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ASSESSMENT OF HIPPOCAMPAL MEMORY AND LEARNING BEHAVIOURS IN ALBINO WISTAR RATS USING PASSIVE AVOIDANCE BOX TECHNIQUE

***[1]Olorunfemi O.J., [2]Asara Azibalua A. and [3]Umana K. T.**

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Port Harcourt, Nigeria.
[2]Bayelsa State College of Health Technology, Otuogidi Ogbia Town, Bayelsa State.
[3]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

***Corresponding Author: Dr. Olorunfemi O. J.**
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Port Harcourt, Nigeria.

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ABSTRACT
The study was designed to assess the hippocampal memory and learning behaviours in rats using passive avoidance box technique and scopolamine after which the rats underwent carry some memory and learning tests such as Barnes maze test, Navigational maze task, elevated plus maze and beam walk test. Twenty albino wistar rats were grouped into four groups which comprises of the control group, mild shock group, moderate shock group and scopolamine group with five rats per group. Group A (control) was given clean water and feed, Group B was given mild shock (2 times) using passive avoidance box, Group C was given moderate shock (3times) using passive avoidance box and group D was given 0.1ml/kg Scopolamine daily after which they underwent a total of nine (9) trials of some memory and learning tests which include: elevated plus maze, Barnes maze, beam walk test and navigational task. The results collected were analyzed using IBM SPSS version 22.0 statistical package. The descriptive characteristics we're expressed as mean and standard deviation. The repeated measured of ANOVA and Post-Hoc analysis technique were used in the comparison of the control group and other groups. The significance level of the groups were set at P>0.05. From result obtained, it can be seen that mild and moderate shock and scopolamine had a positive effect on the hippocampal memory and learning behaviour by increasing the response in the first three trials with the moderate shock having the highest-effect.

KEYWORDS: hippocampal memory, scopolamine, passive avoidance box, shock, maze task.

INTRODUCTION
The PanLab Passive avoidance box is a fear motivated test defined by a white illuminated compartment (by a 75watt light bulb) and a small black dark compartment separated by a guillotine gate with the floor constructed of grids that can pass a small electric shock. The animal position is detected by using high sensitivity weight transducers providing higher effective and reliable detection of animal responses (zone entries) than systems based on photocells beams or on grid floor displacement. (Fasano & Brambilla, 2011). PanLab Passive avoidance box is controlled by the Shut Avoid software, allowing running passive avoidance test experiment in several boxes simultaneously. The link is carried out by one only cable from one box to the other. The dependent variable is the time spent in the lighted chamber during this test trial, typically referred to as Latency. Movement into the dark chamber is interpreted as a failure to recall previously delivered shock. (Silingardi & Angelucci, 2012). Passive Avoidance box technique: has been found to be a very sensitive measure of the effects of drugs that affect memory such as the muscarinic blocker atropine (or scopolamine). Certain types of brain damage including damage to the limbic system and globus pallidus and their transmitters, are similarly sensitive to the passive avoidance task. Learning behavior **occurs** as a result of experience and they are usually less rigid and more adaptive than innate behaviors. Learning behavior occurs in a variety of ways which include habituation (crows), imprinting, classical conditioning (rats), operant conditioning, cognitive learning, observation (monkeys), play (kitten), insight learning (chimpanzee), etc. (Elvander& Addario, 2009)

Assessment of spatial memory and learning behavior include: Fear conditioning, morris water maze, object recognition task, elevated plus maze, 8-arm radial maze, active avoidance apparatus (shuttle box), 2-object novel object recognition, modified barnes maze test, open field habituation, intellicage place learning and cue

discrimination experiments, hand grip, walking on the beam, navigation task, etc. (Solari & Brambilla, 2011)

Hippocampus: gotten from the Greek word "hippo" meaning horse and "campus" meaning sea monster. It is a small organ located within the brain medial temporal lobe which forms an important part of the limbic system. This region is made up of several distinct regions; the information first arrives at the Gyrus dentatus. From here, the neurons transmit to the Cornu Ammonus region CA3 (This is where the memory is stored) which in turns project to CA1. There are two hippocampi, one on each side of the brain of humans and other mammals. The hippocampus is part of the limbic system and plays an important role in the consolidation of information from short-term memory to long-term memory and in spatial memory that enables navigation and also regulates emotion. It contains two main interlocking parts: the hippocampus proper (also called Ammon's horn) and the dentate gyrus. (Blum & Dash, 2009)

MATERIALS AND METHODS

Experimental Animal

A total of twenty make wistar albino rats weighing 180-220g was obtained from animal house. The rats were kept in clean disinfected wooden cages with saw dust as beddings in the animal house, with 12hours light/dark cycle and 50-60% humidity at a temperature of about 30^{o}C and were allowed to acclimatize to the new environment for two weeks, with free access to clean water and animal feed. The rats were weighed using an analytical weighing balance at commencement of the experiment.

Experimental Design

Table 3.1 Experimental Design and Grouping of the Rats.

Group	Number of Rats	Treatment
Group 1	5	Feed +Water ad Libitum
Group 2	5	Feed +Water ad Libitum + Mild Shock (2.0mA)
Group 3	5	Feed +Water ad Libitum + Moderate Shock (3.0mA)
Group 4	5	Feed +Water ad Libitum + 0.1ml Scopolamine

Group 1 (control) was given clean water and feed, Group 2 was given mild shock (twice) using passive avoidance box, Group 3 was given moderate shock (thrice) using passive avoidance box and group 4 was given 0.1ml/kg Scopolamine daily after which they underwent a total of nine (9) trials of some memory and learning tests which include: elevated plus maze, Barnes maze, beam walk test and navigational task. The animals were sacrificed after treatment.

Experimental Procedures

i. A total of twenty (20) rats weighing between 180-220g was bought from the Animal house, Faculty of Pharmaceutical sciences, University of Port Harcourt and will be put in a cage at Animal house for the experiment.
ii. They were weighed and grouped into four (4) groups of five (5) rats per group.
iii. They were allowed to acclimatize for two (2) weeks with free access to clean water and feed.
iv. After which they were exposed to the equipment for trials.

Elevated Plus Maze

This was done according to the modified method of Itoh *et al.*, 1990

i. The animal was placed on an elevated maze of 32cm high having four open arms of 14.2cm (diagonally).
ii. The animal was placed in the centre of the four arms and the stop watch started.
iii. The time taken for the animals to go through the four arms was recorded.
iv. The maximum time of five (5) minutes was set as the longest allowable time for each animal after which it was removed if it could not complete its task within 5 minutes.
v. The animals were subjected to three trials daily.

Beam Walk (This was carried out according to the modified method of Carter *et al*., 2001)

i. The beam was 38cm long and a diameter of 2cm. The animals were placed on one edge of the beam and expected to walk across the beam within five (5) minutes and any animal that did not complete the task within 5minutes were removed.
ii. All the animals in each group passed through this test, one at a time and the process was repeated thrice daily summing up to nine (9) trials.

Navigational Task

i. The animals (one at a time) were placed in a navigational maze which has two doors at opposite ends, but they need to go through a puzzle of complex pathway which the animals were expected to find their ways to the other outlet.
ii. The animals were placed in the navigational maze cabinet and the stop watch immediately starts.
iii. The time taken by the animals to go from side of the maze through the puzzle to the other end was recorded in seconds.
iv. The animal is given a maximum of 5minutes to complete the task and if any animal did not complete the set task in 5minutes, it was removed at the expiration of the 5minutes.

v. All the animals in each group passed through this test, one at a time and the process was repeated thrice daily summing up to nine (9) trials.

Barnes Maze Test (The was done according to the modified method of Barnes, 1979)

i. The Barnes maze consists of a circular surface with up to 20 circular holes around its circumference.
ii. The table surface is brightly lit by overhead lighting. Under one of the holes is an "escape box" which can be reached by the rodent through the corresponding hole on the table top.
iii. The model is based on rodents' aversion of open spaces, which motivates the test subject to seek shelter in the escape box.
iv. A normal rodent will learn to find the escape box within four to five trials and will head directly toward the escape box without attempting to escape via incorrect holes. Various parameters are measured including latency to escape, path length, number of errors, and velocity.
v. These variables help to verify that innate anxiety and cognitive ability differ considerably among mouse strains.

STATISTICAL ANALYSIS

Statistical analysis was done SPSS version 20.0 and the results were expressed as mean ± SEM. One-way ANOVA and Dunnet Post Hoc (multiple comparison) Test was used to compare the mean and P-Value ≤ 0.05 was accepted as statistically significant. Results are presented in tables and chats.

RESULTS

Table 4.1. Results from Barnes Maze Test on the hippocampal memory and learning behavior.

Barnes maze Task performed in nine trials at 24hr Interval (S±SEM)										
Group	**Treatment**	**Trial 1**	**Trial2**	**Trial3**	**Trial4**	**Trial5**	**Trial6**	**Trial7**	**Trial8**	**Trial9**
1	Control (Normal saline)	48.00±32.68	52.80±17.83	42.40±13.67	30.20±11.41	40.20±30.23	34.80±27.40	13.20±5.77	41.80±17.55	33.20±12.89
2	Mild shock (2 times)	11.40±3.31	45.20±18.94	35.80±16.36	57.60±26.05	84.20±36.77	69.60±40.43	53.80±26.87	17.00±35.48	64.40±35.51
3	Moderate shock (3 times)	55.00±26.31	47.40±30.65	16.00±8.57	22.40±9.45	28.80±7.71	74.80±34.13	55.60±28.43	54.20±28.49	20.40±7.36
4	Scopolamine (0.1ml)	21.20±8.48	13.80±6.22	33.00±12.46	19.20±7.39	5.40±2.77	30.60±11.76	17.00±7.68	5.20±1.77	26.00±10.65

Values are presented as mean ± sem. P < 0.05. * means values are statistically significant when compared with the control.

Table 4.2. Results from Beam walk test on the hippocampal memory and learning behavior.

Beam walk Test performed in nine trials at 24hr Interval (S±SEM)										
Group	**Treatment**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**	**Trial 7**	**Trial 8**	**Trial 9**
1	Control (Normal saline)	58.60±36.94	51.80±18.39	28.60±14.14	12.00±4.27	7.00±2.28	15.00±7.98	47.80±23.95	36.80±18.34	13.20±9.346
2	Mild shock (2.0mA)	48.60±24.54	14.60±7.36	88.60±53.31	71.80±43.83	61.60±55.91	72.60±55.86	56.40±46.21	46.00±39.811	72.00±54.81
3	Moderate shock (3.0mA)	11.80±5.22	71.00±51.89	7.40±2.77	63.00±28.22	104.80±29.74	133.00±34.48	55.00±38.47	55.00±38.471	63.40±29.49
4	Scopolamine (0.1ml)	29.20±12.19	26.20±12.88	19.80±6.10	36.00±9.14	22.00±12.41	43.60±27.18	15.80±11.25	15.80±11.253	37.00±21.54

Values are presented as mean ± sem. P < 0.05. * means values are statistically significant when compared with the control.

Table 4.3. Results from Navigational Task on the hippocampal memory and learning behavior.

Navigational Task performed in nine trials at 24hr Interval (S±SEM)									
Group	**Treatment**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**	**Trial 7**	**Trial 8**
1	Control (Normal saline)	77.00± 54.13	109.00±75. 18	27.40±12.0 7	69.20±29 .25	86.60±29.9 9	72.20±32.8 7	87.80±26. 68	17.40±13. 36
2	Mild shock (2.0mA)	22.20± 6.07	144.60±14 3.25	67.00±32.8 1	134.00±3 7.36	28.40±8.13	31.00±12.8 8	176.40±4 8.11	11.00±7.1 4
3	Moderate shock (3.0mA)	110.60 ±31.42	27.40±28.7 5	74.40±40.6 2	125.00±4 1.11	98.40±26.4 7	98.40±26.4 7	128.00±4 3.98	109.40±4 2.43
4	Scopolamine 0.1ml)	97.80± 32.37	204.00±11 8.87	39.80±11.4 9	143.80±4 3.40	34.00±9.80	34.00±9.80	140.00±4 3.01	100.00±6 3.25

Values are presented as mean ± sem. P < 0.05. * means values are statistically significant when compared with the control.

Table 4.4. Results from Elevated Plus Maze Test on hippocampal memory and learning behaviours performed in nine trials at 24hr Interval (S±SEM).

Elevated Plus Maze									
		Trial 1				Trial 2			
Group	**Treatment**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**
1	Control (Normal saline)	14.80±10.54	10.60±6.49	63.80±52.37	147.00±58.89	4.80±2.96	20.20±9.11	104.26±42.43	90.60±34.52
2	Mild shock (2.0mA)	17.20±7.40	7.00±4.43	10.80±1.02	13.20±6.03	21.20±19.73	10.60±1.36	165.00±59.20	62.40±59.42
3	Moderate shock 3.0mA)	20.60±13.33	8.80±3.31	41.60±31.75	35.20±18.98	100.00±41.83	7.00±3.07	167.00±59.62	122.00±53.80
4	Scopolamine (0.1ml)	15.40±9.53	18.40±5.91	23.60±4.76	65.00±40.79	13.00±11.52	9.80±2.65	159.20±57.60	167.00±63.04

Values are presented as mean ± sem. P < 0.05. * means values are statistically significant when compared with the control.

Elevated Plus Maze									
		Trial 3				Trial 4			
Group	**Treatment**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**
1	Control (Normal saline)	9.60±3.20	65.80±57.92	59.20±54.04	73.40±52.02	14.80±4.81	14.80±9.01	108.20±56.05	73.00±56.84
2	Mild shock (2.0mA)	5.80±2.71	3.00±2.00	115.40±65.56	130.60±65.23	5.00±3.26	10.80±5.07	79.80±50.13	116.00±58.55
3	Moderate shock (3.0mA)	3.60±0.93	6.20±2.91	128.40±41.76	163.40±42.74	12.00±3.39	7.40±3.82	123.80±68.04	17.00±7.26
4	Scopolamine (0.1ml)	15.40±6.91	10.60±5.86	130.60±64.82	23.40±9.59	11.20±9.72	8.00±2.17	159.40±58.70	60.60±54.57

Values are presented as mean ± sem. P < 0.05. * means values are statistically significant when compared with the control.

		Trial 5				Trial 6			
Group	**Treatment**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**
1	Control (Normal saline)	10.20±4.75	16.60±7.01	130.20±50.69	74.40±56.91	13.20±6.80	8.40±2.56	143.20±50.87	78.20±56.07
2	Mild shock (2.0mA)	36.80±28.56	5.20±2.42	120.60±67.58	130.00±69.44	11.40±4.79	11.40±4.79	199.40±51.25	117.40±60.20
3	Moderate shock	41.80±39.59	7.20±2.06	62.60±54.72	254.80±42.50	4.40±1.21	11.60±4.68	85.00±54.41	183.80±65.33

	(3.0mA)								
4	Scopolamine (0.1ml)	23.00±7.06	10.40±5.56	84.40±49.47	29.00±7.48	19.60±10.52	13.40±5.26	111.40±43.13	105.00±43.65

Values are presented as mean ± sem. P < 0.05. * means values are statistically significant when compared with the control.

Elevated Plus Maze									
		Trial 7				Trial 8			
Group	**Treatment**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**
1	Control (Normal saline)	15.00±5.48	15.00±5.00	118.80±50.18	72.60±57.23	8.80±6.35	6.00±2.70	99.20±54.02	71.00±50.31
2	Mild shock (2.0mA)	19.00±6.40	8.80±3.87	112.60±63.21	131.40±68.85	6.60±3.68	7.20±3.72	133.00±54.85	115.00±59.27
3	Moderate shock (3.0mA)	12.40±7.19	13.40±4.31	8.00±5.56	239.00±40.155	3.60±0.93	7.40±2.36	83.00±38.85	178.60±63.21
4	Scopolamine (0.1ml)	21.40±10.25	23.00±7.68	48.00±20.35	103.00±27.73	16.60±10.98	7.00±2.10	94.00±39.32	77.00±26.81

Values are presented as mean ± sem. P < 0.05. * means values are statistically significant when compared with the control.

		Trial 9			
Group	**Treatment**	**Right arm**	**Left arm**	**Semi-closed arm**	**Closed arm**
1	Control (Normal saline)	27.00±19.32	2.80±2.80	118.00±50.37	68.80±53.22
2	Mild shock (2.0mA)	5.20±3.32	8.20±3.64	65.80±54.02	168.40±60.30
3	Moderate shock (3.0mA)	17.00±2.00	10.60±4.40	12.00±5.65	204.00±51.15
4	Scopolamine (0.1ml)	21.80±11.97	38.00±14.97	55.00±26.65	68.60±23.15

Values are presented as mean ± sem. P < 0.05. * means values are statistically significant when compared with the control.

DISCUSSION

The present study was designed to examine the effect of electric shock (mild and moderate) and scopolamine on the hippocampal memory and learning behaviour in twenty albino wistar rats using passive avoidance box technique. It was observed that the moderate shock group spent a longer time to locate the escape box when compared to the control with a difference of 7.00±6.37secs mean±sem interval. In the scopolamine group, the group spent 21.20±8.48secs before locating the escape box and when compared to the control, the time difference of 26.90±24.20secs was observed. Therefore, the mild shock group had the least time spent (quickest response) in locating the escape box when compared to the control, moderate shock and scopolamine group. This could be as a result of the stress response with the foot shocks demonstrated. This allowed rapid spatial learning acquisition (i.e. short-term memory) developing within a few seconds mild impairment in hippocampal function resulting in rightward shift in the learning curve. (Eagle, *et al.*, 2016). The difference between the time used in the control group and moderate shock group was 5.60±12.82secs. In the scopolamine group, it was observed that the rats used 13.80±6.22secs to locate the escape box hence when compared to the control group, the mean difference of 39.00±11.61secs was observed. So, the scopolamine group used the least time to locate the escape box when compared to the control, moderate and mild shock. This indicates that in the Barnes maze, scopolamine is a stronger inductor of spatial memory impairments. Moreover, scopolamine appears to be a more potent learning distractor when the process of classical conditioning is involved. (Malikowska, *et al.*, 2017; Malikowska, *et al.*, 2018). Therefore, the moderate shock group has the quickest response when compared to the control, mild shock and Scopolamine group. This signify that moderate shock has an impaired effect on hippocampal memory processes.

From Table 4.2; using the same cohort of animals in a relatively short period of time, the rats underwent trial 1. It was seen that the time taken for the rats to transverse to the other end of the beam was 58.60±36.94secs. The mild shock group spent 48.60±24.54secs to walk through the beam. The mean difference between the mild shock

and control group was 10.00±12.40secs. The moderate shock group spent 11.80±5.22secs to walk to the other end of the beam. When compared to the control group, the difference between the mean of the control group and moderate shock group was 48.60±31.72secs. While the Scopolamine group spent 29.20±12.19secs to reach the extreme and when compared to the control group, the difference between the mean was 29.40±24.75secx. For trial 1, it can be seen that the moderate shock group had the quickest response to walk through the beam to the other end when compared to the control, mild shock and Scopolamine group which could be as a result of the level of shock intensity used which is sufficient to elicit a quick spatial memory and learning in the animal. In summary, the result from beam walk test at P>0.005 is not statistically significant when compared to the control. This result shows that mild and moderate shock has a positive effect on the hippocampal memory and learning behaviour in beam walk test.

From Table 4.3; in trial 1, the control group spent 77.00±54.13secs to go through a puzzle of complex pathway to find their way to the other outlet. Mild shock group spent 22.20±6.07secs in getting to the other outlet through a puzzle of complex pathway. The mean difference between the mild shock and control group was 54.80±48.06secs. For moderate shock group, they spent 110.60±31.42secs in getting to the outlet this the mean difference between the moderate shock and control group was 33.60±22.71secs. But the Scopolamine group spent 97.80±32.37secs in locating the other outlet so the mean difference between the Scopolamine and control group was 20.80±21.76secs. From the results in trial 1, the mild shock group took the least time to locate the other end. At P > 0.05, the result from the navigational maze task is not statistically significant. These results suggest that cholinergic neurotransmitter system differently affects spatial memory (Hojo, & Hattori 2013) and learning behaviour on different memory phases. (Robbins & Everitt, 2008)

The following results exemplify data we have obtained using the elevated plus maze protocol which is delivered in detail above (Table 4.1 - 4.4) to investigate anti-anxiety effect of electric shock and Scopolamine when compared to the control.

It is generally believed that if an animal is afraid, it is most likely to stay at a place and make few movements. If it is not afraid, it uses the available time to mostly explore the environment and find out available means of escape and food. (Ferguson & Fasano, 2016; Ferguson & Fasano, 2018)

It was observed that the rats spent an increased time on the enclosed (semi-closed and closed) arms not regarding what was administered although there was an innate motivation to explore the other arms.

In Trials it was observed that the control group spent more time in the semi-closed and closed arms. After inducing electric shock and administering scopolamine for the other groups, the rats spent an increased time in the semi-closed and closed arms though they explored the open arms when compared with the control group. This suggest that electric shock and scopolamine increased the anxiety level of the animals. This suggest that there was an increased anxiety. Thus, electric shock and scopolamine increase anxiety-like behaviour in the elevated plus maze.

CONCLUSION

This study assessed the effects of electric shock (mild and moderate shock) and scopolamine on the hippocampal memory and learning behaviour of albino wistar rats using passive avoidance box to induce shocks after which they underwent some cognitive and spatial tasks such as Barnes maze, Beam-walk, navigational maze and elevated plus maze. The aim of the study was achieved and it was observed that electric shock (mild and moderate shock) and scopolamine can cause an increase response on the hippocampal memory and learning behaviours with the moderate shock having the highest effect on albino wistar rat.

RFERENCES

1. Fasano, S., & Brambilla, R. Ras-ERK signaling in behavior. *old questions and new perspectives. Front Behav Neuroscience*, 2011: 1–6.
2. Silingardi, D., & Angelucci, A. ERK pathway activation bidirectionally affects visual recognition memory and synaptic plasticity in the perirhinal cortex. *Front Behaviour Neuroscience*, 2012: 87-89.
3. Elvander-Tottie, E., & Addario, C. The role of 5-HT1A receptors in learning and memory. *Behaviourial Brain Receptor*, 2009: 54–77.
4. Solari, N., & Brambilla, RBiological memory in animals and in man. *Memory mass storage,*. 2011: 417–441.
5. Blum, S., & Dash, P. MAP kinase signaling in learning and memory. *Encyclopedia of neuroscience*, 2009: 657–662.
6. Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus maze for the evaluation of memory in mice: Effects of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology., 1990; 101: 27–33.
7. Carter RJ, Morton J, Dunnett SB. Motor coordination and balance in rodents. Curr Protoc Neurosci., (2001); Chapter 8.
8. Barnes CA. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J Comp Physiol Psychol., 1979; 93(1): 74–104.
9. Eagle, L., Kent, M., & Kara, H. Sensitive Assessment of Hippocampal Learning Using Temporally dissociated passive avoindance task. *Bio-Protocol*, 2016: 1-6.

10. Malikowska, N. R., Adrian, P., & Kinga, S. Phencyclidine and Scopolamine for modeling amnesia in rodents: Driect comparison with use of barnes maze test and contextual fear conditioning test in mice. *Neurotoxicity Research*, 2017: 34.
11. Malikowska, N. R., Kinga, S., & Alicja, N. Chemical Biology & Drug Design. *Short term memory in wister rats; disruption from scopolamine*, 2018: 0028-3908.
12. Robbins, T. W., & Everitt, B. J. Cholinergic neurotransmitter system differently affects spatial memory and learning behaviour on different memory phases. *Behavioral Neuroendocrinology*, 2008: 3109-3111.
13. Ferguson, & Fasano Comparative learning and memory defi cits following hippocampal and caudate lesions in mice. *Comp Physiol Psychol*, 2018: 188–194.
14. Ferguson, S., & Fasano, S. Knockout of ERK1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. *Neuropsychopharmacology*, 2016: 2660–2668.
15. Hojo, Y., & Hattori, T.. Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes. *Proc Natl Acad Sci U S A*, 2013: 865-870.