Efficacy of Mediodorsal Thalamic Nucleus Stimulation in a Rat Model of Cortical Seizure

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https://doi.org/10.15017/15638

出版情報：福岡醫學雜誌. 100 (8), pp.274-280, 2009-08-25. 福岡医学会
バージョン：
権利関係：

Original Article

Efficacy of Mediodorsal Thalamic Nucleus Stimulation in a Rat Model of Cortical Seizure

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Abstract **Objective** The mediodorsal thalamic nucleus (MD) has strong connectivity to the limbic systems and frontal lobe. The aim of this study was to determine whether the mediodorsal thalamic nucleus influences seizure induction by electrical stimulation in the rat frontal cortex.

Materials and Methods Juvenile male rats (n = 7) were used in the experiment. At postnatal day 28 (P28), stainless steel skull screws were inserted for stimulating cortices. Depth-stimulating electrodes were stereotaxically inserted into the MD of the bilateral thalamus. Electrical stimulation, which produces afterdischarges, was applied to the frontal cortices once/day for four consecutive days from postnatal day 38. Additional conditioning electrical stimulation to the bilateral MD was fixed at 0.1 mA, and the stimulus frequency was increased from 0 Hz, 1 Hz, and 5 Hz to 10 Hz. All seven rats were stimulated with those frequencies by rotation every other day. The data of animals with 0Hz MD stimulation were defined as controls. Afterdischarge thresholds and durations were measured at each frequency.

Results Seizures were accompanied by bilateral tonic-clonic convulsions and cortical spikes. Seizure behaviors were not different among the groups and there were no statistically significant differences in the cortical afterdischarge (AD) thresholds between animals with MD stimulations and controls in all frequencies. In the cortical AD durations, there were also no statistically significant differences between animals with MD stimulation and controls although the mean duration at 10-Hz stimulation was less than the control animals.

Conclusion These results indicated that thalamic MD stimulation might not suppress epileptic seizure induction. Further studies are needed to analyze other stimulus parameters, altered stimulus locations, and different test paradigms.

Introduction

The thalamic nuclei have been shown to be involved in seizure development and expansion in several studies[1)~6)]. It is well established that thalamo-cortical systems participate in the generation of pathological discharges, such as 3-Hz spike and wave complexes in absence epilepsy. MRI and PET studies recently demonstrated hemodynamic changes within thalamic nuclei associated with ictal activity[7)~10)], suggesting that this area could be an important structure for propagating epileptic discharges and inducing status epilepticus.

Intractable epilepsy is not easily controlled with antiepileptic drugs and is not considered appropriate for resective surgery. Electrical stimulation of deep brain structures (deep brain stimulation; DBS) for epilepsy treatment represents an alternative approach[11)~16)]. More specifically, electrical stimulation of thalamic nuclei for treating intractable epilepsy has been shown to be a minimally invasive therapeutic tool compared to classic resective surgery. The thalamic anterior nucleus and reticular nucleus have, therefore,

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received increased attention as novel targets for DBS [12)~16)].

The mediodorsal nucleus (MD) is part of the medial nuclear group. Afferent and efferent projections to the MD are quite complex. For example, the amygdala, prepiriform cortex, and entorhinal cortex project to the MD. Other GABAergic projections, such as the ventral pallidum, globus pallidus, and pars reticulata, also project to the MD. Connections to the prefrontal cortex, *e.g.*, orbital region, dorsolateral region, and frontal ocular area, are the main source of efferent MD projections. However, studies in macaques have demonstrated that the MD projects to other regions, such as the cingular, insular, parietal, premotor, and supplementary motor cortices[17)~21)]. Therefore, MD may be responsible for seizure development and expansion, similar to the anterior and reticular nuclei. It remains unknown whether MD is involved in the generalization of cortical seizures or whether it exhibits anticonvulsant effects. The present study examined the influence of MD stimulation on seizure induction elicited by cortical electrical stimulation in rats.

Materials and Methods

Placement of stimulation electrodes

All procedures were performed in accordance with Kyushu University's Animal Use Guidelines. Experimental procedures were designed to minimize the number of animals used and to minimize animal suffering. Only male, Sprague Dawley rats (Charles River Laboratories, Quebec, Canada, n = 7) were used in the experiment. At postnatal day 28 (P28), the rats were fixed to a stereotaxic frame, and the head skin was cut to expose the cranium, following general anesthesia with pentobarbiturate (50 mg/kg, intraperitoneal injection). Stainless steel skull screws (length 3 mm, diameter 1.4 mm) were inserted after burr-holes had been drilled at three cranium points : the right fronto-medial point (2 mm anterior to the bregma and 2 mm lateral from the midline), the right fronto-lateral point (2 mm anterior to the bregma and 4 mm lateral from the midline), and the left fronto-lateral point (2 mm anterior to the bregma and 4 mm lateral from the midline). The former two electrodes were used as stimulating-recording electrodes, and the latter one was used as a recording electrode only. Depth-stimulating electrodes, made from twisted nichrome wires (diameter 0.02 mm), were stereotaxically inserted into the MD of the bilateral thalamus (2.8 mm posterior from the bregma, 0.5 mm lateral to the midline, and 5 mm deep from the cortical surface). A reference screw electrode was placed on the frontal bone. The operation protocol was identical for all animals. Each animal was named in alphabetical order (from A to G).

Electrical stimulation

Ten days after surgery (P38), an electrical stimulation test was performed. Animals were electrically stimulated at the cortical surface, with or without MD conditioning stimulation. All electrodes were connected to an 8-channel standard amplifier (MEG-6168, Nihon Kohden, Japan). Electroencephalograms (EEG), which were recorded by screw electrodes, were analyzed and stored using personal computer-based commercial hardware and software (PowerLab/8sp, ADInstruments, Australia ; NB75H, Fujitsu, Japan, respectively). A 5-second train of a biphasic square wave with a 0.3-ms pulse width was administered using an electrical stimulator and isolator (PG4000A and SIU90, Cygnus Technology, USA), between the right fronto-medial and right fronto-lateral points (cortical stimulation). The stimulation frequency was 50 Hz. The electrical current was increased by a 0.2-mA step each minute, starting at 0.2 mA until the appearance of afterdischarges (ADs) in the left frontal cortex. AD was defined as a minimum of three seconds high-voltage discharges on the EEG. The minimum intensity to induce AD was defined as the threshold. Conditioning stimulation to the bilateral MD (MD

stimulation) was 0.1 mA, with a 0.3-ms pulse width, and the stimulation frequency was 0 Hz, 1 Hz, and 5 Hz to 10 Hz. All seven rats were stimulated with those frequencies by rotation every other day. The data of animals with 0 Hz MD stimulation were defined as controls. Testing was performed once per day for four consecutive days. A twenty-four hour rest is enough to avoid the aftereffect of seizure at the previous stimulation because the EEG monitor of animals after twenty-four hour from the previous stimulation showed no epileptic activities.

AD measurement

Thresholds and durations were measured in all animals when clear ADs were elicited. AD duration was defined as the time length from the end of stimulation to the end of cortical spikes.

Analysis of seizure behavior

Seizure behavior was monitored using a digital video camera (DCR-TRV9 NTSC, Sony, Japan). Behavior from the end of each electrical stimulation period was used for analysis.

Tissue processing and histological examination

After the series of electrical stimulations (P41), all rats were sacrificed with a lethal overdose of pentobarbiturate (100 mg/kg). Animals were transcardially perfused with isotonic saline, followed by 4% paraformaldehyde/phosphate-buffered saline (PBS) (pH 7.0, room temperature). Brains were immediately removed, post-fixed in the same fixative for 24 hours, and then processed into paraffin-embedded blocks. Tissues were cut into 4-μm thick coronal sections. Sections were stained using the hematoxylin and eosin (HE) method.

Statistical analysis

AD thresholds and durations were analyzed using student's *t*-test to compare between data of animals with MD conditioning and those of animals without it.

Results

Microscopic findings

Microscopic observation of brains revealed a long scar made by the depth-stimulating electrodes. Analysis confirmed that the electrodes penetrated the cortex, as well as the hippocampal CA1 and dentate gyrus, and reached the MD in all animals (Fig. 1a, b).

Cortical AD thresholds

The mean threshold in the animals with 0Hz MD stimulation (controls) was 2.7 ± 0.4 mA, whereas animals with 1-Hz MD stimulation displayed 2.7 ± 0.4 mA, 5-Hz MD stimulation displayed 2.8 ± 0.3 mA, and 10-Hz MD stimulation displayed 2.9 ± 0.5 mA (mean ± standard deviation ; SD, see Fig. 2a). There was no statistically significant difference between animals with MD stimulations and controls (1-Hz MD stimulation versus control, $p = 0.9$; 5-Hz MD stimulation versus control, $p = 0.58$; 10-Hz MD stimulation versus control, $p = 0.58$; Table).

Cortical AD durations

During early stages of stimulation, EEG findings consisted of bilateral cortical spikes and waves (Fig. 3). In the animals with 0Hz MD stimulation (controls), the mean duration was 10.4 ± 5.0 seconds, whereas in the animals with 1-Hz MD stimulation, the mean duration was 9.8 ± 2.4 seconds. In the animals with 5-Hz MD stimulation, mean duration was 10.1 ± 1.5 seconds, and in the animals with 10-Hz MD stimulation, mean duration was 7.6 ± 2.5 seconds (mean ± SD, see Fig. 2b). Although the mean duration at 10-Hz stimulation was less than the control (non-stimulated) animals, there was no statistically significant difference between animals with MD stimulation and controls (1 Hz MD stimulation versus control, $p = 0.78$; 5-Hz MD stimulation versus control, $p = 0.89$; 10-Hz MD stimulation versus control, $p = 0.2$; Table).

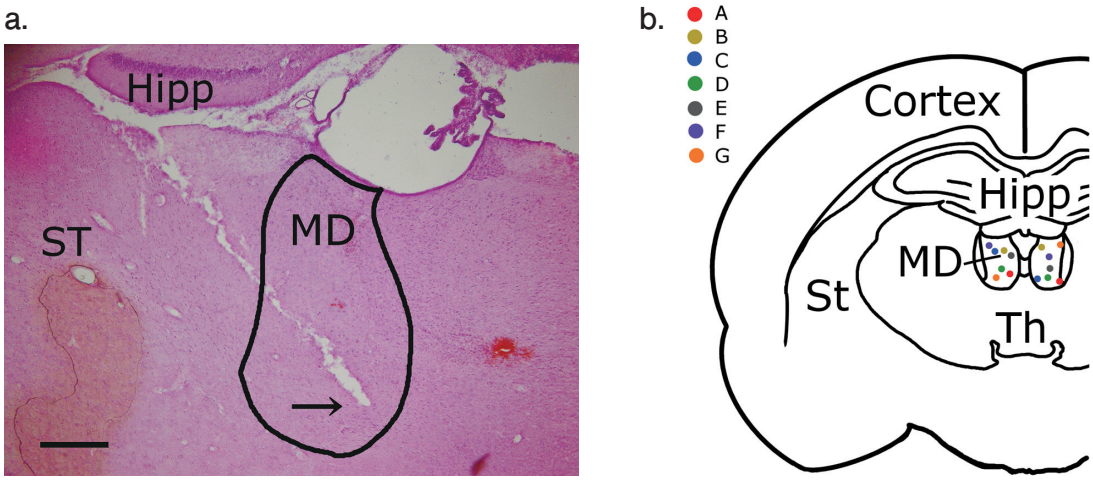

Fig. 1 (a) Microscopic findings in a coronal section of a brain of rat A stained using the hematoxylin and eosin (HE) method. An arrow indicates a stimulating electrode tip location in the right MD. (b) Schematic illustration of the stimulating electrode tip locations in all rats (filled color circles), as determined by histological analysis. Capital letters mean alphabetical order of each rat. Note that the stimulating electrodes are located within the MD in all animals. Cortex, cerebral cortex ; Hipp, hippocampus ; MD, mediodorsal nucleus ; St, striatum ; Th, thalamus. Scale bar : 300 μm.

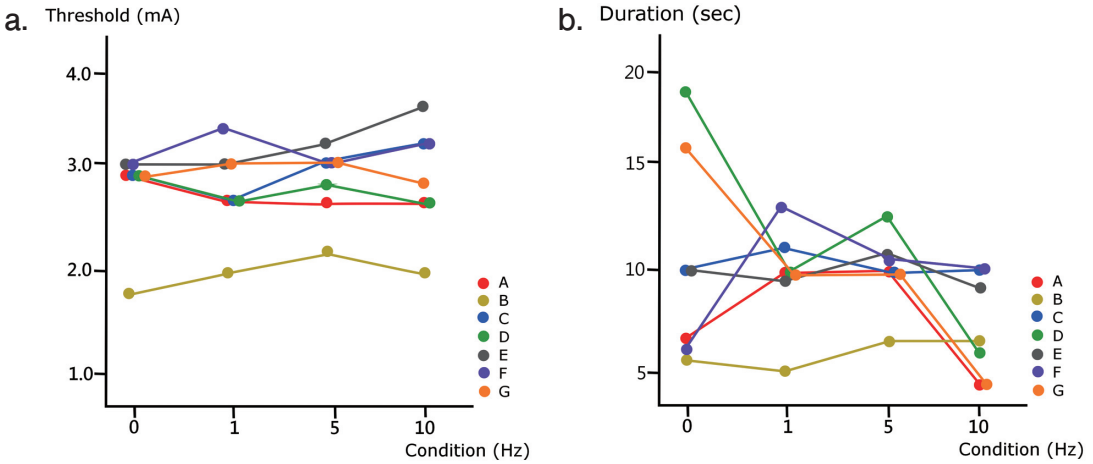

Fig. 2 The raw data of thresholds and durations of animals. (a) Thresholds of each MD condition (0, 1, 5 and 10 Hz) are not changed in all animals. (b) Although two animals (D and G) show shorter durations depending on MD stimulations, other data of animals do not reveal any effect of MD conditioning.

Seizure behaviors

Seizures were accompanied by bilateral tonic-clonic convulsions and cortical spikes. During the postictal stage, animals exhibited confusion (walking around or climbing up the cage wall) for a prolonged period, without cortical spikes. Seizure behaviors were not different among the groups.

Discussion

To our knowledge, this is the first report of electrical cortical stimulation using repetitive MD stimulation in a rat model. The present study demonstrated no statistically significant difference between animals with MD stimulation and without it, with regard to AD thresholds and durations, although mean duration at 10-Hz MD stimulation tended to be shorter than the controls. These results indicated that thalamic MD stimulation might not be effective against cortical electrical stimulation-induced seizures.

Although there was no difference in cortical

Table Threshold and duration of cortical ADs with and without additional MD stimulation (n=7)

MD stimulation +						MD stimulation − (Control)	
1 Hz		5 Hz		10 Hz			
Threshold (mA)	Duration (sec)	Threshold (mA)	Duration (sec)	Threshold (mA)	Duration (sec)	Threshold (mA)	Duration (sec)
2.7 ± 0.4	9.8 ± 2.4	2.8 ± 0.3	10.1 ± 1.5	2.9 ± 0.5	7.6 ± 2.5	2.7 ± 0.4	10.4 ± 5.0

Mean thresholds and durations in the animals with and without MD stimulation. In the animals with 0Hz MD stimulation (controls), the mean threshold was 2.7 ± 0.4 mA, whereas in the animals with 1-Hz MD stimulation, it was 2.7 ± 0.4 mA, in the animals with 5-Hz MD stimulation, it was 2.8 ± 0.3 mA , and in the animals with 10-Hz MD stimulation, it was 2.9 ± 0.5 mA (mean ± standard deviation ; SD). There was no statistically significant difference between MD stimulated and control animals (1-Hz MD stimulated animals versus control, p = 0.9; 5-Hz MD stimulated animals versus control, p = 0.58 ; 10-Hz MD stimulated animals versus control, p = 0.58 ; n = 7).

In the animals with 0Hz MD stimulation (controls), the mean duration was 10.4 ± 5.0 seconds. 1-Hz MD stimulation resulted in 9.8 ± 2.4 seconds, 5-Hz MD stimulation resulted in 10.1 ± 1.5 seconds, and 10-Hz MD stimulation resulted in 7.6 ± 2.5 seconds (mean ± SD). Although mean duration at 10-Hz stimulation was shorter than in the control animals, there was no statistically significant difference between MD stimulated and control animals (1-Hz MD stimulated animals versus control, p = 0.78; 5-Hz MD stimulated animals versus control, p = 0.89 ; 10-Hz MD stimulated animals versus control, p = 0.2 ; n = 7).

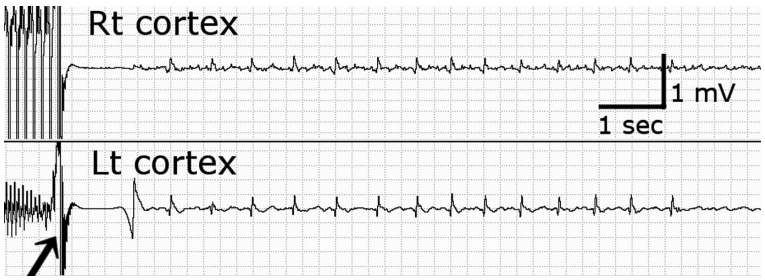

Fig. 3 A representative EEG recording with 10-Hz MD stimulation. The AD form was similar to EEG results of control animals. Total seizure time was 7.4 seconds. The arrow indicates the end point of electrical stimulation.

seizure induction between MD stimulation models and control animals, results stimulated the following questions. First, do other suitable parameters of MD stimulation exist? Previous studies have shown that stimulus frequencies to the anterior and reticular nuclei were between 50 and 100 Hz[12)〜16)]. The present study, however, used 1, 5, and 10 Hz stimulations to the MD. These frequencies were chosen to avoid inducing seizures seizure via the MD.

Second, other than the MD, do appropriate stimulation points or nuclei exist? Thalamic anterior and reticular nuclei are considered to be effective sites for suppressing intractable epilepsy[11)〜16)]. On the other hand, MD has been reported to be closely related to schizophrenic symptoms[17)〜21)]. Therefore, MD stimulation might induce psychiatric symptoms rather than seizure suppression.

Third, was cortical stimulation suitable for test stimulation? The present study utilized an animal model of cerebral cortical stimulation. Previous studies have reported thalamic electrical stimulation in limbic epilepsy animal models[12)13)15)]. This kindling phenomenon can be easily created utilizing electrical or pharmacological stimulation to the limbic areas. Nevertheless, creating a cortical kindling phenomenon in animal models is difficult, and seizure propagation of cortical kindling to other brain structures is quite different[22)]. Therefore, thalamic stimulation might not be effective for suppressing epileptic seizures unless its focus is the limbic structure.

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(Received for publication August 25, 2009)

（和文抄録）

皮質てんかんラットモデルでの視床背内側核刺激の効果

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背景　視床背内側核は前頭葉と辺縁系とに強く結合している．この研究では，前頭葉への電気刺激で励起されるてんかん発作に対し，視床背内側核電気刺激が影響を与えるか否かを検討した．

対象と方法　生後 28 日目の成熟雄ラット（ｎ＝７）の脳表（両側前頭葉）にネジ電極を，両側視床背内側核に深部電極を埋め込み，10 日間休ませた後 38 日目から１日１回前頭葉の電極に電気刺激を行い，全身けいれん発作を出現させた．刺激は明らかな後放電（前頭葉の脳表からのてんかん性放電）が出現するまで，徐々に上昇させ，後放電が出現した時点で刺激を終了した．また，一定の刺激電圧（0.1mA）で視床背内側核電気刺激を０ Hz，１ Hz，５ Hz，10Hz の周波数で各ラットに前頭葉刺激と同時に１日おきに行った．それぞれの群で後放電の閾値と持続時間を計測し比較した．

結果　けいれん発作は両側前頭葉の棘波とそれに伴う全身強直間代性けいれんであった．後放電の閾値には４群間で有意な差を認めなかった．持続時間に関しては 10Hz 刺激でコントロール群より軽度短かったものの，全体として有意差を認めなかった．

結論　視床背内側核の電気刺激は，今回のような低頻度刺激ではてんかん発作を抑制することは難しいと思われる．今後視床刺激の部位や頻度を変更する必要がある．