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Guided motor training induces dendritic spine plastic changes in adult rat cerebellar Purkinje cells

INVESTIGACION Y ESTUDIOS DE POSGRADO

Opción Seminario de Investigación

QUE PARA OBTENER EL TITULO DE:

LICECIADO EN BIOLOGÍA

PRESENTA

DAVID GONZÁLEZ TAPIA

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Coordinación de Carrera de la Licenciatura en Biología

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C. DAVID GONZÁLEZ TAPIA
PRESENTE

Manifestamos a usted, que con esta fecha, ha sido aprobado su tema de titulación en la modalidad de **INVESTIGACIÓN Y ESTUDIOS DE POSGRADO** opción **Seminario de Investigación**, con el título **"Guided motor training induces dentritic spine plastic changes in adult rat cerebellar purkinje cells"**, para obtener la Licenciatura en Biología.

Al mismo tiempo le informamos, que ha sido aceptado como director(a) de dicho trabajo al **Dr. Ignacio González Burgos.**

Sin más por el momento, aprovechamos para enviarle un cordial saludo.

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Las Agujas, Nextipac, Zapopan, Jal., 14 de agosto, del 2012.

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Nos permitimos informar a usted que habiendo revisado el trabajo de titulación, modalidad **INVESTIGACIÓN Y ESTUDIOS DE POSGRADO**, opción **Seminario de investigación** con el título: "Guided motor training induces dentritic spine plastic changes in adult rat cerebellar purkinje cells" que realizó el pasante David González Tapia con número de código 207395639 consideramos que ha quedado debidamente concluido, por lo que ponemos a su consideración el escrito final para autorizar su impresión.

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ded motor training induces dendritic spine plastic changes in adult rat ebellar purkinje cells

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ABSTRACT

The simple cerebellar lobule is involved in several neuromotor processes and it is activated during guided exercise. Although guided exercises are essential for motor rehabilitation, the plastic events that occur in the simple cerebellar lobule during motor training remain unknown. In this study, normal adult rats were intensely trained on a motorized treadmill during a period of four weeks (IT group) varying both the velocity and the slope of the moving belt, and they were compared to a mildly trained (MC) group and an intact control group (IC). Dendritic spine density and proportions of the different spine types on Purkinje cells was assessed in the cerebellar simple lobule, as was drebrin A expression. Both dendritic spine density and drebrin expression increased in the MC and IT groups. Stubby spines were more abundant in the MC animals, while there was an increase in both stubby and wide spines in IT rats. In addition, mushroom spines were more numerous in the IT group. Increases in stubby and wide spines could be related to regulation of the excitability in Purkinje cells due to the motor training regime experienced by the MC and IT rats. Moreover, the observed increase in mushroom spines in the IT group could be related with the motor adjustments imposed by training.

notor training involves the activation of several brain regions as the sensorimotor cortex, lateral ventral thalamic nucleus, cerebellum, all of which are integrated into the cerebellar-mic-cortical circuit (CTCc). Functional activity of the CTCc has implicated in somatosensory integration [28] and information ing [5] in order to achieve adequate motor coordination. In on, CTCc activity provides the motor cortex with information ding the timing, velocity and force associated with movement n particular, the simple lobule of the cerebellum is involved ntrolling the execution of movement through feedback com-on with information coming from the spinal cord [3].

otor-skill learning and not just voluntary motor activity e an increase in the number of granule cells' parallel fibers ses in the cerebellum [4,22], as well as synaptogenesis in the cortex [21,38]. However, normal rats which exercised on a d treadmill activated CTCc-related cerebral regions, includ-e left simple lobule of the cerebellum [16], suggesting that ive plastic changes of dendritic spines may also occur in cere-bellar Purkinje cells. Dendritic spines have been shown to undergo plastic changes that are dependent on the nature of the presynaptic stimulation [11]. Thus, since Purkinje cells integrate all the afferent information to cerebellar cortex and their dendritic spines receive most of the excitatory inputs from parallel fibers, changes in afferent information to Purkinje cells could be integrated through morphological plastic changes at the dendritic spine level. Considering that these cells represent the only corticocerebellar efferent output, we have evaluated the possible plastic changes to Purkinje cell dendritic spines in the simple lobule of the cerebellum in normal exercised adult rats.

Male Sprague–Dawley adult rats ($n = 30$) weighing 250–300 g were used in this study and they were assigned to one of three study groups: an intensely trained group (IT; $n = 10$), a mildly trained control group (MC; $n = 10$), and an intact control group (IC; $n = 10$). Only the IT and MC groups were submitted to the motor training protocol. Rats from both the IT and MC groups were placed on a motorized treadmill (Panlab) for 15 min at a speed of 15 m/min/day during 7 days. Throughout this period, a 0.4 mA current was provided from a shock grid, forcing the rat to run on the treadmill's rolling belt. The rats learned to avoid the shock grid between the first and second day. The exercise protocol was only continued for the IT rats for a further three weeks, 5 days a week, and when electric shocks were no longer used. The rats were trained for 30 min at a speed of 16.2 m/min/day during the first week and in the follow-

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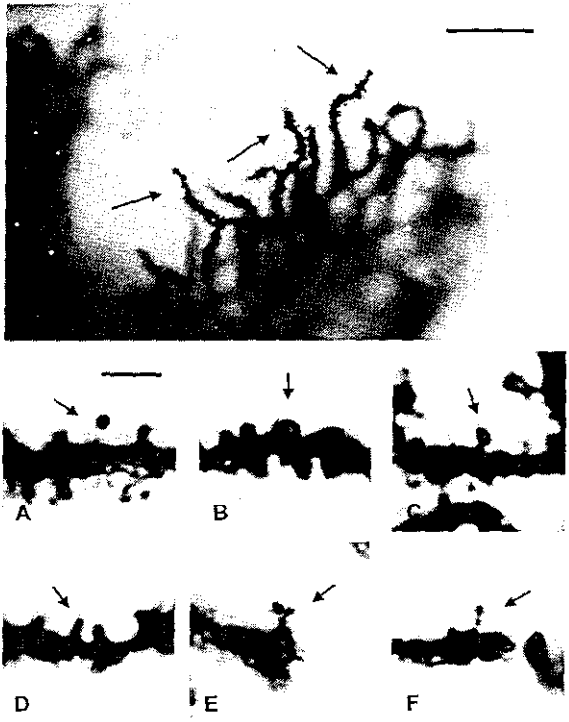
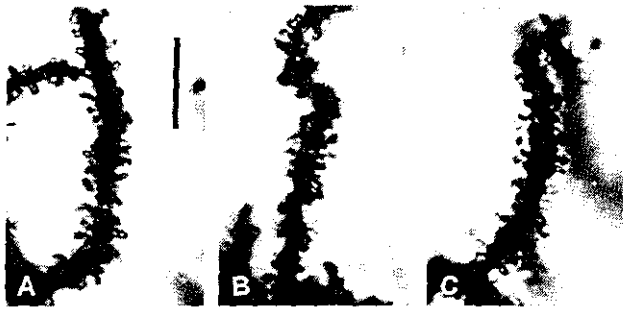
ek, the rats were trained to run at 18 m/min for 30 min/day. , during the last five days the rats ran at a speed of 18 m/min min/day on a 5 slope.

e day after the behavioral training ended, six animals were mly selected from each group. The animals were anaes- ed with ethyl ether, and then perfused with 200 ml of hate-buffered saline (PBS; 0.01 M, pH 7.4) containing sodium in (1000 IU/L) and procaine hydrochloride (1 g/L) [9]. The rats subsequently perfused with 200 ml of a phosphate-buffered rmaldehyde solution, also at a rate of 11.5 ml/min. The ani- brain was then removed and fixed for 48 h in 100 ml of fresh on, and a tissue block from the left cerebellar hemisphere con- g the simple lobule [30] was impregnated using a modified on of the Golgi technique [12]. Sagittal slices (75 μm thick) mounted on slides, and 6 impregnated and clearly visible nje neurons per animal were studied "blind" from each rat. lensity of the spine-like protrusions was quantified, as was the ortion of thin, stubby, mushroom, wide, branched and double es [11,13,37]. These spines were counted in a total stretch of m from 3 to 4 apical terminal dendritic branchlets distal to the a (Fig. 1), in each of the six cells studied per rat. Counting was ormed by direct observation at 2000× using a magnification ger coupled to a light microscope.

The remaining four animals in each group were used to determine the drebrin content. Total protein extracts were prepared by homogenization of the left cerebellar simple lobule of the IC, MC and IT animals in a sodium dodecyl sulfate (SDS) buffer (20% glycerol, 4.0% SDS, 125 mM Tris–HCl, 10% mercaptoethanol, pH 6.8). The protein content was determined according to the Lowry method using bovine serum albumin (BSA) as the standard. The samples were boiledin SDS buffer (3 min, 100 C) and 30 μg of protein was loaded in each lane of a 10% polyacrylamide gel. Electrophoresis was carried out at 200 mA, and the proteins in the gel were subsequently electroblotted onto nitrocellulose membranes (Hybond™-C pure, Amersham Pharmacia Biotech). The membranes were incubated for 1 h at room temperature with a blocking solution (TTBS) containing: 10 mM Tris (pH 7.4), 150 mM NaCl, 0.01% Tween 20, 2% BSA, and 2% skim milk. They were then incubated overnight at 4 C with an antibody against drebrin (1:500, Santa Cruz Biotech, Inc.) and β-actin (1:1000, Sigma) followed by incubation with a secondary biotinylated anti-rabbit IgG generated in horse (1:2000, Vector Laboratories Vectastain, Burlingame, CA, USA). Antibody binding was detected and visualized with 3,3-diamino-benzidine and the size of the drebrin protein detected was 120 kDa. Proteins were visualized and analyzed using Kodak Digital Science 1D software, ver. 3.0.2. (Eastman Kodak Co., Rochester,



. Upper panel: photomicrograph of apical dendritic branchlets (arrows) of a Purkinje cell, where spines were counted. Scale bar = 15 μm. In the lower panel, pho- rographs show thin (A), stubby (B), mushroom (C), wide (D), branched (E) and double (F) spines (arrows), representative of those counted in the present study. Scale 5 μm.



Photomicrographs of representative Purkinje cell apical dendritic branchlets from intact control (A), mildly trained control (B) and intensively trained (C) rats. Note crease in spine density in B and C when mildly and intensively trained rats were compared with intact animals (A). Scale bar: 10 μm.

to evaluate the intensity of the complete spot area, which was essed as arbitrary units of intensity. All measurements were e in duplicate.

or the statistical analysis of spine density, data from the six : per rat were averaged, and the average of the six animals per ıp was compared using the one-way ANOVA, followed by the ey *post hoc* test. The same tests were used to compare the data n Western blots. Finally, one-way ANOVA followed by the Bon- oni correction *post hoc* test was used to analyze the density of different spine types.

The dendritic spine density on Purkinje cells differed in each of three groups of animals studied ($F = 10.624$, $p < 0.001$; Fig. 2). dendritic spine density in Purkinje cells from both IT and MC was greater than that in IC animals ($p < 0.001$, and $p < 0.03$; pectively), and there was no difference in the dendritic spine sity in Purkinje cells from MC and IT animals (Table 1). arding the different spine types, stubby ($F = 13.6$, $p < 0.0001$), shroom ($F = 6.4$, $p < 0.01$) and wide ($F = 5.0$, $p < 0.02$) spine den- es were different in the three groups studied, while the density hin, branched and double spines remained unchanged. Stubby nes were more numerous on both IT and MC Purkinje neurons n on those of the IC group ($p < 0.0001$, and $p < 0.01$; respectively), the density of mushroom spines was greater in IT animals than C rats ($p < 0.008$). Finally, wide spines were more numerous in ats when compared with IC animals ($p < 0.02$; Table 1).

The levels of drebrin differed between the three groups studied 7.58, $p < 0.02$) and the IT group contained more drebrin than n the IC ($p < 0.02$) and MC ($p < 0.04$) rats. There was no signifi- t difference in drebrin content between the IC and MC animals . 3).

e 1
e density and proportional density of the different types of spines, in the simple e of Purkinje cells from intact control (IC), mildly motor-trained control (MC) ntensively motor-trained (IT) rats.

	IC	MC	IT
ne density	133.7 ± 5.1	150.0 ± 3.6[a]	159.8 ± 3.0[b]
ne types			
n	52.2 ± 1.7	53.9 ± 1.6	51.7 ± 2.8
bby	25.0 ± 1.5	31.0 ± 0.6[a]	33.9 ± 1.3[b]
shroom	44.1 ± 2.6	51.3 ± 3.1	57.3 ± 4.4[b]
de	10.6 ± 0.9	11.5 ± 1.1	14.6 ± 0.6[b]
nched	1.1 ± 0.1	1.2 ± 0.2	1.3 ± 0.1
uble	0.6 ± 0.07	0.7 ± 0.1	0.7 ± 0.1

± SEM.
05.
IC vs. IC.
T vs. IC.

The vermis and intermediate hemispheric lobes of the cerebellum are strongly involved in motor coordination [2]. They receive movement-related information through spinal excitatory afferents such as mossy fibers, which excite the dendritic spines of Purkinje cells through the parallel fibers of granule cells [10]. Thus, the dendritic spines of Purkinje neurons would detect any significant change in the motor coordination-related synaptic activity.

Plastic changes in dendritic spines, including modifications in their distribution, density and/or shape, are related to the differential processing of synaptic information. In general, thin and mushroom spines have been considered to be the most efficient spines in transmitting synaptic impulses, attributed in part to their narrow neck [23]. On the other hand, stubby and wide spines have generally been related to the regulation of excitability [8] by virtue of the fact that they have no neck to restrict the current flow from the postsynaptic density to the parental dendrite [23].

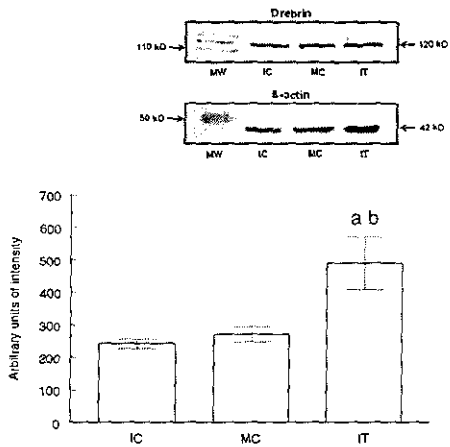


Fig. 3. The upper panel shows a representative Western blot of drebrin and β-actin expression in the intact control (IC), mildly trained control (MC), and intensively trained (IT). MW, molecular weight marker. The lower panel shows the expression of drebrin in arbitrary units. Mean ± SEM. $p > 0.05$. (a) IT vs. IC and (b) IT vs. MC.

ıdritic spines may undergo geometrical transformations that ı lead to changes in the processing of afferent information. cytoarchitectural adaptive changes in part depend on the laptic stimulatory activity [34]. Small pulses of glutamate en spines [25] while pulses of greater magnitude [25] or ive stimulation [19] induce their retraction to stubby-type or pearance. In addition, spine plasticity concurs with variations expression of some cytoskeletal actin-associated proteins, as drebrin [7,35]. Drebrin overexpression has been strongly ated with plastic changes in spine shape [15,24] as well as in genesis [1]. In the present study, spine density increased due tor training, corresponding to an increase in drebrin and β- proteins, closely associated to spinogenesis and to a greater nt of spines, respectively. This suggests, on one hand, that nt parallel fibers could sprout [36] and establish synaptic con- vith the new spines and, on the other, that spine nearness could ase their capability to associate afferent information [14]. Such ic events could help to integrate more efficiently the incom- nhanced motor information in Purkinje cells via parallel fibers, ng in turn to adaptive changes associated with the more and demanding motor challenges imposed.

ne stubby and wide spine types increased specifically in both ly and intensively trained rats. Stubby spines proliferate after ssive synaptic stimulation [25], which agrees with previous rts showing that experimental disinhibition of the prefrontal ex increases the pyramidal cells' multiunitary activity [27], the e density and the proportion of stubby spines in the proxi- dendritic segments of those prefrontal cells [31]. These results led to suggest that spines lacking a neck, such as stubby vide spines, could be involved in the regulation of neuronal tability [8,31]. Accordingly, the observed increase in stubby and e spines could be related to the regulation of the excitability in kinje cells during mild or intensive motor activity. Further elec- hysiological and/or immunohistochemical studies are needed est this hypothesis.

Thin spines have been associated with information acquisition ng learning [6,20,29]. In agreement, Purkinje cell's thin spines n the paramedian cerebellar lobe of rats have been reported ncrease after chronic training in a complex motor learning ıdigm [26]. The task performed here has no relationship with or learning but with the force of movement while perform- the same task imposed from the beginning of the training. intensively trained rats showed an increase in the density of hroom spines which have been related with the storage of rmation [6,20,29]. The modifications in both velocity and slope osed over the four weeks of training could force the animals to st their motor activity; if this continued over an extended time od it could then provoke the consolidation of new motor infor- ion patterns. Thus, such motor adjustments could represent behavioral bases for the development of additional mushroom es.

n summary, both mild and intensive motor training induces an ease in spine density, specifically of those spine types related ne excitability regulation (stubby and wide) and/or adaptive stments (mushroom). Coordinative motor training has shown e useful to improve rehabilitation after cerebellar injury [33], enerative cerebellar disease [18] or Parkinson disease [32]. erstanding the neurobiological mechanisms underlying such or training patterns could help to refine both their design and ations, favouring patient rehabilitation.

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