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Research report

Sensorimotor recovery from cortical injury is accompanied by changes on norepinephrine and serotonin levels in the dentate gyrus and pons

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HIGHLIGHTS

- Spontaneous and sensorimotor activities are reduced three days after cortical lesion.
- Behavioral recovery is observed since day tenth after cortical injury.
- Norepinephrine and serotonin levels in the dentate gyrus increase ten days after injury.
- Twenty days after lesion rats show full behavioral recovery.
- Monoamines hippocampal increase is maintained twenty days after cortical lesion.

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ABSTRACT

Monoamines such as norepinephrine (NE) and serotonin (5-HT) have shown to play an important role in motor recovery after brain injury. The effects elicited by these neurotransmitters have been reported as distal from the area directly affected. Remote changes may take place over minutes to weeks and play an important role in post-stroke recovery. However, the mechanisms involved in spontaneous recovery have not been thoroughly delineated. Therefore, we determined the NE and 5-HT content, in the pons and hippocampal dentate gyrus (DG) as well as motor deficit and spontaneous activity in rats after 3, 10 and 20 days cortical iron injection. Three days post-lesion the pontine NE content diminished, this effect was accompanied by deficient spontaneous activity and impaired sensorimotor evaluation. Ten and twenty days after lesion the NE levels were similar to those of control group, and animals also showed behavioral recovery. Monoamines content on DG 3 days post-lesion showed no differences as compared to controls. Interestingly, ten and twenty days after cortical injury, animals showed increased NE and 5-HT. These results suggest that behavioral recovery after brain damage involve changes on monoamines levels on DG, an important structure to plastic processes. In addition, the results herein support evidence to propose these neurotransmitters as key molecules to functional recovery in the central nervous system.

1. Introduction

In certain pathological conditions, iron accumulation takes place in specific brain areas related to neurodegenerative disorders, including Amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and Huntington's disease [1]. Furthermore, intracortical iron injection causes focal epileptiform discharges and brain edema [2,3]. Both iron and lipid peroxidation (LP) also have important roles in hemoglobin-induced brain injury. In this context, the subpial injection of $FeCl_2$ produces edema and LP in the brain [3]. Moreover, iron potentiates the formation of free radicals, promoting neuronal damage; for example, ferric (Fe^{3+}) and ferrous (Fe^{2+}) iron react with lipid hydroperoxides to produce free radicals [4]. However little is known about the underlying mechanisms for functional recovery after brain injury, which may be associated with clinical application in humans [5,6]. Thus, in this study, we examined part of the molecular mechanisms related to brain recovery after damage.

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Currently, it is widely demonstrated that the damaged central nervous system (CNS) has potential for functional recovery [5,7,8]. The mechanism of functional recovery following brain injury varies according to the site and size of the lesions as well as the time after injury. It has been proposed that functional impairment after brain injury is caused by inhibition of parts of the brain that are remote from, but anatomically related to the injured region [9]. This remote inhibition is known as diaschisis [10]. It is possible that functional recovery could result from a restoration of the basal conditions that were present prior to the injury. In this framework, it has been reported that during recovery following transection of the medial forebrain bundle the levels of serotonin (5-HT) in the *substantia nigra* increase [11]. This lesion can subsequently lead to 5-HT hyper-innervation in the adult rat ventral mesencephalon [12]. This series of results suggest that a compensatory response is generated by the axotomy. However the changes in serotonergic systems after cortical injury have not been completely elucidated. To address this gap, in this study we determined the 5-HT content in the pons and hippocampal dentate gyrus (DG) after cortical iron injection. We considered important the evaluation in DG since the serotonergic raphe-hippocampal pathway has powerful effect on hippocampal electric activity, depression-associated synaptic plasticity, and cognitive behavior [13].

Among other important molecular agents that participate in the recovery from motor deficits after brain insult, norepinephrine (NE) has received considerable attention [5,14]. D-Amphetamine administration enhanced recovery [15], and the intraventricular infusion of NE [16] or L-threo-3,4-dihydroxyphenylserine, an NE precursor, protected against the effects derived from motor cortex injury [17]. Previously, we reported remote decreased noradrenergic activity after iron injection into cortex, which involves LP affecting both sides of the pons [18]. However, the effects in the cerebral noradrenergic system after brain injury have not been completely elucidated. In this regard, the anatomical relationships between the locus coeruleus (LC) and the hippocampus have been described; particularly, the DG receives prominent noradrenergic input from the LC [19,20]. Furthermore, the DG contains high density of NE receptors and receives NE input via projections from the perforant path [21].

The hippocampus plays a fundamental role in learning and memory [22]. Animals and humans display memory impairment after ischemic injury to the hippocampus [23], and cell transplants can reduce memory deficits [24] as potentially newly formed granule cell neurons [25,26]. It is important to point out that alterations in dentate granule cells appear to be a common hallmark in different neurodegenerative diseases [27–29]. Thus, the DG represents a relevant structure to evaluate the impact of plastic events on functional and morphological reorganization after damage, given its cellular proliferative potential [30]. Furthermore, adaptive processes that contribute to recovery after stroke [31] or local damage [32] occur in the DG. Thus, considering the anatomical interactions between the LC and DG and the plasticity of DG in response to brain damage, in the present study we also analyzed the noradrenaline levels in both areas after cortical injury.

2. Materials and methods

2.1. Animals

Forty-three male adult Wistar rats weighing 280–320 g (provided by the vivarium of our institution) were used in the present study. The animals were fed LabDiet® rodent laboratory chow and maintained on a 12–12 h light–dark schedule. Rats were adapted to the laboratory conditions at least 1 week prior to surgical procedures. During this time, rats were handled daily to habituate them to the experimental manipulations. Rodents were treated according to the Guide for the Care and Use of Experimental Animals [33], the guidelines of mexican regulation (Norma Oficial Mexicana, NOM-062-ZOO-1999), and the approval of the local research committee (approval number 106/14). We used the minimum number of animals as possible, according to the bioethical and statistical criteria provided by Festing, [34].

2.2. Spontaneous motor activity

Spontaneous activity was recorded using an Auto-Track Opto-Varimex activity monitoring system (Columbus Instruments, Columbus, OH, USA) placed inside an anechoic chamber. The system was comprised of one chamber (42.2 × 42.5 × 20.5 cm). Activity recording was monitored in five sessions, each one lasting five minutes. The first session was carried out before surgery. The second to fourth sessions were performed when solutions were administered; 3 h before injections (2nd session), 0 min after injections (3rd session), and 3 h after injections (4th session). Fifth session was carried out 3, 10 or 20 days after injections. Distance travelled (cm), resting time (sec.), stereotypic time (sec.), and ambulatory time were recorded. Resting time was equivalent to total time in the chamber minus ambulatory and stereotypic time. Results of distance travelled were normalized and converted into percentages.

2.3. Sensorimotor deficit

Sensorimotor deficits were measured using neurological evaluation as described by García [35]. We recorded: (1) symmetry in the movement of four limbs, (2) climbing, (3) body proprioception and (4) response to vibrissae touch. The score given to each rat at the completion of the evaluation is the summation of all four individual test scores. The minimum score is 3 and the maximum is 12, with a higher score indicating less deficit. Animals were evaluated in five sessions, each one carried out after activity motor recordings.

2.4. Surgery

After the first session of motor evaluation animals were anesthetized with a ketamine–xylazine mixture (100–5 mg/kg) [36] and were mounted in a stereotaxic frame (Stoelting Corp., Wood Dale, IL, USA). A 22-gauge stainless steel guide cannula (gauge 18, Plastics One, Boston, MA, USA) was placed on the meninges over the motor cortical representation of the hind limb [37] and fixed with dental acrylic. The wounds were sutured with catgut 00, and topical healer (furazolidone) was sprayed over the scar. Eight days after surgery, rats were randomly assigned into six groups: sham 3 days (SH3D, $n = 7$), injured 3 days (INJ3D, $n = 7$), sham 10 days (SH10D, $n = 7$), injured 10 days (INJ10D, $n = 7$), sham 20 days (SH20D, $n = 7$) and injured 20 days (INJ20D, $n = 8$). Sham groups received an intracortical injection (10 μl) of artificial cerebrospinal fluid (aCFS, in mM: 125 NaCl, 3 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, and 2.3 $NaHCO_3$, pH 7.2) in free-moving conditions. Injured groups received an intracortical injection of aCFS (10 μl) containing ferrous chloride ($FeCl_2$, 50 mM) in free-moving condition [38]. In order to analyze the time-course of the lesion, animals in the different groups were sacrificed 3, 10 or 20 days after injections. Solutions were administered by inserting 30-gauge microinjectors through the cannula to a depth of 1 mm into the cortical parenchyma (Fig. 1). The microinjectors were attached by polyethylene tubing to a 10 μl Hamilton syringe driven by a microinfusion pump (Stoelting Corp., Wood Dale, IL, USA).