Neurobiological substrates of recovery of fine motor function in a monkey model of ischemic stroke

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Introduction

- Brain damage resulting from stroke produces significant permanent disability.
- Scientists have focused considerable effort on limiting damage following stroke via neuroprotective strategies, which require prompt “ clot-busting” treatment followed by neuroprotective treatments to limit degeneration.
- Even under optimal neuroprotective conditions, however, residual neurological damage usually occurs. This leaves the patient disabled even after the recovery of function that plateaus after about three months.
- Therefore, improved function after stroke requires not only prevention of damage, but also development of treatments to enhance brain plasticity and boost recovery of function.
- Both neuroprotective and enhancement-of-recovery strategies have been used in different models of ischemic stroke.
- However, other than neuroprotection via the clot-buster tissue plasminogen activator, or tPA, these strategies have thus far failed to translate well to humans, especially for recovery of function.
- This failure has been attributed both to the complexity of the human brain, and to humans’ significantly larger brain mass, which allows the cortical center to play a relatively larger role than it does in smaller-brained animals.
- c-Fos is a gene that is a member of the immediate early gene family of transcription factors. Because c-Fos is transiently upregulated following neuronal activation, it can be used as a proxy for neuronal activity.
- Although plasticity and reorganization have been observed in the ipsi- and contralateral motor and premotor cortex in animal models, the goal of this project was to assess neuronal activity at the level of the spinal cord following recovery after an ischemic stroke. Activation of neurons in the cervical spinal cord may also be indicative of reorganization at the level of spinal cord.

Methods: Stroke surgery

- Macaque monkeys were trained to perform two behavioral tasks: the hand dexterity task and the digit coordination task. This training occurred prior to surgery.
- Animals in the experimental group received a subtotal aspiration in the hand area of the motor cortex, mimicking an ischemic stroke in human patients. Control animals did not undergo the surgery.
- Monkeys were re-trained to perform the behavioral tasks, then tested for one hour, three hours prior to perfusion. This is when c-Fos activity peaks, since its expression is transiently upregulated in activated neurons.
- The brains and spinal cord were fixed, frozen and stored at -80°C until used.
- The cervical portions of the spinal cord were sliced into 40 um sections using a freezing microtome.
- Following optimization of a protocol, c-Fos was labeled using immunohistochemistry on a series of sections that spanned the cervical spinal cord.
- Animals without Stroke

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Methods: Tract lesion

- The corticospinal tract is a bundle of axons whose motor neurons originate in the primary motor cortex. Most neurons originate in the primary motor cortex; most neurons originate in the primary motor cortex, terminating in the contralateral spinal cord as a corticospinal tract. This tract terminates in the ventral horn of the spinal cord.
- This tract is the largest projection from the brain to the spinal cord.
- Following damage to the motor cortex, the corticospinal tract is lesioned. This is when c-Fos expression peaks, since its expression is transiently upregulated in activated neurons.
- Animals with Stroke

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Methods: C-Fos staining

- Immunohistochemical staining for c-Fos showed mainly dark-stained nuclei, indicative of neuronal activity. c-Fos expression was visualized using the ABC Elite kit. The ABC Elite kit was used for the visualization of the c-Fos reaction.
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Analysis

- Less staining was evident in the dorsal horn of the spinal cord, as compared to the ventral horn.
- The qualitative assessment of c-Fos staining in the neurons of the spinal cord was approximately equal bilaterally in the ventral horn of the gray matter, where the corticospinal tract terminates.
- Overall, c-Fos staining was more significant in monkeys that did not undergo stroke surgery than in the animals that did have the stroke surgery. This is indicative of some loss of function at the level of cervical spinal cord in animals that had a stroke.
- Because the monkeys only underwent ischemic stroke on one side of the brain, and were tested with only the “impaired” hand, bilateral activation of neurons in the ventral horn does not indicate neurons on both sides contribute to the impaired hand.
- This contrasts the hypothesis that c-Fos activation would occur mainly on the side contralateral to the damaged motor cortex.

Conclusions

- C-Fos can be used as a marker for neuronal activation in the spinal cord following a motor task.
- While there were activated neurons in the ipsilateral cervical spinal cord in animals that underwent the stroke surgery, the level of activation was more intense in animals that did not have a surgery. This may be indicative of a “partial” recovery at the level of the spinal cord.
- Interestingly, the increase in c-Fos activity was evident bilaterally in animals that had surgery, indicating neuronal reorganization may have contributed to the post-ischemic stroke recovery of function.
- Therefore, our results indicate that neuronal activation does not occur solely along the tract corresponding to the side of the brain that underwent an ischemic stroke.
- Additionally, damage from an ischemic stroke does affect c-Fos activation.
- These findings provide evidence to further evaluate reorganization and plasticity at the level of spinal cord as well as the brain.