L-DOPS-Accelerated Recovery of Locomotor Function in Rats Subjected to Sensorimotor Cortex Ablation Injury: Pharmacobehavioral Studies

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KIKUCHI, K., NISHINO, K. and OHYU, H. L-DOPS-Accelerated Recovery of Locomotor Function in Rats Subjected to Sensorimotor Cortex Ablation Injury: Pharmacobehavioral Studies. Tohoku J. Exp. Med., 1999, 188 (3), 203-215 —— Central norepinephrine (NE) has been shown to play a beneficial role in amphetamine-facilitated recovery of behavior. To give insight into understanding the mechanism, the present studies were conducted to examine (a) the effects of L-threo-3,4-dihydroxyphenylserine (L-DOPS) combined with benzerazine (BSZ; a peripheral aromatic amino acid decarboxylase inhibitor) and L-3,4-dihydroxyphenylalanine (L-DOPA), precursors of NE and dopamine (DA), respectively, on the recovery from beam-walking performance deficits in rats subjected to unilateral sensorimotor cortex ablation injury, and (b) the relationships between the behavioral recovery and the frequency of postoperative training and the size of ablation injury. It was found that the combined treatments with L-DOPS and BSZ promoted the recovery of locomotor function as early as 24 hours after injury. L-DOPA alone, however, did not facilitate behavioral recovery. The results of assay for the tissue levels of NE and its major metabolite (3-methoxy-4-hydroxyphenylethylene glycol; MHPG) in the brain using high-pressure liquid chromatography showed MHPG, but not NE, significantly increased in the cerebellum and the hippocampus. The behavioral recovery was also significantly correlated with the frequency of training subsequent to injury, but inversely with the size of cortex ablation. These results suggest that NE is likely to modulate functional recovery in this rodent model. —— behavioral recovery; brain injury; norepinephrine; sensorimotor cortex  © 1999 Tohoku University Medical Press

A number of investigations have shown that the central nervous system (CNS) possesses an ability of functional plasticity and compensation, and yet the mechanism still remains to be elucidated (Davis et al. 1987; Feeney and Sutton 1987; Kolb 1990). The anatomical process of repair and regeneration of injured neurons by itself is not necessarily associated with the functional restoration of neurons (Davis 1985; Lipton 1989; Kolb 1990). Pharmacobehavioral approach

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Address for reprints: Kenji Kikuchi, M.D., Department of Neurosurgery, Yuri Kumiai General Hospital, 38 Yago Kawaguchi, Honjo, Akita 015-8511, Japan.
for comprehensive evaluations of the CNS function is useful in screening neurochemical factors or substances influencing functional recovery (Mason 1984). It is recently demonstrated that neurotransmitters such as norepinephrine (NE) and neurotrophic factors contribute toward functional recovery after neuronal injury in the CNS (Feeney and Sutton 1987; Lipton 1989; Feeney et al. 1993). Of particular interest is dextro-amphetamine (d-amphetamine), which proves to have beneficial effects upon functional recovery in both experimental animals and human stroke hemiplegic patients (Small 1994). A single dose of d-amphetamine augments postsynaptic catecholamines including NE and dopamine (DA) by increased release from storage (Small 1994). It is also suggested that the beneficial effect of d-amphetamine may be due to its ability to increase post-synaptic NE rather than DA. Therefore, NE may play an important role in the amphetamine-accelerated recovery (Pearson and Robinson 1981; Feeney et al. 1985; Davis et al. 1987; Boyeson and Feeney 1990). However, because of abuse potential, even experimental use of d-amphetamine is restricted in Japan.

The current investigation was conducted to gain further insight into the role of catecholamines such as NE and DA in functional recovery of locomotor function. Using a rodent model subjected to unilateral sensorimotor cortex ablation injury, we examined the effects upon behavioral recovery of a) the lesion size, b) the frequency of postoperative training, and c) the treatments with either L-threo-3,4-dihydroxy-phenylserine (L-DOPS) combined with benzerazide (BSZ) or L-3,4-dihydroxyphenylalanine (L-DOPA), which are the precursors for NE and DA, respectively. BSZ, an aromatic amino acid decarboxylase inhibitor, was simultaneously used with L-DOPS to enhance delivery of L-DOPS into the brain and therefore to produce a significant and prolonged increase in the extracellular NE concentration of the brain by inhibiting L-DOPS from being converted into NE in the circulating blood (Brannan et al. 1990).

Materials and Methods

Surgical procedures for cortical lesions

Male Sprague-Dawley rats weighing 290–350 g were used in the present studies. The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and mounted in a stereotactic holder. Their crania were exposed under aseptic conditions, and a square craniotomy, 5 to 7 mm in length, was made over the right sensorimotor cortex. According to the brain mapping by Neafse (1990), the right sensorimotor cortex was identified and a cortical lesion was then made by gentle aspiration with a fine-tipped Pasteur pipette. The standard lesion was created extending 2.0 mm anterior to and 4.0 mm posterior to the bregma, and 1.0 to 5.0 mm lateral midline toward the right side, and this 4 × 6 mm aspiration cavity was defined as a medium-sized lesion (n = 14). Similarly, small (n = 4) and large (n = 10) lesions were also created as the aspiration cavities of the size of 4 × 4 mm and 5 × 8 mm, respectively. Upon
completion of the lesions by aspiration, a piece of absorbable gelatin sponge, 4 × 4 mm in size, soaked in saline was placed in the cavity and the scalp was sutured closed.

**Behavioral training and testing procedure**

The locomotor deficits resulting from the cortical injury were quantified on a beam-walking task as described by others (Feeney et al. 1982; Goldstein and Davis 1990). Briefly, prior to surgery the rats were preliminarily trained twice a day for 5 days to traverse a wooden beam 2.5 cm wide and 122 cm long at the height of 90 cm. At the starting point on the beam, there was a 160-W bulb and a tape recorder generating white noise at 62 dB. At the other end of the beam, there was a darkened goal box measuring 25 × 20 × 18 cm. The animal was placed on the beam, and the light and the noise were turned on until the animal traversed the beam. Each beam-walking performance was scored according to the rating scale by Feeney and Sutton (1987, Table 1). All rats were tested at 24 hours after surgery, and only those rated as a score of 1 were then chosen for subsequent postoperative evaluations for spontaneous functional recovery over a period of up to 14 days. Rating scores on the beam-walking task were compared among the three groups of rats with small, medium and large cortical lesions.

To evaluate the effects of frequency of postoperative additional training on functional recovery, the following experiments were performed. Twenty-four hours after a medium-sized aspiration cavity was created, the rats were divided into two groups: One group was subjected to postoperative training which consisted of 6 trials on the following day and 1 trial daily for subsequent 6 days (n = 8), and another had training once daily alone for 7 days after surgery (n = 4). The former group of rats were arbitrarily referred to here as a “regularly trained” group, and the latter group as a “less trained” group. To assess whether or not restoration of locomotor function after injury was correlated with the frequency of

<table>
<thead>
<tr>
<th>Scale</th>
<th>Performance characteristic</th>
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<tbody>
<tr>
<td>1</td>
<td>Animals fail to traverse the beam and do not place the left hindlimb on the horizontal surface of the beam.</td>
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<tr>
<td>2</td>
<td>Animals fail to traverse the beam, but place the left hindlimb on the horizontal surface of the beam and maintain balance.</td>
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<tr>
<td>3</td>
<td>Animals traverse the beam while dragging the left hindlimb.</td>
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<td>4</td>
<td>Animals traverse the beam and place the left hindlimb at least once during traverse.</td>
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<tr>
<td>5</td>
<td>Animals traverse the beam using the left hindlimb to aid less than 50% of its steps on the beam.</td>
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<tr>
<td>6</td>
<td>Animals traverse the beam using the left hindlimb to aid more than 50% of its steps on the beam.</td>
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<tr>
<td>7</td>
<td>Animals traverse the beam with no more than two foot slips.</td>
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training and the time which elapsed after cortical injury, the animals were tested for beam-walking performance after each trial over a period of up to 7 days.

Measurement of lesion size

Seven days after the end of the experiments by a postoperative beam-walking task, the animals were given an overdose of sodium pentobarbital and decapitated. The brain was removed immediately and stored in 0.1 M phosphate-buffered 10% formalin fixative at pH 7.4 for 7 days at 4°C. The fixed brain was then photographed to measure the size of cortical lesion cavity. A paper weight method was used to calculate the area (in mm²) of aspiration cavity.

Administration of l-DOPS combined with BSZ and l-DOPA

The effects of l-DOPS and l-DOPA, precursors of NE and DA, upon functional recovery were analyzed in the following experiments. Twenty-four hours after a medium-sized lesion cavity was created, the rats were given an intraperitoneal injection of either l-DOPS (400 mg/ml) pretreated with BSZ (2 mg/kg) (n = 10) or l-DOPA (30 mg/kg) alone (n = 8), and were subjected to behavioral quantification of beam-walking performance at every 24 hours after injection. Animals treated with either l-DOPS without BSZ (n = 10) or BSZ alone (n = 5) were also tested in the present studies. Either 0.5% methylcellulose (n = 9) or normal saline (n = 8) was used as a control vehicle injection substituting for l-DOPS or l-DOPA, respectively. BSZ was given to enhance l-DOPS delivery into the brain by inhibiting peripheral l-DOPS from being converted into NE, and to alleviate the side effects of l-DOPS such as pulmonary and renal hemorrhages.

Measurements of brain NE and MHPG after l-DOPS administration

For evaluation of any relationships between l-DOPS-facilitated locomotor recovery and the tissue level of NE, quantitative measurement was conducted of the tissue levels of NE and its major metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in discrete regions of the brain. A group of rats treated with an intraperitoneal injection of l-DOPS and BSZ were sacrificed under deep anesthesia and decapitated 30 (n = 10) and 90 minutes (n = 5) after injection for measurements of MHPG and NE, respectively. The brains were removed immediately and placed on a glass over ice, and the cerebral cortex, hippocampus and cerebellum were dissected, and the tissues were stored at −70°C until assayed. On the day of assay, 1 g of the tissues was homogenized at 4°C for 30 seconds in 5 ml of 0.4 N perchloric acid. The homogenate was centrifuged at 3500 rpm and 0°C for 20 minutes, and the supernatant was repeatedly homogenized at the same condition and recentrifuged. The supernatant was then removed and assayed for NE and MHPG by high-pressure liquid chromatography with electrochemical detection as described elsewhere (Ueda et al. 1977).
Statistical analysis

Statistical analysis was performed using IBM's SAS Statistical Software. All behavioral data obtained from the beam-walking task in small, medium and large lesion groups were analyzed using Tukey's non-parametric statistical test. The results are presented as the mean values ± standard error of the mean (s.e.m.), and the differences were considered to be statistically significant at the $p < 0.05$ level. The difference among the lesion size was also analyzed using Tukey's test. The NE and MHPG data obtained from the cortex, hippocampus and cerebellum were analyzed by t-test and analysis of variance was used for intergroup comparisons. The rating scores on beam-walking task were also analyzed by t-test between the animals treated with L-DOPS with BSZ or L-DOPA and those treated with vehicle. For analysis by t-test $p$ values of 0.05 or less were regarded as statistically significant.

Results

Behavioral recovery after cortex injury

Lesion size. The size of cortical lesions were documented by the area of brain injury on the cortex, and the mean area ($\pm$ s.e.m.) of small, medium and large lesions, as defined in Materials and Methods, were $15 \pm 1.5$ mm$^2$, $24 \pm 0.7$ mm$^2$, $38.2 \pm 5.2$ mm$^2$, respectively. These values were statistically different from one another using Tukey's test ($p < 0.01$). The recovery from beam-walking performance deficits was inversely correlated with the size of cortical injury. Two groups of rats with small and medium-sized cortical lesions both scored 4.3 on the rating scale at the second postoperative day, and became capable of traversing the beam in 2 to 4 days after ablation. The rats with small lesions quickly reached the preoperative state scoring 7 in 5 days, and those with medium-sized lesions showed a similar, but more delayed course of motor recovery on beam-walking task. By contrast, the rats with larger lesions scored 2.0 at the third day and 3.7 at the 6th postoperative day at which time traversing the beam barely became possible, and did not recover to the level of 6 on rating scales of beam-walking performance until 9 days after surgery. Their behavioral recovery was found to be significantly delayed in comparison to the other groups (Fig. 1). In addition, one third of these rats with large lesions eventually did not accomplish a recovery to the preoperative and baseline level of beam-walking performance.

Frequency of training. The regularly trained rats, which had received training 6 times on the first postoperative day and once daily for the next 6 days, became capable of traversing almost normally at the 12th trial on the 7th day after injury. However, the less trained rats, which had received beam-walking training postoperatively only once daily for 7 days, were able to keep their balance but failed to traverse the beam. Postoperative status of beam-walking performance
on the 7th day after injury apparently differed between these two groups with different frequency of training. However, the graphs, which also demonstrated the relationship between the frequency of postoperative training and the degree of behavioral recovery, appeared to be quite similar in their patterns for both groups during the first 7 trials of postoperative training. This clearly indicated that the recovery was correlated with the frequency of training, rather than the time which elapsed after injury (Fig. 2).

Effects of L-DOPS with BSZ and L-DOPA

Behavioral recovery. The rats treated with both L-DOPS and BSZ had an average score of 3.8 in the rating scale at 24 hours after surgery, and more rapidly recovered to be able to traverse the length of the beam than the control rats treated with vehicle. The control rats required 6 days after injection to obtain the rating score of more than 4. During these initial 6 days the rate of behavioral recovery demonstrated by the rats treated with L-DOPS and BSZ was statistically different from that of the control (p < 0.05). However, the final status of beam-walking performance on the 14th day after injury was not significantly different between these two animal groups (Fig. 3). All rats given L-DOPS alone died of acute pulmonary edema, and those administered BSZ did not demonstrate functional recovery of beam-walking performance. Therefore, the treatment with L-DOPS combined with BSZ was shown to facilitate the rate of motor recovery after cortex ablation. In addition, an average score was 4.5 on the third day after injury in the rats treated with L-DOPA, which was higher than, but not statistically

Fig. 1. The effect of lesion size upon recovery of beam-walking performance in 3 groups of the animals with small, medium and large cortical lesions. Data are presented as the mean scores ± S.E.M. The animals with large lesions made a significantly delayed recovery until 9 days after surgery in comparison to those with small- and medium-sized lesions (p < 0.05). ○, small lesion (n = 4); ●, medium-sized lesion (n = 14); △, large lesion (n = 10).
Fig. 2. The effect of the frequency of postoperative trainings on recovery of beam-walking performance over a period of 7 days after surgery. "Regularly trained" rats indicated by open circles represent a group of animals which received postoperative training consisting of 6 trials on the following day and 1 trial daily for subsequent 6 days. "Less trained" rats indicated by closed squares represent another group of animals subjected to postoperative training once daily for 7 days after surgery. Data are presented as the mean scores ± s.e.m. ○, regularly trained rats (n = 8); ■, less trained rats (n = 4).

Fig. 3. The effect of L-DOPS and BSZ on recovery of beam-walking performance. L-DOPS was given intraperitoneally together with BSZ 24 hours after a medium-sized lesion was made. As a control vehicle 0.5% methylcellulose solution was administered. Vertical bars indicate s.e.m. Statistical analysis of data shows that the rate of recovery demonstrated by the animals treated with L-DOPS and BSZ is significantly different from the control treated with vehicle during the initial 6 days (*p < 0.05). ○, vehicle (n = 9); ■, L-DOPS + BSZ (n = 10); L-DOPS, L-threo-3,4-dihydroxyphenylserine; BSZ, benserazide. * indicates statistical significance.
Fig. 4. The effect of l-DOPA on recovery of beam-walking performance. l-DOPA was given intraperitoneally 24 hours after a medium-sized lesion was made. As a control vehicle normal saline was administered. Vertical bars indicate s.e.m. There is no statistically significant difference between these two groups. □, vehicle (n = 8); ■, l-DOPA (n = 8); l-DOPA, L-3,4-dihydroxyphenylalanine.

Fig. 5. The effect of l-DOPS and BSZ on tissue levels of NE (a) and MHPG (b) in the brain. These tissue levels were measured by high-pressure liquid chromatography after an intraperitoneal injection of l-DOPS together with BSZ. Data are presented as the mean values ± s.e.m. Treatment with l-DOPS and BSZ resulted in a significant increase of MHPG levels in the cerebellum and hippocampus (*p < 0.05). However, there was no significant difference in the NE levels. □, vehicle (n = 5); ■, l-DOPS + BSZ (n = 5); l-DOPS.

different from, that of 3.2 in the control rats treated with vehicle. At the 7th day after injection the rats with l-DOPA treatment scored 6.8, whereas the control scored 6.7. There was no statistically significant difference between these animal
groups during the entire course after surgery (Fig. 4). \textit{L}-DOPA did not demonstrate any beneficial effect on the rate of motor recovery.

\textbf{NE and MHPG level.} To analyse for the effects of intraperitoneally administered \textit{L}-DOPS upon tissue concentrations of NE and MHPG in the rat brain, the cerebral cortex, hippocampus and cerebellum were compared. It was found that the NE levels in these brain tissues at 90 minutes after administration of \textit{L}-DOPS and BSZ had a tendency of increasing both in the hippocampus and cerebellum, but not statistically different from those of the control rats treated with vehicle (Fig. 5a). In contrast, the MHPG levels were high in the cerebellum for both the treated and the control rats, and they significantly increased in the treated rats by 20\% in the hippocampus and 30\% in the cerebellum. However, the MHPG levels were not elevated in the cerebral cortex 30 minutes following intraperitoneal administration of \textit{L}-DOPS with BSZ (Fig. 5b). The MHPG/NE ratio was then calculated to determine NE turnover. Both the hippocampus and cerebellum had significantly increased ratios in the treated rats, and the ratio in the cerebral cortex was decreased.

\textbf{Discussion}

In the present studies, we have demonstrated that the recovery from beam-walking performance deficits was accomplished in 14 days after cortical injury, and that the rate of functional recovery was associated directly with the frequency of training and inversely with the lesion size. In addition, \textit{L}-DOPS combined with BSZ significantly promoted the restoration of locomotor function, and yet by contrast \textit{L}-DOPA alone had no beneficial effect upon functional recovery. These studies replicated the results of several others, confirming that the rat brain has a plasticity of motor function following cortical injury (Feeney and Sutton 1987; Kolb 1990).

Previous studies documented that \textit{d}-amphetamine, which augments postsynaptic catecholamines including NE and DA, promoted behavioral recovery in a variety of animal models (Feeney et al. 1982; Feeney and Hovda 1983, 1985; Feeney and Sutton 1987), and it is confirmed more recently that \textit{d}-amphetamine facilitates the restoration of locomotor function 24 hours after injection both in the animal model of sensorimotor cortex ablation and in patients with cerebral infarction (Feeney et al. 1985; Davis et al. 1987; Hurwitz et al. 1989; Sutton et al. 1989; Small 1994). As the locus coeruleus is the origin of diffuse noradrenergic arborizations throughout the cortex, the role of the locus coeruleus in recovery was studied (Feeney et al. 1985). It is suggested that a massive release of NE from the locus coeruleus by \textit{d}-amphetamine is associated with the functional recovery, and the underlying mechanism for this phenomenon may be explained in part by a neuroprotective action of NE, which contributes toward improving cross cerebellar diaschisis, extensive functional impairment, and disturbed level of consciousness all of which result from damaged brain (Feeney and Hovda 1983; Feeney et
al. 1985; Feeney and Barron 1986; Lipton 1989; Boyeson and Feeney 1990; Feeney 1991). However, it is also true that d-amphetamine alone without subsequent training does not have beneficial effect upon behavioral recovery (Hovda and Feeney 1984), and that the recovery persists for a prolonged period of time even by a single dose of d-amphetamine. These observations further need to be clarified.

Beam-walking performance is an integrated form of behavior necessitating pertinent levels of consciousness, memory, sensorimotor and cerebellovestibular functions. The results of this behavioral testing are reproducible and therefore this testing is useful in detection of latent locomotor deficits and evaluation of motor recovery (Feeney et al. 1982; Davis et al. 1987; Hurwitz et al. 1989). Several lines of evidence suggest that the recovery of the ability of rats to traverse a narrow beam after unilateral injury to the sensorimotor cortex is noradrenergically mediated (Boyeson et al. 1992; Goldstein 1995). However, little is known about the anatomical background for motor recovery from beam-walking performance deficits after injury. The responsible sites for recovery currently may include the medial portion of the sensorimotor cortex and the contralateral cerebellum to which the noradrenergic axons from the locus coeruleus project (Boyeson et al. 1986; Goldstein and Davis 1990). More recently Goldstein and Bullman (1997) suggested the effect of NE on recovery of beam-walking ability may be partially exerted in the cerebral cortex contralateral to the injury.

In view of a massive release of NE and DA by d-amphetamine, behavioral recovery was also comparatively examined in the present studies after a single intraperitoneal injection of L-DOPS (precursor of NE) or L-DOPA (precursor of DA). BSZ was simultaneously used to enhance delivery of L-DOPS into the brain (Karai et al. 1987). L-DOPS with BSZ, but not L-DOPA alone resulted in acceleration in motor recovery at 24 hours after injury. These results are consistent with those of the previous reports that NE rather than DA is associated with enhancement of the recovery (Feeney and Sutton 1987; Boyeson and Feeney 1990). However, there is a likelihood that a high dose of L-DOPA could also enhance recovery by increasing NE synthesis. Boyeson and Feeney (1990) suggested that NE may be synthesized in the brain from L-DOPA via DA.

NE possesses a multiplicity of neurochemical actions, and among them, especially pertinent to functional recovery, are those of enhancing synaptic plasticity (Kasamatsu and Pettigrew 1976; Lipton 1989) and of potentiating synaptic transmission at N-methyl-D-asparate (NMDA) receptors (Marshall and Tsai 1988). The present report demonstrated recovery from beam-walking performance deficits was related to the frequency of training, rather than the time which elapsed after injury, and may also be accelerated by the treatments with L-DOPS with BSZ. It is therefore postulated that the motor recovery on a beam-walking task may rather be a form of learning process substantiated by reconstruction of neuronal circuit and more efficient synaptic transmission as a result of training.
and an increased level of catecholamines such as NE.

The mechanism of repair and regeneration in damaged brain varies according to the type and site of neuronal cells (Davis 1985; Feeney and Sutton 1987). NE is originated from the locus coeruleus, and widely distributed in the CNS including the cerebral cortex, hippocampus, cerebellum, and spinal cord (Dahlstrom and Fuxe 1964). Amphetamine-accelerated recovery involves not only locomotor function but also sensory and visual functions, learning, and memory as well (Feeney and Sutton 1987). It is therefore implicated that NE is one of the nonspecific neuromodulators favorably influencing functional recovery regardless of the site of injury. In fact, d-amphetamine has recently proved to be effective on the recovery from locomotor deficits and aphasia in patients with cerebral infarction (Walker-Batson et al. 1992), and NE may also play a beneficial role as a neuromodulator on functional recovery in humans.

In the current studies quantitative measurements of NE and its metabolite MHPG contents in the rat brain demonstrated an increase in both the hippocampus and cerebellum following intraperitoneal administration of L-DOPS and BSZ. An increase of MHPG alone was statistically significant, but still remains to be relevant since MHPG content is the most reliable indicator of NE release (Stone 1973), and electrostimulation on noradrenergic neurons of the locus coeruleus leads to a significant increase in MHPG content in the brain (Crawley et al. 1980). It was also demonstrated in the behavioral testing that the treatment with L-DOPS and BSZ significantly accelerated recovery of locomotor function as evidenced by beam-walking performance. The combined results from these biochemical and behavioral studies highly support a significant role of NE in beam-walking recovery after cortical injury in this rodent model, and further indicate that L-DOPS could become a promising candidate for pharmacotherapeutic studies for hemiplegic stroke patients.

References


