

# Behavioral Effect of Oleoylethanolamide on Perinatal Asphyxia

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**Abstract:** Perinatal asphyxia (PA) is still a serious health problem associated with neuronal loss and morbidity. PA pathophysiology implies oxidative stress, cell damage and over activation of inflammatory response. The absence of an established treatment for PA encourages research on neuroprotective mechanisms. Oleoylethanolamide (OEA), a cannabinoid agonist that exerts anti-inflammatory actions through PPAR $\alpha$  activation, could be a possible target for neuroprotection. However, its role in perinatal hypoxic brain injury remains still unknown. In this study, we evaluated the behavioral consequences of OEA treatment in 30 days-old asphyctic rats. Results indicated that rats subjected to OEA administration showed an improvement in exploratory locomotion. This data suggests a possible neuroprotective role of OEA in severe perinatal asphyxia modifications.

**Keywords:** Neuroprotection, Anti-inflammatory effect, Exploratory locomotion, Perinatal asphyxia.

## 1. INTRODUCTION

Perinatal asphyxia (PA) is a neonatal complication associated with an impaired gas exchange [1]. The most common reasons for this health problem are: abruption of the placenta, compression of the umbilical cord, abnormal uterine contractions or failure of the neonate to begin breathing [2]. The incidence of severe PA is estimated at 1/1000 live birth in developed countries and 5-10/1000 live births in developing countries [3]. Cerebral palsy, mental retardation, and epilepsy are among the most common complications of PA [4-7], which continues to be a determinant of neonatal mortality and neurological morbidity [8, 9]. Although hypothermia and estradiol therapy have shown favorable results [10, 11], there is not an established treatment for PA.

Regarding the pathophysiology of PA, a decrease of oxygen saturation implies a severe energetic crisis: a shift to anaerobic metabolism takes place in order to

preserve the fundamental demands of energy. However, anaerobic metabolism is inefficient for providing enough energy for development of neuronal networks and is associated to lactate accumulation and acidosis. These functional consequences of the primary insult are exacerbated by and during the re-oxygenation period, which is necessary for survival but implies free radicals accumulation, oxidative stress, cell damage and over activation of defense mechanisms such as inflammatory cascades [12].

OEA is present in human and rat brain in considerable amounts [13-17]. This lipid amide is a PPAR $\alpha$  (alpha-type peroxisome proliferators activated receptor) agonist [13, 14, 18]. In experimental studies, OEA attenuated inflammation in wild-type mice but had no effect in mice deficient in PPAR $\alpha$ . This finding suggests that PPAR $\alpha$  mediates the anti-inflammatory action of OEA [13, 14]. In fact, the alpha-type receptor is expressed in several immune cells [19, 20] and regulates the expression of large sets of genes, thereby modulating important metabolic processes [21], such as inflammatory reactions. In addition to its anti-inflammatory properties, OEA showed cytoprotective effects both *in vitro* and *in vivo* models of substantia

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nigra dopamine neuron degeneration [22]. Besides, a substantial body of evidence has suggested that cannabinoid agonists could exert its neuroprotective effect modulating the inhibitory and excitatory neurotransmission or inducing hypothermia [23]. However, the neuroprotective properties of OEA on PA remain still unknown. Therefore, the aim of this experimental work was to elucidate this issue by analyzing the behavioral effects of OEA administration in asphyctic rats.

In the present study, we used a rat model that induces global severe PA [11, 24, 25]. The advantages of this model are 2-fold: first, it mimics the asphyxia just in the moment of delivery; second, it allows studying both the short as well as the long-term effects, since it is a non-invasive procedure [26, 27]. The most obvious and serious short-term consequence of PA observed in this model is mortality. At 37°C, an asphyctic period longer than 20 min was inevitably associated with death probably induced by glutamate overactivation and excessive free radicals release [26, 27]. Rats subjected to 20 min of asphyxia at 37°C showed chronic defects in neurotransmitters such as decrease in dopamine, aspartate and glutamate release monitored by microdialysis [28]. F-actin striatal cytoskeleton changes were observed in post-synaptic densities of asphyctic rats at postnatal day 30 P30 [29] and an increase in the nitric oxide in neostriatum and neocortex at short- and long term period was also found [30, 31]. Alterations in cytoskeletal organization were observed after 4 months of PA [11]. Recently, an increase in the ubiquitination level and neurodegeneration after 6 months of PA in neostriatum was also registered in our laboratory [25]. Finally, behavioral deficit in exploration was observed after 3 months of PA [32]. The present study was focused on earlier behavioral effects of PA and its possible recovery through OEA treatment. To accomplish this purpose, exploratory locomotion and anxiety levels were measured in the Elevated Plus Maze test at postnatal day 30 (P30).

## 2. MATERIALS AND METHODS

### 2.1. Animals

Subjects consisted of 8 pregnant *Sprague Dawley* rats obtained from the School of Veterinary Sciences' central *vivarium* at the Universidad de Buenos Aires.

The sample (N=24) consisted of male rat pups and was divided into 4 groups: asphyctic rats undergoing to vehicle treatment (PA20), asphyctic rats subjected to

treatment with OEA (PA20 OEA10), control of cesarean delivery undergoing to vehicle treatment (C+), control of cesarean delivery subjected to treatment with OEA (C+ OEA10).

### 2.2. Induction of Perinatal Asphyxia

PA was induced in newborn rat pups delivered by cesarean operation. Gestational age was determined by vaginal smear. After the surgical site was prepared for surgery (shaved, cleaned, antiseptic applied), rats within the last day of gestation were deeply anesthetized with Nembutal (30 mg/ml) by i.p. injection of 0.1 cc per 100 grams of body weight. Their entire uteri containing the fetuses were taken out by hysterectomy, and placed in a water bath at 37°C for 20 min (severe asphyxia). The mother rat was euthanized. Cesarean-delivered control and asphyctic pups were obtained from the same mother. Following PA, the uterus horns were rapidly opened and the pups were removed and stimulated to breathe on a heating pad by cleaning off the delivery fluid and by tactile stimulation with small pieces of medical wipes. In all of the pups the umbilical cord was tied (previous antiseptic applied) and left to recover on a heating pad for 1 h. After that the pups were given to the surrogate's mothers, which had delivered few hours before the experiment, mixing their normal litters with previously marked asphyctic and control pups. Experimental and control pups were weaned at 24 days, and transferred to cages with free access to food and water [11, 24, 25]. All experiments were conducted according to the principles of the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996), and approved by the Institutional Animal Care and Use Committee at the University of Buenos Aires (School of Medicine) No. 4081/04.

### 2.3. Oleoylethanolamide Administration

The respective treatment was performed within the first hour of life by subcutaneous injection. Vehicle used was 1:1:9 DMSO, Tween 80 y NaCl.

OEA dose administered was 10 mg/kg (OEA 10 mg/kg).

### 2.4. Elevated Plus Maze

This behavioral test is validated to evaluate anxiety and exploration [33]. The maze consists of a black central platform from which four black arms radiate in the form of a cross. Two of the arms have a wall all around its perimeter and are called closed arms. The other two arms are open. The maze is elevated one

meter from the floor. Rats are placed onto the central platform facing an open arm. The test lasts 5 minutes [32].

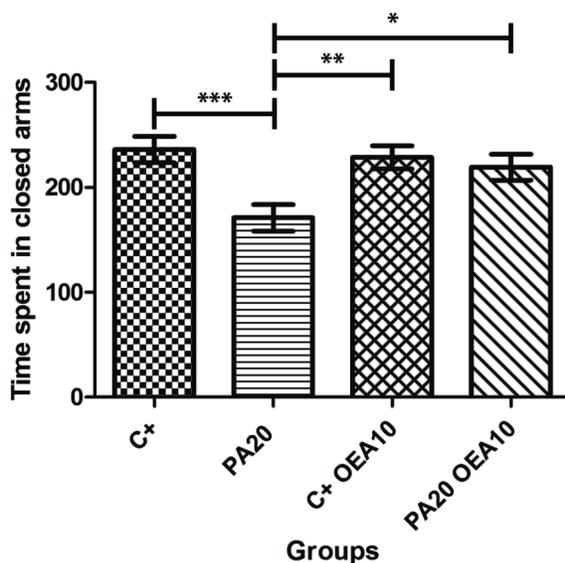
In the present study, the elevated plus maze test was carried out at P30. Dependent variables were measured: Time spent in open arms (index of anxiety) and time spent in closed arms (index of exploratory locomotion). Time was measured in seconds.

### 3. RESULTS

Results were expressed as means  $\pm$  SEM. One-way ANOVA followed by post-hoc comparisons (Fisher's test) were carried out. Homoscedasticity was previously tested. A probability was considered to be significant at 5% or less. Statistical analyses were performed using the SPSS 18.0 for windows (SPSS Inc., Chicago, IL, USA).

#### 3.1. Exploratory Locomotion

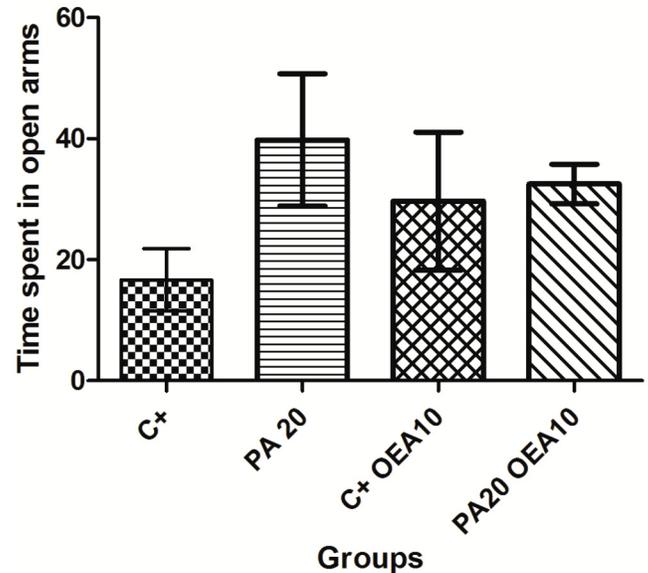
When time spent in closed arms was analyzed, a significant effect of group was found ( $F_{(3,20)}=4.949$ ;  $p=0.01$ ). Post hoc analyses revealed that time exploration of PA rats was significantly lower than the other groups: C+ Veh ( $p=0.001$ ), C+ OEA10 ( $p=0.007$ ) and PA20 OEA10 ( $p=0.033$ ). Figure 1 illustrates these results.



**Figure 1:** Graph showing the performance of different group of animals during Elevated plus Maze test (close arms). Perinatal asphyxia animals show a significant decrease in the exploration locomotion activity. This activity was recovered with OEA treatment. C+ = Control cesarean; PA20 = 20 minutes of Perinatal Asphyxia; C+ OEA10 = Control Cesarean & OEA treatment (10 mg/kg); PA20 OEA10: 20 minutes of Perinatal Asphyxia & OEA treatment (10 mg/kg). \*\*\*  $p \leq 0.001$ , \*\*  $p \leq 0.01$ , \*  $p \leq 0.05$  compared with PA20 group.

#### 3.2. Anxiety

No significant differences between groups in terms of time spent in open arms were found ( $F_{(3,20)}=1.554$ ;  $p=0.232$ ), as shown in Figure 2.



**Figure 2:** Graph showing the performance of different group of animals analyzed during Elevated plus Maze test (open arms). No differences were observed between different groups. C+ = Control cesarean; PA20 = 20 minutes of Perinatal Asphyxia; C+ OEA10 = Control Cesarean & OEA treatment (10 mg/kg); PA20 OEA10: 20 minutes of Perinatal Asphyxia & OEA treatment (10 mg/kg).

### 4. DISCUSSIONS AND CONCLUSION

The main finding of this study is that OEA treatment (in a dose of 10 mg/kg) would be associated with an improvement in exploratory locomotion in asphyctic rats. Our behavioral results could be related to the apparent anti-inflammatory action of OEA, which is mediated by PPAR- $\alpha$  [13, 14]. Consistent with this data, a recent study of our laboratory has shown that the effects of OEA could prevent neuroinflammation in asphyctic rats<sup>1</sup>. Substantial body of evidence suggests that in neurodegenerative diseases, the inflammatory process, which attempts a tissue recovery through microglial action, could lose its regulation and become a powerful factor of neuronal destruction [34]. In fact, damage elicited by PA implies Poly (ADP- ribose) polymerase 1 (PARP-1) overactivation, promoting expr-

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ession of proinflammatory cytokines and microglial migration toward the site of neuronal injury. But excessive PARP-1 activation could lead to energy crisis and to apoptosis [12]. Therefore, the apparent anti-inflammatory actions of OEA would be neuroprotective for perinatal hypoxic brain damage.

In addition, we observed behavioral modifications at P30. This data is consistent with previous studies from our laboratory using the same model of PA [11, 24, 25]. Asphyctic animals showed a decrease in exploration without an alteration in anxiety at post-natal day 90 (P90) [32]. These behavioral modifications associated with PA could be related to F-actin neostriatal cytoskeleton changes observed in post-synaptic densities of asphyctic rats at P30. These morphological modifications described in our laboratory might be one of the mechanisms involved in the neuronal loss induced by PA, since we observed dramatic synaptic changes with ubiquitination, degeneration and inflammatory modifications 6 months after induction of PA [25, 29]. Further studies will be carried out to determine if OEA might have some protective effects on synaptic structure and functions.

In conclusion, OEA treatment (10 mg/kg) in asphyctic rats would be accompanied by an improvement in exploratory locomotion at P30. A more thorough understanding of neuroprotective mechanisms of OEA and its behavioral effects on PA may contribute to obtain effective treatments for this neonatal complication.

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