FOOTSHOCK-INDUCED STRESS EFFECTS ON MOTOR FUNCTION AND GAIT PATTERNS IN WISTAR RATS

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ABSTRACT - Exposure to unpredictable and uncontrollable conditions causes animals to perceive stress and stress hormones affect a variety of behaviors and motor functions. This research was carried out to investigate the effect of foot shock induced stress on motor functions and gait in rat using beam walking test, gait test, balance test and inverted screen test. The experimental procedures were carried out using healthy wistar albino rats which were allowed to acclimatize for one week. After acclimatization, the rats were divided into four different groups. The group one (control) were fed with water and animal feeds and did not undergo foot shock stress before carrying out experimental tests. Group two had their front and limbs shocked with a mild shock of pulse width, 0.5 msec; 100 Hz of 100 ma administered for 0.2 seconds and no blocker was administered. The third group was shocked using the passive avoidance machine and received0.3ml/100gintraperitoneal (i.p.) of Amilodipine administered and were made to perform the experimental procedure 30min after drug administration. Group four received similar shock as group two and 0.01 mg/kg intraperitoneal (i.p.) of epinephrine drug administered. The animals were allowed to undergo all experimental tasks. The results were presented as a mean value standard error of means (±s.e.m) and statistical analysis was done using the SPSS and excel. From the results obtained, shock induced stress may significantly modulate gait and motor system function.

Keywords: beam walking test, gait test, balance test and inverted screen test, foot shock.

Introduction

Stress is an organism's response to a stimulus that disturbs the physical and mental equilibrium. Stress typically describes a negative condition that can affect a person's mental and physical well-being (Wood et al, 2010; Walter and Levitsky, 1926). It is a strain or interference that disturbs the normal functioning of an organism.All somatic motor activity ultimately depends on control exerted by motor neurons in the nuclei of the cranial nerves and spinal cord. In all motor activity, the brain plans the movement, coordinates and synchronizes the contractions of different muscles involved and continuously adjusts the movement.Gait analysis is the systematic study of locomotion, using the eye and the brain of observers, augmented by instrumentation for measuring body movements, body mechanics, and the activity of the muscles (Levine *et al*, 2012; Kondziela, 1964). Gait analysis is used to assess, plan, and treat individuals with conditions affecting their ability to walk.Research evidence shows that footshock of varying intensity produces behavioral and neurochemical changes reflecting depression, anxiety, and post-traumatic stress disorder (PTSD). Animals, generally, do not habituate to footshock in comparison to other stressors.Previous studies have revealed that stress represents a major modulator of motor function (Rudorferet al, 2001; jadavji, 2008).

Materials & Methods

Experimental Animals

Twenty (20) healthy albino wistar rats, male and female, weighing between 40-60g were used in the experimental research and were housed in the animal house, College of Basic Medical Science, University of Port Harcourt, Nigeria. They were kept and maintained under good laboratory conditions of temperature, humidity and light and were constantly fed with standard rat diets and water.The experimental protocols and procedures used in this study were approved by Ethical Committee, University of Port Harcourt, Rivers State, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH Publication No. 85-93, revised 1985).

Acclimatization

After identification, the animals were housed in a wire mesh cage of standard condition for one week to acclimatize under normal temperature, humidity. They were exposed to 12hours light/dark cycle with constant access to food and water. The beddings, feed and water were hygienically handled and changed daily throughout the period of one month (31 days).

Experimental design

The experimental animals (albino wistar rats) were divided into four groups of five rats per group.

Group 1(Control): This group consists of five rats. They received normal poultry feed and water throughout the study and they were not administered with any analgesic or induced with shock.

Group 2: This group consisted of five rats. They were made to undergo foot shock using the ECT machine and received no drug administration before carrying out the experimental tests.

Group 3: This group consists of five rats. Each of these rats were induced with shock using the passive avoidance machine. They were administered 0.1ml of epinephrine drug and made to carry out the experimental tests.

Group 4: This group consisted of five rats. They were made to undergo foot shock using the ECT machine and then injected with 0.3ml/100g amlodipine drug dissolved on distilled water.

Drugs: Amilodipine and Epinephrine were purchased (NORVASC). Oral administration of dose amlodipine (0.3ml/100g), NORVASC) was dissolved in 333 ml of normal saline effectively without any residue. The animals also received either 0.01 mg/kg intraperitoneal (i.p.) epinephrine [(−) epinephrine bitartrate, Sigma Chemical, Madrid, Spain. For group three, experimental tests were carried out 30minutes after drug administration of 0.1ml epinephrine drug. For the fourth group, experimental tests were performed immediately after the injection of 0.3ml amlodipine drug.

Experimental Test: To determine the effects of stress and drug used on animals, the following experimental tests were carried out;

Beam Walking Test: The balance beam is a test of motor coordination. This test essentially examines the ability of the animal to remain upright and to walk on an elevated and relatively narrow beam (Goldstein and Davis,2006). The beam balance consists of an elevated cylindrical beam of about 30cm long on which the rat was placed. Quantification of performance for this test is determined by the time it takes the rat to walk across the beam.

Inverted Screen Test: The inverted screen is a 43 cm square of wire mesh consisting of 12 mm squares of 1 mm diameter wire. It was devised and published in 1964 by Kondziela.The inverted screen test is a test used in assessing muscle strength using all four limbs.

Balance Test: The Rotarod is designed to test balance and motor coordination. Experimental animals were placed onto a horizontal rotating rod which accelerates over the course of a trial. Trial lasts from the time the animal is placed on the rod until it falls off or until 5 minutes have elapsed. Animals can also be tested chronically to examine learning or motor degeneration.

Gait Test: The gait test is used to examine the quality of movement. The paws of the experimental animals were stained with black dye and the animals placed on plain sheets of paper. Quantification of test performance was done based on pattern and direction of movement

Stress Inducing Machines

Electro convulsive Therapy (ECT)

The ECT apparatus is devised specifically for neurochemical and neuropharmaclogical disorders. It is a psychiatric treatment in which seizures are electrically induced in patients to provide relief from psychiatric illnesses (Rudofer*et al,* 2001).

The Electro Convulsive shock was applied through bilateral clip electrodes. The shock, a square wave pulse (pulse width, 0.5 msec; 100Hz) of 100Ma, was administered for 0.2 seconds to induce shock.

Passive Avoidance Box

The passive avoidance chamber or light/dark box is partitioned into two sections, one light and one dark. As the mouse moves into the dark section a mild footshock is delivered through the floor of the chamber.

Statistical Analysis

The statistical analysis of the results obtained from the study was done using SPSS and the results were expressed as mean value Standard Error of Means (±S.E.M) and relative percent change. Results were presented in tables, charts and plates.

RESULT

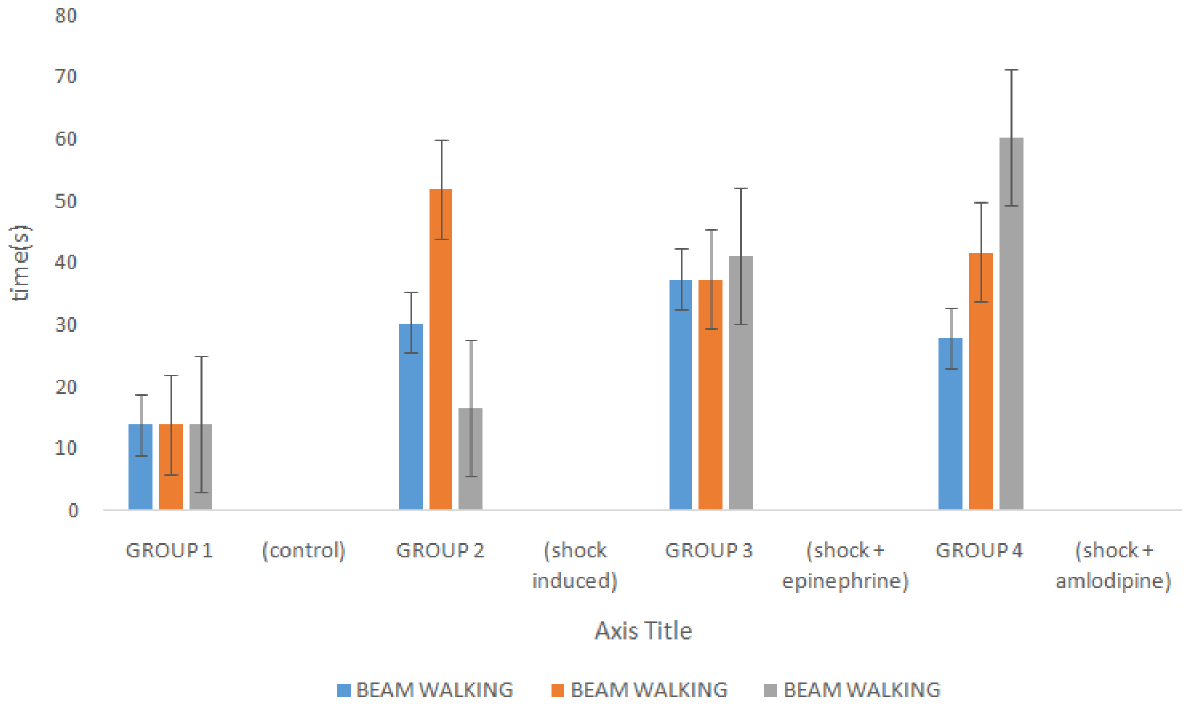


Figure 1: bars showing results from beam walking test across the test groups and the control

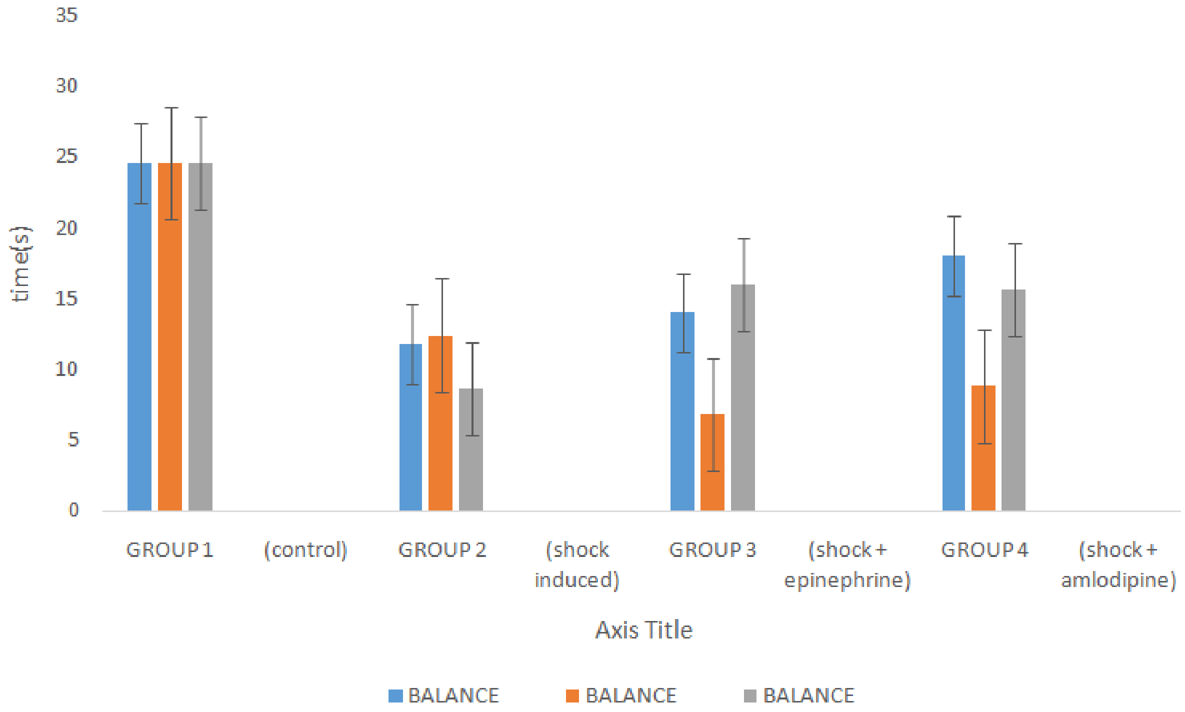


Figure 2: bars showing results from balance test across the test groups and the control

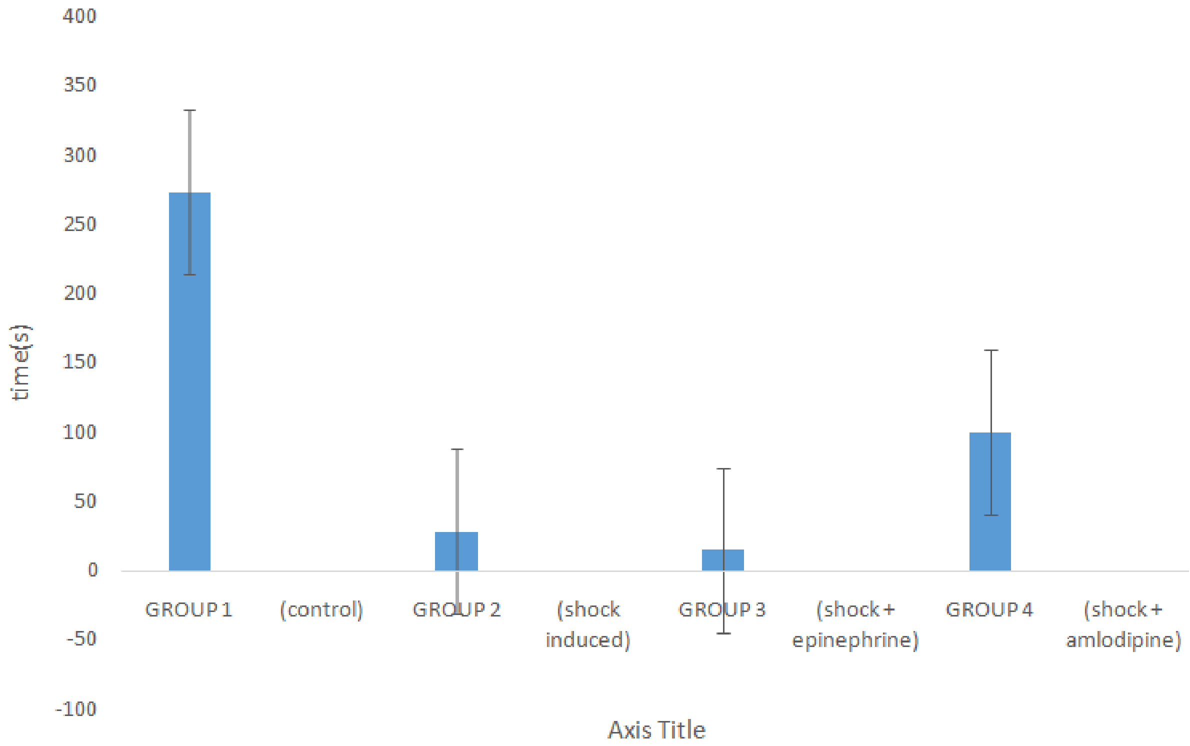
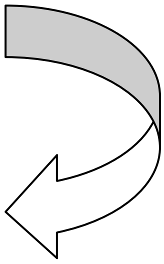
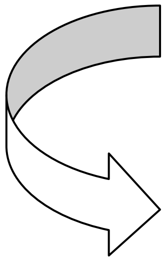
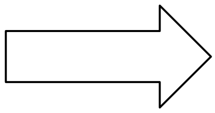
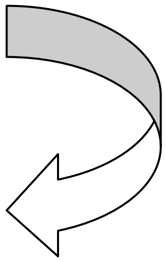
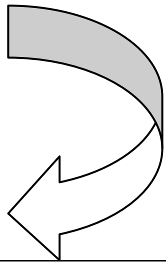
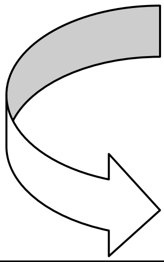
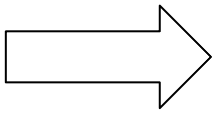
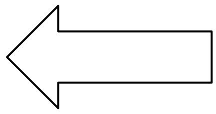
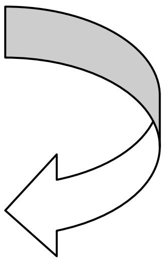
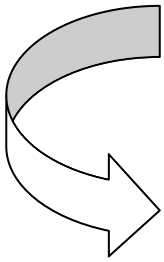
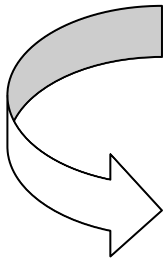
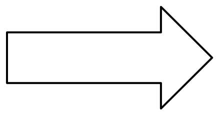


Figure 3: bars showing results from inverted screen test across the test groups and the control

Table 1: showing gait pattern result across the Test groups in comparison with the control

	Control	Group 2 (Stress Induced)	Control	Group 3 (Stress + Epinephrine)	Control	Group 4 (Stress + Amlodipine)
Trial 1	Left To Right	Right To Left	Left To Right	Right	Left To Right	Left To Right
Trial 2	Left To Right	Right	Left To Right	Right	Left To Right	Left
Trial 3	Left To Right	Right	Left To Right	Right To Left	Left To Right	Right

Table 2: Recorded arrow representation of gait pattern results in the test and control groups

	CONTROL	GROUP 2 (STRESS INDUCED)	GROUP 3 (STRESS + EPINEPHRINE	GROUP 4 (STRESS + AMLODIPINE
TRIAL 1				
TRIAL 2				
TRIAL 3				

Discussion

This study evaluated the possible effect of foot shock induced stress on gait and motor functions. The experimental procedures were carried out on wistar albino rat using the following tasks; beam walking, balance test, inverted screen and gait test. The research was conducted one group per day.

The balance beam task tests motor coordination and balance. Comparatively, the results as shown in table 1 indicated a significant (p≤0.05) change across the test and control group. Group one traversed the beam the fastest, followed by the shock induced group (group two), the epinephrine group (group three) and finally the amlodipine group (group four).According to the results, shock improved fine motor coordination and balance as reported earlier (Gomez-Lazaro et al, 2011).

From the results obtained in the mean frequency of balance as shown in table 2, significant (p≤0.05) change across the test and control groups were observed. Compared to the control group, result obtained from the fourth group (amlodipine) was higher followed by the third group (epinephrine) and finally the second group (shock). This could indicate that electric shock inhibits gross motor skills. According to weaver, 2001, gross motor skills involve the use of large muscles(Finnellet al, 2017; Wood et al, 2015). These skills include simple strength skills or skills involving symmetric movement(Goldstein& Davis, 2006).

Amlodipine improved balance and motor coordination and reduced the effect of shock. This could be due to its action as a calcium channel blocker. Amlodipine relaxes blood vessels thereby reducing blood pressure and thus decreasing stress effects.

Observations from the inverted screen test as shown in table 4.3 showed a significant (p≤0.05) change in the time it took for the study animals to grasp the screen using their forepaw and hind paw strength.

In group two, the fore and hind paw strength were revealed to be significantly (p≤0.05) affected by stress. This indicated that electric shock could disrupt normal functioning and loss of control in muscles of the limbs (Bonnie and Wen, 2012).

It was observed that both the epinephrine groups and amlodipine group performed the inverted screen task at almost same time with the group three (epinephrine) showing the least performance. This could indicate that epinephrine reduces strength of the fore and hind limbs. Early REA plasma assays indicated that epinephrine and total catecholamine rise late in exercise mostly when anaerobic metabolism commences (Perez-Tejada et al, 2013; Galbo and Kiecolt,1978). However, the result contrasts with Williams, 2010 who stated that the epinephrine pathway for stress response activates within seconds when the sympathetic branch of the autonomic nervous system carry alarm signals from the hypothalamus to the adrenal medulla which releases epinephrine. This results in involuntary use of muscles beyond limitation and voluntary use.

It was observed that foot shock and injection of epinephrine showed a significant ($p \leq 0.05$) change in gait. Based on the above results, shock affected gait. Epinephrine caused a change in gait pattern. This could be because epinephrine leads to loss of fine motor skills (Mellie and Tartakovsky, 2013). Amlodipine showed no significant ($p \leq 0.05$) effect on gait.

Conclusion

This study demonstrated a possibility of both psychological and psycho-somatic interference of electric shock on gross cognito-motor activities involving movement and balance. The study also showed the impact of shock induced stress on some motor functions and gait in rats. The results suggest that fine motor coordination and balance can be enhanced by shock and amloodipine. It also suggests that shock and epinephrine causes a change in normal gait.

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