Exp Brain Res (1984) 56: 257–262

Experimental Brain Research

Reversible cooling of the brainstem reveals areas required for mesencephalic locomotor region evoked treadmill locomotion

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Summary. The evidence suggests that the mesencephalic locomotor region (MLR) may not be a unitary region since anatomical and functional variations in the descending projections are clearly indicated. Reversible cooling of midline reticular structures can effectively block locomotion evoked by stimulation of lateral MLR (L3.5–4) sites while not significantly affecting the locomotion evoked from more medial MLR (L2–2.5) sites. In contrast, locomotion evoked by stimulation of the medial MLR sites is blocked by cooling of the ipsilateral lateral brainstem region which corresponds to the pontomedullary strip (PLS). Ipsilateral PLS cooling was not effective for blocking lateral MLR evoked locomotion, and contralateral PLS cooling was not effective for blocking either medial or lateral MLR evoked stepping. The evidence indicates that the lateral MLR relays through medial reticular nuclei while the medial MLR sites relay largely through the lateral brainstem structures often referred to as the PLS.

Key words: Locomotion – Mesencephalic locomotor region – Pontomedullary strip – Reversible cooling

Introduction

It has been established for some time that the spinal cord contains the neuronal circuitry necessary to generate the rhythmic alternating movements associated with locomotion (Sherrington 1910). Various brainstem regions which can effectively turn on the spinal central pattern generator for locomotion have been identified (Shik and Orlovsky 1976; Grillner 1975). Included in these regions is the mesencephalic locomotor region (MLR) which, when electrically stimulated, can produce coordinated quadruped locomotion in the precolliculuar, postmammillary decerebrate cat (Shik et al. 1966, 1967). Although the classical MLR region (P1, H4.5, L4) (Shik et al. 1967) is commonly used in such preparations during both treadmill and fictive locomotor studies, a more medial region, also referred to as the MLR (P1, H4.5–6, L2), has recently been investigated both anatomically and electrophysiologically (Garcia-Rill et al. 1983b, c).

A comparison of the work done on these two MLR regions indicates that, although they both can initiate stepping activity when stimulated, their anatomical projections differ significantly. Steeves and Jordan (1984) examined the descending projections from the lateral MLR region using anterograde labelling techniques and found that the majority of the descending projections travelled to the medial reticular nuclei, specifically the n. gigantocellularis, magnocellularis and raphe magnus, with the heaviest labelling on the ipsilateral side and much less contralateral labelling. There was no indication of any descending projections past this point. Garcia-Rill and coworkers, using anterograde and retrograde labelling, found projections from the more medial MLR sites to a lateral area corresponding to Probst's tract (Garcia-Rill et al. 1983b). They suggested that Probst's tract and the pontomedullary locomotor strip (PLS) are coextensive. The PLS has been reported to produce locomotion when stimulated electrically (Shik and Yagodnitsyn 1978, 1979) but the organization of the PLS and its projections past the uppermost cervical levels have not been defined.

The purpose of this study was to determine the functionally important relay sites in the brainstem for MLR evoked locomotion, and to examine the hypothesis that locomotion evoked by lateral MLR stimulation is relayed via midline brainstem nuclei, while locomotion evoked by medial MLR stimulation

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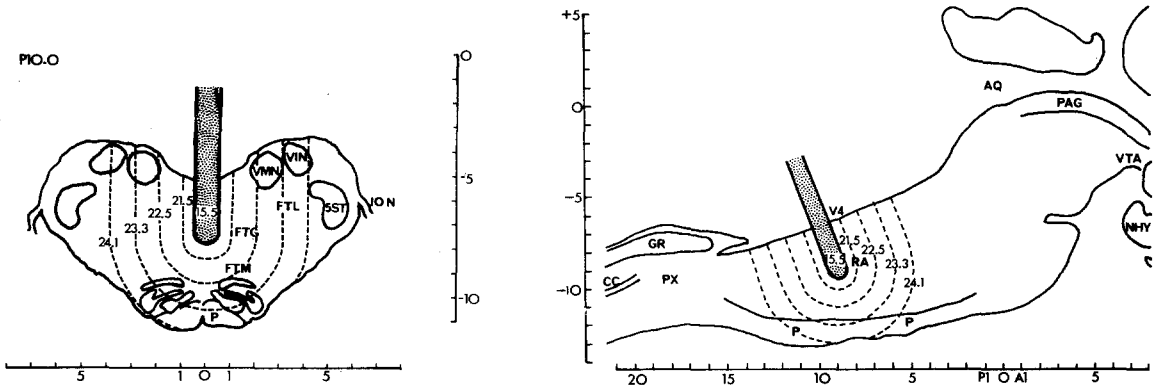

Fig. 1. Temperature at cooling probe tip and the estimated surrounding isotherms. A cross section (left panel) and sagittal view (right panel) of the brainstem where reversible cooling was done. The brainstem tissue was assumed to be homogeneous and the area for effective synaptic blockage was localized to within 1 mm of the cooling probe

travels mainly through lateral brainstem regions. Local cooling of the various brainstem areas was used to produce reversible block of synaptic transmission.

Methods

Fourteen cats were studied using the precollicular, postmammillary decerebrate preparation. The cerebellum of each cat was removed in order to provide visual access to the floor of the fourth ventricle, and the obex was used as a landmark for the placement of the cooling and exploring thermocouple probes. The animal's head was fixed in a stereotaxic headframe over a treadmill with all four limbs free to step on the moving belt. The cervical vertebrae were rigidly clamped, and the hindquarters of the animal were suspended using either a sling under the belly of the cat or pins placed on the iliac crests. Stepping was induced by electrical stimulation (0.5 ms duration, 10–60 Hz, 25–150 μA) of the MLR at stereotaxic coordinates P2–4, H4.5–6.5, L4 (Berman 1968). Locomotor activity in both fore- and hindlimbs was monitored using bilateral intramuscular electromyographic (EMG) electrodes from the following muscles: tibialis anterior (TA), lateral gastrocnemius (LG), biceps brachii (BB), and triceps brachii (TB). All eight EMGs were amplified with a bandpass of 100 Hz to 10 KHz and recorded on analog magnetic tape. Stimulus sites used for experimental trials did not produce any observable side effects during locomotion. Cooling of the various brainstem regions was carried out with a coaxial 18 gauge stainless steel probe through the inner shaft of which ice water was circulated at a rate of 15 ml/min. A subminiature thermocouple was glued to the outside surface of the probe tip. The temperature at the probe tip reached that which would effectively block synaptic (20° C) but not axonal transmission (10° C). Mapping of the tissue temperatures around the tip of the cooling probe was undertaken using a hypodermic needle thermocouple to provide information pertaining to the extent of cooling. Temperatures were read out on a digital thermometer. After a cooling trial was completed, the temperature of the region was returned to control by circulating water at body temperature through the probe.

Locomotor trials followed one of two protocols. In the first, MLR stimulation was applied for 10 s at 1 min intervals. After a 4 to 5 min control period cooling was started and continued for a maximum of 13 min followed by rewarming for a maximum of 6 min. The other protocol consisted of continuous MLR stimulation during which the cooling and rewarming procedures were executed and the effects on the locomotion observed.

In preparation for computer analysis, the taped EMG records of appropriate 10 s trials were replayed with analogue fullwave rectification and integration (20 ms time constant) of each channel. All eight integrated EMG channels were digitized simultaneously at 100 Hz per channel, the digital data being stored on floppy disk.

Abbreviations: AQ, aqueduct; BC, brachium conjunctivum; CB, cerebellum; CC, central canal; CNF, cuneiform nucleus; FTG, gigantocellular tegmental field; FTL, lateral tegmental field; GR, gracile nucleus; IC, inferior colliculus; LG, lateral geniculate; LLD, dorsal nucleus lateral lemniscus; LLV, ventral nucleus lateral lemniscus; MLB, medial longitudinal bundle; NHY, neurohypophysis; 5N, trigeminal nerve; 1ON, vagus nerve; P, pyramidal tract; PAG, periaqueductal gray; PX, pyramidal decussation; RA, raphe nucleus; 5ST, spinal tract of trigeminal; VIN, inferior vestibular nucleus; VMN, medial vestibular nucleus; V4, fourth ventricle.

Results

The cooling technique used in this series of experiments produced temperatures at the tip of the probe that were below the temperature necessary to block synaptic transmission but not below that needed to block axonal transmission (Brooks 1983). Figure 1 illustrates the temperature at the cooling probe tip and shows isotherms at 1 mm intervals away from the center of the probe. It is apparent that temperatures below about 20° C, producing effective blockage of synaptic transmission, were produced only within a radius of 1 mm from the tip of the probe.

Lateral MLR (L3.5–4.0) stimulation

In each of the 4 cats where locomotion was evoked using a lateral MLR (L4) region, cooling of the midline region in the medulla, 5 mm rostral to the

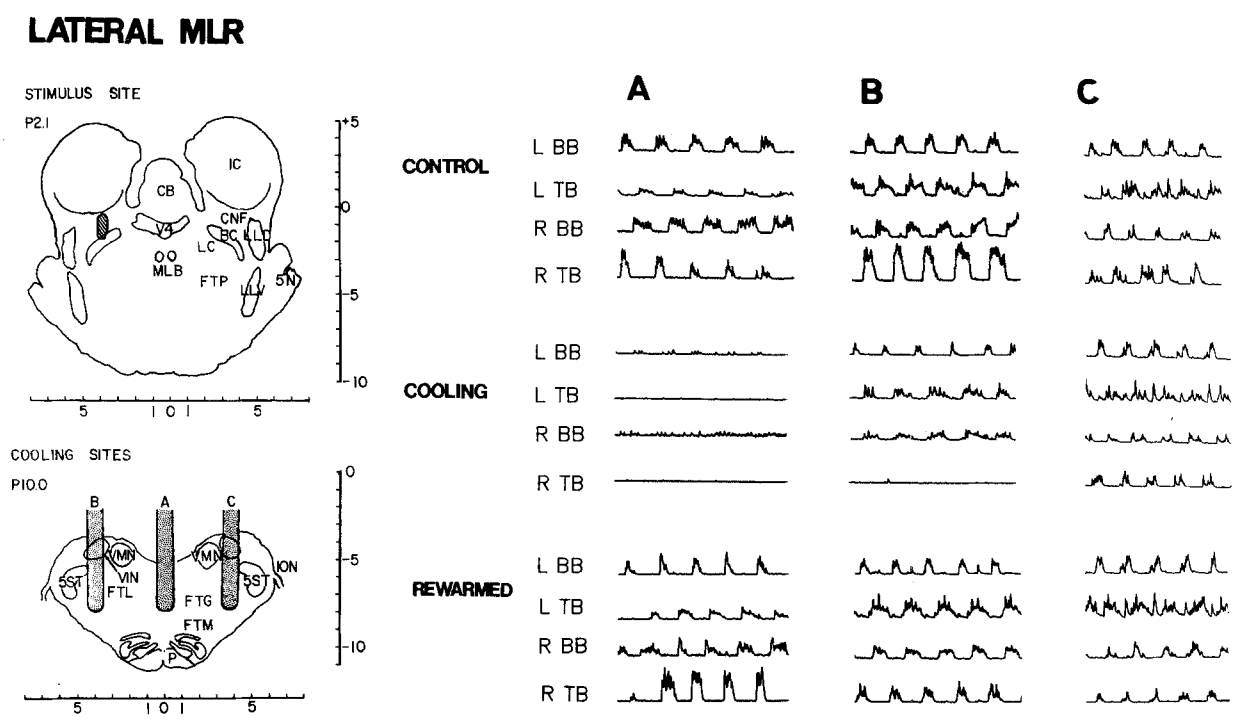

Fig. 2A–C. Lateral MLR (L3.5–4) stimulus site and cooling sites with forelimb EMG (BB-biceps brachii, TB-triceps brachii) records before and during cooling as well as after rewarming the region. Midline cooling effectively blocked evoked locomotion **A** while ipsilateral PLS cooling decreased the EMG burst amplitude on the contralateral side as well as in the ipsilateral BB (flexor) **B.** The extensor activity (TB) on the ipsilateral side was abolished. Contralateral PLS cooling was without a significant effect **C.** The step cycle length varied from 706 ms to 730 ms

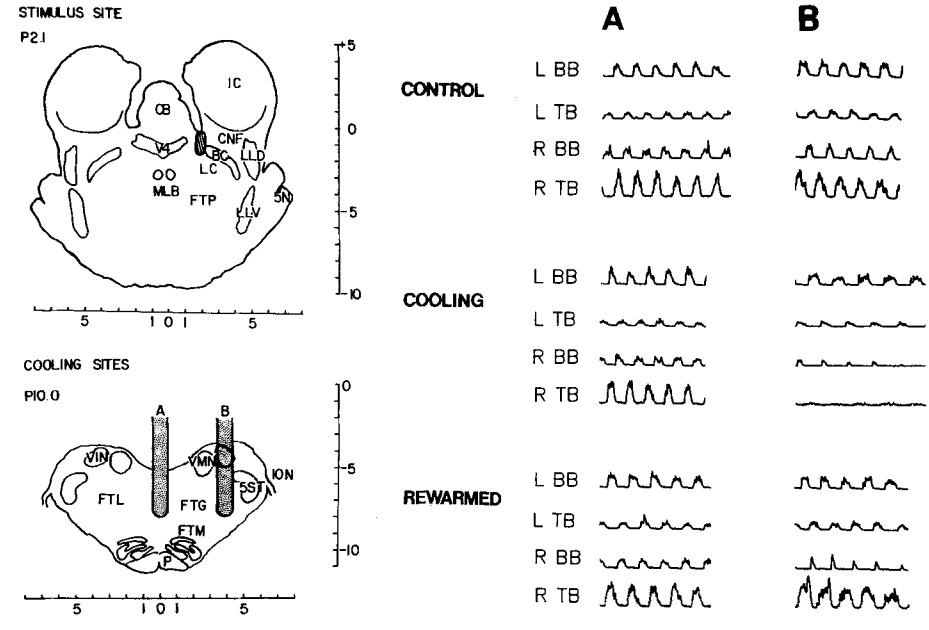

Fig. 3A and B. Medial MLR site (L2.0–2.5) and cooling sites with corresponding EMG activity before and during cooling as well as after rewarming. Midline cooling **A** was without significant effect on evoked locomotion while ipsilateral PLS cooling **B** decreased the amplitude and rate of the EMG activity bilaterally. The step cycle length ranged from 461 ms to 580 ms

obex and 3 mm below the floor of the fourth ventricle could effectively abolish all locomotor activity in both hind and forelimbs (Fig. 2A). Complete recovery was observed with rewarming. The cooled region corresponded to an area including portions of the gigantocellular tegmental field and the magnocellular tegmental field.

Cooling of brainstem regions 4 mm lateral to the previously described midline site in the medulla, both ipsilateral and contralateral to the site of MLR stimulation, was also performed (Fig. 2B and C). In this case the effective cooling area included the rostral region of the lateral tegmental field just dorsal to the caudal portion of the nucleus of cranial nerve 7. This area corresponded to the ponto-medullary strip (PLS) (Mori et al. 1977). Cooling of the ipsilateral PLS did not consistently affect lateral MLR evoked stepping. At best, such cooling appeared to alter the stepping in the ipsilateral side but did not significantly change contralateral stepping. Cooling of the contralateral PLS was without effect on stepping (Fig. 2C).

Medial MLR (L2–2.5) stimulation

With stimulation of a more medial MLR region, cooling of the midline (5 mm rostral to obex, 3 mm below floor of the fourth ventricle) was without effect in 3 out of 4 animals. In the fourth animal there was a slight decrease in the amplitude and vigor of locomotion but no abolition of the stepping pattern (Fig. 3A). Cooling of the ipsilateral PLS altered locomotion with the effects varying from the complete abolition of all stepping activity to a decrease in EMG amplitude and stepping rate in all limbs engaged in stepping activity (Fig. 2B).

Since Garcia-Rill et al. (Garcia-Rill et al. 1983b) reported a small but significant projection from the more medial MLR sites to a region contralateral to the MLR and about 1 mm lateral to the midline, this site was cooled to evaluate the effects it had on evoked locomotion. The site corresponded to a portion of the magnocellular tegmental field, and cooling here affected the vigor of stepping in all four legs. Nevertheless, it was not observed to abolish MLR evoked stepping.

Spontaneous locomotion

In two animals the effects on spontaneous locomotion of cooling several brainstem locations were examined. In both animals, cooling the region at the midline at the junction of the superior and inferior colliculi at a depth of about 3 mm abolished spontaneous locomotion. Recovery was complete in all cases. In one cat, cooling of a midline region 5 mm rostral to the obex and 3 mm below the surface of the fourth ventricle abolished all spontaneous stepping activity, as did cooling of either of the more lateral PLS regions. In both cats the spontaneous locomotion eventually deteriorated to the extent that stimulation of the MLR was necessary to produce stable locomotion and the midline and PLS cooling 5 mm rostral to the obex were repeated using MLR evoked stepping, the results of which have been described in the previous sections.

Discussion

Since the first work done by the Russians (Shik et al. 1967) on the region of the brainstem that was capable of evoking locomotion in the mesencephalic cat, the term MLR has been used extensively in a variety of experimental procedures related to locomotor mechanisms. The originally reported coordinates for the MLR were given as P2, H4.5, L4, although the literature has extended the region of the MLR to include P1–2, H4.5–6.0, L2–4 (Garcia-Rill et al. 1983a, Shefchyk and Jordan 1983). Recent anatomical work investigating the projections of the MLR revealed that cells in medial MLR coordinates (L2–2.5) terminate in lower brainstem areas that differ from the projections observed for cells in the lateral extent of the MLR (L4). This anatomical work, paired with the results presented in this paper, support anatomical and functional separation between these two subdivisions of the mesencephalic locomotor region.

Earlier work investigating the details of the lateral MLR as originally described (P2, H4.5, L4) (which will be referred to as the lateral MLR from this point on) showed that the majority of the descending efferent projections from the MLR were directed to the ipsilateral medial reticular nuclei (Steeves and Jordan 1984). These reticular nuclei have been demonstrated to project to all levels of the spinal cord by way of the ventrolateral quadrant of the spinal cord (Kuypers and Maisky 1977). The ventrolateral region of the spinal cord has been demonstrated to be the only region of the cord necessary for the initiation of evoked locomotion from stimulation of the lateral MLR (Steeves and Jordan 1980). In addition, the ventrolateral quadrant of the spinal cord is necessary for hindlimb stepping in the spontaneously walking cat (Eidelberg et al. 1981). The evidence for these medial reticular nuclei being functional in the locomotor "command" relay

includes the evidence for rhythmicity of these cells during locomotion (Orlovsky 1970) and the electrophysiological data indicating a functional synaptic connection from the MLR to these reticular nuclei (Selioniv and Shik 1981). In addition, the results obtained in these cooling experiments also support the role of these midline reticular nuclei as relays for MLR signals since cooling of the midline region can effectively block lateral MLR evoked locomotor activity.

Previous work (Mori et al. 1977; Shik et al. 1979) described the PLS as a column of cells extending from the MLR through the brainstem at a location about 4 mm lateral to the midline and 2 mm below the floor of the fourth ventricle, to the upper cervical levels (Mori et al. 1977). The proposal of a multineuronal system composing the PLS was based on electrophysiological data and lesion studies which demonstrated that lesioning along the strip did not prevent locomotion evoked by stimulation above or below the lesion along the PLS. It was also demonstrated electrophysiologically that the cells of the PLS projected to cells of the medial and lateral reticular formation (Budakova et al. 1980). Hence, the possibility exists that some of the effects evoked by stimulation of the PLS could in fact be mediated through the reticular formation in a pathway separate from but parallel to the PLS. The results from the cooling of the ipsilateral PLS support this, since lateral MLR stimulation evoked locomotion that was not significantly perturbed by ipsilateral PLS cooling, although some changes were observed in the vigor of the stepping on the ipsilateral side.

Investigations by Garcia-Rill and coworkers (Garcia-Rill et al. 1983b and c) examining more medial areas of the MLR indicated that the heaviest descending projections were to the ipsilateral lateral region which they claimed corresponded to Probst's tract and the PLS. A lesser amount of labelling of the contralateral midline nuclei was observed. The results of cooling the ipsilateral PLS during medial MLR stimulation again supported these anatomical findings. It would appear that although the medial MLR is also quite adequate for evoking stepping, it forms part of an anatomically and functionally distinct pathway. The latter point is supported by data describing the recruitment of the forelimbs prior to the hindlimbs with medial MLR stimulation (Garcia-Rill et al. 1983a) and hindlimb recruitment prior to forelimbs with lateral MLR stimulation (Shik et al. 1967). Mori et al. (Mori et al. 1978, 1982) examined two midline regions in the pons which they described as the dorsal inhibitory region and the ventral facilitatory region. Stimulation of the former could decrease muscle tone and inhibit stepping while

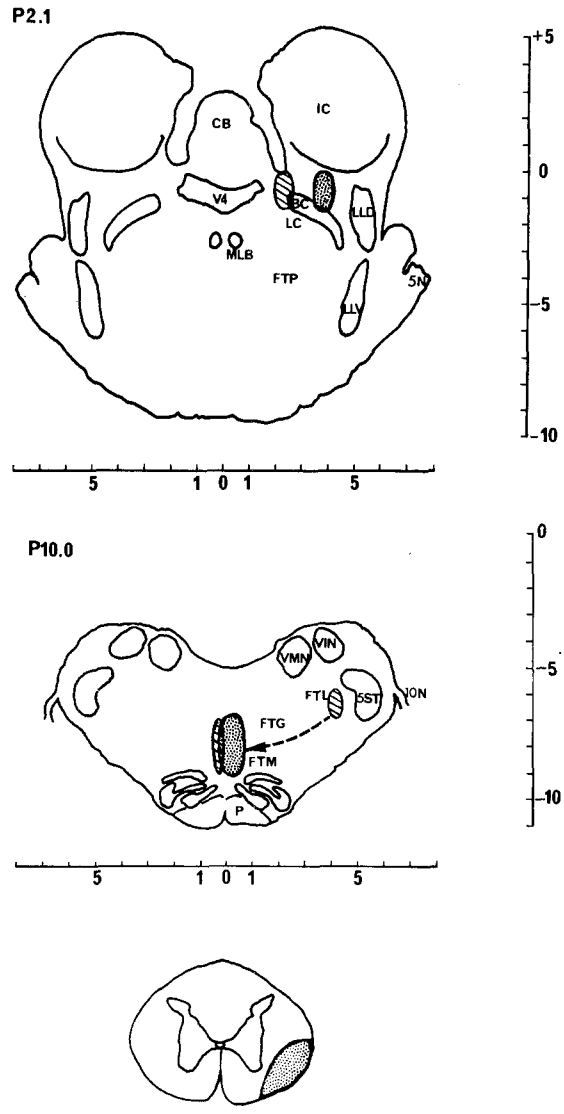

Fig. 4. Summary of the medial and lateral MLR sites and their proposed trajectory. The lateral MLR and related regions are represented with dots while the medial MLR and related regions are indicated with hatched lines. The middle section illustrates the separation of the relays in the brainstem for the two pathways. The hatched arrow represents the suggested connection between the lateral brainstem sites (PLS) related to the medial MLR and the medial reticular nuclei which are relays for the lateral MLR. The ventrolateral quadrant of the spinal cord at the upper cervical level is illustrated in the lower panel and the dotted area represents the funiculus in which the fibers from the midline reticular nuclei project to the lower levels of the spinal cord. This same hatched area corresponds to the region of the spinal cord that must be intact in order to evoke locomotion from the lateral MLR. In addition, this ventrolateral area is necessary for hindlimb stepping in the spontaneously walking cat

stimulation of the latter could increase tone and facilitate stepping. From the results obtained with midline cooling in this series of experiments as well as from the data reported by Mori and coworkers (Mori

et al. 1982), it is not possible to distinguish between the tone setting and locomotion initiation systems with any degree of confidence.

Figure 4 summarizes the connections of the brainstem locomotor regions suggested by our results. We propose that the lateral MLR relays its information through midline reticular structures, while the medial MLR appears to relay largely through the region corresponding to the PLS. There is also evidence for connections between the PLS and the medial reticular formation. The degree of independence of the two systems is not clear, and information describing retrograde labelling from injections within the midline reticular nuclei and PLS is required in order to evaluate the possibility of distinct subregions of the MLR.

Acknowledgements. We thank Brian Noga, Sharon Nault and Jack Kettler for their technical assistance, and Peter Hortensius for software development for the EMG analysis. This research was supported by a Medical Research Council of Canada (MRC) grant to LMJ and a Manitoba Health Research Council grant to RMJ. SJS is the recipient of a MRC studentship.

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Received December 8, 1983 / Accepted February 27, 1984