

Comparison of the Effects of Electrolytic and Chemical Destruction of the Red Nucleus on the Compensatory Capacity of Rats with Rubrospinal Tract Lesions

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SUMMARY

Transection of the rubrospinal tract in rats, performed before lesion of the red nucleus, resulted in the facilitated recovery of motor activity and operantly conditioned reflexes. Such facilitation was absent when the red nucleus is lesioned alone. This phenomenon is explained by the switching of descending influences on the corticospinal tract through the participation of the following system: red nucleus—inferior olive—cerebellum—ventrolateral thalamic nucleus—cerebral cortex. The above mentioned facilitating influence on the recovery process was particularly prominent in rats with quinolinic acid-induced lesion of the red nucleus. Under these conditions, the cerebellar ascending fibers to the ventrolateral thalamic nucleus were preserved. Decreased facilitated recovery following electrolytic lesion of the red nucleus suggests the existence of additional cerebello-cortical pathways for the realization of the switching phenomenon.

KEYWORDS

Plasticity, operantly conditioned, switching of descending influences

INTRODUCTION

Transection of the rubrospinal tract (RST) before lesion of the red nucleus (RN) has been shown to facilitate the recovery of operantly conditioned reflexes and the compensatory-rehabilitation process in rats. RN lesion alone, however, not preceded by transection of RST, leads to prolonged disturbances of motor activity and operantly conditioned reflexes (Kennedy, 1990). On this basis, it has been proposed that the quick recovery of motor activity after the transection of RST is a consequence of activation of the rubro-olivary projection (Rossi & Strata, 1997), leading to switching of motor activity under control of the corticospinal tract (Kennedy & Humphrey, 1987). This switching is realized by the participation of the cerebellum and the ventrolateral thalamic nucleus (VL), which transmit the information to the cerebral cortex. During this process, VL functions as a basic relay center for the ascending cerebellar influences on the cerebral cortex, leading to the switching of cortical control for the new context of motion (Kennedy & Humphrey, 1987; Kennedy, 1990). In a previous paper (Fanardjian et al., 1995), we demonstrated the role of VL in the process of descending motor switching in the operantly conditioned rat; the preliminary destruction of VL was shown that to greatly hamper the process of switching. Comparison of the effects of electrolytic and chemical lesion (by quinolinic acid) of RN may clarify the role of VL in the mechanisms of switching of the supraspinal influences. Electrolytic lesion of RN not only leads to

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damage of rubral cells, but also results in the interruption of the cerebello-thalamic fibers passing through RN to VL that give collaterals to rubral neurons (Toyama et al., 1970; Shinoda et al., 1988). Therefore, electrolytic lesion of RN leads to disturbance of activity of RST and VL systems. On the other hand, injection of quinolinic acid into RN destroys the somata of cells, whereas the cerebello-thalamic fibers are preserved (Kennedy & Humphrey, 1987). We have addressed the role of the cerebello-thalamic system in the switching mechanism of descending motor systems. The results of the two experiments mentioned above on operantly conditioned rats are presented below.

METHODS

Predominantly male albino rats ($n=42$), 2 to 3 months old and weighing 210 to 250 g, were trained to move and balance on a slowly rotating horizontal bar (9 rotations per minute), which was 30 cm in length and 3 cm in diameter. The horizontal bar was fixed at a height of 90 cm above a soft cushion. Conditioning for the instrumental reflex of equilibrium was determined as the time spent on the rotating bar. The maximum time of each trial was 5 min. The trial was repeated 10 times per session, with a 1-min interval between each trial. The criterion for conditioned performance was the rat's ability to balance on the rotating bar for no less than 250 s (Kennedy & Humphrey, 1987).

After conditioning for stable instrumental reflexes, rats were anesthetized intraperitoneally using sodium pentobarbital (Nembutal, Tallinn) (40 mg/kg body weight), and then the surgical procedures were performed. After several sequential experimental procedures, the vast majority of rats survived and underwent further experiments, which included two basic groups with electrolytic and chemical destruction. Each group underwent the following experimental procedures:

1. operant conditioning;
2. isolated electrolytic and chemical destruction of RN;
3. preliminary transection of the spinal cord dorso-lateral funiculus (DLF) and further electrolytic and chemical destruction of the red nucleus;
4. injection of horseradish peroxidase (HRP) into VL of rats with chemically lesioned RN.

In 21 rats, the DLF (which contains RST) was transected unilaterally at the upper cervical spinal cord (C 3). Contralateral to the above mentioned transection, RN was lesioned electrolytically or chemically, guided by the stereotaxic coordinates (Paxinos & Watson, 1986). In 11 animals, RN alone was lesioned. Thus, after the experimental procedure, 32 rats were taken for further analysis. After each procedure, reconditioning of instrumental reflexes was performed, and a general picture of the changes in dynamic behavior was constructed.

Electrolytic lesion of RN was effected by passing direct anodic current (2.5 mA) for 10 to 15 s through a steel unipolar electrode, which was insulated except for the tip (100 μm) (14 rats). The neurons of RN were destroyed chemically by injecting a solution of 2% quinolinic acid (8 rats). The solution was prepared by sonicating 100 mg of acid powder (Sigma) in 5 mL of phosphate-buffered saline (pH 4.0). Quinolinic acid was injected under pressure with the by a glass micropipette connected to a Hamilton syringe. The Hamilton syringe was filled with high-vacuum silicone grease and later by a solution of 2% quinolinic acid. The syringe with the micropipette was fixed to an electrode carrier. The tip of the micropipette was oriented onto RN according to the stereotaxic coordinates (Paxinos & Watson, 1986) and 0.8 to 1.0 μL of quinolinic acid was slowly injected, as determined by the meniscus of the silicone grease.

Preservation of the cerebello-thalamic fibers passing through RN was determined in the rats operated on by the above mentioned methods. In two control experiments, 6 to 7 days after the unilateral injection of quinolinic acid into RN, HRP was injected into VL. For this purpose, a

solution of 5% HRP (Sigma, Type VI) in 0.1 M Tris-buffered saline, 0.5 M KCL and 0.5 M DMSO (pH 8.3) was injected into VL of two anesthetized rats. The injection was made with a glass micropipette (tip diameter = 20 to 30 μ m) that was stereotaxically guided to VL. HRP was injected microiontophoretically (positive current of 16 to 20 nA, 200 Hz, 5 min series duration, having a 5-min interval at a four times recurrence period). During the injection, the micropipette remained for 30 min in the brain. Anesthesia was repeated 48 h later, and brain perfusion was carried out. The brain was divided into blocks from which serial sections (75 μ m) were made and HRP was histochemically revealed using tetramethyl benzidine (Mesulam, 1978).

In all experimental animals, neurological investigations of motor disorders were conducted in parallel with the study of instrumental reflexes. Characteristic postoperative disorders, such as impairment of the limbs, tilt of the head, and reflex of bar grasping, reflex of the limb flexion

and extension to squeezing, reflex of the body and head rectification at the moment of fall, were tested. Under standard conditions of vivaria, all animals were segregated into groups of 4 to 5 rats. After the above experiments were concluded, morphological investigations of the damaged structures were conducted. Experimental animals were sacrificed with a lethal dose of sodium pentobarbital, and brains were extracted and fixed in 10% neutral formalin. Sections of the brain were stained by Nissl's method. The Student's t-test was used to determine the extent of the difference in the recovery of motor activity and instrumental reflexes (Pollard, 1977).

RESULTS

In all rats that were operantly conditioned, the defined criterion for stable reflexes was reached on the 2nd to 4th experimental day (mean days 2.5 ± 0.2 ; SD; n=42) (Fig. 1, In). Unilateral transection of DLF

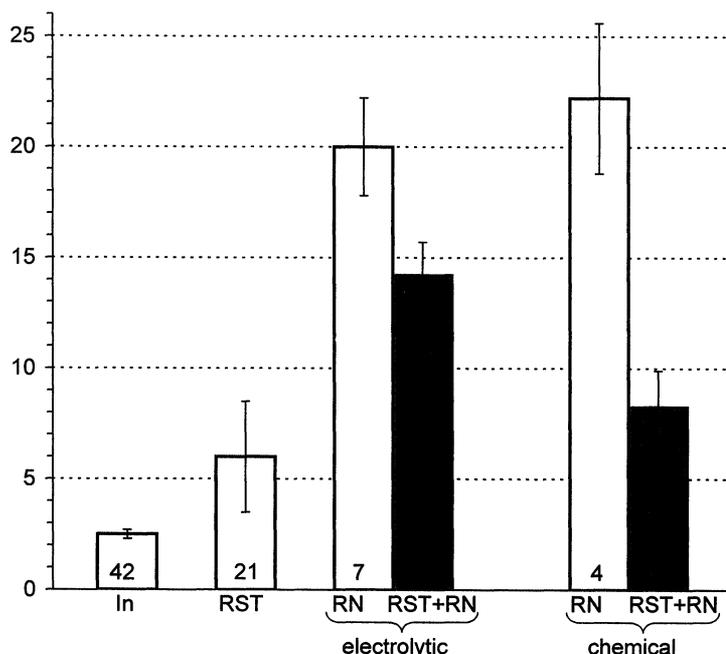


Fig. 1: Histogram comparing mean values \pm SD from the mean in the number of days required to reach criterion values of performance of instrumental reflexes (ordinate). Abscissa indicates the training of reflexes in intact rats (In), after transection of the dorsolateral funiculus (here called the rubrospinal tract, RST), electrolytic lesion red nucleus (RN) alone, transection of RST and subsequent electrolytic lesion of RN (RST+RN, electrolytic), chemical lesion of RN alone (RN), transection of RST and subsequent chemical lesion of RN (RST+RN, chemical). The numerical value within each column indicates the number of rats in this series of experiments.

at the third cervical vertebra (C 3) was performed (n=21). This procedure led to paresis of the homolateral forelimbs and hindlimbs, which subsided in a few days. Rats were taken for the experiments on the 3rd to 7th postoperative days. Stable instrumental reflexes were developed after 3.5 to 8.5 experimental days (mean days 6.0 ± 2.5 ; n=21) (Fig. 1, RST). Then the animals with stable instrumental reflexes underwent either electrolytic or chemical lesion of RN.

Effects of electrolytic lesion of the red nucleus

In seven rats, electrolytic lesion of the contralateral RN was performed from the 16th to

17th day after unilateral transection of DLF. The lesion resulted in paresis of the forelimbs and hindlimbs, contralateral to the lesioned side. Motor disorders were head tilt to the same side and wide positioning of the limbs during movement. On the 7th to the 10th day after the second operation, the conditioning of instrumental reflexes resumed and then became stable by 12.6 to 16.0 experimental days (mean days $=14.2 \pm 1.5$; n=7) (Fig. 1, RST + RN electrolytic, and Fig. 2B).

In another series of experiments (seven rats), RN alone was electrolytically lesioned. The resultant motor disorders were similar to those observed in the preceding series. Motor deficit was compensated for on the 5th to 10th postoperative day. It should be

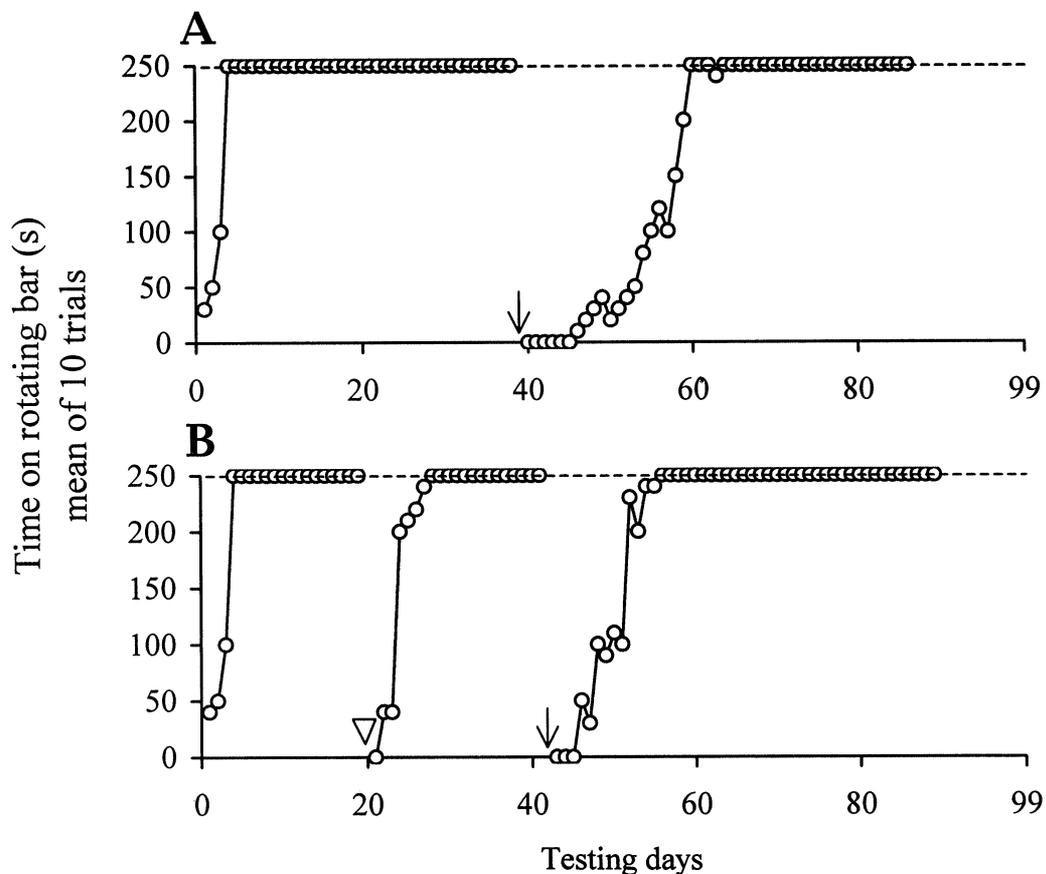


Fig. 2: Effects of transection of the dorsolateral funiculus and electrolytic lesion of the RN on the performance of learned instrumental reflexes in two rats. (A) RN alone was lesioned. (B) RN lesion was made after transection of the DLF. The progression of the reflexes is shown in days (abscissa). The mean value (in seconds) of 10 trials on the rotating bar is plotted for each training day (ordinate). The dashed line is the 250-s criterion level of performance. The open arrowhead designates the day of the DLF transection; the arrow indicates the day of RN lesion.

noted that the intensity of motor deficit was dependent on the degree of lesion (see Fig. 3). Conditioning of instrumental reflexes resumed, reaching 100% by 17.5 to 22.2 experimental days (mean days, 20.0 ± 2.2 ; $n=7$) (Fig. 1, RN, electrolytic, and Fig. 2A). Thus, following RN lesion alone, motor activity and instrumental reflexes recovered in 20 d. In contrast, however, recovery was faster (14.2 d) in animals that underwent transection of DLF before RN lesion (statistically significant at $p < 0.005$).

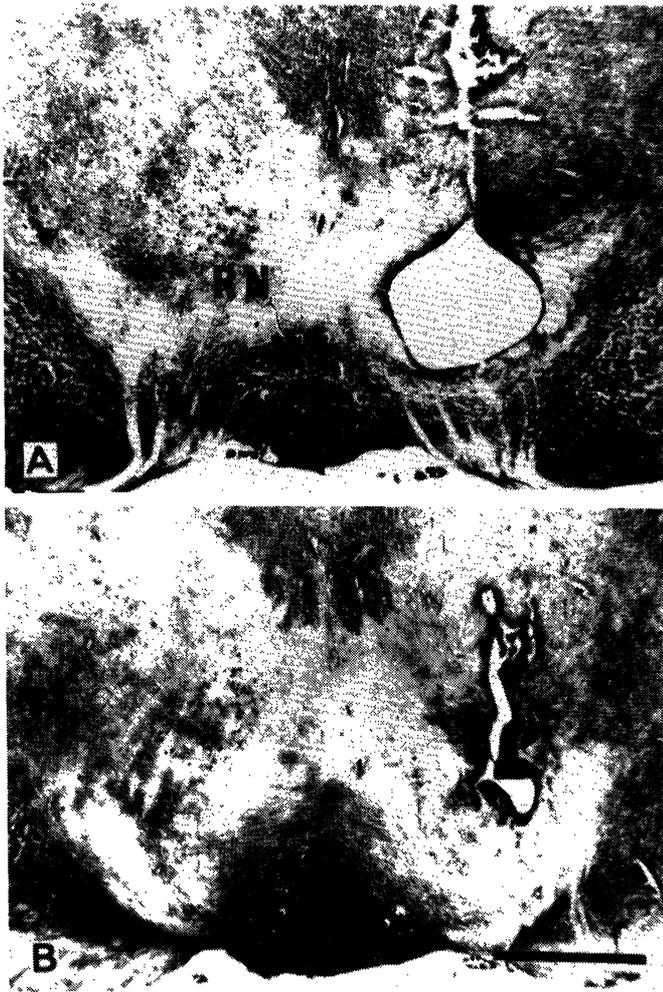


Fig. 3: Microphotographs of the frontal sections from the midbrain of rats with unilateral electrolytic completely (A) and incompletely (B) lesioned RN. (A) The cells in the RN are easily recognized on the unaffected side. On the opposite right side, no nerve cells are left in the area. (B) The incompletely lesioned RN, with some unaffected nerve cells. Stained by Nissl's method. Bar = 1 mm.

Effects of chemical lesion of the red nucleus

After compensation of motor disorders and recovery of instrumental reflexes (18.0 to 19.0 experimental days) following unilateral transection of DLF, the contralateral RN was chemically lesioned in four rats. After the injection of quinolinic acid into RN following recovery from anesthesia, tonic spasms, such as extension of the neck, back, and tail, occurred in response to pinch and to loud sound. During this period, the limbs ipsilateral to the lesion were flexed, while those contralateral were extended. This response was markedly intense in the forelimbs. Occasionally, small oscillatory movements of the head were observed. After 3 to 4 h, when the rats had recovered from the anesthesia, these phenomena disappeared. A clinical picture characteristic of lesion of RN developed. A characteristic gait was observed in the animals, as they moved with their limbs apart and dragged their hindlimbs. Rats were taken for the experiments on the 7th to 8th day after the second operation. Stable instrumental reflexes developed by experimental days 6.0 to 10.0 (mean days 8.25 ± 1.65 ; $n=4$) (Fig. 1, RST + RN chemical, and Fig. 4C, 4D).

In another group of four animals, the unilateral RN alone was chemically destroyed. Motor disorders developed that were similar to those observed in the experimental rats from the previous series of experiments. On the 6th to 11th postoperative day, the rats were taken for further experiments. Stable instrumental reflexes developed by 18.0 to 27.0 experimental days (mean days 22.2 ± 3.4 ; $n=4$) (Fig. 1, RN, chemical, and Fig. 4A, 4B). Similar statistically significant differences ($p < 0.005$) in the times of recovery of motor activity and of instrumental reflexes were observed during the experiments involving chemical lesion of RN alone (22.2 d) and lesion of RN after transection of DLF (8.25 d).

HRP injection demonstrated that quinolinic acid destroyed the somata of rubral neurons, whereas the cerebello-thalamic fibers passing through RN were preserved. Figure 5 shows that unilateral destruction of RN was accomplished by

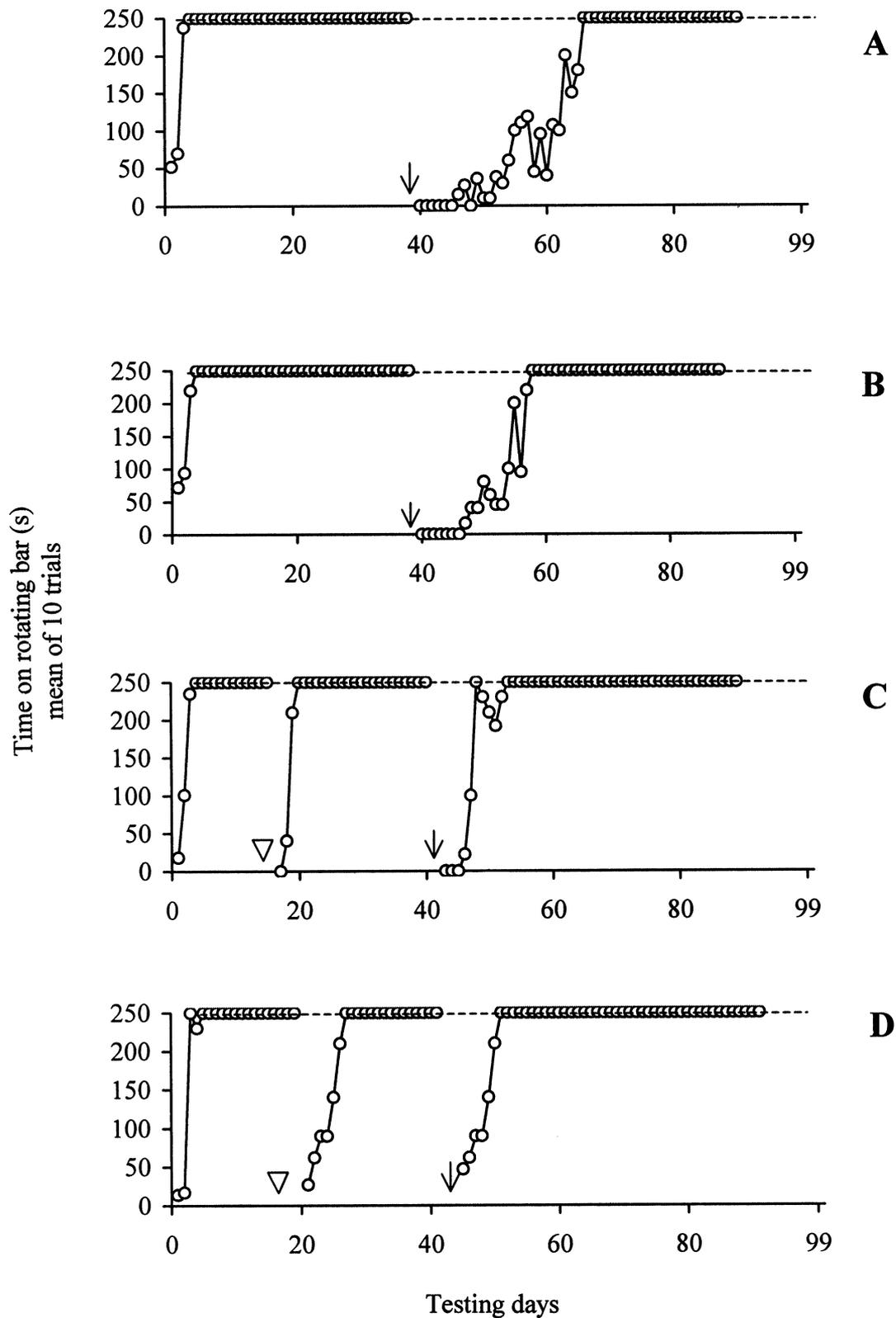


Fig. 4. Effects of transection of the dorsolateral funiculus and chemical lesion of RN on performance of learned instrumental reflexes in four rats (A, B, C, D). A and B: RN alone was lesioned. C and D: RN lesion was made after transection of the DLF. The designations are the same as those in Fig. 2.

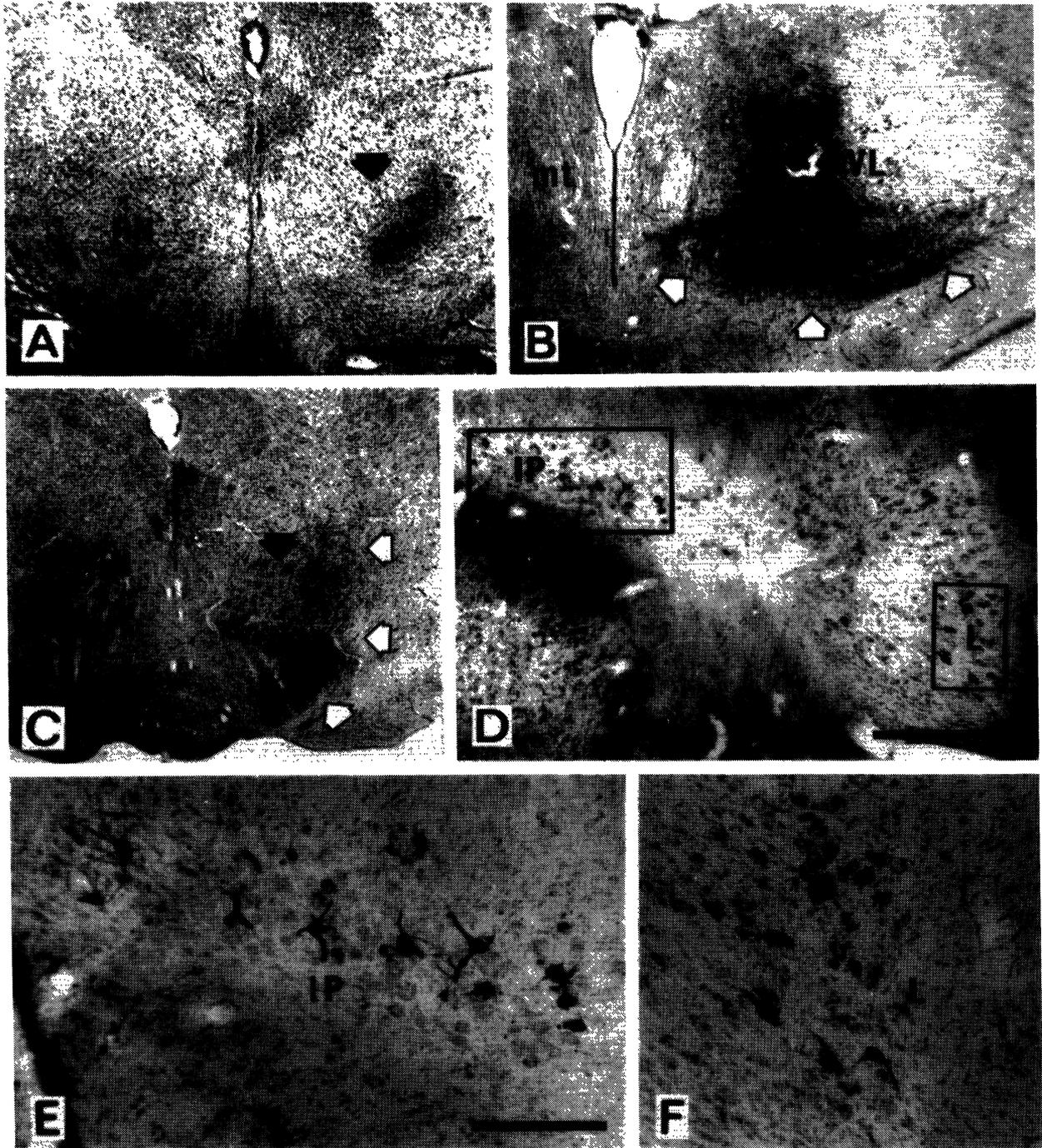


Fig. 5: Microphotographs of the frontal sections of the (A,C) rat midbrain, (B) the thalamus, and (D,E,F) cerebellar nuclei. The effects of chemical lesion of RN by injection of (A,C) local quinolinic acid and (B) local microiontophoretic HRP into the VL, on day 7 after chemical lesion. Microphotographs of HRP-labeled efferent neurons of nucleus interpositus (IP) and nucleus lateralis (L) of the cerebellum (D). Magnifications of the insets shown in (D) for IP and L (E and F, respectively). The closed arrowheads designate destroyed neurons of RN; the open arrowheads designate retrogradely labeled cerebello-thalamic fibers passing through RN (C). Section (A) was stained by Nissl's method; in sections (B to F), the enzyme was histochemically revealed using tetramethyl benzidine. Bar: 1mm=500 in A to C, 1mm=200 in D; mt = mammillo-thalamic tract.

quinolinic acid. In contrast, the opposite side of RN was completely unaffected (5A, 5C). Labeling of neurons in the nucleus interpositus and nucleus lateralis of the cerebellum (5D), following HRP injection into VL (5B), indicated that the cerebello-thalamic fibers passing through RN (5C) were preserved. The higher magnification (5E, 5F) emphasizes this effect. Thus, the unilateral injection of HRP into VL after unilateral RN destruction clearly demonstrated the absence of rubrospinal and other somatic structures in the ipsilateral RN, whereas cerebello-thalamic fibers were largely preserved.

DISCUSSION

The experiments presented here show the facilitating influence of preliminary transection of RST on the recovery of motor activity and instrumental reflexes in rats after lesion of RN, which agrees with the finding of Kennedy and Humphry (1987). It should be noted that the facilitating influence is prominent in rats with chemically lesioned RN (specifically, under conditions of cerebello-thalamic fiber preservation). The facilitation time was calculated as the difference between the times of recovery after RN lesion alone and after RN lesion preceded by transection of RST. The respective facilitation times for electrolytic and chemical lesions were 5.8 d (compare 20.0 and 14.2 d) and 14 days (22.2 and 8.25 d).

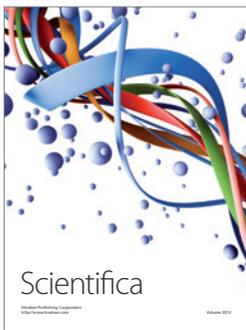
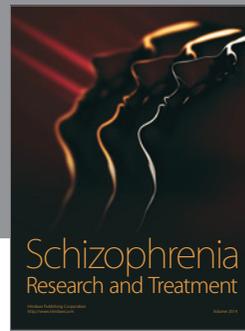
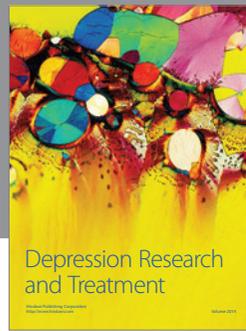
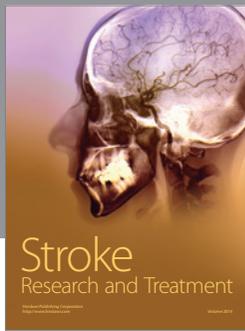
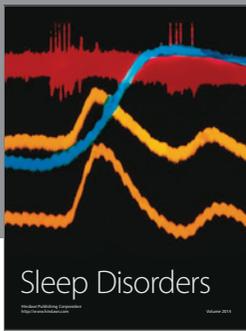
As previously hypothesized, VL is a major relay nucleus for cerebellar influences on the cerebral cortex and leads to a switching of cortical control for the new context of motion. Earlier studies conducted in our laboratory (Fanardjian et al., 1995) showed that preliminary lesion of VL strongly hampers the switching of motor activity that is under the control of the corticospinal tract in rats with damaged RST and RN. In some animals, however, unstable instrumental reflexes remained after three consecutive operations. The findings of the present study agree with those results. Our results,

obtained by electrolytic and chemical lesion of RN, confirm that the recovery of motor activity and instrumental reflexes occurs even after the interruption of the cerebello-thalamic fibers. Therefore, we predict that transmission of the ascending cerebellar influences on the cerebral cortex can be realized even through alternative cerebello-cortical pathways (see Mauk, 1997). Such pathways may involve different thalamic structures, as well as the reticular formation of the brainstem (Ito, 1984). Among the latter, the tectal structures of the midbrain, namely, the ventral tegmental region, the pars compacta of substantia nigra, and the retrorubral region, require great attention. The above mentioned structures contain a higher number of dopaminergic neurons (Dahlstrom & Fuxe, 1964), receive direct connections from the lateral and interpositus cerebellar nuclei (Perciavalle et al., 1980) and send ascending projections to cortical structures, thus, taking part largely in motor activity (Oades & Halliday, 1987; Piazza et al., 1987). Therefore, a comparison of the results of chemical and electrolytic lesion of RN confirms the presence of additional cerebello-cortical ascending pathways that might explain the switching effect. Such switching is, indeed, one of the basic compensatory mechanisms of destroyed motor function.

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