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BEYOND THE BASAL GANGLIA: cFOS EXPRESSION IN THE CEREBELLUM IN RESPONSE TO ACUTE AND CHRONIC DOPAMINERGIC ALTERATIONS

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Abstract—The suggestion of an anatomical and functional relationship between the basal ganglia and cerebellum is recent. Traditionally, these structures were considered as neuronal circuits working separately to organize and control goal-directed movements and cognitive functions. However, several studies in rodents and primates have described an anatomical interaction between cortico-basal and cortico-cerebellar networks. Most importantly, functional changes have been observed in one of these circuits when altering the other one. In this context, we aimed to accomplish an extensive description of cerebellar activation patterns using cFOS expression (cFOS-IR) after acute and chronic manipulation of dopaminergic activity. In the acute study, substantia nigra pars compacta (SNc) activity was stimulated or suppressed by intra cerebral administration of picrotoxin or lidocaine, respectively. In addition, we analyzed cerebellar activity after the induction of a parkinsonism model, the tremulous jaw movements. In this model, tremulous jaw movements were induced in male rats by IP chronic administration of the dopamine antagonist haloperidol (1.5 mg/kg). Acute stimulation of SNc by picrotoxin increased cFOS-IR in the vermis and cerebellar hemispheres. However, lidocaine did not produce an effect. After 14 days of haloperidol treatment, the vermis and cerebellar hemispheres showed an opposite regulation of cFOS expression. Chronic dopaminergic antagonism lessened cFOS expression in the vermis but up-regulated such expression in the cerebellar hemisphere. Overall, the present data indicate a very close functional relationship between the basal ganglia and the cerebellum and they may allow a better understanding of disorders in which there are dopamine alterations.

Key words: cerebellum, dopamine, haloperidol, substantia nigra, picrotoxin.

INTRODUCTION

Traditionally, basal ganglia and cerebellar circuits were considered as working separately to organize and control goal-directed movements and cognitive functions (Doya, 2000; Graybiel, 2005). It was accepted that these independent loops interacted at the cortical level in motor and nonmotor areas (Middlenton and Strick, 2000; Kelly and Strick, 2003, 2004; Clower et al., 2005; Akkal et al., 2007; Coffman et al., 2011). Even though four decades ago some sparse data had already supported the interactions between the basal ganglia and cerebellum (Bratus' and Moroz, 1978; Fox and Williams, 1968; Ikai et al., 1992), the proposal of anatomical and functional relationships between them is up-to-date. Several studies in rodents and primates have described anatomical relationships between these apparently segregated networks, which are independent of the cerebral cortex (Hoover and Strick, 1999; Ichinohe et al., 2000; Hoshi et al., 2005; Akkal et al., 2007; Yu et al., 2007; Bostan and Strick, 2010). Moreover, it is known that the ventral tegmental area (VTA) sends dopaminergic afferents to the cerebellar cortex, mainly distributed in lobules Crus I and Crus II (Ikai et al., 1992). The existence of a dopaminergic direct pathway to the cerebellum has also been supported by the presence of molecular elements of dopaminergic neurotransmission (Panagopoulos et al., 1991; Melchitzky and Lewis, 2000; Glaser et al., 2006). Remarkably in rats, detectable DA levels were found in posterior lobules of the vermis VII–X, right and left hemispheres and the fastigial, dendate and interpositus deep nuclei (Glaser et al., 2006).

In addition, a functional relationship between both networks may be evident in conditions of injury or alteration (Centonze et al., 2008; Koch et al., 2008; Rossi et al., 2008; Cutuli et al., 2011). Right hemicerebellectomy abolishes contralateral striatal LTD. Also, in human neuroimaging studies the degeneration of the nigrostriatal dopaminergic pathway that occurs in Parkinson's disease (PD) induces a hyperactivation of the cerebellum (Rascol et al., 1997; Cerasa et al., 2006; Yu et al., 2007). Supporting human studies, a recent study in nonhuman primates treated with the

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Abbreviations: 6-OHDA, 6-hydroxydopamine; DN, dentate nucleus; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PN, pontine nuclei; SNc, substantia nigra pars compacta; SNr, SN reticulata; STN, subtalamic nucleus.

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neurotoxine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce nigrostriatal degeneration described a persistent activation of the Purkinje neurons that correlates with the degree of dopamine loss (Heman et al., 2012). Additional support has been reported using MPTP or 6-hydroxydopamine (6-OHDA) as models of PD (Rolland et al., 2007). In these PD models, cerebellar and basal ganglia–thalamic territories of ventrolateral and ventromedial thalamus respectively, reduced their neuronal metabolism by about 50%, suggesting a loss of neuronal activity in the cerebello-thalamic and basal ganglia–thalamic circuitry. Recently, it has been suggested that one of the most important sources of the parkinsonian tremor might be the cerebellum and its thalamic projections (Rolland et al., 2007; Bostan and Strick, 2010; Lewis et al., 2013).

The present study explored cerebellar activity after acute and chronic manipulations of the basal ganglia. In the acute study, substantia nigra pars compacta (SNc) was stimulated or inactivated by intra-cerebral administration of picrotoxin or lidocaine (a local anaesthetic), respectively. Picrotoxin is a non-competitive antagonist of $GABA_A$ that prevents opening of Cl (−) channels and so activates dopaminergic neurons of the SNc by disinhibition (Wood et al., 1982). In addition, we analyzed cerebellar activity after the induction of a parkinsonism model, the tremulous jaw movements (Tarsy, 1983; Salamone et al., 1998) (for further review Collins-Praino et al., 2011). This is one of the parkinsonism models that meets all validation criteria for animal models (Willner, 1986; Collins-Praino et al., 2011). Taken together, the present findings strongly suggest a close functional relationship between the basal ganglia and the cerebellum. Remarkably, cerebellar activity patterns could be affected even after short-lasting modifications in the activity of the basal ganglia.

EXPERIMENTAL PROCEDURES

Subjects

Male Wistar rats were obtained from the colony of CICE (Centro de Investigaciones Cerebrales, Universidad Veracruzana, Xalapa, Mexico) with a body weight of 250–300 g. Animals were housed in standard collective, clear acrylic boxes, under light–dark reverse cycle conditions during 12 × 12-h periods and fed with food and water on demand. A total number of 54 animals were used, 24 for acute experiments and 30 for the chronic study. Animals were handled every day for a week and then randomly assigned to the control or the experimental groups. All animal procedures were performed in accordance with the Official Norms in Mexico (NOM-062-ZOO-1999 and NOM-087-ECOL-SSA1-2003), the NIH Guide for the Care and Use of Laboratory Animals, and the principles presented by the Society for Neuroscience.

Surgery

Animals were anesthetized with ketamine (80 mg/kg, i.p) and xylazine (10 mg/kg, i.p). After anesthesia, subjects were implanted into the SNc with a unilateral steel guide cannula 12-mm long (left–right side randomized). The stereotaxic coordinates were as follows: AP = −5.3 mm, ML = ±2.2 mm and DV = +7.2 mm (Paxinos and Watson, 2007). The guide cannula (0.025 mm in diameter) (A-M Systems, INC) was fixed to the skull with acrylic. After surgery, the animals were treated with antibiotic and analgesic drugs and housed in individual plastic cages 1 week for recovery.

Cannula placement was checked by Nissl staining. Coronal sections at the level of the SNc stereotaxic coordinates were immersed for 2 min in distilled water and stained by 0.5% Cresyl Violet for 15 min. They were rinsed in water for 2 min and dehydrated in 70% alcohol, 95% alcohol, 100% alcohol, and 100% alcohol. They were then put in xylene and finally the sections were cover slipped with Permount.

IC Microinjections

All drugs were administered intracranially (i.c). Picrotoxin (0.50 μg/0.25 μl) (0.125 μl/min) (Sigma–Aldrich Chemistry, St Louis, USA) and Lidocaine 2% (Sigma–Aldrich Chemistry, St Louis, USA) were dissolved in a Ringer Solution (NaCl 8.60 mg, $CaCl_2$ 133 mg, KCl 298 mg). Ringer solution was used as vehicle control.

Infusions were made through an injector (A-M Systems, INC, Greenville, USA) of 13 mm in length (DV = +8.2 mm) and 0.012 mm in diameter connected by a polyethylene tubing to a 5 μl Hamilton syringe placed in an infusion pump programed to release a volume of 0.25 μl to 0.125 μl/min rate for 2 min in awake animals. Three groups were i.c injected: the sham control received Ringer Solution (n = 6) into SNc; the stimulation group was treated with picrotoxin (0.50 μg/0.25 μl) (n = 6) and the inactivation group received lidocaine 2% (n = 6). In addition, an intact group (n = 6) was used in order to estimate the effects of the cannula placement. Intact rats was also videotaped for 20 min and returned to their cages. They were anesthetized and perfused. Half of the animals were injected into the left side and half into the right one. The injector was left in place for 1 min after infusion for diffusion into the tissue. Immediately after the injection, the rats were individually placed in a plastic cage and were videotaped over a period of 20 min and then returned to their cages.

Protocol for the induction of the tremulous jaw movements

Thirty male rats were used in the chronic study. To establish the tremulous jaw movement model described by Salamone et al. (1998), a haloperidol dose of 1.5 mg/kg (1 ml/kg) was IP injected daily for 14 days (n = 17). Haloperidol (RBI Research Biochemicals International, Natick, MA 01760, USA) was dissolved in 0.3% DL-tartaric acid in warm saline (Sigma–Aldrich Chemistry, St Louis, USA). The control group received 0.3% DL-tartaric acid dissolved in saline (n = 8). Jaw tremor was characterized and videotaped for the posterior