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ORIGINAL PAPER

Pontine and Cerebellar Norepinephrine Content in Adult Rats Recovering from Focal Cortical Injury

Rigoberto Gonzalez-Pina · Antonio Bueno-Nava · Sergio Montes · Alfonso Alfaro-Rodriguez · Angelica Gonzalez-Maciel · Rafael Reynoso-Robles · Fructuoso Ayala-Guerrero

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Abstract Norepinephrine (NE) plays an important role in motor recovery after brain damage. Most studies concerning NE activity have been performed in the cerebellum, while the role of the pons, the site where the norepinephrinergic locus coeruleus is located, has not yet been elucidated. For this work, we studied the changes in cerebellar and pontine NE content in sham-operated ($n = 17$), motor cortex injured ($n = 6$) and recovered rats ($n = 12$). Motor effects were assessed by means of footprint analysis and sensorimotor evaluation. It was found that after cortical brain damage, the stride length decreases while the stride angle increases after 6 h post-surgery, while the sensorimotor evaluation showed an increase in the motor deficit. Recovery was observed after 24 h. NE content increased in the pons after 6 h and returned to normal levels in recovered rats, with no significant changes observed in the cerebellum. Based on the functional remote inhibition, it is possible that NE exerts an autoinhibitory effect in the pons after motor cortical ablation. On the other hand, the absence of an effect in the cerebellum suggests that cerebellar NE activity related to damage and/or recovery is limited to discrete areas of the structure.

Keywords Norepinephrine · Brain injury · Pons · Cerebellum · Diaschisis

R. Gonzalez-Pina (✉) · A. Bueno-Nava
Laboratorio de Neuroplasticidad, Torre de Investigación, Instituto Nacional de Rehabilitacion, Calz. Mexico-Xochimilco 289 Col. Arenal de Guadalupe, Del. Tlalpan, C.P. 14389 Mexico City, Mexico
e-mail: higgo65@salud.gob.mx

S. Montes
Instituto Nacional de Neurologia y Neurocirugia M.V.S. SSA, Mexico City, Mexico

A. Alfaro-Rodriguez
Laboratorio de Neuroquimica, INR SSA, Mexico City, Mexico

A. Gonzalez-Maciel · R. Reynoso-Robles
Instituto Nacional de Pediatria, SSA, Mexico City, Mexico

F. Ayala-Guerrero
Facultad de Psicologia, UNAM, Mexico City, Mexico

Introduction

Recovery from hemiplegia has been documented in both experimental animals [1, 2] and humans after stroke [3]. It has been suggested that norepinephrine (NE) plays an important role in the recovery after brain insult [4]. Administration of D-amphetamine enhances recovery [5], and intraventricular infusion of NE [6] or l-threo-3,4 dihydroxy-phenyl-serine, a NE precursor [7], protects from the effects of motor cortex injury. The cerebellum has been the structure most often studied [8] in which a NE decrease after motor cortex ablation has been observed [9, 10]. This fact seems to be part of a functional depression of metabolic processes in the cerebellum, a remote and intact brain area related with the injured site, a phenomena known as diaschisis. Since NE seems to be involved in such a depression, the NEergic locus coeruleus (LC) is then also implicated. The LC is located adjacent to the fourth ventricle in the pontine brainstem [11] and it is the primary source of NE in the central nervous system [12]. Several lines of evidence indicate that spontaneous functional

recovery from hemiplegia is mediated, in part, by LC NEergic mechanisms [13, 14]. For example, lesions in the LC result in a difficult recovery period after brain injury [15], while administration of yohimbine and idaxozan, two α2-adrenoceptor antagonists that enhance LC NEergic input, accelerate the rate of recovery [16]. Conversely, clonidine, prazosin and phenoxibenzamine, drugs that decrease LC NEergic transmission, result in a difficult recovery and restore deficits in animals previously recovered from cortical injuries [16, 17]. Moreover, anatomical relationships between the LC and cortex [18] and the LC and cerebellum [19] have been described, suggesting that cerebellar NE depletion found in cortically injured animals may be mediated by NE changes in the LC. However, the condition of the cerebellar and pontine NE contents after cortical ablation and after recovery is achieved is not currently known. Thus, the aim of this work was to measure the cerebellar and pontine NE levels in non-injured, injured and recovered rats after motor cortical ablation.

Experimental procedure

We used 35 male wistar rats (weight 280–300 g) provided by the vivarium of Instituto Nacional de Neurologia y Neurocirugia MVS, where they were reared (courtesy of MVZ Rodolfo Perez Madrigal). Animals were fed with Purina rodent laboratory chow and maintained on a 12–12 light–darkness schedule.

Rats were adapted to the laboratory conditions at least 1 week prior to the surgeries. During this time, rats were handled daily in order to habituate them to the experimental manipulations, and a training protocol was performed in order to obtain their footprints, as described by Gonzalez-Pina et al. [20]. Rats were treated according to the Guide for the Care and Use of Experimental Animals [21]. We used the minimal possible number of animals using bioethical and statistical criteria, according to Festing [22].

Once training was completed, a basal recording of the footprint was performed. Immediately after that, animals were anesthetized with Ketamine–Xylazine (100 mg/kg-5 mg/kg). Then, the rats were mounted in a stereotaxic frame (David Kopf). The skull was exposed by means of an incision on the scalp and a trephine hole was made (1 mm diameter, approximately). The meninges were cut using the tip of a syringe needle. Seventeen rats were sham-operated, while 18 were injured by means of the aspiration of the tissue of the right motor cortex representative of the hindlimb [23], located in the following coordinates: posterior 2 mm and lateral 2 mm with respect to the bregma. Aspiration was performed using a vacuum pump (Stoelting 6C) coupled to a glass pipette (1 mm diameter). Finally, the scalp was sutured with catgut No. 00 and postoperative care was provided as recommended by Gonzalez-Pina and Escalante-Membrillo [24]. Nearly 1 h after the ablation was performed, the rats were fully awakened and the motor evaluations were performed.

Six of the injured rats and six of the sham-operated were recorded from one time before surgery and again 3 h after that the surgery was performed. Then, records and scores were taken every hour for 4 h, which corresponded to 6 h post-surgery. These animals were evaluated using a sensorimotor evaluation protocol according to García et al. [25]. These methods evaluations were used to assess the animals because the recording time is shorter than that required for footprint analysis and therefore are more adequate to measure the early sensorimotor effects of the cortical ablation. Sensorimotor evaluation consisted in the use of four tests [25]. Symmetry in the movements of four limbs (SMFL) and climbing (C) evaluated motor performance, while response to vibrissae touch (RVT) and body propioception (BP) evaluated sensory aspects (see ref. [25] for details). The feasibility and reliability of this examination have been described by Pantoni et al. [26]. Finally, animals were sacrificed in order to analyze NE content.

The remaining 12 injured and the 11 sham-operated rats were recorded exclusively for the footprint analysis 6 h after surgery and every 6 h after that for 48 h, according to the method of Gonzalez-Pina et al. [20]. After the behavioral assessment was performed, all rats were sacrificed by decapitation. Animals were currently sacrificed between 18:00 and 19:00 h in order to avoid NE variations due to the circadian rhythm. The brain was removed and placed on a cool plate in order to extract the pons and cerebellum, which was divided into right and left sides each. Tissue was collected in teflon tubes and a solution of 0.5 ml of perchloric acid 0.4 N containing 0.1% w/v of sodium metabisulfite was added. Then the tissues were homogenized and centrifuged at 15,000 rpm for 10 min at 4°C. The supernatant was filtered and stored at –70°C for chromatographic analysis. The content of NE was analyzed by high performance liquid chromatography (HPLC, Perkin-Elmer LC-250) coupled to a metrohom electrochemical detector. The peaks were sent to a computer with a Perkin-Elmer 1020 Plus program. In order to determine the concentrations of NE, the chromatograms of the samples were interpolated with

the chromatogram obtained from four standard samples, whose NE concentrations were known. An analytical column for catecholamines was used (ALLTECH, adsorbosphere C18 100 × 4.8 mm, 3 μm of particle size). The mobile phase utilized consisted of a phosphate buffer solution (0.1 mM, pH 3.2) that contained (in mM): sodium octyl sulfate (0.2), EDTA (0.1) and methanol (15% v/v). The flow rate employed was 1.2 ml/min while the potential was set at 0.65 V against a reference electrode of Ag/AgCl.

The encephalus was placed in a 10% formalin-buffer solution in order to delineate the lesion by means of the Kluver–Barrera stain. Following observation by light microscopy, the cavity was outlined on schemes taken from the Paxinos and Watson's stereotaxic atlas [27].

From the sensorimotor evaluation, comparisons were made between sham and motor ablation groups using a non-correlated Mann–Whitney's U test. From the footprints, we analyzed the percent of change with respect to baseline in the length, width and the angle of the stride. Comparisons between groups were also analyzed by means of a non-correlated Mann–Whitney's U test. NE analysis was performed by means of a one-way ANOVA and a pos hoc Tukey test. In all the cases, significant levels were set to $P \leq 0.05$.

Results

After the cerebral coronal slices were processed, we observed that the cavity corresponding to the lesion started –1.6 mm from the bregma (Fig. 1A), increased to a maximum between –2.56 mm and –2.80 mm (Fig. 1B), and then decreased to a final size between –3.14 mm and –3.60 mm (Fig. 1C), covering the entire motor hindlimb cortical representation, according to Hall and Lindholm [23].

Sensitive assessment did not show any additional changes (data not shown). However, motor assessment showed a notorious and sustained increase in the motor deficit in injured animals. Such changes started 3 h after the surgery ($P \leq 0.001$), achieving a maximal score 5 ($P \leq 0.0001$) and 6 ($P \leq 0.0001$) hours after that the lesion was performed (Fig. 2).

Footprint analysis showed that injured animals had a significant decrease in the stride's length after 6 h (–8.00% ± 2.4; $P \leq 0.001$; Fig. 3A), with a concomitant increase in the stride's angle (22.11% ± 4.65; $P \leq 0.003$; Fig. 3B), while the stride's width did not show any significant change. As shown in the figures, recovery was observed 24 h after injury, since the mean percentage of change with respect to the

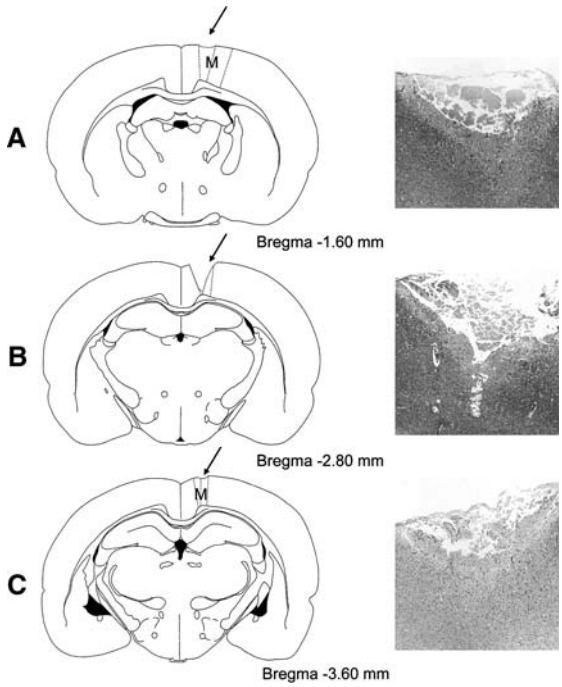

Fig. 1 Schematic representation showing the extent of the lesion (left), and microphotographs (10×) taken from the equivalent places in one typical slice (right). After the microscopic observations were performed, the cavity was outlined on the equivalent schemes taken from the Paxinos and Watson's stereotaxic atlas [27]. It is observed in both, schemes and photomicrographs, that the lesion was circumscribed to the motor cortex in the hindlimb representative area. In **A** and **C** the primary motor cortex border is affected (M), while in the injured area, a portion of the somatosensory cortex (**B**) was also affected. However, the lack of somatosensorial effects suggests that the size of the lesion in such an area is not significant

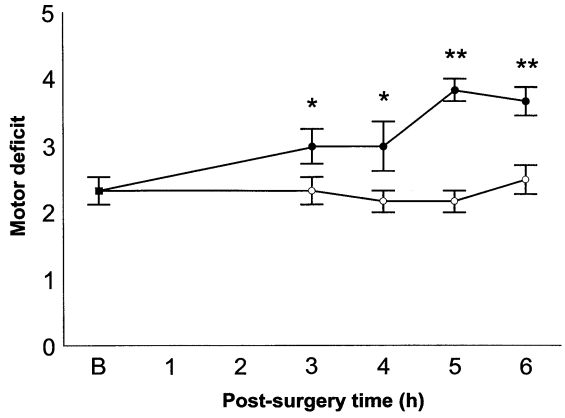

Fig. 2 Early motor deficit found in injured rats and compared with sham-operated rats. After 3 h of surgery, the injured animals showed important increases in motor deficits. Such effects were observed for 4 h until 6 h post-surgery had passed. Data are expressed as mean ± s.e.m. ■ Basal record (B) prior to either sham or injury surgeries; ○ Sham operated rats; ● injured rats; Mann–Whitney's U test *$P \leq 0.001$, **$P \leq 0.0001$

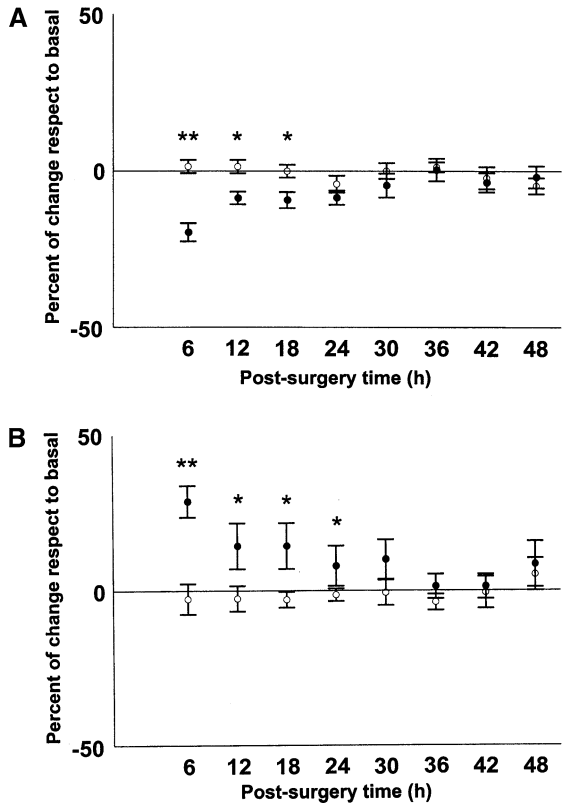

Fig. 3 Parameters measured in the footprints. Since the stride's width did not show any significant differences, such data are not presented. (**A**) Percent of change with respect to baseline in the stride's length, measured every 6 h for 48 h post-surgery. An important decrease after 6 h in injured animals was observed. Recovery was observed 24 h post-lesion and was maintained over the next 24 h. Data are expressed as mean ± s.e.m. Mann–Whitney's U (*$P < 0.05$, **$P \leq 0.01$). ○ Sham operated rats; ● injured rats. (**B**) Percent of change with respect to baseline in the stride's angle, where a significant increase after 6 h post-lesion was observed. Recovery was observed 30 h post-lesion, although after 24 h a marginally significant difference was observed. Recovery was sustained for at least 48 h. Results are expressed as mean ± s.e.m. Mann–Whitney's U (*$P < 0.05$, **$P \leq 0.01$) .○ Sham operated rats; ● injured rats

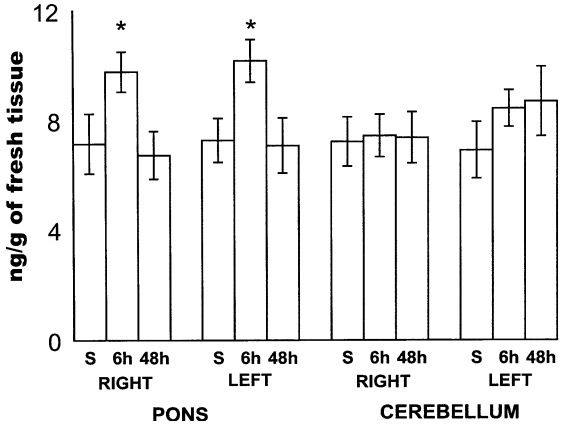

Fig. 4 Mean total content of pontine and cerebellar norepinephrine found in sham-operated rats sacrificed 48 h after of surgery (S), rats injured and sacrificed 6 h post-lesion (6 h) and rats injured and sacrificed 48 h post-lesion (48 h). Determinations were performed separately in the right and left side of the pons and in the left and right hemispheres of the cerebellum. The right and left sides in the pons of injured rats (6 h) showed an increase in NE, while such levels in recovered rats (48 h) were close to those of the sham-operated rats (One-way ANOVA, *$P < 0.05$). On the other hand, significant differences in the cerebellar hemispheres (Right $P = 0.791$, left $P = 0.522$) were not detected. Data are expressed as mean ± s.e.m

baseline was not significant when the sham group was compared to the injured one. Such a recovery was sustained for 48 h post-lesion.

When the mean total content of NE was analyzed in either the right or left pontine sides, it was found that rats 6 h after injury showed a significant increase (in ng/g of fresh tissue) in NE (9.76 ± 0.72) in the right hemisphere when they were compared with sham-operated after 6 h of surgery (6.54 ± 0.79, not shown in plot), sham-operated after 48 h of surgery (6.76 ± 0.87) and recovered rats (6.32 ± 0.71). The same pattern of change was found in the left hemisphere ($P \leq 0.017$; Fig. 4). There were no significant differences between NE levels found in both pontine sides in each individual rat.

Therefore, cerebellar NE content did not show any significant difference between the right ($P \leq 0.723$) and left ($P \leq 0.565$) hemispheres.

Discussion

Footprint analysis showed that motor performance is recovered after just 24 h, unlike most of the literature, in which motor recovery is achieved 4–6 days post-lesion [1]. This could be due to the extent of the discrete lesion that we used, limiting damage to the somatosensory area. This is a considerable advantage because it allowed us to study the recovery mechanisms in a short period of time. In this context, footprint analysis and early motor assessment showed that when rats expressed the strongest injury-induced effects, pontine NE content was elevated, and when animals recovered, pontine NE content was found to have returned to levels similar to these found in sham-operated rats after 48 h of surgery.

It is known that innervation from the LC to the motor cortex (MCX) is ipsilateral [19]. So, it is to be expected that an injury in the MCX will produce an ipsilateral effect on the LC. However, we found an increase in NE in both sides of the pons. There is strong evidence that behavioral effects of motor cortical injury results from functional depression in

remote but intact brain areas related with the injured site [28]. If NE increase in pons is part of such functional depression, we propose that this fact represents a norepinephrinergic pontine autoinhibitory mechanism. Pharmacological observations suggest that inhibition of NE release on postsynaptic target cells could be mediated by α2-adrenoceptors [29, 30], widely distributed in the LC neurons. Thus, the binding of pontine NE to such receptors in the LC, enhanced by the observed NE increase, could produce the decreased NE release previously reported in cerebellum after MCX injury, as assessed by microdialysis [10]. Specifically, the α2A-receptor subtype could be involved, since it is found in 100% of the rat's LC neurons [31], and, it has been reported that an α2-receptor agonist inhibited release of NE by 96% [32]. The inhibitory action of NE has also been observed in other brain areas, such as the acetylcholine NE-induced inhibition in the tegmental nuclei [33].

Although we found that the pontine NE content is altered 6 h after injury, we provide evidence that remote inhibition of the pons could start as soon as 3 h after brain injury, since early motor evaluation showed motor deficits during this time period. Thus, remote inhibition of the pons could be almost immediate to the lesion. However, a limitation to the method employed for early motor evaluation is that the assessment is less quantitative than the footprint. Instead, the footprint is a long-term duration assessment. This is why it was necessary to use both complementary tests.

On the other hand, it is unclear how unilateral cortical injury results in bilateral increase of total NE levels in the pons, since innervation between the LC and cortex is predominantly ipsilateral [19]. It is possible that the effects of the unilateral MCX injury have an influence on both pontine sides [10]. Instead, the LC innervation to the cerebellum is bilateral [34] and the effects of MCX injury in the cerebellum may lead to bilateral retrograde effects within the LC neurons. However, we not observed changes in the cerebellar NE levels. Most of the literature has shown that cerebellar NE is involved in the mechanisms leading recovery. A decrease in cerebellar NE has been reported after cortical injury [35, 36], and such levels return to normal values when amphetamine is administered [34]. It has also been reported that cerebellar NE infusions facilitate recovery after sensorimotor cortex injury [35]. Such facts strongly support a cerebellum NE-mediated mechanism to achieve recovery after brain injury. The absence of changes in cerebellar NE content in our experiments indicates that NE activity in the cerebellum related to recovery from motor cortical ablation is confined to discrete regions, such as the cerebellar cortex. It is known that the ventral and dorsal portions of the LC innervate all of the neurons of the cerebellar cortex and, to a lesser extent, the cerebellar nuclei [37, 38].

Our results suggest that diaschisis is involved because we have the presence of a cerebral injury circumscribed, a neuronal basis for the functional depression, the participation of a remote structure related with the lesion site (the pons), anatomical relationships between the pons and the cortex and the process is reversible [39], as was observed by the footprint analysis and the NE content after 48 h of brain injury surgery.

Thus, we demonstrate that pontine NE is increased in animals after brain damage and this is concomitant with an increase in the motor deficit, suggesting that remote functional inhibition could be mediated by NE increase and no decreases, as it could seems. On the other hand, we found that when motor performance is recovered, animals showed pontine NE levels very similar to these found in sham-operated rats after 48 h of surgery, suggesting an important role of the pons in the cerebellar inhibition after cortical injury, previously reported by others. Therefore, lack of effects on cerebellar NE content opens the need to search for specific cerebellar areas related to recovery after cortical brain injury.

The relevance of this study of the biochemical mechanisms leading to the recovery after brain injury is that this information is a determining factor for the development of pharmacological strategies that enhance recovery in humans. It must be noted that the results of clinical experience are very similar to those observed in experimental animals [4], suggesting that the mechanisms observed in the basic research are comparable between rats and man. Then, it must be regarded that the clinical use of NE agonists and antagonists such as antidepressants and ansiolytics in humans after stroke could alleviate or extend to the remote neuronal depression, facilitating or hindering functional recovery. Most studies are needed in order to elucidate the detailed mechanisms underlying brain recovery after injury. Particularly, the specific role of LC in the norepinephrinergic functional depression must be clarified. We have provided here evidence of that pontine NE increase is closely related to the motor deficit produced by motor cortical injury. However, it is needed to provide evidence about a more precise correlation between NE and recovery using other neurochemical approaching, such as in vivo microdialysis sampling.

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References

1. Brailowsky S, Knight RT, Blood K (1986) gamma-Aminobutyric acid-induced potentiation of cortical hemiplegia. Brain Res 362:322–330
2. Hovda DA, Feeney DM (1984) Amphetamine with experience promotes recovery of locomotor function after unilateral frontal cortex injury in the cat. Brain Res 298:358–361
3. Katz DI, Alexander MP, Klein RB (1998) Recovery of arm function in patients with paresis after traumatic brain injury. Arch Phys Med Rehabil 79:5488–5493
4. Goldstein LB (2000) Effects of amphetamines and small related molecules on recovery after stroke in animals and man. Neuropharmacology 39:852–859
5. Sutton RL, Hovda DA, Chen MJ, Feeney DM (2000) Alleviation of brain injury-induced cerebral metabolic depression by amphetamine: a cytochrome oxidase histochemistry study. Neural Plast 7:109–125
6. Boyeson MG, Feeney DM (1990) Intraventricular norepinephrine facilitates motor recovery following sensorimotor cortex injury. Pharmacol Biochem Behav 35:3497–3501
7. Kikuchi K, Nishino K, Ohyu H (2000) Increasing CNS norepinephrine levels by the precursor L-DOPS facilitates beam-walking recovery after sensorimotor cortex ablation in rats. Brain Res 860:130–135
8. Boyeson MG, Krobert KA, Grade CM, Scherer PJ (1992) Unilateral, but not bilateral, locus coeruleus lesions facilitate recovery from sensorimotor cortex injury. Pharmachol Biochem Behav 43:771–777
9. Gonzalez-Pina R, Bueno-Nava A, Escalante-Membrillo C, Montes S, Gonzalez-Maciel A, Ayala-Guerrero F (2003) Cerebellar and pontine norepinephrine contents after motor recovery in rats. Res Neurol Neurosc 21:219–220
10. Krobert KA, Sutton RL, Feeney DM (1994) Spontaneous and amphetamine-evoked release of cerebellar noradrenaline after sensorimotor cortex contusion: an in vivo microdialysis study in the awake rat. J Neurochem 62:2233–2240
11. Berridge CW, Waterhouse BD (2003) The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Rev 42:33–84
12. Aston-Jones G, Rajkowski J, Cohen J (2000) Locus coeruleus and regulation of behavioral flexibility and attention. Prog Brain Res 126:165–182
13. Gladstone DJ, Black SE (2000) Enhancing recovery after stroke with noradrenergic pharmacotherapy: a new frontier? Can J Neurol Sci 27:97–105
14. Boyesson MG, Scherer PJ, Grade CM, Krobert KA (1993) Unilateral locus coeruleus lesions facilitate motor recovery from cortical injury through supersensitivity mechanisms. Pharmacol Biochem Behav 44(2):297–305
15. Goldstein LB, Bullman S (1997) Effects of dorsal noradrenergic bundle lesion on recovery after sensorimotor cortex injury. Pharmacol Biochem Behav 58:1151–1157
16. Sutton RL, Feeney DM (1992) α-noradrenergic agonist and antagonist affect recovery and maintenance of beam-walking ability after sensorimotor cortex ablation in the rat. Restor Neurol Neurosci 4:1–11
17. Dunn-Meynell AA, Yarlagadda Y, Levin E (1997) Alpha 1-adrenoceptor blockade increases behavioral deficits in traumatic brain injury. J Neurotrauma 14:43–52
18. Waterhouse BD, Lin CS, Burne RA, Woodward DJ (1983) The distribution of neocortical projection neurons in the locus coeruleus. Comp Neurol 217:418–431
19. Steindler DA (1981) Locus coeruleus neurons have axons that branch to the forebrain and cerebellum. Brain Res 223:367–373
20. Gonzalez-Pina R, Bueno-Nava A, Montes S, Alfaro-Rodríguez A, Gonzalez-Maciel A, Reynoso-Robles R, Ayala-Guerrero F (2005) Pontine norepinephrine content after motor cortical ablation in rats. Proc West Pharmacol Soc 48:73–76
21. Olfert ED, Cross BM, McWilliam AA (1993) Guide for the care and use of experimental animals. Can Council Animal Care 1:211
22. Festing MFW (1994) Reduction of animal use: experimental design and quality of experiments. Lab Anim Sci 28:212–221
23. Hall RD, Lindholm EP (1974) Organization of motor and somatosensory neocortex in the albino rat. Brain Res 66:23–38
24. Gonzalez-Pina R, Escalante-Membrillo C (2000) Algunas consideraciones bioéticas en los estudios de sueño simultaneos al muestreo neuroquimico en animales con libertad de movimientos. Animales de experimentación 6:26–32
25. García JH, Wagner S, Liu KF, Hu XJ (1995) Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 26:627–35
26. Pantoni L, Bartolini L, Pracucci G, Inzitari D (1998) Interrater agreement on a simple neurological score in rats. Stroke 29:871–872
27. Paxinos G, Watson C (1982) The rat brain in stereotaxi coordinates. Academic Press, Australia
28. Serteser M, Ösben T, Gümüslü S, Balkan S, Balkan E (2001) Biochemical evidence of crossed cerebellar diaschisis in terms of nitric oxide indicators and lipid peroxidation products in rats during focal cerebral ischemia. Acta Neurol Scand 103:43–48
29. Washburn M, Moises HC (1989) Electrophysiological correlates of presynaptic alpha 2-receptor-mediated inhibition of norepinephrine release at locus coeruleus synapses in dentate gyrus. J Neuroscice 9:2131–2140
30. Nasseri A, Minneman KP (1987) Relationship between alpha 2-adrenergic receptor binding sites and the functional receptors inhibiting norepinephrine release in rat cerebral cortex. Mol Pharmacol 32:655–662
31. Smeets WJ, Gonzalez A (2000) Catecholamine systems in the brain of vertebrates:new perspectives through a comparative approach. Brain Res Brain Res Rev 33:308–379
32. Bucheller MM, Hadamek KK, Hein L (2002) Two alpha(2)-adrenergic receptor subtypes, alpha(2A) and alpha(2C), inhibit transmitter release in the brain of gene-targeted mice. Neuroscience 109:819–826
33. Hobson JA, McCarley RW (1977) The brain as a dream state generator: an activation-synthesis hypothesis of the dream process. Am J Psychiatry 134:1335–1348
34. Room P, Postema F, Korf J (1981) Divergent axon collaterals of rat locus coeruleus neurons: demonstration by a fluorescent double labeling technique. Brain Res 221:219–230
35. Boyeson MG, Krobert KA (1992) Cerebellar norepinephrine infusions facilitate recovery after sensorimotor cortex injury. Brain Res Bull 29:435–439

36. Krobert KA, Sutton RL, Feeney DM (1994) Spontaneous and amphetamine-evoked release of cerebellar noradrenaline after sensorimotor cortex contusion: an in vivo microdialysis study in the awake rat. J Neurochem 62:2233–2240
37. Schambra UB, Mackensen GB, Stafford-Smith M, Haines DE, Schwinn DA (2005) Neuron especific alfa-adrenergic receptor expression in human cerebellum: implication for emerging cerebellar roles in neurologic disease. Neuroscience 135:507–523
38. Schweighofer N, Doya K, Kuroda S (2004) Cerebelar aminergic neuromodulation: towards a functional understanding. Brain Res Brain Res Rev 44:103–116
39. Feeney DM, Baron JC (1986) Diaschisis. Stroke 17:817–830