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Research article

Different patterns of motor activity induce differential plastic changes in pyramidal neurons in the motor cortex of rats: A Golgi study

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HIGHLIGHTS

- Dendritic spines of pyramidal cells in motor cortex increased after motor activity.
- Different regimes of motor activity induced differential plastic changes in spines.
- Thin spines increased when velocity changed during motor activity.
- Mushroom spines increased when velocity and incline changed during motor activity.

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ABSTRACT

Rehabilitation is a process which favors recovery after brain damage involving motor systems, and neural plasticity is the only real resource the brain has for inducing neurobiological events in order to bring about re-adaptation. Rats were placed on a treadmill and made to walk, in different groups, at different velocities and with varying degrees of inclination. Plastic changes in the spines of the apical and basal dendrites of fifth-layer pyramidal neurons in the motor cortices of the rats were detected after study with the Golgi method. Numbers of dendritic spines increased in the three experimental groups, and thin, mushroom, stubby, wide, and branched spines increased or decreased in proportion depending on the motor demands made of each group. Along with the numerical increase of spines, the present findings provide evidence that dendritic spines' geometrical plasticity is involved in the differential performance of motor activity.

1. Introduction

After a disabling injury, guided motor activity serves as the basis for rehabilitation schemes in the brain. Likewise, physical training works towards the perfection of psychomotor skills. Voluntary movement can promote permanent changes in patterns of psychomotor activity, which are mediated by the integration of information in the pyramidal neurons in layer V of the motor cortex. The dendritic spines of the pyramidal neurons process afferent information, resulting in its integration.

Dendritic spines are cytoplasmic protrusions that exhibit highly dynamic plastic activity. New spines may form, old spines may retract, and they may transform their geometric structure from one to another of the five generally recognized types, which are thin, mushroom, stubby, wide, and branched [1].

Recent studies have shown that motor training induces electrophysiological changes in the neurons of the primary motor cortex in rats, which suggests increased excitatory neurotransmitter activity [2]. In agreement, there exists evidence that motor activity induces plastic changes in the dendritic spines of the motor cortex in rodents, and these changes have been shown to occur during normal motor activity [4,6]. While spines' interconversion of their geometric structure is a phenomenon characteristic of their plastic capacity, as of yet there are no studies that analyze the role that varying density of spines play in the motor cortex under different paradigms of motor activity.

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CONTROL	E1	E2	E3
• 15' / day / 7 days	• 15' / day / 7 days • 1575m total • 15m / min • 0° inclination	• 15' / day / 7 days • 2025m total • - 15m / min (days 1-3) - 20m / min (days 4-5) - 25m / min (days 6-7) • 0° inclination	• 15' / day / 7 days • 1575m total • - 15m / min (days 1-3) - 15m / min (days 4-5) - 15m / min (days 6-7) • - 0° inclination (days 1-3) - 5° inclination (days 4-5) - 10° inclination (days 6-7)

Fig. 1. Experimental design used.

2. Material and methods

2.1. Animals

This study used forty male Sprague–Dawley adult (60 days) rats born to 10 dams. We maintained the subjects under standard conditions of regular 12-h light–dark cycles (07:00–19:00 h), 45–50% environmental humidity, a temperature of 22 ± 2 °C, with free access to food and water.

All experimental procedures were carried out in accordance with the NIH guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996 revision), and they were approved by the Research Ethics Committee of the Instituto Mexicano del Seguro Social, Mexico.

2.2. Experimental design

The rats were assigned to one of four groups of study: three experimental groups (E1–E3; n = 10 per group) and one control group (C; n = 10). The animals from groups E1–E3 were exposed to different paradigms of motor activity in a treadmill apparatus (Panlab, model LE8700C) over 7 days. E1 animals ran a total distance of 1575 m in 15 min/day, at a constant speed of 15 m/min in a horizontal plane. E2 animals ran 675 m in 3 days, at a speed of 15 m/min in 15 min/day; 600 m in the following 2 days, at a speed of 20 m/min, and; 750 m in the last 2 days, at a speed of 25 m/min; for a total distance of 2025m, which they ran in a horizontal plane. E3 animals were subjected to the same scheme as to E1, but in days 4 and 5 the plane was inclined by 5°, and in days 6 and 7 was 10°. The increase in velocity in E2 group would provoke the activation of rapid contraction motor units (type II), while in E3, the increased incline would contribute to the activation of slow contraction motor units (type I) [3]. Control animals were placed into the apparatus 15 min/day over 7 days but no motor activity was induced (Fig. 1).

2.3. Golgi study

Six animals per group were selected at random for the Golgi study. They were anesthetized with lethal doses of pentobarbital (30 mg/kg), and perfused with 200 ml of a washing phosphate-buffered solution (pH 7.4; 0.01 M) containing 1000 IU/l of the anticoagulant sodium heparin and 1 g/l of the vasodilator procaine hydrochloride. Subsequently, 200 ml of a phosphate-buffered 4% formaldehyde fixative solution was perfused. Both solutions were perfused at a rate of 40 ml/minute. The rats' brains were removed and maintained for 48 h in 100 ml of a fresh fixative solution. The motor cortex was dissected out following the atlas of Paxinos and Watson [4] and impregnated using a modification of the Golgi method [5]. Six pyramidal neurons from the fifth-layer were studied per rat. Spines were counted in a 50 μm segment from two dendrites —one secondary to the apical, and one basal- per neuron. They were then classified as thin, mushroom, stubby, wide, or branched spines (Fig. 2). We performed counts by direct observation at 2000× using a magnification changer coupled to a light microscope.

Table 1
Numerical density of dendritic spines in secondary branchlets to the apical dendrite and primary basal dendrites of fifth-layer pyramidal neurons from the motor cortex of the rats in the groups studied.

Spine density	Group			
	C	E1	E2	E3
Apical	64.1 ± 1.1	73.7 ± 1.6 [a]	83.3 ± 1.0 [ab]	73.4 ± 1.3 [abc]
Basal	65.2 ± 1.5	68.7 ± 1.0	77.3 ± 1.6 [ab]	72.7 ± 1.1 a

Mean ± SEM.
$p < 0.05$.
a: vs. C; b: vs. E1; c: vs. E2.

2.4. Statistics

We averaged and compared both spine density and the proportions of spine types between groups. We analyzed spine density with one-way ANOVA and Tukey *post hoc* tests and determined the proportional density of the spine types using one-way ANOVA and Bonferroni *post hoc* tests.

3. Results

3.1. Dendritic spine density

3.1.1. Apical arborization

In dendrites secondary to the apical, spine density was different among the groups ($F = 34.785, 3, p < 0.0001$). E1 ($p < 000.1$), E2 ($p < 0.0001$), and E3 ($p < 0.0001$) had more spines than control group C, and E2 showed more spines than both groups E1 ($p < 000.1$) and E3 ($p < 0.0001$) (Table 1).

3.1.2. Basal arborization

In the rats' basal dendrites, spine density was differentiated ($F = 16.260, 3, p < 0.0001$). E2 ($p < 0.0001$) and E3 ($p < 0.002$) showed spine densities that were different from that of control group C. In addition, E2 showed more spines than E1 ($p < 0.002$) (Table 1).

3.2. Proportional density of spine types

3.2.1. Apical arborization

The proportional density of thin ($F = 9.801, 3, p < 0.0001$), mushroom ($F = 46.062, 3, p < 0.0001$), stubby ($F = 4.181, 3, p < 0.01$), wide