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Acute inactivation of the contralesional hemisphere for longer durations improves recovery after cortical injury☆

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ABSTRACT

A rapidly growing number of studies using inhibition of the contralesional hemisphere after stroke are reporting improvement in motor performance of the paretic hand. These studies have used different treatment onset time, duration and non-invasive methods of inhibition. Whereas these results are encouraging, several questions regarding the mechanisms of inhibition and the most effective treatment parameters are currently unanswered. In the present study, we used a rat model of cortical lesion to study the effects of GABA-mediated inactivation on motor recovery. In particular, we were interested in understanding better the effect of inactivation duration when it is initiated within hours following a cortical lesion. Cortical lesions were induced with endothelin-1 microinjections. The contralesional hemisphere was inactivated with continuous infusion of the GABA-A agonist Muscimol for 3, 7 or 14 days in three different groups of animals. In a fourth group, Muscimol was infused at slower rate for 14 days to provide additional insights on the relation between the effects of inactivation on the non-paretic forelimb behavior and the recovery of the paretic forelimb. In spontaneously recovered animals, the lesion caused a sustained bias to use the non-paretic forelimb and long-lasting grasping deficits with the paretic forelimb. Contralesional inactivation produced a general decrease of behavioral activity, affected the spontaneous use of the forelimbs and caused a specific reduction of the non-paretic forelimb function. The intensity and the duration of these behavioral effects varied in the different experimental groups. For the paretic forelimb, increasing inactivation duration accelerated the recovery of grasping function. Both groups with 14 days of inactivation had similar recovery profiles and performed better than animals that spontaneously recovered. Whereas the plateau performance of the paretic forelimb correlated with the duration of contralesional inactivation, it was not correlated with the spontaneous use of the forelimbs or with grasping performance of the non-paretic hand. Our results support that contralesional inactivation initiated within hours after a cortical lesion can improve recovery of the paretic forelimb. In our model, increasing the duration of the inactivation improved motor outcomes but the spontaneous use and motor performance of the non-paretic forelimb had no impact on recovery of the paretic forelimb.

Introduction

Several studies have shown that following a lesion, there is an increase of cortical excitability in the contralesional hemisphere (Meyer et al., 1985; Mohajerani et al., 2011; Sakatani et al., 1990). The hyperexcitability in the contralesional cortex (Buchkremer-Ratzmann et al., 1996) is associated with a diminution of GABAergic inhibition (Witte and Stoll, 1997) and a reduction of GABA-A receptor binding (Lee et al., 2011; Qu et al., 1998), suggesting that it may be related to a decreased potency of the inhibitory GABAergic system. Longitudinal imaging studies in humans have shown that the contralesional activity is typically maximal early after the injury and progressively diminishes with time and recovery (Carey et al., 2006; Jaillard et al., 2005; Marshall et al., 2000).

In the last few years, several modulatory approaches using non-invasive stimulation techniques to favor adaptive plasticity and recovery after stroke have been proposed and are currently being tested. In particular, many groups are intensively investigating the effects of inhibition of the contralesional hemisphere on behavioral recovery (Hummel and Cohen, 2006). The rationale behind the treatment strategy used in most studies is based on the concept of

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interhemispheric imbalance (Liepert et al., 2000; Nowak et al., 2009). According to this hypothesis, hyperexcitability in the contralesional hemisphere results in an augmented inhibitory influence on the ipsilesional hemisphere. In this manner, the contralesional hemisphere would interfere with function and adaptive plasticity in the ipsilesional hemisphere and with recovery of the paretic arm. However, this hypothesis is far from being universally accepted.

While some studies using protocols of inhibition of the contralesional cortex with non-invasive stimulation techniques show improvements in motor performance of the paretic hand (Khedr et al., 2009; Nowak et al., 2008; Takeuchi et al., 2005), inhibition of contralesional areas with atypically high activity in chronic stroke patients is also shown to interfere with performance of the paretic hand (Johansen-Berg et al., 2002; Lotze et al., 2006). To date, only a few studies have used multiple treatments sessions, many of them with different treatment duration and onset (Boggio et al., 2007; Fregni et al., 2006; Khedr et al., 2009). Therefore, the effect of inhibition duration on behavioral recovery is virtually unexplored. No study has yet has tested the effect of contralesional inhibition initiated within hours following the lesion, when the interhemispheric imbalance should be maximal. Moreover, the mechanisms through which non-invasive stimulation methods can induce cortical inhibition and to what extent they act through GABA are not fully understood, leaving open the question if potentiating GABA-mediated inhibition of the contralesional hemisphere can improve recovery.

To provide some insight on these issues, we used a well-established method of inactivation consisting of continuous infusion of the GABA-A agonist Muscimol (Martin, 1991) in a rat model of cortical lesion. In particular, our objectives were to confirm that GABA-mediated inactivation and very early inactivation could favor recovery, and to study the effect of duration of contralesional inactivation on motor outcomes. Of course, interpretation of our results and their translation to humans should be done with caution. The inherent differences in the methods used to inactivate the contralesional hemisphere in our model and humans, and the much longer durations and intensity of the inactivation we used, as revealed in the behavior of the non-paretic forelimb, should all be kept in mind.

These data increase our understanding of the basic interactions between inactivation of the contralesional hemisphere, behavior of the non-paretic forelimb and recovery of the paretic forelimb and may provide useful cues for the development of treatments based on contralesional inhibition after stroke.

Methods

Animals

A total of 53 Sprague–Dawley rats (Charles River, QC, CA) of approximately 2 months of age and weighing 250–300 g were included in the study. All animals were housed in solitary standard Plexiglas cages with reverse day–night cycle (7 am–7 pm). They were handled only during the dark cycle. Upon their arrival at our facility, animals were familiarized with banana food pellets in the Montoya staircase test (Biernaskie and Corbett, 2001; Montoya et al., 1991) for 10 work-days (Fig. 1). To incite reaching behavior in the Montoya test, food access was carefully monitored during the two weeks of familiarization. For each rat, the daily food minimum corresponded to 5% of its body weight. Rats had free access to 85% of their daily minimum in the home cage. They could obtain more food to surpass the 100% value in the Montoya staircase apparatus. On any given day, if the animal did not attain its daily minimum in the Montoya, additional food was supplied in its home cage to reach the daily minimum. Baseline data were collected at the end of the familiarization period. Spontaneous use of forelimbs in exploratory behavior was documented with the cylinder wall test (Schallert et al., 2000) and grasping function with the Montoya Staircase test (Montoya et al., 1991). Animals that performed above the inclusion criteria (see Montoya Staircase test below) were randomly assigned to an experimental group. During the post-lesion period, food was restricted for 12–14 h prior to each behavioral testing session and animals were given free access to food after testing. The weight of the animals was recorded daily during the 2 weeks prior to the lesion and weekly after the lesion. If an animal lost more than 10% of its original body weight at any point during the experiment, it was excluded. Two animals from Group 14D were excluded from the study during the recovery period because of weight loss and seizures. Animals had ad libitum access to water at all times. Our experimental protocol followed the guidelines of the Canadian Council on Animal Care and was approved by the Comité de Déontologie de l'Expérimentation sur les Animaux of the Université de Montréal.

Cylinder test: measurement of spontaneous forelimb use

To detect spontaneous asymmetrical use of forelimbs, rats were placed in a transparent cylinder of 19 cm diameter and 33 cm height for 3–30 min or until 60 touches to the cylinder wall was achieved (Schallert et al., 2000). Animals were videotaped from above using a high definition digital video camera (30 frames/s). The videos were analyzed frame-by-frame offline to count the use of paretic versus non-paretic limbs during vertical exploration of the cylinder wall. The forelimb asymmetry score was calculated using the following equation:

$$\textit{Asymmetry score} = \frac{\textit{touches with paretic forelimb} - \textit{touches with nonparetic forelimb}}{\textit{total number of touches}}$$

Montoya Staircase test: Grasping and retrieving performance

Rats were placed in a Plexiglas chamber (6-cm wide, 12-cm high and 30-cm long) with a central platform (2.3-cm wide, 6-cm high and 19-cm long) that supports the weight, separating the right and left forelimbs (Biernaskie and Corbett, 2001; Montoya et al., 1991). A pair of staircases with seven steps on each side was loaded into the Plexiglas chamber on both sides of the central platform. Each step had a smooth well that can hold one to three standard 45 mg banana flavored food pellets (Bioserve Inc., Frenchtown, NJ, USA). During the familiarization period, animals had a session of Montoya staircase in the morning and one in the afternoon, the two separated by 3 to 4 h. In a session, the rats had 4 trials with each hand (8 trials per day in total). Initially, for each trial, every well on one side of the staircase was filled with 3 food pellets and 15 min were given to retrieve the pellets. In the following days, the number of pellets in each well and the time provided was progressively tapered according to the performance of the rat. However, by the 8th day, only one pellet per well and 3 min per trial were given to all rats. On the 9th and 10th days of the familiarization period, the performance in terms of the number of eaten pellets was recorded and used to establish if the animal reached our inclusion criteria. To be included in the study, rats needed to eat 4 out of 7 pellets in 3 of the 4 trials on both days with one of the two arms. Based on these criteria, 9 animals were excluded from the study. The lesion was induced in the cortex contralateral to the arm with the best performance score.

Surgical procedures

All surgical procedures were conducted aseptically. Anesthesia was induced with Ketamine (80 mg/kg, intra-peritoneal) and maintained with isoflurane (~2% in 100% oxygen) delivered via a custom-made facial mask adapted to our stereotaxic frame. Pulse rate and oxygen saturation were monitored and documented during the surgery. A self-regulating heating blanket (Harvard Apparatus, Holliston, MA) was used to maintain body temperature during the surgery. A midline incision was made to expose the skull and neck muscles. A small