2.07.01 – Fisiologia / Fisiologia Geral.

ASSESSMENT OF SEROTONERGIC SYSTEM IN FORMATION OF MEMORY AND LEARNING.

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Abstract:
OBJECTIVE: We evaluated the involvement of the serotonergic system on memory formation and learning processes in healthy Wistar rats. **METHODS**: Fifty-seven rats weighing between 220-250 grams were divided in 5 groups and had one serotonergic nuclei damaged by an electric current. Electrolytic lesion was carried out using a continuous electric current of 2mA during two seconds by stereotactic surgery. After seven days of recovery, animals were submitted to learning and memory tests. Tests were performed in 3 consecutive days and the time given to the animal achieve the objective was measured in seconds. Data are presented as mean (m) ± standard deviation (SD) and was used the Paired T Test. Differences were considered statistically significant when the p-value was ≤ 0.05. **RESULTS**: Rats presented different responses in the memory tests depending on the serotonergic nucleus involved. Surprisingly, our results showed that both explicit and implicit memory may be affected after serotonergic lesion although some groups showed significant difference and others did not**. CONCLUSION**: Despite the intact limbic system, the damage in the serotonergic nucleus was able to cause impairment in the memory of Wistar rats, since nuclei projections could have been secondarily affected by electric stimulation. The formation of implicit and explicit memory is impaired after injury in some serotonergic nuclei.

Legal autorization: Procedures were executed in 2002 and experimental Ethical Committee there wasn't exist.

Key-words: Memory; serotonin; neuronal plasticity.

Financial support: This work was supported by grants from the Fundação de Amparo à Pesquisa do Estado de Alagoas – FAPEAL (Brazil).

Introduction:

The main subcortical input to the hippocampal region comes from fimbria-fornix pathway, which is involved in explicit and implicit memory processes *(*Guillazo-Blanch et al., 2002; Morrell, 2011). The hippocampi are often reported as the generator of rhythms related with learning and memory due to its involvement with neuronal plasticity known as short- and long-term potentiation (Beierlein et al., 2003; Ghaderi et al., 2016; Dittman et al.,2000; Sheikhzadeh et al., 2015). Facilitation of synaptic plasticity may occur by repeated activations and increase of neurotransmitter release, either by saturating the calcium buffer or increasing its concentration in the presynaptic terminal of the neuron. Studies show that plasticity of nervous system may occur throughout life and appears to be widespread in areas such as hippocampus, several cortical areas and thalamic-cingulate pathway (Sheikhzadeh et al., 2015; Emptage et al., 2001; Shyu Bai-C et al., 2009; Nudo, 2006). In this paper, we describe the involvement of the serotonergic system with memory formation and learning processes in the rat.

Methods:

Fifty-seven adult male Wistar rats weighing between 220-250 grams were housed under environmentally controlled conditions (light/dark cycle with lights on from 07:00 to 19:00 hours, 22-24.8°C) and permitted free access to food and water throughout the experiment. The procedures involving animals and their care followed Institution's guidelines, which comply with the international Ethical Guidelines for Biomedical Research (Howard-Jones, 1985).

Experimental procedures

Experimental animals (n=50) were divided into 5 groups according to serotonergic nucleus included in this research (B5, B6, B7, B8 and B9). Rats were anesthetized with a mixture of ketamine 90 mg/kg, and xylazine 12 mg/kg (i.p.), after exposure of the skull, bipolar electrodes (stimulating) stainless steel were implanted into one of the serotonergic nuclei using stereotactic surgery according to Paxinos and Watson atlas (1997). Each group of ten animals received injury in a specific serotonergic nucleus. Damage was done by continuous electric current of 2mA during 2sec using a stimulator device/lesioner (*EFF 341 - Insight, Brazil*).

After seven days to recovery animals were evaluated through the classic maze with food and Morris water maze to assess spatial memory and learning. The time spent for each animal to complete the tests was measure in seconds. All tests were performed in 3 consecutive days and each animal was assessed individually.

Statistical analysis

Data are presented as mean (m) ± standard deviation (SD). The comparison of the time (in second) spent for the animal to reach the food on the 1st compared to the 3rd day (same group) was executed using the Paired T Test. Differences were considered statistically significant when the p-value was ≤ 0.05. GraphPad Software Prism 4.0, San Diego, USA, was used to analyze the data.

Results and Discussion:

Our findings showed that the time spent for the rat to reach the food on the classic maze and the platform in the Morris water maze tests were different among groups. Animals who suffered lesion in the B5 nucleus showed improvement in implicit and explicit memory tests when the 1st and 3rd day was compared (Table 1).

Table 1. Results of the classic maze and platform in the Morris water maze to learning and memory

Groups	Implicit memory (m±SD)			Explicit memory (m±SD)		
	1st day	3rd day	P value	1st day	3rd day	P value
B5	1,930.8±899.61	1,165.4±512.86	P<0.05	65.6±21.56	13.6±13.51	P<0.05
B6	996.3±805.91	811.2±1,078.43	-	22.62±15.24	16.00±9.49	-
B7	1,139.46±807.66	1,271.69±1,031.93	-	19.39±9.19	12.38±5.25	-
B8	1,413.7±565.86	1,343.6±609.92	-	65.6±21.56	13.6±13.51	P<0.05
B9	547.5±486.06	1,594.1±1,215.16	-	27.36±10.90	10.53±4.87	-

Mean±Standard deviation: m±SD; 1st: First; 3rd: Third.

B8 group of rats also had a significant reduction in the time spent to reach the objective in the explicit memory tests, showing an improvement in its execution. Concerning the implicit memory test, B7 and B9 groups spent more time to have it completed, but statistical analysis was not significant. On the other hand, animals who suffered damage in B6 and B8 nuclei showed tendency to reduce the time to complete the implicit memory test. In the explicit memory test, animals with lesion in B6 nucleus and also those with B7 and B9 nuclei damaged, showed a tendency to a improvement in the test, although results did not show significance.

The understanding of the relationship between declarative (explicit) and nondeclarative (implicit) memory systems would be a great advance to neurobiology and behavioral neurosciences (Weiss et al., 2015). In this work we analyzed implicit and explicit memory of rats submitted to lesions in serotonergic groups (B5, B6, B7, B8 and B9) using classic maze with food and Morris water maze. Although we expected an impairment of both memories (declarative and nondeclarative), in the most part of the nuclei damaged, results showed that lesion into serotonergic nucleus of rats induced to different responses, depending on the nucleus injured.

Tumbull et al. (1994) showed that lesions in fornix, fimbria, as well as on medial septal nuclei, might affect theta rhythm in the hippocampus and also memory impairment. On the other hand, after stimulating electrically the hippocampus in rats with the fornix damaged, researchers found normal hippocampal theta rhythm and also improvement in memory (Turnbull et al., 1994). Researchers have observed that patients who suffered a lesion, or received stimulation in a specific area of the brain show different responses after that. A study made with patients after they had thalamotomy surgery, did not show reduction in cognitive level when memory tests were applied. Whereas, stimulation into the thalamus showed increased responses in memory tests. Lesion on the left side of the thalamus showed a decrease in the number of correct responses given by the patient when evaluated by a listening test. Parkinsonian patients submitted to thalamotomy were tested to investigate the verbal memory and did not present cognitive deficit. In this paper authors showed that high-intensity stimulation in the left side of the thalamus decreased the performance into the test (Wester et al., 1997). In our work we saw that rats who had a specific serotonergic group damaged (B5-implicit and explicit or B8- explicit only) showed a better response in these memory tests, which may not be related to an improvement in this type of memory. Other groups (B6 and B8) for implicit memory test and (B6, B7 and B9) for explicit memory test showed a tendency to improve its execution, but results was not significant. On the other hand, animal with B7 and B9 serotonergic nuclei damaged, showed a tendency to worse its response in implicit test, but results did not show significance between the 1st and 3rd day of execution. Many disorders seen in patients without an effective treatment have encouraged basic researchers to look for novel approaches in the use of animal models of cognitive impairment, which is important for the understanding of its physiopathology and also the development of new therapies (*Gornicka-Pwlak et al., 2015;* Dalrymple-Alford et al., 2015; Huijgen et al., 2015; Danet et al., 2015).

Conclusion:

This work suggests that serotonergic nuclei together with Papez's circuit might be an important target in the study and understanding of the mechanisms involved in memory impairment. These data represent an approach to study the paradigms of mechanisms involved in the damage of serotonergic nuclei. Additional studies are needed for further understanding of the neurophysiology of serotonergic system and its relationship with Papez's circuit.

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