Difusão do Conhecimento Através das Diferentes Áreas da Medicina

Lais Daiene Cosmoski
(Organizadora)

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APRESENTAÇÃO

Cada vez mais percebemos, que no mundo da ciência, principalmente da área da saúde, nenhuma profissão trabalha sozinha, é necessário que vários profissionais estão envolvidos e engajados em conjunto, prezando pela, prevenção, diagnóstico e tratamento de diversas patologias, visando sempre a qualidade de vida da população em geral.

A Coletânea Nacional “Difusão do Conhecimento Através das Diferentes Áreas da Medicina” é um *e-book* composto por 4 volumes artigos científicos, que abordam relatos de caso, avaliações e pesquisas sobre doenças já conhecidas da sociedade, trata ainda de casos conforme a região demográfica, onde os locais de realização dos estudos estão localizados em nosso país, trata também do desenvolvimento de novas tecnologias para prevenção, diagnóstico e tratamento de algumas patologias.

Abordamos também o lado pessoal e psicológico dos envolvidos nos cuidados dos indivíduos, mostrando que além dos acometidos pelas doenças, aqueles que os cuidam também merecem atenção.

Os artigos elencados neste *e-book* contribuirão para esclarecer que ambas as profissões desempenham papel fundamental e conjunto para manutenção da saúde da população e caminham em paralelo para que a para que a ciência continue evoluindo para estas áreas de conhecimento.

Desejo a todos uma excelente leitura!

Lais Daiene Cosmoski

SUMÁRIO

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CAPÍTULO 9

COGNITIVE IMPAIRMENTS INDUCED BY EARLY ANESTHESIA WITH SEVOFLURANE ARE REVERSIBLE BY INTERMITTENT EXPOSURE TO ENRICHED ENVIRONMENTS

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ABSTRACT: Early exposure to inhalational anesthetics are linked with neurodegeneration, and recent findings show that the exposure to enriched environments can improve neurogenesis. In this work, we tested whether exposure to enriched environments could revert the negative effects induced by sevoflurane. When reached 14 days of age, rats from the SEVO group were subjected to deep anesthesia by sevoflurane 3%, while rats from the oxygen group (O2) were only exposed to oxygen and controls were kept in the animal facility. Rats from the stimulus groups (STIM and SEVO+STIM) were placed together in large cages with tube labyrinths for one hour three days per week for 18 days. On day 40, animals were subjected to behavioral tasks. Even though in the open field task no differences were observed in the locomotor activity, exploratory behavior or anxiety, the Morris water maze showed that the

SEVO group took more time to perform the task in all five training days, while rats from the SEVO+STIM group performed similar to controls. Memory impairments could also be observed in the inhibitory avoidance task, where SEVO animals were the only ones to show a significant reduction in the freezing time. BDNF levels were significantly increased in the prefrontal cortex of animals of both stimulated groups, while it was higher only in the hippocampi of SEVO+STIM rats. This work provides further evidence on the impairments caused by early exposure to sevoflurane, and shows the therapeutic effects of recreation, exercise, and social interaction, even when intermittent.
KEYWORDS: development; inhalational anesthetics; learning; memory.

1 I INTRODUCTION

The use of general anesthesia is recommended or required for a number of surgical procedures in individuals of every age group. However, findings from the last decades strongly suggest that general anesthetics, when administered at early stages of the development of the central nervous system (CNS), can lead to neurodegenerative effects associated with important long-lasting deficits in learning and memory, thus making their use not as innocuous as previously thought (Fang, Xue, & Cang, 2012; Satomoto et al., 2009; Yu, Jiang, Gao, Liu, & Chen, 2013). Considering that in the United States alone more than 4 million pediatric surgeries are performed every year, the clinical importance of this subject demands special attention, looking for a much deeper understanding of the mechanisms of action and possible consequences of early subjection to anesthetics (Vesna Jevtovic-Todorovic, Bushnell, & Paule, 2017).

The precise mechanisms by which inhalational anesthetics might cause neurotoxicity are still uncertain. Many hypotheses were raised, but it is known that there are two critical factors behind neuronal injury: the stage of brain development in the moment of exposure to the drug and the magnitude of this exposure, which includes both frequency and intensity (Vesna Jevtovic-Todorovic et al., 2013). Animal models and children repeatedly exposed to high concentrations of anesthetics at early age are more susceptible to develop CNS damages, because of the enhanced synaptogenesis of earlier brain development stages (Yon, Daniel-Johnson, Carter, & Jevtovic-Todorovic, 2005). What is known for sure is that the exposure to anesthetics can induce neuronal apoptosis both in the hippocampi and in the brain cortex, leading to long lasting effects (Loepke & Soriano, 2008; Shen et al., 2013).

The search for new alternatives to attenuate or completely avoid the negative effects of early exposure to anesthetics is much needed. Among the proposed treatments, stimulation via exercise, social interaction, and recreation are some of the most promising approaches, since they are affordable and lack contraindications.

This kind of stimulation was already reported to raise the levels of the brain-derived neurotrophic factor (BDNF), induce neuronal growth, and increase the formation of dendritic spines and new synapses (Bekinschtein, Oomen, Saksida, & Bussey, 2011).

This study aims to evaluate the cognitive functions and the structural integrity of the brain of juvenile rats subjected to deep anesthesia with sevoflurane and whether exposure to an enrichment environment can revert these effects.

2 I METHODS

2.1 Experimental model

Six progenitor adult Wistar rats, two males and four females, were bred in the central animal facility of the Federal University of Rio Grande do Sul (UFRGS), and were transferred and kept in the animal facility of the Institute of Biological Sciences (ICB) of the Federal University of Rio Grande (FURG). The day the progenies were born was considered as day 0 of the experiment. On day 21, the progenies were weaned and separated in cages by brood and gender. The animals were kept in a light/dark cycle of 12 h, with controlled temperature (23±1 °C) and humidity (55±5%), and with free access to food and water. All protocols were previously approved by FURG Ethics Committee on Animal Use (CEUA) under the certification number P030/2016.

2.2 Experimental design

Sixty newborn animals were randomly assigned to five groups of 12 animals each: Control, O2, SEVO, stimulus (STIM) and SEVO+STIM, with care to distribute a similar number of males and females for each group. Animals from the SEVO and SEVO+STIM groups were subjected to deep anesthesia by sevoflurane 3% for 3 h under a glass dome attached to the anesthetic apparatus (Omeda 200 with a Tec 7 sevoflurane vaporizer), with continuum oxygen liberation at a rate of 2 L/min. The temperature of the surgical room during the experiment was kept at 36 °C to avoid hypothermia during the exposure to the inhalational gases. Rats from the oxygen group (O2) were only exposed to oxygen, while animals from the Control group were kept in the animal facility. Animals from the STIM and SEVO+STIM groups were placed together in large cages enriched with tube labyrinths for one hour during the morning, three days per week (Mondays, Wednesdays and Fridays) from days 21 to 39, resulting in a total of 9 sessions. Animals from all groups were then subjected to behavioral tasks starting on day 40, being these tests the only activities experienced by the animals of the Control group.

2.3 Behavioral assessment

2.3.1 Open field

The open field task is used to evaluate the locomotor activity and exploratory behavior of the animals. The apparatus consisted of a wooden box with the floor measuring 30 x 22 cm. The activity of each animal was recorded for 5 min using a camera placed on the ceiling of the room. Locomotor activity and area of predominance (central vs. peripheral) were measured using a video analysis system (Smart 3.0, Harvard Apparatus). After each trial, the number of fecal pellets was counted and the box was cleaned with alcohol 70%.

2.3.2 Morris Water Maze

The Morris Water Maze (MWM) task was performed to evaluate the spatial memory of the animals. A circular water tank measuring 168 cm diameter was filled with water until 70 cm of depth was reached. During all sessions, the water temperature was maintained at 24±2 ºC. The water was stained black using an odorless non-toxic colorizer, and visual cues were placed on the walls of the test room. Test sessions were filmed using a camera fixed on the ceiling, and image data was analyzed using the same video analysis system used for the open field task. The tank was virtually divided into four numbered quadrants. A platform was placed in the four quadrant, having its surface hidden just below water level.

The training sessions consisted of four trials per animal. For each trial, the rat was placed in the tank starting from a different quadrant, starting by the first one, with an interval of 60 s between trials. The time taken to find the platform in each trial was measured, with a time limit of 2 min. If the platform could not be found, a researcher placed the animal on the platform for 20 s before the next attempt. After five days of training, the test was carried out. In this session, platform was removed, the animal was placed in the second quadrant and the time spent on the fourth quadrant (target quadrant, TQ) was measured, with more time in the TQ meaning that the animal remembered the original position of the platform.

2.3.3 Inhibitory avoidance task

This test was performed to evaluate the aversive memory. The apparatus consisted of a steel box (30 x 5 x 15 cm) with steel bars on the floor. One of the walls was made of glass, to allow visualization. For the training session, animals were allowed to explore the box for three min, then being subjected to three quick electric shocks (0.5 mA) with 20 s intervals between them. The animals remained in the box

until the completion of 5 min. There were no electrical shocks in the test session, and the time of freezing behavior was measured.

2.4 Biochemical parameters

After the behavioral assessment, the animals were decapitated for the collection of the brain and further isolation of hippocampi and PFC for the measurement of BDNF levels. Following isolation, the structures were weighted, homogenized in a buffer solution, and centrifuged at 4500 rpm for 10 min. BDNF levels were determined using a kit (Rat BDNF ELISA Kit, Sigma-Aldrich), and the adopted protocols followed the instructions provided by the manufacturer.

2.5 Statistical analysis

Obtained data was tested for normality and homocedasticity using the D'Agostino & Pearson and Bartelett tests, respectively. When applicable, data was analyzed using ANOVA followed by Tukey's post-hoc test. In all box-plots, all data points are shown, whiskers represent min and max values, the edges of the boxes represent the lower and higher quartiles (Q_1 and Q_3, respectively), and the central line represents the median. In the XY figures, points and error bars represent mean and standard error of the mean (SEM), respectively. Differences were considered as statistically significant when $p < 0.05$.

3 I RESULTS

Total traveled distance in the open field task was not different among the experimental groups (Fig 1A). Similarly, the two parameters used to evaluate stress were not statistically different between groups, with similar time spent in each zone (central or peripheral) (Fig 1B) and number of fecal pellets (data not shown).

In order to evaluate if the animals were able to recall the localization of the platform from the previous training sessions in the MWM, we have quantified the time spent to find the platform in the first training session of each day (starting at quadrant 1). With this approach, we could observe that animals from the SEVO group needed more days than the other groups to learn the task (Fig 2A). We have also quantified the time needed to find the platform in the last session of each training day, and animals from the SEVO group needed more time to find the platform than some of the other groups in the first day of training (Fig 2B). Interestingly, no significant differences were found in the test session performances, as time spent in the TQ was not significantly different from the other groups (Fig 2C).

Freezing time was significantly lower in animals from the SEVO group in comparison to all other groups (Fig 3). This once again shows that animals from this

group had more difficulty to recall past events, in this case by not evocating the fear memory associated with the previous experiences within the box.

Hippocampal BDNF levels were significantly higher in animals from the SEVO+STIM group in comparison to all other groups (Fig 4A), and it was higher in both stimulated groups when BDNF was quantified from the PFC (Fig 4B). These results suggest an important effect of the enriched environment on the biochemistry of the brain, with greater effect on animals subjected to anesthesia at early age.

4 I DISCUSSION

In this study we evaluated the effects of early anesthesia with sevoflurane in rats and whether exposure to an enriched environment could revert the negative neurobehavioral effects.

Total traveled distance in the open field task was similar among all groups, suggesting that the different treatments did not induce differences in the exploratory behavior or to motor impairments. This second point is important, once differences in the locomotor activity could have led to misinterpretations of results observed in the MWM task, designed to assess cognitive outcomes only. Open field results also did not evince anxiety-like behavior (as assessed by preference for the peripheral region or increased number of fecal pellets). Both these results are in accordance with previously published data (Chung et al., 2015).

Even though SEVO animals needed more time to find the platform in early MWM training sessions, the latency time became increasingly shorter until no more differences could be identified among groups. The difficulty to find the platform during the training session suggest an impairment in learning. The working memory has been receiving special attention due to its impact on learning (Wang & Gathercole, 2013). It is a system of limited capacity that allows the temporary storage and manipulation of verbal or visual information necessary for complex tasks, such as comprehension, learning, reasoning, and planning (Baddeley, 2003). The process of redeeming information from long-term memories and establish associations with the new information is the reason why it is denominated as working memory (Baddeley, 2003). Malfunctioning of one of more components of the working memory is associated with learning disabilities and poor school performance (Uehara & Landeira-Fernandez, 2010). Children with learning disabilities may have limitations to properly and precisely store or organize information to perform motor or academic tasks (Uehara & Landeira-Fernandez, 2010). The association between working memory and the academic progress in language and mathematics is well established (Gathercole, Pickering, Ambridge, & Wearing, 2004; Schuchardt, Maehler, & Hasselhorn, 2011). The PFC is anatomically associated with the working memory,

while the hippocampus is associated with long-term memories (Lent, 2004).

Even though animals from the SEVO group showed more difficulties during the MWM training sessions, their performance was not significantly different from animals from the other groups on the test day. This finding suggests that even with the memory impairments, the task could be learnt after enough repetition. In fact, animals from this group performed increasingly better after each day of training. It is possible that the repeated exposure to a problem situation that demanded reasoning, like the MWM training, could have served as a stimulus for the brain to recover, at least partially. Brain stimulation through cognitive training was already identified to play an important role for the recovery of different brain injuries, including stroke (Zeiler & Krakauer, 2013).

The MWM training sessions make extensive use of the working memory, while the long-term memory is used to remember the sessions from the previous days. The long-term memory is also used in the inhibitory avoidance task, where SEVO animals showed more difficulty to remember the events from the previous sessions. Similar amnesic effects in aversive memory were also reported in other works (Liu et al., 2010). Some anesthetics may lead to hypoalgesia, which could be a confounding factor for the interpretation of this result. However, hypoalgesia due to opioid treatment is temporary, and would not have lasted from the exposure to the test session, 26 days later (Abreu, Aguado, Benito, García-Fernández, & Segura, 2015). Moreover, the SEVO+STIM group was able to learn the task, indicating that sevoflurane treatment did not interfere with the perception of pain in the tested conditions.

Animals from the SEVO+STIM group performed similar to controls in all behavioral tasks, suggesting that stimulation in an enriched environment could successfully reduce the neurobehavioral impairments caused by early anesthesia. Similar beneficial effects were already observed in children with brain injuries, that were able to perform equally or even better than the average in cognitive tests after treatment with repeated sensory stimuli in an enriched environment (Izquierdo, 2011). This effect is possibly a combination of physical exercise, social interaction, recreation and exposure to a complex environment, serving as stimuli for the growth and ramification of axons and dendritic spines and the formation of new synapses via increase of BDNF (Bekinschtein et al., 2011; Izquierdo, 2011). In accordance to that, our findings give evidence on the association between enriched environment, increase in BDNF levels and improvement of cognitive performance.

Animals from both groups exposed to the enriched environment showed BDNF levels in the PFC many times higher than what was observed in the other groups. These findings are in accordance to the literature, and environmental stimuli are consistently associated with a better cognitive performance. The physical exercise

and social interaction provided by the enriched environments were seen to increase the levels of synaptic proteins (synapsin I and synaptophysin), regulated by BDNF (Bekinschtein et al., 2011). The activation of these signaling pathways modulate proliferation, survival, and maintenance of the neuronal system, suggesting that the increase in BDNF levels leads to the functional improvements described in studies with enriched environments (Bekinschtein et al., 2011; Frey et al., 2006; Santos, Comprido, & Duarte, 2010).

Differently from other reports (Bekinschtein et al., 2011; Santos et al., 2010) the exposure to the enriched environment in our study was not continuous, but intermittent (1 hour per day 3 times per week). Nevertheless, this exposure protocol was enough to revert all complications detected in the non-stimulated animals exposed to sevoflurane.

5 I CONCLUSIONS

It could be confirmed that early anesthesia with sevoflurane is associated with neurodegeneration, leading to the apoptosis of brain cells along with important deficits in learning and memory. However, stimulation with enriched environments that allow exercising and social interactions, even when limited to few times per week, leads to a method that is effective, low-cost, and without contraindications that can be used as a treatment for the neurological impairments triggered by sevoflurane and other similar anesthetics in pediatric patients.

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FIGURE CAPTIONS

Figure 1. Neither early anesthesia with sevoflurane nor environmental stimulation led to alterations in locomotor activity or in anxiety-like behavior in the open field task. Locomotor activity was measured by the total traveled distance within 5 min (A), while anxiety-like behavior was assessed by the time spent in the central zone of the box, with more time spent in the peripheral zone meaning more anxiety (n=12-13) (B).

Figure 2. Spatial learning and memory impairments caused by early anesthesia with sevoflurane, as tested with the Morris water maze (MWM), were ameliorated following stimulation with environmental enrichment. Animals were trained for five days in the MWM task, and each training session consisted of four trials. Non-stimulated animals exposed to sevoflurane (SEVO) needed more days to learn the task (*** = $p < 0.001$ vs. all groups) (A) and performed worse in the last trial of the first training session (* = $p < 0.05$ vs. SEVO+STIM) (B). In the test day, however, animals from all groups spent a similar amount of time in the target quadrant (TQ), suggesting that the task could be learnt by animals of all groups after the 5-day training program (Two-way ANOVA followed by Tukey's post-hoc test, n = 12-13) (C).

Figure 3. Aversive memory acquisition was impaired after early exposure to sevoflurane. Animals anesthetized with sevoflurane at early age not exposed to environmental enrichment (SEVO) showed a reduced freezing time in the test session in the inhibitory avoidance task. On the other hand, animals exposed to sevoflurane that were stimulated (SEVO+STIM) performed similar to controls (*** = $p < 0.001$ vs. Control and SEVO+STIM, ANOVA followed by Tukey's post-hoc test, n = 12-13).

Figure 4. Early anesthesia with sevoflurane followed by intermittent exposure to an enriched environment led to higher BDNF levels both in the hippocampi and in the pre-frontal cortex (PFC). While the increase in BDNF was only observed in the hippocampi of stimulated animals anesthetized with sevoflurane (SEVO+STIM) (A), this effect was observed in the PFC of these animals and the ones only exposed to the enriched environment (STIM) (B) (* = $p < 0.05$ and *** = $p< 0.001$ vs. Control and SEVO, ANOVA followed by Tukey's post-hoc test, n = 6).

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