent neonatal hyperoxia model, damage to the brain tissue occurs globally. For this reason, it would be more appropriate to carry out early functional evaluation by considering the physical development of the offspring. For the evaluation of the rodent hyperoxic brain injury model, it may be a more accurate approach to use motor developmental tests in the first twenty days and then behavioral and learning memory tests in accordance with brain maturation in the following period (Figure 2) (Eltokhi et al., 2020).

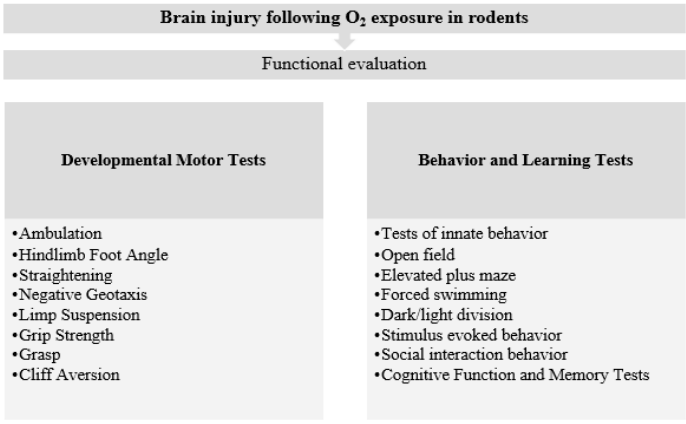

Figure 2. Functional Evaluation of Brain Damage Caused by High Oxygen Exposure in Neonatal Rodents

Şekil 2. Yenidoğan Kemirgenlerde Yüksek Oksijen Maruziyeti sonucu Oluşan Beyin Hasarını Fonksiyonel Değerlendirilmesi

A- Evaluation of Physical Development

The gestation period for mice and rats is 20–24 days, and they have multiple births per pregnancy. Newborn mice and rat pups typically weigh between 1-5 g. Mouse and rat pups are born toothless, hairless, and with their eyes and ears closed. Their movement abilities are limited. Maternal care in mice and rats includes nest preparation, cleaning the young and frequent breastfeeding sessions. In addition to breastfeeding, licking and cleaning the baby by the mother is a crucial part of the maternal care, which is considered as an immature form of parental care in humans. This maternal care affects physical development, growth and brain development in the offsprings (Cameron et al., 2008). There are 5–6 pairs of nipples in mice and rats, and they are located anatomically in the thoraco-inguinal region. Pups suckle 50–80 times daily and remain attached to the udder for a total of 18 hours each day (Heyser, 2003). In the newborn period, the hormonal and thermoregulatory balances of the offspring are mainly provided by the mother (Champagne, 2009). The time of the anterior teeth's eruption, the separation of the auricle from the scalp, and the opening of the eyes should all be monitored as part of a newborn rodent's physical development. Figure 3 shows the postnatal (PN) physical development, motor reflex development, and behavioral assessments of mice and rat pups. Although the procedures for testing behavioral and learning abilities as well as the development of motor reflexes in mice and rats are comparable, the dimensions of the test platforms utilized vary due to the different body sizes. The dimensions of the mouse platforms are therefore less than those of the rat platforms.

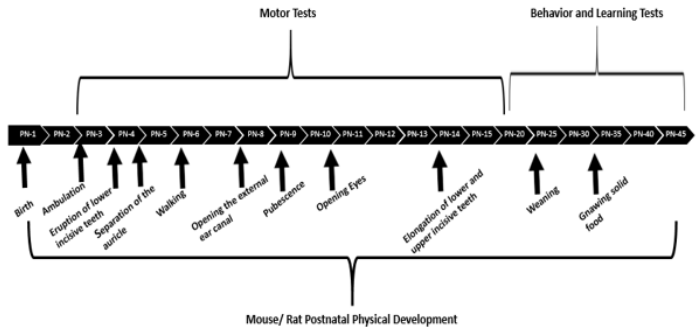

Figure 3. Evaluation of neonatal hyperoxic brain İnjury over time in rodents. (PN: Post Natal)

Şekil 3. Kemirgenlerde neonatal hiperoksik beyin hasarının zaman içinde değerlendirilmesi. (PN: Doğum Sonrası)

B. Developmental Motor Assessment Tests

Higher cortical networks that go through neuronal migration, myelination, and synaptogenesis as the brain matures are necessary for the development of motor skills and neurodevelopmental reflexes. Damage to the central nervous system impairs brain development and causes aberrant cortical connections, lack of function, and myelination, which delay or prevent neurodevelopmental reflexes. (Farber et al., 1985; Zafeiriou, 2004). Therefore, reflex delays in experimental models of neurodevelopmental disorders should be carefully assessed. Because cortical development happens after birth and rodent pups' brains are not yet developed enough to handle sophisticated motor, sensory, and cognitive tasks when they are born, functional evaluation in the neonatal rodent brain injury model is meaningless. In newborn rat pups, PN1 corresponds to a stage of brain development comparable to that of a preterm human infant aged 23–28 weeks. However, it has been suggested that mouse and rat pups, at PN 7–10, have brain maturation similar to that of term human newborns (Clancy et al., 2007; Dobbing & Sands, 1973, 1979; Semple et al., 2013). This correlation was defined according to anatomical and histological analyzes as well as electroencephalography(EEG) findings (Dean et al., 2011; Tucker et al., 2009). Pre-oligodendrocytes are the predominant cells in the fetal brain, developing between 23-32 weeks in humans, and this maturation stage corresponds to PN 1-3 in rodents (Back et al., 2001, 2007; Dean et al., 2011; Semple et al., 2013). In rats, myelination begins in utero life and the level

of myelination at PN10 is similar to that of human term infants (Tucker et al.). According to EEG data, PN1 in rats is equivalent to 23 weeks of gestation in humans, while PN7–10 is equivalent to 30-32 weeks. These factors make neonatal reflex tests on newborn rat pups suitable for evaluating brain development, ontogeny, and injury (Tucker et al., 2009).

Developmental motor tests in rodents were described for the first time in the literature by WM Fox and A. Lubics (Fox, 1965; Lubics et al., 2005). Newborn motor development tests are ambulation, hindlimb foot angle, stance correction, negative geotaxis, anterior and posterior limb suspension, grasp reflex, four-limb grip strength, and cliff avoidance tests. These tests are used to monitor the motor development of mouse and rat pups through PN2-14 (Feather-Schussler & Ferguson, 2016). These tests, which are used in the evaluation of neonatal brain damage, can also be used in the evaluation of functional deficiency in brain regions that are responsible for learning-memory-motor-sensory functions such as the hippocampus, amygdala, and cortex (sensory-motor) affected by hyperoxia. The motor reflex development times of mouse and rat pups are given in Figure 4.

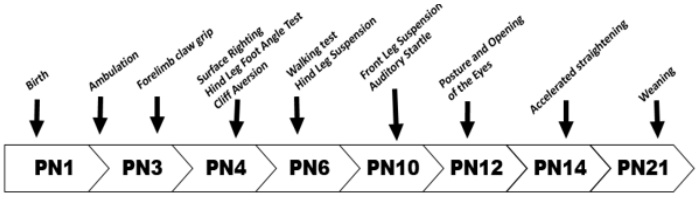

Figure 4. Time periods for the evaluation of neonatal motor function in mice and rats

Şekil 4. Fare ve Sıçan Yenidoğan Yavrularında Motor Fonksiyonunun Değerlendirilme Zamanları

1. **Ambulation Test:** In rodent pups with closed eyes, ambulation is the first form of locomotion that emerges. Since hyperoxia causes brain hypomyelination and cell death, its effect on crawling can be tested. Rodent pups start to crawl between PN 0-5 and walk between PN 5-10. Since there is no learning potential, this test can be repeated as needed throughout the experiment. During the test, the pup is placed in an open space where it can be seen from the sides and from above, and is motivated by gently touching the tail (Figure 5). Ambulation of puppies is scored for three minutes. In terms of scoring, 0 corresponds to no movement, 1 to asymmetrical limb ambulation, 2 to slow symmetrical ambulation, 3 to fast ambulation/walking (Balasubramaniam et al., 2005; Williams & Scott, 1954).

Figure 5. Ambulation

Şekil 5. Ambulasyon

2. **Hind Leg Foot Angle Test:** Placing the hind legs properly on the ground is important for walking function in newborn rodents. With this test, gait abnormalities are tested. As pups transition from crawling to walking, there is a marked developmental change in hind leg posture (Figure 6). In normal development, the hind legs are positioned under the body and the angle between the hind legs is less. Although the hind leg foot angle changes over time, mouse pups of the same age with different injuries or diseases can be compared. This test has no learning potential, so it can be repeated as needed throughout the experiment. Test duration is two minutes. Three to five measurements are made and the average angle is calculated for each pup tested (Feather-Schussler & Ferguson, 2016).

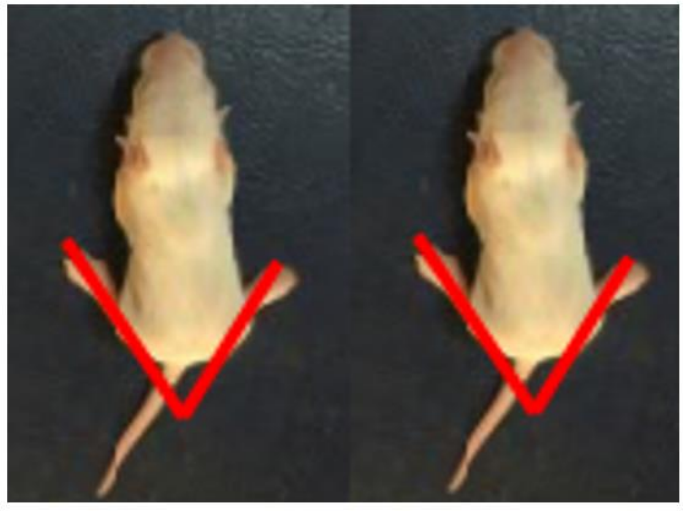

Figure 6. Hind leg angle test

Şekil 6. Arka ayak açısı testi

3. Surface Righting: Being able to stand up straight after turning from the supine posture and moving into the standard stance position is a crucial skill in the motor development of a pup. The righting reflex normally appears in rodents between PN1 and 10. It begins to form about PN5. Since this test is a reflex, there is no learning component and can be repeated throughout the experiment. Pups are placed on their backs on a cotton cloth and held in this position for five seconds (Figure 7). The time from pup release to emergence is recorded. A total of one minute is given for each trial. A total of three repetitions can be made.

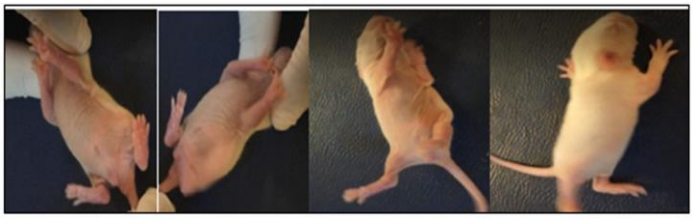

Figure 7. Surface righting

Şekil 7. Yüzey düzeltme

4.Negative Geotaxis Test: In newborn rodents, vestibular functions allow movement on an inclined surface in the opposite direction of the slope against gravity. When puppies are placed upside down, they turn up thanks to their vestibular tips. This test, which evaluates motor coordination, can be done between PN3-15 (Figure 8). During practice, the pup is placed head down on a 45-degree incline and held for five seconds and then released. The elapsed time is recorded when the pup faces upwards. Total test time is two minutes. A total of three trials are repeated. Mice that fall off a slope or fail to turn may be retested, eliminated or given a zero score (Heyser, 2003).

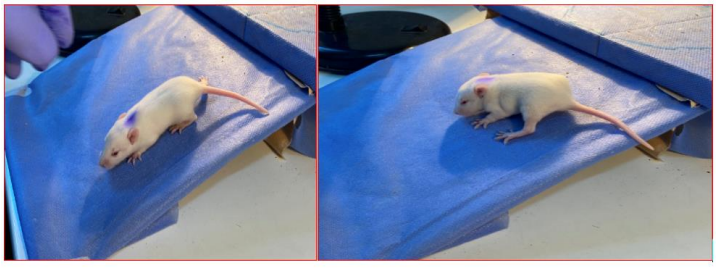

Figure 8. Negative geotaxis

Şekil 8. Negatif geotaxis

5. Front Limb Suspension: Front legs in newborn rodents are important in terms of functions such as grasping, holding and shredding. Testing foreleg strength, including arm and paw strength, this test is not recommended for puppies smaller than PN10 (Corti et al., 2008; Grondard et al., 2005). Puppies are allowed to hold a wire stretched to a fixed object and hook it with both front paws (Figure 9). There is no learning and negative reinforcement in this test, which allows to detect right/left paw strength differences. It can be repeated three times in total.

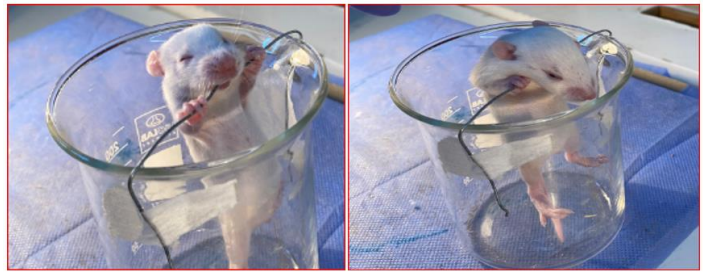

Figure 9. Front Limb Suspension

Şekil 9. Ön uzuv süspansiyonu

6. Hindlimb Suspension: In newborn rodents, hind legs constitute the main thrust of the movement and are the main factor for walking. This test, which determines hind leg strength, is especially designed for newborns. This test, which can be applied between PN2-12, can detect right/left hind leg strength differences as well as neuromuscular function. For this test, a standard cylindrical tube with a volume of 50 ml filled with cotton is used. Similar to the front leg suspension test, this test can be learned, especially since there is no negative result from a fall (Figure 10). Therefore, increased non-joining, as seen with litters that drop as soon as they are released, or non-adherence when placed at the rim of the tube can be noted. The cub is released, the hind leg posture is observed, and the pup's posture is scored. The test is repeated three times (El-Khodor et al. 2008).

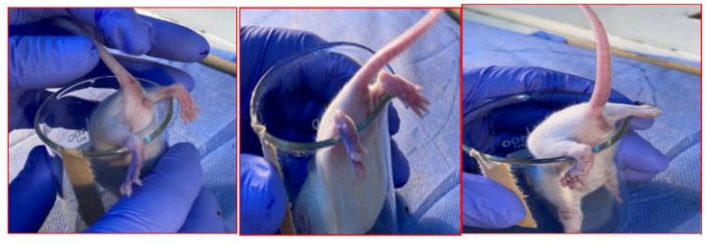

Figure 10. Rear leg Suspension

Şekil 10. Arka bacak süspansiyonu

7. Grip Strength: It is a test for grasping the front and hind legs together and carrying the weight of the whole body in newborn rodents and examines the strength of all four

paws at the same time (Figure 11). For the test, 16x18 cm fiberglass mesh is used. For a rodent to grasp a horizontal screen, it is made between PN5–15. The test starts from the standard horizontal position and first changes to 45°, 90° and 180° degrees. The pup is allowed to adapt to this environment for about five seconds. The wire platform is slowly turned to 180 degrees. The approximate angle of the screen is recorded when the pup falls. It is repeated for a total of three trials and the average of the trials is taken (Corti et al., 2008; Fox, 1965; Heyser, 2003; Venerosi et al., 2009).

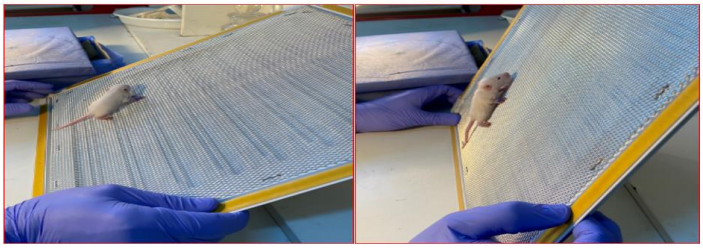

Figure 11. Grip Strength

Şekil 11. Kavrama gücü

8. Grasping Reflex: The front and hind claws of newborn rodents are tested one by one for grasping, holding, climbing and holding functions. The grasping reflex is usually performed between PN3-15, on the 7th day after birth on average (Figure 12). Each paw is tested individually. Since it is a reflex, this test can be repeated until the reflex appears. He is not inclined to learn. Each paw is tested separately. The presence or absence of coupling is recorded. 1 point is awarded for each paw grasped by the pup (Heyser, 2003).

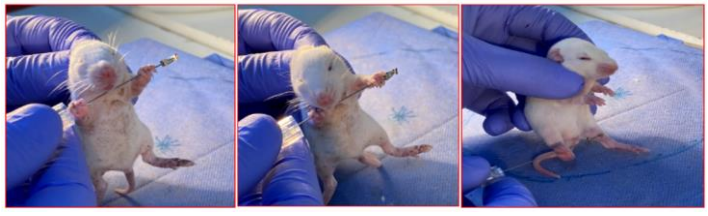

Figure 12. Grasping Reflex

Şekil 12. Kavrama refleksi

9. Cliff Aversion: It is an important test that develops in newborn rodents while their eyes are closed (Figure 13). It can be used to test pups between PN1-14. The pup is placed on the flat raised ledge with the front paw and nose pointing out of the cliff. The pup is expected to move away from the cliff and remove its paws and nose from the edge. The test is repeated for a total of three trials and is terminated if no response is received after a total of 30 seconds (Hill et al. 2008).

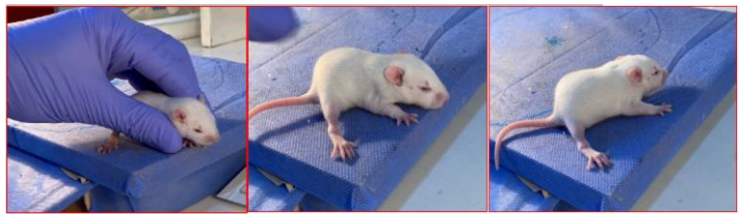

Figure 13. Cliff aversion test

Şekil 13. Uçurumdan kaçınma testi

C- Rodent Behavior Tests

Behavior can be defined as the whole of the organism's responses to stimuli. In humans, great changes and maturation in synaptic connections and neural networks are observed in parallel with physical development from infancy to adolescence. The 20-day period of rodents provides a rapid brain maturation as well as their physical development. This brain development provides the opportunity to measure and evaluate innate and acquired behaviors. Although the timing of these behavioral and learning-memory assessment tests varies, the approximate assessment time is given starting from the twentieth day after birth (Figure 14). Considering the brain regions affected by hyperoxic brain damage, behavioral, learning and memory tests can be preferred. The age of the animals should also be considered in test preferences.

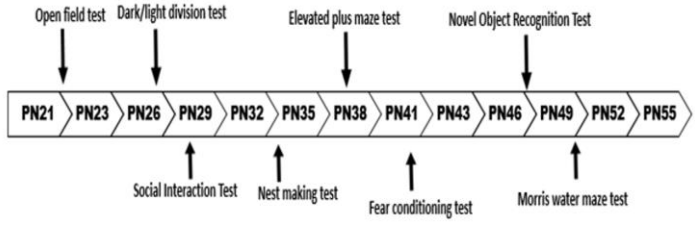

Figure 14. Earliest times to perform learning behavior tests in rodents

Şekil 14. Kemirgenlerde Öğrenme Davranış Testlerin En Erken Yapılma Zamanları

1. Innate Behaviors: Innate behavior tests are a measure the functional integrity of the hippocampus and assess actions such as eating, drinking, and self-care in rodents. The nesting test, instinctively evaluates the nesting behavior of the animal, can be done at PN35-40 days during adolescence. Likewise burrowing which is an innately

important behavior and can be evaluated at PN30-40 days (Eltokhi et al. 2020).

2. Open field test: It is one of the most widely used tests to evaluate behavior. This brief and simple test yields information on the animal's behavior as well as information on its general locomotion and level of depression. (Seibenhener & Wooten, 2015). The foundation of the test is based on the contradiction of exploring the test space and avoiding the open space. Open field testing can be evaluated from the fourth week after birth (Eltokhi et al. 2020).

3. Elevated plus maze test: The elevated plus maze consists of four arms. Two of the arms are open and the other two are closed. Animals are placed on the middle platform of the maze facing the open arm. The total number of entries into the open and closed arms and the total time spent in each arm type are measured. This test can be done at nine weeks after birth (Eltokhi et al. 2020; Hager & Dringenberg, 2010).

4. The forced swim test: This test is one of the most commonly utilized tests to study depression-like behavior in rodents. This test can be done from 6-8 weeks after birth. It is based on the assumption that when animals are placed in a bowl of water, they will first try to escape, but will eventually display inactivity as a measure of behavioral despair (Eltokhi et al. 2020).

5. Evoked Behavior Test: Fear conditioning is tests in which animals learn to associate with stimuli that they do not hear, such as a sound stimulus, electric shock (conditioned stimulus). This test can be done from 7-8 weeks after birth.It has been found that the hippocampus is involved in learning and memory in fear conditioning (Rogan et al. 1997) and the amygdala in sensory fear conditioning is involved in learning (Hager & Dringenberg, 2010; Wiltgen et al. 2006).

6. Passive Avoidance Test: Passive avoidance is a fear-guided test used to evaluate negative reinforcement-based long-term memory in laboratory animals (rat, mice). This test can be done from 7-8 weeks after birth. (Eltokhi et al. 2020).

7. Social Interaction Behavior Test: Social interaction testing can be done at 6-8 weeks after birth. It is done as described by Bandstra et al for social interaction testing. The social interaction test is a learning and memory test that evaluates the social interaction between pairs in normal room lighting designed in accordance with mouse and rat sizes. Each rat can be randomly assigned to partners with animals from different groups. Each pair stays in the arena for 10 minutes. In a couple's social interaction, sniffing and grooming, crawling under or over the partner, chasing, climbing and crawling are recorded (Bandstra et al. 2010).

8. Cognitive Function and Memory Test: Different tests are performed to measure learning ability and memory function during brain maturation period in rodents. For this purpose, evaluation is made with Morris water maze, T and Y maze, puzzle test and object recognition tests. Since learning and memory tests are related to brain development and maturation, it is more suitable to be done at 6-8 weeks.

9. Morris Water Maze: This test is another common test in small rodents. In this test, water acts as a motivator and stimulant for quick search in animals. A useful aspect of many memory tests is that they can be spread out over days or even weeks. This test facilitates comparison of pre- and post-treatment results (Lueptow, 2017).

10. T-maze and Radial Arm Labyrinths Test: This test is based on encouraging a space to learn and remember its layout with a reward (food pellet). The animal needs to find the handle with the food pellet and remember it in the next attempt. In memory deficiency, it cannot remember and results in the test animal always entering the wrong arm (Lueptow, 2017).

11. Y Maze Innovation Preference test (NPT) : The Y maze can be used to assess short-term memory. It is a behavioral test used to measure rodents' willingness to explore new environments. Many parts of the brain are involved in this task, including the hippocampus, septum, basal forebrain, and prefrontal cortex.

12. Object Recognition Test: It is a test that works on the basis of rodents' curiosity about new objects. The animal will investigate and engage with an object when it sees it for the first time. But if the same thing is shown later, it won't arouse the same attention. The greater the test's success can be interpreted, the more the subjects are drawn to the novel thing and less to the familiar object. The test is simple and can be completed in three days. It can be shortened to study short-term memory or extended to investigate long-term memory (Rogan et al. 1997).

Conclusion

In conclusion, in order to further our understanding of the mechanisms underlying the effects of supplemental oxygen on developing brains, experimental models offer significant information. Translational researchers can benefit from using the best evaluation methods to increase their knowledge of the subject. As the discipline of translational neonatology develops, extensive and standardized tests for the evaluation of newborn experimental motor function in mouse models are needed. These studies can measure the effectiveness of treatments and corroborate experimental patterns of brain disease or injury in rodents. This study seeks to provide light on the behavioral and learning tests that were used by researchers to evaluate the effects of supplementary oxygen on developing brain. When studying neonatal hyperoxic brain injury, it is important to adopt an animal model that is appropriate for the brain's developmental processes and more accurately represents human brain damage. Additionally, it is important to choose the right assesment test and apply it at the appropriate time. The use of motor development, behavior, and memory tests in the functional assessment of the impacted brain regions is crucial. Motor development, behavior, and memory tests should be evaluated in connection to cellular and molecular changes in mouse models of hyperoxic brain injury.

Yazar Katkıları: Fikir- C.Y., O.Y.; Tasarım - C.Y.,D.E., A.K.; Denetleme- O.Y.; Kaynaklar-C.Y.; Veri Toplanması ve/veya İşlemesi – C.Y.; Analiz ve/veya Yorum – C.Y, D.E., A.K.; Literatür Taraması – C.Y., O.Y.; Yazıyı Yazan – C.Y., O.Y.; Eleştirel İnceleme – O.Y.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.
Finansal Destek: Yazarlar, bu çalışma için finansal destek almadığını beyan etmiştir.

Author Contributions: Concept – C.Y.,O.Y.; Design- C.Y.,D.E., A.K ; Supervision – O.Y.; Resources- C.Y.; Data Collection and/or Processing- C.Y.; Analysis and/or Interpretation- C.Y, D.E., A.K.; Literature Search- C.Y., O.Y .; Writing Manuscript- C.Y., O.Y. ; Critical Review-O.Y.
Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.

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