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Chronic electrical stimulation of the contralesional lateral cerebellar nucleus enhances recovery of motor function after cerebral ischemia in rats

Andre G. Machado, MD, PhD,

Assistant Professor of Surgery, Center for Neurological Restoration, Department of Neurosurgery, Departments of Biomedical Engineering and Neuroscience, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Ave, Desk S31, Cleveland, OH 44195, Fax: 216 636 2989, Phone: 216 444 4270, machada@ccf.org

Kenneth B. Baker, PhD,

Staff, Department of Neurosciences, Lerner Research Institute, Cleveland Clinic

Daniel Schuster,

Center for Neurological Restoration, Cleveland Clinic

Robert S. Butler, MS, and

Senior Biostatistician, Quantitative Health Sciences

Ali Rezai, MD

Center for Neurological Restoration, Jane and Lee Seidman Chair in Functional Neurosurgery, Department of Neurosurgery, Cleveland Clinic, Cleveland, OH USA

Abstract

Novel neurorehabilitative strategies are needed to improve motor outcomes following stroke. Based on the disynaptic excitatory projections of the dentatohalamocortical pathway to the motor cortex as well as anterior and posterior cortical areas, we hypothesize that chronic electrical stimulation of the contralesional dentate (lateral cerebellar) nucleus output can enhance motor recovery after ischemia via augmentation of perilesional cortical excitability. Seventy five Wistar rats were pre-trained in the Montoya staircase task and subsequently suffered left cerebral ischemia with the 3-vessel occlusion model. All survivors underwent stereotactic right lateral cerebellar nucleus (LCN) implantation of bipolar electrodes. Rats were then randomized to 4 groups: LCN stimulation at 10

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Robert Butler has no conflicts with this research other than his employment at the Cleveland Clinic. Daniel Schuster is employed by the Cleveland Clinic. IntElect Medical is a spin-off company of the Cleveland Clinic.

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pps, 20 pps, 50 pps or sham stimulation, which was delivered for a period of six weeks. Performance on the Montoya task was re-assessed over the last four weeks of the stimulation period. On the right (contralesional) side, motor performance of the groups undergoing sham, 10 pps, 20 pps and 50 pps stimulation was, respectively, 2.5 ± 2.7 ; 2.1 ± 2.5 ; 6.0 ± 3.9 ($p < 0.01$) and 4.5 ± 3.5 pellets. There was no difference on the left (ipsilesional) side motor performance among the sham or stimulation groups, varying from 15.9 ± 6.7 to 17.2 ± 2.1 pellets. We conclude that contralesional chronic electrical stimulation of the lateral cerebellar nucleus at 20 pps but not at 10 or 50 pps improves motor recovery in rats following ischemic strokes. This effect is likely to be mediated by increased perilesional cortical excitability via chronic activation of the dentatothalamocortical pathway.

Keywords

Stroke; Ischemia; Cerebellum; Dentate Nucleus; Electrical Stimulation; Plasticity; Deep Brain Stimulation

Introduction

Stroke is a disease of epidemic proportions in the industrialized world and a leading cause of long term disabilities. Approximately 795,000 people in the United States only suffer strokes every year (Lloyd-Jones et al., 2009). While the majority of patients will survive the acute phase, persistent neurological sequelae likely will jeopardize quality of life and productivity, with approximately 50% of survivors hemiparetic at 6 months after stroke and 30% requiring assistance with activities of daily living (Kelly-Hayes et al., 2003). According to the American Heart Association Statistics Committee and Stroke Statistics Subcommittee, the estimated indirect and direct cost of stroke for 2009 is US\$ 68.9 billion (Lloyd-Jones et al., 2009). These numbers underscore the need for translational research aimed at enhancing motor outcomes after stroke.

Spontaneous improvement of function occurs in stroke survivors and has been linked to changes at the cellular level, including synaptic reorganization and axonal migration (Carmichael, 2006). The extent of this recovery has further been shown to be influenced by several factors, including lesion volume (Chen et al., 2000; Pan et al., 2006), age (Saucier et al., 2007), lesion location (Shelton and Reding, 2001) and environmental enrichment (Hicks et al., 2007; Johansson, 2004) in the period following ischemia. Inflammation and angiogenesis (Carmichael, 2006; Cramer and Riley, 2008) also have been recently linked to recovery.

Cortical excitability has been linked to rehabilitation from stroke in the chronic phase (Butler and Wolf, 2007; Liepert, 2005). The cerebellum is one major source of excitatory input to the cortex through the dentatothalamocortical pathway, and indeed a lack of cerebellar input has been shown to result in reduced cortical excitability (Liepert et al., 2004). In the case of cerebral ischemia, a concomitant reduction in the tonic cerebello-cortical facilitation as a result of cerebellar pathology or hypometabolism may negatively impact natural recovery. In crossed cerebellar diaschisis (CCD), contralateral cerebellar hemispheric activity (metabolism) is reduced as a consequence of massive cortico-ponto-cerebellar deafferentation, most commonly due to cerebral ischemia. Takasawa et al (Takasawa et al., 2002) have demonstrated that patients presenting with CCD in the subacute phase present with worse 60-day motor outcomes than controls, regardless of the initial severity of neurological deficits. While lesions to the dentatothalamocortical pathway reduce excitability in the contralateral cortex, stimulation of the dentate nucleus enhances cortical excitability (Rispa-Padel et al., 1981) and motor facilitation (Iwata et al., 2004; Iwata and Ugawa, 2005). Based on widespread excitatory projections from the dentate nucleus to the contralateral cerebral hemisphere, extending from the premotor to the parietal regions (Dum et al., 2002; Dum and Strick, 2003), a novel method

for rehabilitation of motor function following cerebral infarctions has been rationalized. We propose that chronic electrical stimulation of the dentate nucleus will enhance gains of motor function following strokes beyond natural recovery. Previous experience from our group has demonstrated that stimulation of the rodent dentate nucleus with implanted depth electrodes generates evoked responses in the contralateral hemisphere not limited to the motor cortex but, rather, extending to the premotor and posterior cortical areas (Machado et al., 2005). Chronic stimulation with implanted electrodes initiated in the subacute phase is expected to increase ipsilesional cortical facilitation and enhance motor outcomes. To test our hypothesis, we evaluated the effects of chronic deep cerebellar electrical stimulation at varying frequencies in a rat model of ischemic strokes. The ideal dentatothalamocortical stimulation frequency for achieving chronic enhancement of excitability is not known. Previous experience with chronic subcortical stimulation in movement disorders indicate that high frequencies (100 pps and above) cause a lesion-like effect and lower frequencies enhance the activity of the affected neuronal pathway (Benabid et al., 1998; Constantoyannis et al., 2004). Since our intention is to enhance the output of the dentatothalamocortical pathway, stimulation frequencies at 50pps and lower were tested.

2. Results

2.1. Histology

All animals had strokes as confirmed by histological examination. Stroke volume was calculated by integrating the stroke areas measured in individual coronal slices at 320 μ m intervals. The strokes affected the sensorimotor territory, extending from the anterior most part of the cortical area corresponding to M1 to the somatosensory cortex posteriorly. The cortical surface anterior to the coronal suture was, on average, affected across the entire medial-lateral extension, whereas at the level of the bregma, the lateral third of the cortex was typically preserved. Posterior to bregma, only a small section of cortex was affected, typically in the medial third of the medial-lateral extension. Hence the majority of the parietal cortex, including a part of the somatosensory territory, was preserved, as were the thalamus and basal ganglia. Figure 2 presents a diagrammatic representation of a typical stroke. The average stroke volume was 106 mm³ (SD $\pm$ 52.9mm) and individual stroke volume was found to be correlate to motor outcome ($p = 0.0037$). Therefore, to reduce the bias of stroke volume on outcomes, the results presented below were regressed against both stroke volume and stimulation frequency (as determined per randomization group). There were no significant differences in stroke volume between treatment groups.

Electrode location was verified by histology in all animals. Prior to sacrificing, an electrolytic lesion was created through the electrode in order to facilitate identification of the location of the electrode's tip. The location of implantation was confirmed by comparing the location of the electrolytic lesion and implantation track to the topography of the LCN in the Paxinos and Watson atlas of the rat brain (figure 1). The histology did not allow for identification of discrete variance in electrode location in sub-regions of the nucleus. All rats included in the data analysis had adequately located electrodes.

2.2. Motor outcomes

Forty eight rats completed the experimentation. Table 1 summarizes the outcome data collected in the Montoya staircase task from each of the four groups studied: LCN stimulation at 10 pps, 20pps, 50 pps or sham stimulation. The data represents the average of the last 10 trials in the Montoya staircase, obtained during the last five days (2 Montoya trials per day) of the six-week course of post-stroke LCN stimulation. Data are shown for the side of the body ipsilesional (left) and contralesional (right) to the infarcted hemisphere. Regression analysis of the data shows that task performance for the ipsilesional limb was not significantly different

between the groups. The mean pellet consumption with the ipsilesional limb for sham, stimulation at 10 pps, 20 pps and 50 pps was, 16 ± 5.0 ; 15.9 ± 6.7 ; 17.2 ± 2.1 and 16.7 ± 1.9 , respectively. Contralateral (Figure 3) motor performance was markedly reduced with a mean consumption of 2.5 ± 2.7 pellets in the group undergoing sham stimulation; 2.1 ± 2.5 pellets in the group stimulated at 10 pps, 6.0 ± 3.9 at 20 pps and 4.5 ± 3.5 at 50 pps. The effect of 20 pps stimulation on the right side was significantly different from sham stimulation ($p < 0.01$).

2.3. Time course of motor training and recovery

Pre-stroke training in the Montoya staircase was performed over a period of three weeks. Stimulation was initiated two weeks after the stroke (one week after electrode implantation) and lasted for six weeks. Post-stroke assessments in the Montoya task lasted for four weeks, corresponding to the last four weeks of LCN stimulation.

. In the pre-stroke training phase, rats reached the motor function plateau during the last week of training (figure 4). Figure 5 represents the time course of motor recovery in the group stimulated at 20 pps. This is the only group with significantly enhanced recovery compared to sham stimulation. Motor recovery reached a plateau at the end of the third week of post-stroke Montoya assessments.

Discussion

Reciprocal connectivity between sensorimotor cortical territory and the contralateral cerebellar hemisphere has been well established (Asanuma et al., 1983b; Brodal and Bjälie, 1997). This input is not limited to the motor cortex, but rather extends to premotor and parietal regions (Dum and Strick, 2002; Dum and Strick, 2003). As such, we hypothesized that stimulation of the output of the dentate nucleus will modulate cortical excitability, not only in those cortical areas damaged by MCA cerebral ischemia (i.e. motor and adjacent cortex) but also in perilesional areas. In the present study, we evaluated the effects of chronic electrical stimulation of the lateral cerebellar (dentate) nucleus region upon motor recovery of rats that had undergone cerebral ischemia with the 3-vessel occlusion method. We have shown that electrical stimulation at 20 pps significantly enhances motor recovery as indexed by performance in the Montoya staircase task. Our data also indicates that the observation period of four weeks of staircase testing was sufficient to capture the peak and plateau of motor recovery.

3.1 Functional interdependence of the cerebral cortex and contralateral cerebellar hemisphere

The large pathways connecting the cerebral hemisphere and contralateral cerebellar hemisphere are a reflection of the interdependence of function between these two structures. The cerebellar hemisphere receives massive projections through the corticopontocerebellar pathway. Disruption of this pathway, as in ischemic infarcts of the territory of the middle cerebral artery results in deafferentation (Gold and Lauritzen, 2002) such that a reduction in metabolism (or blood flow) of the contralateral cerebellar hemisphere can be detected in functional neuroimaging studies in the acute and subacute phases (Lin, 1997; Meneghetti et al., 1984; Pantano et al., 1987). Perpetuation of CCD into the chronic phase may result in crossed cerebellar atrophy (Pantano et al., 1986; Tien and Ashdown, 1992), illustrating the functional impact of the corticopontocerebellar pathway on activity of the contralateral cerebellar hemisphere. While CCD illustrates functional dependence of the cerebellar hemispheres on the contralateral cerebral cortex, the function of the cerebral cortex, in turn, depends on the facilitatory effects of the dentatohalamocortical pathway. The excitatory effect of this two-synaptic pathway has been demonstrated non-invasively and with depth electrodes in humans and non-human primates, respectively (Iwata et al., 2004; Rispa-Padel et al., 1981).

Stroke patients who present with CCD can be expected to have reduced excitability of the ipsilesional hemisphere not only due to the ischemic process but also because of diaschisis related hypoactivity of the dentatohalamocortical pathway. Takasawa et al (Takasawa et al., 2002) have demonstrated that CCD is associated with worse long-term outcomes after stroke, regardless of the initial severity. In the present study, we intended to generate an effect opposite to CCD related cortical hypoexcitability. Our goal was to chronically augment the output of the dentatohalamocortical pathway, expecting to enhance contralateral cerebral cortical excitability and, consequently, motor recovery.

Projections from the dentatohalamocortical pathway extend beyond the motor cortex—

Lesions generated by our model affected most of the motor cortex of the rat with relative sparing of the somatosensory cortex (figure 2). In our experience, temporary ligation of the carotid arteries for 30 minutes generates strokes with average volumes (and SD) of 106 mm³ (SD $\pm$ 52.9mm), approximately 10% smaller than strokes generated with 60 minute ligations (Yanamoto et al., 2003). These lesions proved to be severe enough to generate chronic, severe motor deficits, as seen in the sham stimulation group which did not recover beyond an average consumption of 2.5 pellets per session with the affected side (compared to 15 pellets in the non affected side). Our rationale for facilitation of motor recovery was to enhance cortical excitability and plasticity, which is unlikely to be accomplished in the core of the ischemic lesion but, rather, in the perilesional cortex. Hence, the rationale relies more on the preserved perilesional areas than on the affected motor region. Although the dentate nucleus has well established projections to the motor cortex via the motor thalamic nuclei (Asanuma et al., 1983a), its projections have been shown to extend to premotor and non-motor parietal regions as well (Dum et al., 2002). An unfolded map of the dentate nucleus has been developed by Dum and Strick (Dum and Strick, 2003), demonstrating its projections to the arcuate sulcus and premotor areas 9L and 46 as well as to the parietal cortex and area 7. These projections indicate the stimulation of the dentate nucleus is likely to influence not only areas affected by the ischemic damage in this stroke model but also to the perilesional regions expected to be involved in post-injury reorganization. Reorganization of the perilesional cortex has been pointed as a likely mechanism of recovery of motor function following focal cerebral ischemia, in both animals and humans (Dijkhuizen et al., 2003; Nudo and Milliken, 1996; Tombari et al., 2004).

Cortical excitability and stroke rehabilitation—Cortical excitability has been linked to potential for motor recovery in several experimental paradigms. Evaluation with transcranial magnetic stimulation has shown increased cortical excitability following rehabilitation exercises (Liepert, 2006), coupled with improvements in motor function. Although a causative relationship cannot be established, it indicates a link between excitability and plasticity.

Electrical or magnetic stimulation of the perilesional cortex has been shown to facilitate cortical plastic reorganization (Plautz et al., 2003) and enhance motor recovery in animal models of stroke (Adkins-Muir and Jones, 2003) and in patients (Hummel et al., 2005). In the present study, our rationale was to enhance perilesional cortical excitability not with stimulation directly over the ipsilesional cortex but, rather, through stimulation of a large natural neuronal pathway with projections to the motor, premotor and posterior cortical regions. Lateral cerebellar nucleus stimulation at 20 pps but not at 10 pps or 50pps was effective in enhancing motor function. The selective efficacy of 20 pps stimulation may be due to this frequency's proximity to natural beta band oscillations found in the dentate nucleus during movement in the awake state (Aumann et al., 1998). Electrical stimulation of the dentate nucleus region after cerebral ischemia may serve to restore dentatohalamic output lost due to cerebellar hypoactivity secondary to crossed cerebral diaschisis. Although speculative, the selective efficacy of LCN stimulation in the beta band is further indication that, in the setting of massive deafferentation in the cortico-ponto-cerebellar-thalamo-cortical circuit, deep cerebellar

electrical stimulation may have rehabilitative effects mediated by restoration of patterns of neural activity in the dentatothalamocortical pathway.

In conclusion, this study corroborated our central hypothesis that stimulation of the dentatothalamocortical pathway at its origin in the dentate nucleus region can enhance recovery of motor function after ischemic strokes. Although further elucidation of the mechanisms underlying these effects is necessary, it is possible that cortical reorganization was facilitated by activation of the dentatothalamocortical pathway.

4. Experimental Procedures

Experiments were performed using Sprague-Dawley rats weighing 220–250 grams at study onset. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved animal facility in a controlled environment, including a 12-hour light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Cleveland Clinic.

Animals were pre-trained in the Montoya staircase task twice per day, five days per week, for a period of 3 weeks. Upon completion of training, all animals underwent left side ischemic strokes using the 3-Vessel occlusion model. Following a one week period of recovery with ad libitum access to food and water, surviving animals underwent stereotactic implantation and fixation of electrodes targeted to the right lateral cerebellar nucleus. Subsequently, animals were randomized to one of the following groups: 50pps, 20 pps, 10 pps and no (sham) stimulation. The treatment phase consisted of stimulation delivered for a total of 12 hours per day, during the animals' active, dark cycle, for a period of 6 weeks. Beginning on the third week and continuing until the end of the sixth week of stimulation, animals were re-acquainted and evaluated with the Montoya staircase test, twice per day, five days per week. Subsequently, animals were deeply anesthetized pentobarbital (100 mg/kg), followed by transcardiac perfusion prior to removal of the brains for histological analysis.

Seventy-five Wistar rats weighing 220–250g were included in the study (average 231g). Data are reported on 48 animals. Twenty-seven animals were excluded from the study either because of failure to achieve the minimal pellet consumption during baseline performance on the Montoya staircase task (6) or death (21). The most common cause of death was respiratory distress, marked by stridor in the early recovery phase after removal from mechanical ventilation. Stridor was associated with immediate natural death or precipitated the investigators to sacrifice the animals. The higher mortality rate associated with respiratory distress is likely related to subsequent procedures for stroke and lead implantation/fixation under mechanical ventilation.

4.1. The Montoya staircase test

The Montoya staircase test is a well established measure of forepaw motor function in rats and has been demonstrated to be reproducible and sensitive to motor impairments (Colbourne et al., 2000; DeBow et al., 2003; Hudzik et al., 2000). Briefly, animals are underfed (free water access) to 85% of their predicted weight over three days prior to the initial training day and subsequently receive a smaller ration of food/day (~12 grams) throughout the training/baseline period. During the testing, the animal is placed on a central base with two staircases on either side, designed such that only the ipsilateral paw can reach a given staircase (Montoya et al., 1991). Each of the seven steps contains a food-well loaded with three pellets (45 mg precision pellets, Research Diets, NJ). A lip on the platform edge prohibits the animal from dragging the pellet up along side the platform, thus requiring it be grasped and brought up and around the platform edge. Outcome is measured as the number of pellets recovered with each paw. During training, the results of the final five days (out of fifteen total) of testing were averaged to serve

as baseline score. Animals that did not reach a baseline of at least 13 pellets consumed per side were excluded from the remainder of the study. Similarly, during the trial phase evaluation (at the end of the experimentation), the final five (out of twenty) test days were averaged for final reporting.

4.2. Three-Vessel Stroke Model Procedure

Rats underwent cerebral infarctions by microsurgical coagulation and sectioning of the middle cerebral artery combined with temporary occlusion of the common carotid arteries for 30 min, as described by Yanamoto et al (Yanamoto et al., 2003). Anesthesia was initiated in a chamber saturated with isoflurane at 5% and subsequently maintained under mechanical ventilation with continuous isoflurane (1.5% – 3.0%) and oxygen. The animal was then placed in a supine position and each appendage secured to a rodent operating board. The hair along the ventral cervical region and left side of the head was clipped. A cervicotomy was opened and the common carotid arteries isolated bilaterally. A 4-0 Silk ligature was looped around each artery but not yet tied. Once the ligatures were in place, the rat was positioned laterally. An incision was opened on the left side of the head, followed by exposure and removal of the zygoma. A small craniotomy, approximately 4 mm in diameter, was created over the middle cerebral artery (MCA), using a high-speed drill. With microsurgical technique, the dura mater was opened and the MCA coagulated with bipolar forceps and sectioned lateral to the olfactory tract. Both carotids were then immediately tied. Non-Invasive arterial pressure and rectal temperature monitoring were performed during the ischemia period, with temperature controlled by means of a closed-loop heating pad. The carotids were re-perfused after 30 minutes and the incisions approximated with 3-0 Monocryl sutures. Post procedure analgesia was maintained with buprenorphine 0.05 mg/kg SQ.

4.3. Cerebellar Electrode Implantation

Anesthesia was initiated in a chamber saturated with isoflurane at 5% and maintained under mechanical ventilation with continuous isoflurane (1.5% – 3.0%) and oxygen. The rat was positioned on a stereotactic frame (David Kopf, Tujunga, CA), fixed at the external auditory canals and maxilla. An incision was opened over the calvaria, exposing the bregma, lambda and occipital region. A small (~2 mm) burr hole was created over the posterior calvaria, through which a 0.28 mm diameter concentric bipolar stimulating electrode (Model MS306, Plastics One Inc., Roanoke, VA, USA) with 0.3 mm of exposed tip was inserted to the pre-calculated target of the lateral cerebellar nucleus (LCN) based on Paxino's atlas (Paxinos and Watson, 1998): -11.0 mm (posterior) to Bregma, 3.7 mm lateral and 6.0 mm ventral (figure 1). The electrode was fixed to the skull using dental cement and small stainless steel screws (MX-0090-2; Small part Inc., Miami Lakes, FL). The plastic connector at the proximal end of the electrode remained exposed to allow for attachment to a commutator system (Model SL2C, Plastics One, Inc., Roanoke, VA USA) (Burgess et al., 2003). The skin edges were approximated around the implant with 3-0 Monocryl sutures. The animals were allowed to recover from anesthesia, with food and water provided ad-libitum and buprenorphine was used for analgesia.

4.4. Chronic Stimulation

The treatment phase was initiated one week after cerebellar implantation, with each animal being randomized to one of the four treatment groups. All animals were individually housed and tethered, by means of a spring-reinforced wiring system, to a commutator (Model SL2C, Plastics One Inc., Roanoke, VA, USA) mounted above the home cage. The system enabled unrestricted movement of the animal throughout the cage. Charge-balanced, pulsed square-wave stimulation was delivered using a constant-current stimulus isolator (Model SIU-102, Warner Instruments, Hamden, CT, USA) controlled by a Grass stimulator (Model S88). All

animals were freely moving. Stimulus frequency was determined by treatment group, with pulse width maintained at 400 microseconds. The amplitude of stimulation was adjusted for each rat by determining the threshold for ipsilateral motor response (i.e., motor threshold). Titration of the motor threshold was performed in a transparent cylinder so that the rat could be observed from all directions. The threshold was defined as the lowest current to produce visible cervical contractions or ipsilateral vibrissae or limb movement. For chronic stimulation, the pulse amplitude was set at 75% of the motor threshold, ranging from 50 to 100 microamperes. Charge densities never exceeded $30\mu\text{C}/\text{cm}^2/\text{phase}$. Rats were excluded or sacrificed if electrodes or tethering cables broke or presented high impedances.

Rats were randomized to one of the treatment groups or sham stimulation only after the stroke and electrode implantation. Our chronic stimulation housing unit allows for concomitant stimulation of multiple animals. Although amplitudes can be adjusted individually, up to three stimulation frequencies can be tested in different groups simultaneously. The experimentation was carried out in two consecutive cohorts. In the first cohort, animals were randomized to stimulation at 20 pps, 50 pps or sham stimulation. In the second, rats were randomized to stimulation at 10 pps, 20 pps or sham stimulation. Data from both cohorts are shown combined in table 1.

4.5. Histology

Following the experiments, histological analysis of all animals was performed for the purpose of verifying the location of the cerebellar electrode and for determination of stroke volume. Immediately prior to sacrifice, a lesion was generated at the distal tip of the deep cerebellar electrode by electrocoagulation (1.0 mA, DC current, 15 seconds duration). Under deep anesthesia with pentobarbital (50 mg/kg), the rats were transcardially perfused with saline followed by 4% paraformaldehyde. The brains were removed and immersed in 4% paraformaldehyde. All brains were blocked in paraffin and sliced at $40\mu\text{m}$. Every eighth slice (from the frontal pole to the tentorium) was mounted so that the interspacing of each mounted slide was $320\mu\text{m}$. Slices were Nissl stained to optimize visualization of the anatomical structures and subsequent calculation of hemispheric areas on either side. Determination of stroke volume was calculated by taking the difference between the hemispheric volumes for the affected and non-affected hemispheres (Corbett et al., 2000). These volumes were calculated by integrating the hemispheric areas, using ImageJ software (NIH, public domain).

The cerebellum was sectioned at $40\mu\text{m}$ and every other slice was mounted so that the spacing between slides was $80\mu\text{m}$. The Pearls/DAB stain was used to facilitate recognition of the iron deposits in the trajectory of the electrode. The greater resolution in the cerebellum was chosen to allow for more precise localization of the electrode location.

4.6. Statistical analysis

The data were analyzed using multivariate regression techniques. The number of pellets eaten was measured for various combinations of stimulus (pps), stroke (before and after) and stroke volume over time. The test combinations of these variables allowed the construction of a regression model that included these variables as well as the interaction of stroke and stimulation condition. The model was used to assess the effects of any one of the variables independent of the others. Multiple pair-wise comparisons were made using the Tukey-Kramer adjustment.

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References

- Adkins-Muir DL, Jones TA. Cortical electrical stimulation combined with rehabilitative training: enhanced functional recovery and dendritic plasticity following focal cortical ischemia in rats. *Neurol Res* 2003;25:780–8. [PubMed: 14669519]
- Asanuma C, et al. Anatomical evidence for segregated focal groupings of efferent cells and their terminal ramifications in the cerebellothalamic pathway of the monkey. *Brain Res* 1983a;286:267–97. [PubMed: 6189562]
- Asanuma C, et al. Distribution of cerebellar terminations and their relation to other afferent terminations in the ventral lateral thalamic region of the monkey. *Brain Res* 1983b;286:237–65. [PubMed: 6189561]
- Aumann TD, et al. The relationship between monkey dentate cerebellar nucleus activity and kinematic parameters of wrist movement. *Exp Brain Res* 1998;119:179–90. [PubMed: 9535568]
- Benabid AL, et al. Long-term electrical inhibition of deep brain targets in movement disorders. *Mov Disord* 1998;13(Suppl 3):119–25. [PubMed: 9827607]
- Brodal P, Bjaalie JG. Salient anatomic features of the cortico-ponto-cerebellar pathway. *Prog Brain Res* 1997;114:227–49. [PubMed: 9193147]
- Burgess R, et al. Implementation of a rodent monitoring system for large-scale animal testing in epilepsy research. *Epilepsia* 2003;44:213.
- Butler AJ, Wolf SL. Putting the brain on the map: use of transcranial magnetic stimulation to assess and induce cortical plasticity of upper-extremity movement. *Phys Ther* 2007;87:719–36. [PubMed: 17429003]
- Carmichael ST. Cellular and molecular mechanisms of neural repair after stroke: making waves. *Ann Neurol* 2006;59:735–42. [PubMed: 16634041]
- Chen CL, et al. Brain lesion size and location: effects on motor recovery and functional outcome in stroke patients. *Arch Phys Med Rehabil* 2000;81:447–52. [PubMed: 10768534]
- Colbourne F, et al. Prolonged but delayed postischemic hypothermia: a long-term outcome study in the rat middle cerebral artery occlusion model. *J Cereb Blood Flow Metab* 2000;20:1702–8. [PubMed: 11129786]
- Constantoyannis C, et al. Tremor induced by thalamic deep brain stimulation in patients with complex regional facial pain. *Mov Disord* 2004;19:933–6. [PubMed: 15300659]
- Corbett D, et al. Persistent neuroprotection with prolonged postischemic hypothermia in adult rats subjected to transient middle cerebral artery occlusion. *Exp Neurol* 2000;163:200–6. [PubMed: 10785459]
- Cramer SC, Riley JD. Neuroplasticity and brain repair after stroke. *Curr Opin Neurol* 2008;21:76–82. [PubMed: 18180655]
- DeBow SB, et al. Constraint-induced movement therapy and rehabilitation exercises lessen motor deficits and volume of brain injury after striatal hemorrhagic stroke in rats. *Stroke* 2003;34:1021–6. [PubMed: 12649509]
- Dijkhuizen RM, et al. Correlation between brain reorganization, ischemic damage, and neurologic status after transient focal cerebral ischemia in rats: a functional magnetic resonance imaging study. *J Neurosci* 2003;23:510–7. [PubMed: 12533611]
- Dum RP, et al. Motor and nonmotor domains in the monkey dentate. *Ann N Y Acad Sci* 2002;978:289–301. [PubMed: 12582061]
- Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. *Physiol Behav* 2002;77:677–82. [PubMed: 12527018]
- Dum RP, Strick PL. An unfolded map of the cerebellar dentate nucleus and its projections to the cerebral cortex. *J Neurophysiol* 2003;89:634–9. [PubMed: 12522208]
- Gold L, Lauritzen M. Neuronal deactivation explains decreased cerebellar blood flow in response to focal cerebral ischemia or suppressed neocortical function. *Proc Natl Acad Sci U S A* 2002;99:7699–704. [PubMed: 12032346]
- Hicks AU, et al. Enriched environment enhances transplanted subventricular zone stem cell migration and functional recovery after stroke. *Neuroscience* 2007;146:31–40. [PubMed: 17320299]

- Hudzik TJ, et al. Long-term functional end points following middle cerebral artery occlusion in the rat. *Pharmacol Biochem Behav* 2000;65:553–62. [PubMed: 10683498]
- Hummel F, et al. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain* 2005;128:490–9. [PubMed: 15634731]
- Iwata NK, et al. Facilitatory effect on the motor cortex by electrical stimulation over the cerebellum in humans. *Exp Brain Res* 2004;159:418–24. [PubMed: 15558250]
- Iwata NK, Ugawa Y. The effects of cerebellar stimulation on the motor cortical excitability in neurological disorders: a review. *Cerebellum* 2005;4:218–23. [PubMed: 16321876]
- Johansson BB. Functional and cellular effects of environmental enrichment after experimental brain infarcts. *Restor Neurol Neurosci* 2004;22:163–74. [PubMed: 15502263]
- Kelly-Hayes M, et al. The influence of gender and age on disability following ischemic stroke: the Framingham study. *J Stroke Cerebrovasc Dis* 2003;12:119–26. [PubMed: 17903915]
- Liepert J. Transcranial magnetic stimulation in neurorehabilitation. *Acta Neurochir Suppl* 2005;93:71–4. [PubMed: 15986730]
- Liepert J. Motor cortex excitability in stroke before and after constraint-induced movement therapy. *Cogn Behav Neurol* 2006;19:41–7. [PubMed: 16633018]
- Liepert J, et al. Motor cortex excitability after cerebellar infarction. *Stroke* 2004;35:2484–8. [PubMed: 15375297]
- Lin WY. Crossed cerebellar diaschisis: related to lesion location or disease duration? *J Nucl Med* 1997;38:2006. [PubMed: 9430487]
- Lloyd-Jones D, et al. Heart disease and stroke statistics -- 2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2009;119:e21–181. [PubMed: 19075105]
- Machado, AG., et al. Society for Neuroscience. Society for Neuroscience; Washington, DC: 2005. Effects of cerebellar stimulation on motor cortex in rats.
- Meneghetti G, et al. Crossed cerebellar diaschisis in ischemic stroke: a study of regional cerebral blood flow by ¹³³Xe inhalation and single photon emission computerized tomography. *J Cereb Blood Flow Metab* 1984;4:235–40. [PubMed: 6609930]
- Montoya CP, et al. The “staircase test”: a measure of independent forelimb reaching and grasping abilities in rats. *J Neurosci Methods* 1991;36:219–28. [PubMed: 2062117]
- Nudo RJ, Milliken GW. Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *Journal of Neurophysiology* 1996;75:2144–9. [PubMed: 8734610]
- Pan SL, et al. Location and size of infarct on functional outcome of noncardioembolic ischemic stroke. *Disabil Rehabil* 2006;28:977–83. [PubMed: 16882637]
- Pantano P, et al. Crossed cerebellar diaschisis. Further studies. *Brain* 1986;109(Pt 4):677–94. [PubMed: 3488093]
- Pantano P, et al. Crossed cerebellar diaschisis in patients with cerebral ischemia assessed by SPECT and ¹²³I-HIPDM. *Eur Neurol* 1987;27:142–8. [PubMed: 3497808]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; 1998.
- Plautz EJ, et al. Post-infarct cortical plasticity and behavioral recovery using concurrent cortical stimulation and rehabilitative training: a feasibility study in primates. *Neurol Res* 2003;25:801–10. [PubMed: 14669522]
- Rispal-Padel L, et al. Contribution of the dentato-thalamo-cortical system to control of motor synergy. *Neurosci Lett* 1981;22:137–44. [PubMed: 7231805]
- Saucier DM, et al. Enriched environment and the effect of age on ischemic brain damage. *Brain Res* 2007;1170:31–8. [PubMed: 17714698]
- Shelton FN, Reding MJ. Effect of lesion location on upper limb motor recovery after stroke. *Stroke* 2001;32:107–12. [PubMed: 11136923]
- Takasawa M, et al. Prognostic value of subacute crossed cerebellar diaschisis: single-photon emission CT study in patients with middle cerebral artery territory infarct. *AJNR Am J Neuroradiol* 2002;23:189–93. [PubMed: 11847040]

- Tien RD, Ashdown BC. Crossed cerebellar diaschisis and crossed cerebellar atrophy: correlation of MR findings, clinical symptoms, and supratentorial diseases in 26 patients. *AJR Am J Roentgenol* 1992;158:1155–9. [PubMed: 1566683]
- Tombari D, et al. A longitudinal fMRI study: in recovering and then in clinically stable sub-cortical stroke patients. *Neuroimage* 2004;23:827–39. [PubMed: 15528083]
- Yanamoto H, et al. Evaluation of MCAO stroke models in normotensive rats: standardized neocortical infarction by the 3VO technique. *Exp Neurol* 2003;182:261–74. [PubMed: 12895438]

Figure 1.

a) Diagrammatic representation of a coronal slice of the cerebellum at bregma = -11.0 mm (Paxinos and Watson, 1998) showing the indented target for implantation of the electrode in the lateral cerebellar nucleus (arrow). b) coronal Pearls/DAB stained section of the cerebellum showing the artifact from the trajectory and the location corresponding to the tip of the implanted electrode (arrow), where there electrolytic lesion was created.

Figure 2.

Diagrammatic representation of the typical stroke. The cortex anterior to the bregma was predominantly affected, with relative sparing of the areas posterior to the bregma. Limited injury to the anterior part of the somatosensory cortex was observed, mostly anterior to segments 2 mm posterior to the bregma. The subcortical white matter was affected proportionately to the cortex but with near complete sparing of the basal ganglia and complete sparing of the thalamus).

Figure 3.

The box plot represents the data from the last week of post-stroke assessment in the Montoya staircase task for all groups: sham stimulation, 10pps, 20 pps and 50 pps stimulation. For reference, the performance of the last week of pre-stroke training in the Montoya task is also shown on the left. The top and bottom of the box represent, respectively, the second and third quartiles. Inside each box, the median (second quartile) is represented. A marked suppression in motor performance was observed across all groups after strokes and was maintained throughout the observation period. Although a trend for improvement could be observed in the 50 pps group, only stimulation at 20 pps resulted in a significant difference in motor outcomes compared to sham stimulation.

Figure 4.

Time course of the average right sided motor training (pre-stroke). All animals included. Each column of data in the graph represents a session in the Montoya staircase (2 sessions per day). Each dot represents data derived from the first cohort. Each circle represents data from the second cohort of animals. Circles with a dot in the center indicate data overlapping from animals from more than one cohort. The learning curve of the motor outcome task is steep during the first 10 days and then approximates a plateau near an average of 16 pellets per side. Only animals consuming at least 13 pellets per side continued in the study.

Figure 5.

Time course of right sided motor recovery of rats undergoing chronic 20 pps stimulation. This is the only stimulation group that presented with motor outcomes significantly improved in relation to sham stimulation. There is an ascending slope of motor performance up to 15 days into the post-stroke Montoya assessment period, followed by a plateau in motor performance. This corresponds to the end of the third week of assessments (5 assessment days per week).

Table 1

Summary of the data, comparing sham stimulation to stimulation at 10 pps, 20 pps and 50 pps. Only stimulation at 20 pps resulted in motor recovery of the affected (right) side which was significantly enhanced compared to sham stimulation ($p < 0.01$). A trend for improvement was observed at 50 pps but without significance. There was minimal variation of motor outcomes on the non-affected (left side) regardless of the stimulation group.

Stimulation Group	LimbSide	N	Mean	Standard Deviation
0 (Sham)	Right	15	2.5	2.7
10 pps	Right	7	2.1	2.5
20 pps	Right	18	6.0	3.9
50 pps	Right	8	4.5	3.5
0 (Sham)	Left	15	16.0	5.0
10 pps	Left	7	15.9	6.7
20 pps	Left	18	17.2	2.1
50 pps	Left	8	16.7	1.9