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Research report

Voluntary forced use of the impaired limb following stroke facilitates functional recovery in the rat

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HIGHLIGHTS

- Intracortical and intrastriatal endothelin-1 microinjections produce unilateral deficits in forelimb function in the rat.
- Appetitively-motivated forced use combined with repetitive task practice accelerates functional recovery.
- Rehabilitation increases doublecortin positive cell numbers in SVZ and peri-infarct regions.
- Rehabilitation alters the cellular origins of BDNF in peri-infarct zones.

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ABSTRACT

Constraint induced movement therapy (CIMT), which forces use of the impaired arm following stroke, improves functional recovery. The mechanisms underlying recovery are not well understood, necessitating further investigation into how rehabilitation may affect neuroplasticity using animal models. Animal motivation and stress make modelling CIMT in animals challenging. We have shown that following focal ischemia, voluntary forced use therapy using pet activity balls could engage the impaired forelimb and result in a modest acceleration in recovery. In this study, we investigated the effects of a more intensive appetitively motivated regimen that included task specific reaching exercises. Adult male Sprague Dawley rats were subjected to focal unilateral stroke using intracerebral injections of endothelin-1 or sham surgery. Three days later, stroke animals were assigned to daily rehabilitation or control therapy. Rehabilitation consisted of 30 min of generalized movement sessions in activity balls, followed by 30 min of voluntary task-specific movement using reaching boxes. Rats were tested weekly to measure forelimb deficit and recovery. After 30 days, animals were euthanized and tissue was examined for infarct volume, brain derived neurotrophic factor expression, and the presence of new neurons using doublecortin immunohistochemistry. Rehabilitation resulted in a significant acceleration of forelimb recovery in several tests, and a significant increase in the number of doublecortin-expressing cells. Furthermore, while the proportion of cells expressing BDNF in the peri-infarct region did not change, there was a shift in the cellular origin of expressed BDNF, resulting in significantly more non-neuronal, non-astrocytic BDNF, presumed to be of microglial origin.

1. Introduction

Almost 850,000 North Americans experience a stroke each year [1,2]. The majority of stroke survivors are left with motor disabilities including upper extremity impairments [2], and it has been suggested that stroke has a greater disability impact than any other chronic disease [3]. Patients can remain chronically impaired for months to years following stroke, which vastly impacts quality of life and is associated with a higher rate of post-stroke depression [4,5]. Presently, recombinant tissue plasminogen activator (tPA) is the only approved drug to administer to someone experiencing a stroke, however, it is associated with a number of limitations [6–8]. As such, many stroke patients continue to rely critically on post-stroke rehabilitation, and continued efforts towards progression of rehabilitative techniques are warranted to improve treatment of the devastating effects of a stroke.

Rehabilitation has historically focused on preventing the impairment from worsening and learning to compensate for loss

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of function [9]. However, with an evolving understanding of the brain's post-injury plastic potential, emphasis has shifted to non-invasive techniques aimed at maximising tissue reorganization, with the goal of reinstating function [10]. One rehabilitative technique that has been developed to increase use and improve function of the upper limb in survivors of stroke is constraint induced movement therapy (CIMT) [11]. The therapy discourages 'learned non-use' [12], whereby movement is initially suppressed due to failure and adverse consequences encountered when a subject attempts to use the affected limb. This results in persistent compensatory behaviours and subsequent suppression of use of the impaired limb, even when function may eventually be possible. Through constraint of the unaffected limb and subsequent forced use of the affected one, CIMT encourages positive feedback about the limb's functional potential. Generally, the constraint device is worn for most waking hours during a two week period [13,14], and is accompanied by intensive repetitive task practice (RTP) performed daily using the (unconstrained) impaired limb. RTP is performed in conjunction with shaping, during which participants engage in meaningful functional activities with measurable progressions for which they receive positive feedback as the activities become increasingly difficult. In addition to shaping, other behavioural techniques such as home practice and problem solving sessions are used to aid in the transfer of functional gains to performance of daily activities [15], all of which appears to be crucial for optimal treatment [16]. CIMT has been clinically shown to improve functional outcome [11,13,14,17,18], even when administered to patients with chronic deficits [19].

Although a seemingly effective technique [18], the mechanisms responsible for CIMT-assisted functional recovery are not well understood. As such, valid experimental models can assist further investigation into the underlying processes on a cellular and tissue level. In recent years, several creative animal models of forced limb use therapies have been developed [20–33]. These have provided important insights into the numerous variables that need to be considered (e.g. experimental stroke model to be used, forced vs. voluntary limb rehabilitation), in order to use paradigms that best model particular and appropriate aspects of CIMT [34]. A major challenge faced by researchers when modelling forced use in animals is that of subject motivation. The affected forelimb can be constrained in rats [20,22,23]; however, increased animal stress [35,36] and lack of behavioural pressure to rely on the impaired forelimb [23] may be problematic. Because of this, some researchers have opted to shift focus away from constraint and towards forced use by other means.

We previously reported a modest acceleration of functional recovery using a novel method of appetitively forcing use of the impaired forelimb using commercial pet activity balls [37]. The purpose of the current study was to investigate the effectiveness of appetitively-motivated forced use therapy with a task-specific component (similar to the RTP portion of CIMT), and to characterize the resulting cellular and neurochemical changes that could influence recovery. We hypothesized that rehabilitation would lead to accelerated functional recovery, and increased expression of markers of neuroplasticity in the brain.

2. Methods

2.1. Experimental animals

Adult male Sprague–Dawley rats ($N = 31$) were purchased from Charles River Laboratories (Montreal, Canada) and single housed on a 12 h reverse light/dark cycle (lights off at 08:00, on at 20:00). Activity levels were found to affect participation in the rehabilitation, as shown by others [38], therefore all procedures took place during the more active dark cycle. Unless otherwise stated, animals had ad libitum access to food and water, and weighed between 300 and 350 g at the time of surgery. All procedures were conducted in accordance with the guidelines of the Canadian Council for Animal Care and were approved in advance by the University of Prince Edward Island Animal Care Committee.

Table 1
Stereotaxic coordinates for intracerebral endothelin-1 microinjections.

	Stereotaxic coordinates (mm from bregma)			Volume (μl)
	AP	ML	DV	
Cortex injection 1	+2.5	−2.8	−2.5	1.0
Cortex injection 2	+1.6	−2.8	−2.5	1.0
Cortex injection 3	+0.9	−2.8	−2.5	1.0
Cortex injection 4	+0.4	−2.8	−2.5	1.0
Striatum injection	+0.9	−3.7	−6.0	0.5

400 pmol/μl endothelin-1 was injected at stereotaxic coordinates and volumes indicated. AP—anterior/posterior, ML—medial/lateral, DV—dorsal/ventral.

2.2. Surgical procedure

Endothelin-1 (ET-1), a vasoconstricting peptide, was used to produce focal transient ischemia as described previously [37]. Rats were placed into an induction chamber pre-filled with 3.5% isoflurane in oxygen for 8 min; anaesthesia was later maintained during surgery using 2% isoflurane in oxygen. Once anaesthetized, animals were mounted onto a stereotaxic apparatus (David Kopf Instruments, USA), an incision was made, and the scalp was retracted with clamps. The surface of the skull was cleared of all tissue, and injection coordinates were marked on the hemisphere opposite the preferred paw (determined in training, prior to surgery; see Section 2.4.3). A total of five injection coordinates targeting the forelimb sensorimotor cortex [39] were used as previously described [37], and listed in Table 1. Small holes were drilled through the skull at each injection location using a stereotaxically mounted drill (Stoelting Co., IL, USA). A 26 ga needle with a 10 μl syringe was lowered at each set of coordinates and left undisturbed for 1 min. Endothelin-1 (400 pmol/μl in sterile water; Calbiochem, La Jolla, USA) was injected at a flow rate of 0.5 μl/min (see Table 1 for injection volumes) and the needle was left undisturbed for 4 min before being slowly retracted from the brain. The scalp was sutured and the incision site was treated with topical anaesthetic (Xylocaine, AstraZeneca, Canada). Body temperature was monitored regularly and maintained at 36.0 ± 0.2 °C for the duration of surgery using a heating pad. Following surgery, animals were given a subcutaneous injection of butorphanol tartrate (2.0 mg/kg), returned to their home cage, and allowed to recover. A heating pad remained under the home cage for 1 h post-surgery. Sham-operated rats received the same surgical procedure excluding drilling and injections ($n = 18$ Stroke; $n = 5$ Sham).

2.3. Rehabilitation

Following surgery, stroke animals were assigned to 25 days of either daily rehabilitation (Stroke/Rehab; $n = 9$) or control therapy (Stroke/Control; $n = 9$) beginning on post surgery day (PSD) 3. Rehabilitation sessions consisted of a 30 min activity ball session, followed by 30 min of task specific movement (described below). We previously reported that a similar rehabilitation program had no effect on the performance or post-mortem histology of sham animals [37], therefore sham surgical controls remained untreated, to reduce the number of animals required for the present study. Rats that did not willingly engage in either form of rehabilitation during