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Non-invasive and non-chemical method of stimulating the brain and inducing hypermotility in normal Wistar rats

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Abstract

The effect of diffuse transcranial electrical stimulation (DTES) on motility was investigated in healthy male and female Wistar rats. Diffuse transcranial electrical stimulation (5V, frequency 100Hz, pulse width 1ms), was delivered with the aid of ear clip electrodes, while locomotor activity was measured with the aid of motility counter for 10 seconds before, during and after stimulation. Diffuse transcranial electrical stimulation was found to induce reproducible hypermotility in the rats. Locomotor activity in normal rats, measured for 10 seconds, averaged 10.1±4.1 before stimulation. The activity significantly ($p<0.001$) increased to 81.8±13.5 during first stimulation and significantly ($p<0.05$) dropped after stimulation. When stimulation was repeated 30 minutes later, motility count was insignificantly ($P>0.05$) lower than the former, but followed the same course as the later. Locomotor activity after DTES did not significantly ($p>0.05$) vary from the values obtained before and after electrical stimulation. These results suggest that diffuse transcranial electrical stimulation produces reproducible hyper-motility which is inhibited by diazepam in normal conscious rats. DTES may serve as a simple method of stimulating the CNS and increasing its levels of catecholamines. The inhibition by diazepam further shows that brain catecholamines are raised during stimulation.

Keywords: *Hypermotility, Noradrenergic pathway, Diazepam, GABA Receptors*

Introduction

Diffuse transcranial electrical stimulation (DTES) of the brain of an animal has been suggested as a non-invasive method of stimulating the whole brain of an animal (Osunkwo, *et al.*, 1994). Electrical stimulation of the various centers causes emotional stress-like behavior. DTES as with emotional stress activates higher brain centers and is thought to increase sympathetic outflow to the periphery (Osunkwo *et al.*, 1994). This in turn induces hyperactivity causing restlessness.

Selected areas of the brain (like amygdala and locus coerulus) have been stimulated with a resultant cardio-acceleration and arterial hypertension (Bunag *et al.*, 1975). These workers effected electrical stimulation of discrete brain regions by rupturing the skin, skull and even brain tissue to insert stimulating electrode. The injury inflicted can alter the desired result and thus give false results. Low voltage electric current (through the whole body or specifically the brain) has been used as a form of therapy in the

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management of affective disturbances (anxiety, depression) and in alcoholic patients (Kruptsky *et al.,* 1991). Osunkwo *et al.,* (1994) reported that diffuse transcranial electrical stimulation increases sympathetic outflow and arterial blood pressure in anaesthetized Wistar rats. The increase in sympathetic outflow could be influenced by humoral mechanisms, by intrinsic activity or by electrical stimulation of the brain. Electrical stimulation of the brain modifies levels of humoral activity in the brain to bring about some somatic manifestations (Bunag *et al.,* 1975).

Passage of low DC electric current through various parts of the brain increases the catecholamine levels in the portion of the brain concerned (Hollister, 1971). This in turn may lead to the stimulation of the postsynaptic cell membrane and increase in the activity of the sympathetic out flow to the periphery (Bunag *et al.,* 1975). Diazepam decreases the electrochemical signal for nor-adrenaline (NA) by acting on GABAergic receptors. Diazepam has been reported to also decrease 5-Hydroxytryptamine 5-HT levels in the brain. The most common mechanism of action for diazepam is augmentation of sympathetic nerve inhibition by activation of GABA (gamma amino-butyric acid) mechanisms. Diazepam is used as anxiolytic and anti-panic agents. Diazepam is also useful in the management of Alzheimer's disjoins to treat anxiety, agitation, insomnia, and other behavioral symptoms that frequently accompany these disease states (Alan *et al.,* 1986).

The objective of this study is to find out the effect of diffuse transcranial electrical stimulation on locomotor activity of the conscious parts and to ascertain the effect of some drugs.

Experimental

Materials and Method: Adult male and female Wistar rats (200-250g) inbred in the animal Production Unit of the National Veterinary Research Institute, Vom were used for these experiments. The animals had access to water and feed (24% protein: Pfizer Products, Lagos, Nigeria) *ad libitum*. The rats were housed at 25±2° C and acclimatized on approximately 12 hours light and 12 hours dark cycle in the animal house. They were randomly selected from colony of Male and Female rats.

Delivery of DTES: Diffuse trans-cranial electrical stimulation was delivered via two steel electrodes clipped one on the left and the other on right ear lobe of the rat. Electrical stimulation (5-10V, frequency 90Hz, pulse width 1 ms) was delivered for duration of 10 seconds using a CFP stimulator, model 8048 (C. F. Palmer, London, UK.).

Male and female Wistar rats were divided into groups of ten rats each. Rats from group 1were isolated and placed in the stimulating chamber (individually) and allowed 30 minutes to acclimatize before the experiments. Locomotor activity of the rats in group 1 were recorded with the help of the motility counter (Maurel-Reny, *et al.,* 1995) , 10s before, 10s during and 10s after electrical stimulation. The same procedure was repeated for the same rats 30 minutes after the first stimulation and the effect noted. The rats in groups II and III were pretreated with Diazepam (Pfizer, Switzerland) 0.35mg and 0.45mg/100g respectively, intra-peritoneally 30min prior to the administration of diffuse transcranial electrical stimulation. DTES was delivered and motility count noted.

Results are presented as means ± SEM and were analyzed using Student's t-test. *P* values less than 0.05 were considered significant (Snedecor and Cochran. 1967)

Activity counter: The locomotive activity of the rats was recorded on the motility counter (40Fc, Motron products, Stockholm. Sweden, Longoni *et al.,* 1987). The motility counter counts the number of movements of an

experimental animal. It consists of Photocells (connected to electronic meter) on which the infrared rays from infrared bulb positioned few centimeters from the photocells shines constantly. The photocells are connected to a meter, which counts or reads when any of the photocells are blocked from the infrared rays. These photocells are evenly distributed on the board and the total number of movements by the animal can be read from the meter. The Motility counter is normally placed in a cupboard with a small window (which allowed for observation of the animal) to prevent interference from light in the laboratory. Locomotor activity (which is displayed on the meter) is quantified by totaling the interruptions of the photocells from the infrared rays. Maurel–Reny *et al.,* (1995) used a similar activity counter to study the effect of clozapine and compound MDL 100907 on hyper-motility induced by phencyclidine.

Results

Preliminary experiments were carried out to determine the suitable voltages for brain stimulation. If found that between 5V to 25V produced reproducible actions and activity on the rats. 5V -10V were then chosen for these experiments.

Effect of DTES on the activity of normal Wistar rats.

Locomotor activity in normal rats, measured for 10 seconds, averaged 10 ± 4.1. When the rats were receiving 10sec. DTES, it significantly ($p < 0.001$) increased to 81.8 ± 13.5. Locomotor activity for 10 sec. at the end of DTES, did not significantly ($p > 0.05$) increase from the values obtained before electrical stimulation but when compared with values during stimulation, there was a marked ($p < 0.001$) fall (Table 1,). Thus, DTES induces hypermotility in normal rats.

Effect of repeated DTES (30sec later) on the locomotor activity of normal Wistar rats.

Table 2, shows that DTES repeated after 30 minutes in the same group of rats reproduced the increased motility count earlier observed in (Table 1,), though the degree of increase in hypermotility was lower than the former (Figure 1). Therefore, diffuse transcranial electrical stimulation induces reproducible hypermotility in normal conscious rats.

Effect of Diazepam (0.35mg/100g) *on DTES-induced hypermotility.*

In diazepam (0.35mg/100g) pretreated Wistar rats, it was observed that the motility counts before DTES were significantly ($p < 0.05$) lower than that of the non-pretreated (control) rats. The value during stimulation in pretreated rats was significantly lower than values in the control rats ($P < 0.001$) (figure 4), but significantly ($P < 0.001$) higher than value before DTES in pretreated rats. It was also observed that After DTES the motility count significantly ($P < 0.001$) dropped to 13.38 ± 2.3 which is significantly ($P < 0.05$) higher than the values before DTES but significantly ($P < 0.001$) lower than the values during DTES (Table 3).

Effect of higher dose of diazepam (0.45mg/100g) on DTES-induced hypermotility.

The results obtained when the dose of diazepam was increased to 0.45mg/100g was the same as the one obtained with lower dose except that the motility count during stimulation for 0.45mg/100g was lower than that of 0.35mg/100g. (Table 3)

Discussion

The results from this study have shown that Wistar rats are activated in response to diffuse transcranial electrical stimulation. This activation manifested as increase in locomotor activity of the rats. The rats were observed to be restless during stimulation. These observations mimic the behaviour of animals under stress, and suggest that DTES can be used as a model of stress. Stress has been attributed to the

increased turnover of catecholamines in the brain, which can affect the psychological functioning of the body. For example, it can lead to the development of High blood pressure. Osunkwo *et al.,* reported that DTES causes an elevation in the blood pressure of anaesthetized Wistar rats. In man, the most common cause of hypertension is stress, which is sometimes associated with restlessness.

In this study, it was observed that DTES induces reproducible hypermotility in the rats. Hypermotility is a sign of restlessness in man. It was also observed that the motility count for repeated exposure was lower than the count for first exposure. This may have occurred because of depletion of the stores of noradrenaline during the first exposure. Diazepam, a central acting GABA enhancer was observed to reproducibly attenuate DTES induced hyper motility. Diazepam acts by the augmentation of inhibition by activation of GABA (Gamma-amino butyric acid) mechanisms. GABA is the most common inhibitory neurotransmitter in the brain. GABA binds to at least two subtypes of receptors, $GABA_A$ and $GABA_B$. Activation of either type results in inhibitory postsynaptic potentials (IPSPs), but the receptors have different physiologic characteristics and locations. Drugs that activate GABA mechanisms bind to $GABA_A$ receptors or other receptors that are closely related to GABA receptor. $GABA_A$ receptors are postsynaptic inotropic receptors that are directly linked to chloride channels that shunt current through the membrane, resulting in hyper polarization of the postsynaptic cell. Diazepam has binding sites on the $GABA_A$ receptor that are separate from the GABA binding site. Binding of benzodiazepines to their receptors results in frequent opening of the channel. (Twyman *et al.,* 1989). The net result of GABA activation is to prevent noradrenergic neuronal cells from depolarization and thus stop or decrease neuronal discharge.

Table 1: Effect of DTES (5V) on locomotor activity of normal rats. (Contact time =10s, n =10; Data is presented as mean ± S.E.M)

Duration of exposure	No. of rats	Motility Count/10 s	% Increase in motility	P Values
Before DTES (10 sec)	10	10 ± 4.1	0	
During DTES (10 sec)	10	81 ± 3.5	709.9	< 0.001*
After DTES (10 sec)	10	21.6 ± 6.8	114	NS* < 0.001**

* When compared with values obtained before DTES; ** When compared with values for obtained during DTES

Table 2: Effects of first and repeated exposure (30 minutes later) DTES (5V) on locomotor activity of normal rats. (Data is presented as mean ± S.E.M)

Duration of exposure	No. of rats	Motility count	% Increase in motility	P Values
Before DTES (10 sec)	10	21.3 ± 7.2	0	
During DTES (10 sec)	10	61.3 ± 11.5	187	< 0.05*
After DTES (10 sec)	10	21.3 ± 8.8		NS* < 0.001**

* When compared with values obtained before DTES; ** When compared with values for obtained during DTES

Table 3: Effect of diazepam (0.35mg/100g) on DTES (5V) induced hypermotility. (Data is presented as mean ± S.E.M)

Duration of exposure	No. of rats	Motility count	% Increase in motility	P Values
Before DTES (10 sec)	8	5.62 ± 1.9	0	
During DTES (10 sec)	8	30.75 ± 3.4	446.7	< 0.001*
After DTES (10 sec)	8	13.38 ± 2.3	137.8	< 0.05* < 0.001**

* When compared with values obtained before DTES; ** When compared with values for obtained during DTES

Table 4: Effect of diazepam (0.45mg/100g) on diffuse transcranial electrical stimulation (5v) induced hypermotility. (Data is presented as mean ± S.E.M)

Duration of exposure	Number of rats	Motility count	% Increase in motility	P Values
Before DTES (10 sec)	6	5.55 ± 1.9	0	
During DTES (10 sec)	6	20.5 ± 3.4	369.4	< 0.001*
After DTES (10 sec)	6	14.1 ± 2.3	254.1	< 0.01* < 0.001**

* When compared with values obtained before DTES; ** When compared with values for obtained during DTES

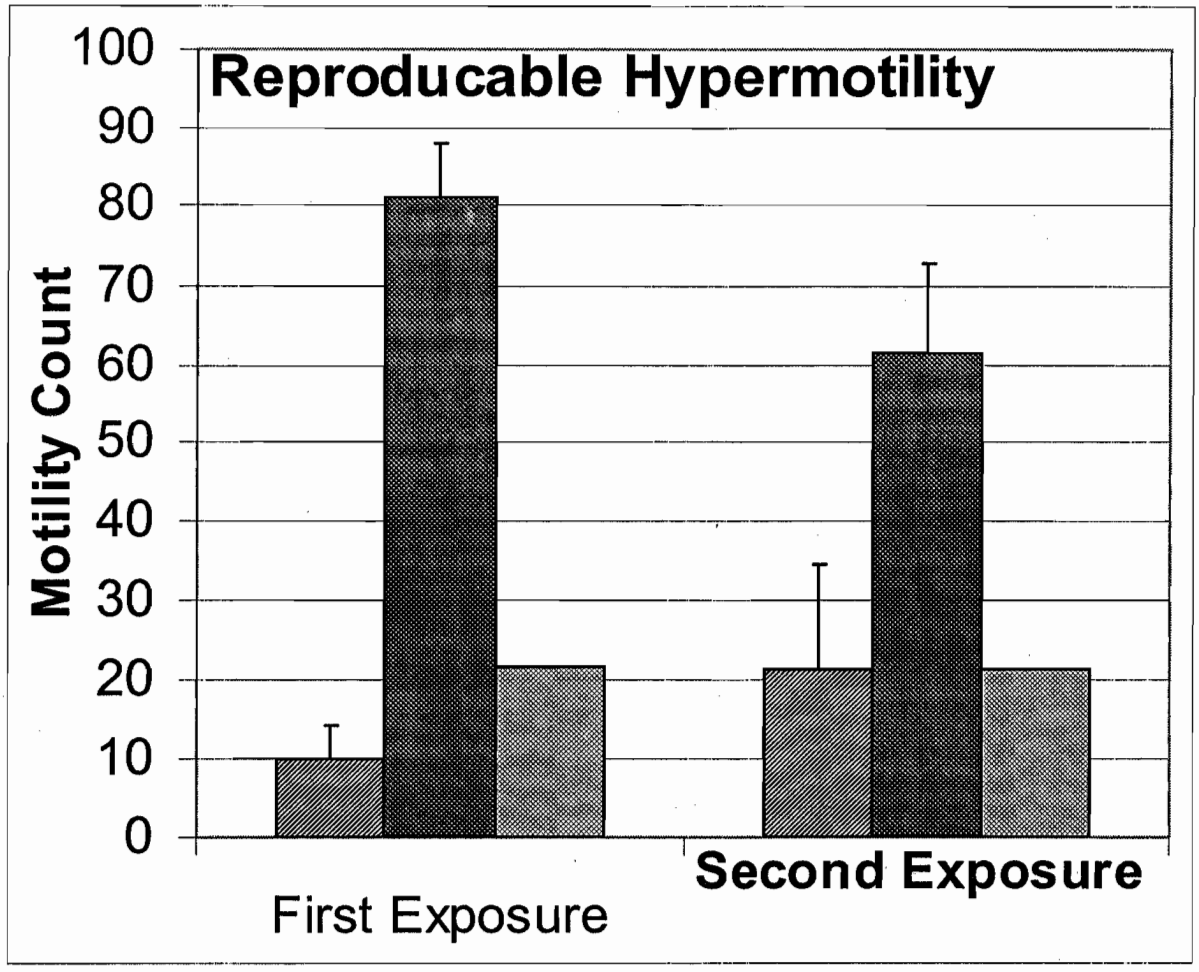

Figure 1: A comparison of the motility count for conscious normal Wistar rats before ( ), during ( ) and after ( ) first and repeated Exposure (30 minutes later) to diffuse transcranial electrical stimulation.

These results may further explain why benzodiazepines are useful in the management of stress related health conditions and in the treatment of stress-induced hypertension. Diazepam is used as anxiolytic and antipanic agent. Diazepam has been prescribed for people with Alzheimer's disease to treat anxiety, agitation, insomnia, and other behavioral symptoms that frequently accompany the disease. (Alan *et al.*, 1986).

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