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AN EXPERIMENTAL STUDY TO EVALUATE THE EFFECT OF INSTRUMENTAL INDIAN CLASSICAL AND WESTERN MUSIC THERAPY ON COGNITIVE AND BEHAVIORAL IMPAIRMENTSIN STRESS INDUCED YOUNG RATS.

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Background: Stress is known to induce alterations in various physiological responses leading to pathological status. Stress is also linked with memory. Long term stress leads to severe of cognitive deficits. A powerful source of auditory stimulation to the human brain is provided by music. Listening to triggers a complexsequelae of cognitive and emotional events in the brain involving distinct neural substrates.

Objectives:To compare the effect of instrumental Indian classical and western instrumental music therapy on anxiety, learning and memory of stress induced young rats.

Methods: Rats of age 30 days were stressed by Tube stress for two hours per day over ten days followed by instrumental music therapy. Four Comparative groups with six rats in each group were studied. Then, the rats were tested for cognitive and behavioral changes by Elevated Plus maze and Passive avoidance, along with age matched rats as controls.

Results: Stress significantly increases anxiety, decreases learning and memory in the rats. However, significant ($p<0.01$) improvement was observed after treatment with instrumental Indian classical music among stress induced rats.

Conclusion : Instrumental Indian classical music is a very cost effective and easily accessible remedy in relieving the negative effects of stress induced cognitive impairments.

INTRODUCTION

Stress is an integral part of life. It refers to the circumstances that place physical or psychological demands on an individual but also to the emotional reactions experienced in these situations (Hazards, 1994).Stress is ubiquitous in our society[1] and has become part of day to day life. It was defined by Hans Selye, 1936 as a pressure or strain exerted upon a material object or person) which resists these forces and attempt to maintain its original state. Stress is known to induce alterations in various physiological responses thereby leading to pathologies[2].It seems to facilitate memory formation when the learning procedure itself is arousing or emotionally valanced, or when learning takes place in a stressful context[3]. Stress is a biologically significant factor that can disturb cognitive processes such as learning and memory[4]by altering brain cell properties, and consequently limit the quality of human life[5].It is known that severe stress lasting weeks to months can impair cell communication in the learning and memory region of the brain[1]. Stress has also been linked with memory. Long term stress leads to increased severity of cognitive deficits. Excess stress can result in reduced quality of work, concentration, memory capacity etc[6].

Amongthe most powerful sources of auditory stimulation in the human brain is by music. Listening to music is a complex process for the brain since it triggers a sequelae of cognitive and emotional components[6]. That music affects the emotions is a well-known phenomenon, andmusic therapy seems to be a powerful non-invasive tool to stimulate brain systems and affective regulation involved in stress and anxiety.

Modern neuroscience has revealed the subcortical areas involved in processing of emotion and emotional disorders[7]. In particular, subcortical limbic and frontal areas of the brain are thought to malfunction in depressive states and even show morphological changes and biochemical dysfunction[7]. The present study was

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under taken to evaluate the effects of music therapy on various learning, memory as well as behavioral parameters in stress induced young rats.

MATERIALS AND METHODS

Thirty days old wistar strain albino male and female, rats were selected for this study. Experiments were carried out after obtaining Institutional Animal Ethical Committee approval (01/03/a/CPCSEA dated 25/10/2010).

The animals were maintained under 12:12 hours dark: light cycle and controlled temperature (25 ± 3°C). Animals were fed with food (Amruth feeds, standard rat pellets) and water ad libitum. All theexperiments were performed between 08:00-16:00 hrs.

Study design:Here, we used a total of 24 rats. Random method was followed to select the animals into four different groups (n = 6).

Group 1: Normal controls (NC)

Group 2: Stress controls (SC)

Group 3: Stressed rats later treated with instrumental Indian music therapy labeled as (SC+ IMT)

Group 4: Stressed rats later treated with instrumental western music therapylabeled as (SC+ WMT)

Rats in the stress group received tube stress forten days of durationtwo hours each day. Rats in group 3rd and 4th received tube stress for the same duration as in group 2nd followed by instrumental Indian classical and instrumental western classical music therapy forone hour respectively[8].

The stress protocol involved placing the ratsin a plexiglas restraining tube (23.4 cm length and 7 cm in diameter)and exposing them tohundread 1.6 mA inescapable shocks for 5 sec each,with an average interval of 60 sec. The shocks wereapplied through electrodes taped to the tail. The animals werestressed between 8:00 and 10:00 A.M. After stressor termination,theywere returned to their homecages. Cages of stressed rats wereplaced on the side of the room (14 × 12 ft) opposite to that of control animals. From days 21 to day 30 after stress induction, the rats in group 2 were tested for cognitive parameters. Similarly, rats in group 3rd and 4th were also studied for the same parameters, from day 21 to day 30after therapeutic intervention[8]. The details of the tests performed are as follows:

1. Elevated Plus Maze:

a. Anxiety Protocol: The rats were placed on the central platform with its head towardsan open arm (OA). The frequency of entries onto the open and closedarms were noted and time spent on the open arms was recordedover 5 min. The time spent in and number of entries into the open arms of the maze provides the measures of anxiety. Decrease in these measures indicates an anxiogenic effect. The number of enclosed arm (EA) entries provides the best measure of locomotor activity in this test. An arm entry was defined as all four paws entering the arm and an arm exit was defined as two paws leaving the arm.

b. Learning protocol: A midline is drawn, so as to divide the closed arm into two equal parts. The rats were placed at the end of an open arm and allowed to explore for 90 seconds. The time for the rat to cross a linehalf way along one of the closed arms was measured (transferlatency) on day 1 and the same procedure is repeated on the day 2 as well. The rat has to have its bodyand four paws on the other side of the midline for it to be considered or having crossed midline. If it didnot cross the line after 90 sec, it was placed beyond midline on the closed arm manually and transfer latency was recorded as 90 sec.After crossing the line, the rat was allowed to spend 30 sec for exploringthe apparatus. Learning was defined as reduced transfer latencyon day 2 compared to day 1. So the normal rats usually take less time to cross the half way midline on closed arm as compared to time taken on day one.

2. Passive avoidance test

The two compartment passive avoidance apparatus was used to assess the associative learning and memory. Essentially the apparatus consists of a square box with a

floor grid of 50 x 50 cm and wooden walls of 35 cm height. A 100 watts bulb illuminates this box. In the center of one of the walls is an opening of 6 × 6 cm which can be opened or closed using a transparent glass sliding door. This opening leads to a smaller (15 × 15 X 15 cm), dark compartment provided with an electrified grid floor that can be connected to a shock source (stimulator). Animals were placed individually in an illuminated chamber facing away and at the farthest distance from the entrance to the dark compartment. On the first day of the test, each rat was allowed to explore both the compartments for 5 min. On the second day, time taken by the rats to enter the dark compartment for the first time was noted and soon two learning sessions followed. At the end of the third day one, as soon as the rat entered the dark compartment, the door was closed and three inescapable foot shocks (50V, 50 Hz, 1sec) were given. Then the animal was returned to its home cage. A day later each animal was placed again in the passive avoidance apparatus as before for a maximum period of 5 min. The latency time required for the animal to enter the dark compartment was measured. Increased latency/absence of entry into the dark compartment indicated positive memory retention[9-14].

STATISTICAL ANALYSIS

The comparisons between groups werecarried out by student's unpaired 'T' test except for neuro-cognitive tests which were analyzed by Bonferroni Multiple comparison test. Differences were considered to be significant at probability value (p) < 0.05.

RESULTS

In the present study, we used a total of24 rats with random method followed to select animals in to four different groups as mentioned above.

A summary of the performance of the experimental animals in the elevated plus maze (EPM) in each group is shown in Table 1.During the test, anxiety protocol showed that the stressed rats spent lesser time in the open arm (OA) and showed less number of entries, when compared to that of normal rats. Statistically significant difference was observed in number of entries in to OA (P = 0.000) between normal control and stress control and the time spent in OA (P = 0.019) between SC+IMT and SC+WMT. Significant difference was also observed in other behavioral (ethological) parameters such as rearing, grooming and number of excreta boli in between NC and SC(P = 0.024),and SC with IMT groups (P = 0.000) but significant difference with WMT therapy.

The learning protocolshows the significant difference in the transfer latencies during the day 1 learning trial session in NC versus IMT (P = 0.000) and NC with WMT (P = 0.000) and SC compared to therapeutic groups *(P* = 0.000). Also there was significant difference in the transfer latencies on day 2 memory retention trial in NC with SC (P = 0.033) and NC with IMT (P = 0.033). Statistically significant difference is observed in transfer latencies on both day 1 and day 2 among animals in NC compared to animals with SC and therapeutic groups shown in Table 2.

In the passive avoidance box (Table 3), 24 hours after the stimuli, retention of memory was significantly different in normal control and stress control group. (P=0.033) and also NC compared to IMT group (P=0.033), and even there was significant difference between SC and therapeutic groups, which showed the reduced transfer latency. Furthermore, significant difference was observed in the transfer latency among the stressed rats with IMT and WMT groups(P=0.964).

Table 1: Effect of Instrumental Indian classical and western music therapy on anxiety levels in normal control and Stress induced young rats in elevated plus maze (n = 6 in each group).

Group	Number of entries		Time spent in OA	Ethological parameters		
	Enclosed arm (EA)	Open arm (OA)	In seconds	Rearing	Grooming	No. of excreta boli
Normal control	4.5 ± 1.05	2.8 ± 0.75	12 ± 3.77	5.1 ± 1.72	2.6 ± 3.77	1.5 ± 0.83
Stress control	7.3 ± 1.05	2.5 ± 1.37	5.2 ± 12.25	6.6 ± 1.36	4.1 ± 1.6	3.3± 1.77
SC with Instrumental classical IMT	8.1± 1.83	4 ± 1.41	20 ± 4.29	5.3 ± 1.36	3 ± 0.89	1.1 ± 0.98
SC with instrumental WMT	6 ± 1.26	2 ± 0.63	12.8 ± 2.56	3 ± 0.63	1 ± 0.63	2.5 ± 0.83
P value	0.003*	0.33*	0.000*	0.001*	0.000*	0.004*
F value	6.48	3.562	10.81	7.786	12.698	6.072

a) NC with SC shows significant value 0.000.

b) NC with IM shows significant value 0.011

c) SC with IM shows significant value 0.000.

d) WM with IM shows significant value 0.019.

(Mann-Whitney non-parametric test is used for statistical analysis)

Table 2: Effect of Instrumental Indian classical and western music therapy on learning and memory in normal control and Stress induced young rats in elevated plus maze (n = 6 in each group).

Group	Transfer latency (in sec.)	
	Day 1	Day 2
Normal control	64 ± 12.65	26 ± 7.54
Stress control	72.61 ± 19.85	44.6 ± 18.93
SC with IM	18.5 ± 6.80	7.6 ± 1.63
SC with WM	27.8 ± 9.68	7.7 ± 1.63
P value	0.000*	0.000*
F value	24.135	14.264

a) NC with SC shows significant value 0.033.

b) NC with IM shows significant value 0.011.

c) SC with IM shows significant value 0.001.

d) SC with WM shows significant value 0.001.

e) IM with WM shows significant value 0.964.

(Mann-Whitney non-parametric test is used for statistical analysis)

Table 3: Effect of Instrumental Indian classical and western music therapy on memory retention in passive avoidance (n = 6 in each group).

Group	Time taken to enter small compartment (sec)
	Day 3 - Transfer latency
Normal control	183 ± 64.05
Stress control	19.5 ± 5.01
SC with IM	29.1 ± 3.65
SC with WM	14.5 ± 2.82
P value	0.000*
F value	38.222

a) NC with SC shows significant value 0.000.

b) NC with IM and WM shows significant value 0.000.

c) SC with IM shows significant value 0.000.

d) SC with IM & WM shows significant value 0.000.

DISCUSSION

Music is one of the greatest forcesuniting humanity.In today's world reaching full of conflict and tension, music plays a significant role towards reaching inner peace and harmony.It is also known to be great healer and has influence both direct and indirect on physical and mental health of an individual. Of late, music therapy is being used as an adjunct to medical line of treatment.

In the present study we have evaluated the effect of the music therapy on stress induced anxiety, learning deficits and memory deficits in rats.Stress impairsthe spatial learning and memory.In our study the stressed animals showed memory impairment, which was evident in the Elevated Plus Maze test and Passive Avoidance test. The novel finding of this study was that music therapy during the post-stress stage can enhancecognitive and behavioral recovery and decreases anxiety.

Music, by its very nature, has strong connections to both attentionand memory systems. Brain imaging studies have shown that listeningto polyphonic music calls for rule-based analysis and combinationof sound patterns from multiple auditory streams, which naturallyrecruits bilateral temporal, frontal and parietal neural circuitsunderlying multiple forms of attention, working memory, semanticand syntactic processing and imagery[15,16]. Recent evidence suggests that listeningto music that is enjoyable temporarily improves performance in tests of spatial-temporal attention, verbal fluency and creativityin healthy subjects[17-19]. Other studies reveal that auditorystimulation by music also temporarily improves performancein tests of autobiographical recall in dementia patients and in tests of visual neglect in strokepatients[20-21]. Listening to music of their choice has been shown to reduce pre-operative anxiety in ambulatory surgery patients[22].

In healthy subjectsas well as multiple sclerosis patients,verbal material presented in a musical modality

is learned and retrieved more efficiently thanone presented verbally[23-24]. Randomized controlled trials havealso shown that active music therapy or music-based exerciseimproves general cognition and verbal fluency in patients with dementia and symptom scores in schizophreniapatients and also improves the communication skills inautistic children[25-27]. Similar studies thathave used a within-subject design have also shown that musicimproves phonological and spelling skills in dyslexic children, speech content and fluency in dementia patientsand verbal fluency in cardiac rehabilitationpatients[28-30]. Collectively, these findingsprovide evidence that music engages and facilitates a wide rangeof cognitive functions. Moreover, listening music was associated with less depressedand confused mood, suggesting that music may help to cope withthe emotional stress brought about by sudden and severe neurologicalillness[6].

Listening to pleasant, relaxing musicenhances the recovery of cardiovascular and respiratoryfunctions and decreases cortisol levels after exposur to stress[31-33]. Music therapyhas been shown to reduce anxiety and depression in patientswith a somatic illness and also in neurological patients[34-35]. Music is also closely linked to emotions and arousal. Evidencesuggests that listening to music modulates emotional arousal asindexed by changes in electrodermal, cardiovascular and respiratoryactivity[36-38]. These findings suggest that music has an effect in reducing anxiety and directing attentionaway from the negative experience, thus helping to cope withemotional stress.

CONCLUSION

Music has profound impact upon the human brain with subsequent effects on mental, physical and spiritual health. Listening to music can facilitate a wide variety ofcognitive and emotional functions. Instrumental Indianclassical music is a very cost effective and easily accessible remedy in relieving the negative effects of stress induced cognitive and behavioral impairments and even helps in decrease of anxiety levels.

LIMITATIONS

An important and difficult question still pertains to the neuralmechanisms that can account for the beneficial effect of musicon cognition.

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