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# BRIEF COMMUNICATION

# Inhibitory Effect of Midbrain Raphe Stimulation on the Maintenance of an Active Avoidance Reflex

GÁBOR L. KOVÁCS AND GYULA TELEGDY

*Department of Pathophysiology, University Medical School, Szeged, Hungary*

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KOVÁCS, G. L. AND G. TELEGDY. *Inhibitory effect of midbrain raphe stimulation on the maintenance of an active avoidance reflex.* PHARMAC. BIOCHEM. BEHAV. 5(6) 709–711, 1976. – Performance of an active avoidance (bench-jumping) reflex has been studied in rats during stimulation of the midbrain raphe nuclei. Raphe stimulation (10 cps, 0.2 msec, 2.5–5.0 V) inhibited the performance of the reflex. A serotonin receptor blocker (methysergide, 2.0 mg/kg IP) increased the reflex performance in non-stimulated animals and prevented the action of raphe stimulation. The data indicate that the cerebral serotoninergic system might have an inhibitory control over the performance of conditioned avoidance reflex.

Midbrain raphe stimulation  Methysergide  Conditioned avoidance reflex

MANY attempts have been made to define the substrates of behavioral arousal and inhibition on a neurochemical and humoral level. There is a great deal of evidences that the cerebral serotoninergic system, which has a well- defined neuroanatomical basis, might be involved in the neuronal control of different central nervous processes. The serotoninergic perikarya, originating from the B 7 (nucleus raphe dorsalis), B 8 (nucleus raphe medianus) and B 9 areas of the midbrain [3], project to various cortical and subcortical sites like hypothalamus, mesencephalon, hippocampus and lateral ventricle [1, 4, 8, 9, 13, 22, 25].

Electrical stimulation of the serotoninergic cell bodies at the raphe level has been shown to result in an increased serotonin (5-HT) turnover and release [2, 15, 26, 28]. Raphe stimulation, by increasing the serotoninergic neuronal activity, inhibited the activity of brain stem reticular neurons [24], induced sleep [15], inhibited the cortical evoked potentials to electrical stimulation of sensory nerves [27] and diminished the stress-induced activation of the hypothalamo-pituitary-adrenocortical axis [20].

The behavioral action of raphe stimulation, however, remains to be further elucidated. In cats, either no effect [10, 21, 26] or a mild arousal [10] was found after stimulation of the raphe system with different stimulation frequencies. In rats, Kostowski, *et al.* [15] described a close relationship between the biochemical, bioelectric and behavioral correlates of raphe stimulation. The stimulated animals showed behavioral signs of calmness. Sheard and Aghajanian [28], on the other hand, observed a lack of habituation to external stimuli in raphe stimulated rats.

The present study was undertaken to investigate the effect of midbrain raphe stimulation on the maintenance of previously trained active avoidance reflex.

## METHOD

Twenty-two adult male albino rats of an inbred CFY Strain, weighing 200–250 g at the beginning of the experiments were used. The animals were kept on standard laboratory diet with drinking water ad lib. Standard illumination schedule of 12 hr light and 12 hr dark (lights on at 6.00 a.m.) was used. The experiments were performed daily between 10.00 a.m. and 2.00 p.m.

### *Behavioral Methods*

Avoidance behavior was investigated in a bench-jumping apparatus described in detail earlier [19,30]. The rats were trained to avoid electric footshock by jumping on a bench during the 5 sec of conditioning stimulus, being the light of a 45 W bulb. The lack of performance during this time was associated with the unconditional stimulus in the next five sec, which were electric footshocks of 1.0 mA, 180 V a.c. Thirty trials were given daily, in a fixed intertrial interval of 60 sec (range: 50–70 sec). After the animals have met the criterion of learning – over 80% conditioned avoidance responses for three consecutive days – the rats were subjected to surgical implantation of electrodes.

After a postoperative period of 7 days, the animals were retrained, until a stabilized reflex performance (between

70–80%) was obtained. The animals were then subjected to different treatments of the following sequence: (a) the performance was tested for two days, with the stimulating wire on head, however, without being stimulated (control period); (b) the animals were stimulated (2.5–5.0 V square waves of 0.2 msec impulse duration, 10 cps) during the 30 min of the behavioral session; (c) methysergide (Deseryl, Sandoz) was injected IP in a dose of 2.0 mg/kg. Ninety min later the reflex performance was tested without stimulation; and, (d) after methysergide treatment, the animals were stimulated during the whole session.

*Electrode Implantation*

The animals were anesthetized with pentobarbital (Nembutal, 'Abbott', 40 mg/kg) and bipolar stainless steel electrodes, insulated with enamel except for the 0.5 mm tip, were stereotaxically implanted into the midbrain raphe nuclei (nucleus raphe medianus or dorsalis) or in the mesencephalic reticular formation. The stereotaxic coordinates of Fifková and Marsala [7] were used.

*Histology*

For histological verification of the electrode tips, the carotid artery was perfused with a 10 percent formol solution containing 3 percent potassium ferrocyanide. The Prussian-blue spots, caused by the iron deposits were controlled on haematoxylin-eosin stained histological sections.

For statistical evaluation of the data analysis of variance was used. A probability level of 0.05 or less was accepted as a significant difference.

## RESULTS

The results are summarized on Fig. 1. Midbrain raphe stimulation significantly ($p < 0.001$ vs control) decreased the performance of the active avoidance reflex. Methysergide treatment in a dose of 2.0 mg/kg increased the performance of avoidance reflex in the nonstimulated animals ($p < 0.05$ vs control) and prevented the effect of raphe stimulation ($p < 0.001$ vs raphe stimulation). Stimulation of the mesencephalic reticular formation, on the other hand, resulted in an increased reflex performance ($p < 0.05$ vs control).

## DISCUSSION

Midbrain raphe stimulation, a procedure which has been shown to increase the activity of central serotoninergic system [2, 15, 28] inhibited the performance of active avoidance reflex. The blockade of serotoninergic receptors by methysergide treatment, on the other hand, resulted in an increased reflex activity. The data seem to exclude the possibility that the effect of raphe stimulation was brought about by changing (facilitating or interrupting) the ongoing neuronal activity in interconnected structures in the vicinity of stimulating electrodes [6,14], since the stimulation of the mesencephalic reticular formation, adjacent to the raphe region, failed to inhibit the reflex activity.

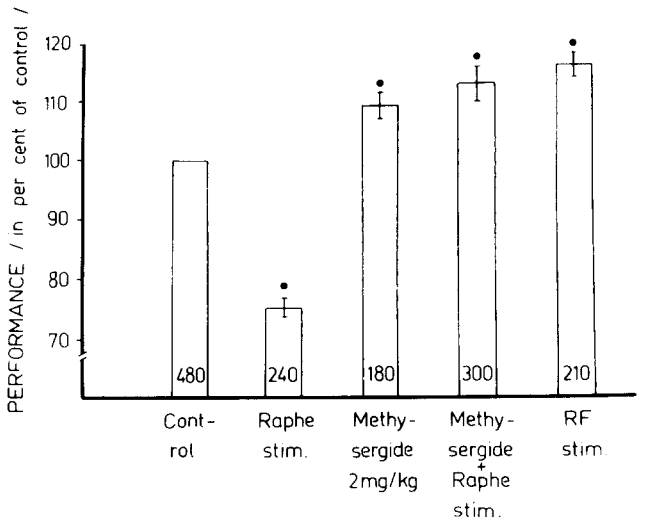


FIG.1. Effect of midbrain raphe stimulation on the maintenance of conditioned avoidance behavior in normal and methysergide pretreated animals. Abbreviation: RF – mesencephalic reticular formation (The vertical lines on the bars represent the standard error of mean, the points show statistacally significant differences).

Failure of raphe stimulation to inhibit the reflex activity after methysergide treatment support that the raphe stimulation-induced behavioral changes were due to the increased release and turnover of 5–HT.

The results confirm the hypothesis that the performance of a fear-motivated avoidance reflex is under the control of cerebral serotoninergic system. Increased activity of this transmitter system resulted in behavioral inhibition [17, 18, 23 etc.], while the impaired serotoninergic activity has a facilitatory effect of behavior [5, 11, 12, 16, 19, 29].

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