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Accelerating recovery of behavioral and cognitive functions via single intracerebral injection of matrigel containing neurotrophic factors after Somatosensory Contusion in Adult Rats

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by

Nariman Arfai

B.A., Psychology, University of California Los Angeles, 1999

M.S., Psychology, University of New Mexico, Albuquerque, 2002

Dissertation

Submitted in Partial Fulfillment of the
Requirements for the degree of

Doctor of Philosophy
in
Psychology

Behavioral Neuroscience

The University of New Mexico
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DEDICATION

This manuscript is dedicated to my undergraduate mentor at UCLA, Dr. Frank Krasne, who taught me that science is limited, but our imagination is infinite; to my brother,Mr. Nooshiravan Arfai; and especially to Dana, Jesse and Erin who brought a higher purpose to my life. Thanks for the smiles.

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ABSTRACT OF DISSERTATION

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Abstract

Unilateral sensorimotor contusion injury results in contralateral hindlimb paralysis, as well as, behavioral & cognitive deficits in rats. In response to cortical injury, adult neural stem cells (NSC) in the subventricular zone (SVZ) proliferate, and generate new neurons & glia that migrate towards injured areas. Thus, they may potentially be harnessed for neural repair. After cortical injury, the number of migratory NSC's to lesioned sites has been shown to be significantly lower than the overall cell loss in rats. To address this issue, in-vitro & in-vivo studies have shown that various growth factors (GF) enhance the rate of proliferation, differentiation & migration of NSC's within the SVZ, as well as the rostral migratory stream (RMS). The present study is an attempt to accelerate recovery of behavioral & cognitive functions from cortical TBI via a single injection of matrigel containing GF immediately after somatosensory contusion in adult rats. Each injection (containing 100ng GF/60mcL matrigel) was made such that a continuous stream of gel was formed from the region of the RMS (rostral to genu of the corpus callosum) to the site of injury. Assessment of behavioral & cognitive tasks began 7 days post surgery. Fourteen days of Morris Water (i.e., moving hidden platform - 8 trials a day) & 7 days of Rod task (4 trials a day) testing revealed that lesioned animal which received the FGF or EGF treatment

performed as well as sham & significantly better than lesioned control groups. Similarly, animals who received combination of all 3 GFs performed better than lesion control groups on both tasks, however not as well as sham, the FGF or EGF-treated groups. VEGF- treated animal performed similarly to lesion/no treatment animals. Overall, this study shows that the intracerebral injection of certain neurotrophic factors accelerates the recovery of behavioral & cognitive skills in cortical injured rats. Potential mechanisms for acceleration of functional recovery could involve their recruitment & directed migration of NCSs from the RMS to the site of cortical injury and /or local neuroprotective effects of the growth factors.

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Accelerating Recovery of Behavioral and Cognitive Functions via Single Intracerebral Injection of Matrigel Containing Neurotrophic Factors after Somatosensory Contusion in Adult Rats

Neurogenesis significantly increases following stroke (Yamashita et al., 2006; Zhang et al., 2003; Arvidsso et al., 2002; Jim et al., 2001, Liu et al., 1998), seizures (Parent et al., 2008-09a, Kernie et al., 2010), brain tumors (Aboody et al., 2000), focal apoptosis (Magavi et al., 2000), and various cortical lesions (Sun et al . 2007, 2010; 2004; Gage et al., 2009; Chirumamilla et al., 2002; Fallon et al., 2000). For more than a decade, adult neuronal stem cells (NCS) have been investigated as a potential source for treatment of various brain diseases and injuries (Gage et al., 2009; Kempermann et al., 2002; Aarum et al., 2003; Khun et al, 1998). However, the behavioral and cognitive recoveries of such treatments after traumatic brain injury (TBI) have not been well investigated. The current experiment examines the possible role for enhancing neurogenesis as a treatment for recovery of functions after cortical injury. Specifically, the present study is an attempt to accelerate recovery of behavioral and cognitive functions via a single injection of biodegradable matrigel containing three growth factors (i.e., epidermal growth factor (EGF), basic- fibroblast growth factor -2 (FGF-2) and vascular epidermal growth factor (VEGF), alone or their combination) immediately after somatosensory contusion in adult rats.

Adult neuronal stem cells are categorized as cells with a potential for prolonged self-renewal (Kempermann., et al., 2002; Gage et al., 1999; Shamblott, et al., 1998), in addition to the capability of producing at least one type of highly differentiated and functional neurons and glial (Alvarez-Buylla et al., 2001, 1994; Gage et al., 1999;

Thomson, et al., 1998). These studies also documented different characteristics of adult neuronal stem cells (Lois & Alvarez-Buylla., 2001). Gage et al. (1999) reconfirmed two locations/reservoirs of NCS (i.e., the subventricular zone-SVZ and the dentate gyrus-subgranular zone- SGZ) in adult rats.

An important characteristic of adult stem cells is that they can migrate from regions where they are born to other brain regions where they may be needed (e.g., from subventricular zone to olfactory bulb). Target regions are often characterized by their plastic functions (Alvarez-Buylla et al., 2001; Gould et al., 1999; Yandava et al., 1999; Paton et al., 1984) or due to their recent cell loss (Jiang et al., 2006; Lois & Alvarez-Buylla., 2001; Snyder et al., 1994). For example, Lois et al., 1994 showed that SVZ stem cells give rise to neuroblasts that migrate in chain along the rostral migratory stream (RMS). Eventually, these neuroblasts integrate into circuitry of the olfactory bulb (Alvarez-Buylla et al., 2001; Lois et al., 1994). However, neuronal stem cells may also disperse away from the RMS during brain injury (Jin et al., 2010; Magavi et al. 2000). Using synchronized laser bursts to induce focal apoptosis, Magavi, Leaveitt and Mackilis (2000) eloquently showed that endogenous neural precursors can be attracted and/or motivated to migrate out of SVZ/RMS regions toward the injured neurons.

Traumatic brain injury (e.g., stroke or somatosensory contusion) causes rapid cell death, a disruption of neuronal circuits (Yamashita et al., 2006; Ramaswamy et al., 2005) in addition to behavioral and cognitive deficits (Kolb et al., 1983; Sutherland, et al., 1982). The injured cortical areas began to recover as regenerative processes are activated (Chirumamilla et al., 2002; Eriksson et al., 1998a). Increased rates of NCS proliferation

and migratory deviations from the rostral migratory streams toward injured site are among few contributors to post-injury recovery (Magavi et al., 2000; Kernie et al., 2010; Chen et al., 2003; Ramaswamy 2005; Yamashita et al., 2006; Urrae et al., 2007; Richardson et al., 2007; Parent et al., 2009). For example, Yamashita et al. (2006) showed that NCSs proliferate from SVZ and give rise to neuroblasts in the striatum after transient middle cerebral occlusion (i.e., a rodent stroke model). Moreover, they also showed that these neuroblasts formed elongated chain-like cells that aggregate, similarly to those in the normal SVZ of intact animals. Furthermore, these chains were shown to be closely associated with thin astrocytic processes providing a physical path toward the injured site. In contusion model of cortical injury, Ramaswamy et al. (2005) demonstrated that a focal cortical impact lesion can lead to a marked increase of NCS proliferation and lateral migration from SVZ/RMS into the severely damaged area of the somatosensory cortex in mice. Altogether, these studies also claim that without exogenous treatments, endogenous NCS contributions to regional recovery via neurogenesis produces minimal and perhaps transient affect (Yamashita et al., 2006; Ramaswamy et al., 2005; Richardson et al., 2007; Gage et al., 2002).

In order to investigate and compare the effect of multiple growth factors on mammalian adult neurogenesis, Kuhn et al. (1998) administered (i.e., intracerebroventricular) basic fibroblast and/or epidermal growth factors close to SVZ and dentate gyrus-subgranular zone (DGS) in intact rodent. They clearly demonstrated that EGF and FGF-2 can have different and site-specific effects on proliferation and differentiation of NCS, *in vivo.* Both basic fibroblast and epidermal growth factors

expanded the neuronal stem cell population of SVZ after 2 weeks, however only FGF-2 increased the number of newborn cells (Ninomiya et al., 2008; Kempermann et al., 1997, Grill et al., 1997; Kuhn et al., 1998). Their histology also documented that EGF reduced the total number of newborn neurons reaching the olfactory bulb, however EGF substantially enhanced the generation of glial cells such as, astrocytes in the olfactory bulb (Kuhn et al., 1998). Recent studies have been exploiting the therapeutic potential of growth factors to further enhance neurogenesis after stroke **(**Kernie et al., 2010; Wu et al., 2008; Jin et al., 2009; Ohab et al., 2006; Yamashita et al., 2006) and TBI (Sun et al., 2007, 2010)**.** Sun et al. (2007) showed that continues intraventricular administration (i.e., via miniature osmotic pump) of basic fibroblast growth factor (FGF) enhance production of new neurons and glia cells, and enhanced recovery of cognitive function after fluid percussion lesion of somatosensory cortex in rats. Similarly, Wu, et al. (2008) also showed that intraventricular injection of vascular epidermal growth factor VGEF increase neurogenesis and angiogenesis after traumatic brain injury in adult rodent. Altogether, such studies reinforce the usage of growth factor such as EGF, FGF, and VEGF as treatment drugs for our research.

It was believed that brain injury such as stroke (Broca, 1861) and contusion result in permanent loss of neurons. These dogmas are put to rest by extensive evidence that suggests that certain brain areas retain the capability to generate new neurons into adulthood (Yamashita et al., 2006; Kornack et al., 2001; Pencea et al., 2001) and, enhances after TBI (Fallon et al., 2000; Jin et al., 2009; Sun et al., 2007, 2010). Such findings prompt this investigation to test recovery of behavioral and cognitive functions

after somatosensory cortical impact contusion of manipulating migration of adult stem cells of SVZ/RMS using bridge surgical method in rats (Arfai et al., 2002). The bridge treatment involved single injection of biodegradable matrigel containing FGF, EGF, and VGEF (alone and combination with each other), which connects the site of injury to the rostral migratory stream.

Method

Subjects

The subjects were 95 male hooded rats of the Long-Evans strain (350-450 gm) obtained from Harlan Laboratories. They were housed in hanging wire single mesh cages on a 12:12 light/dark cycle with free access to food and water. All animals were tested during the light phase.

Experimental Schedule

After 5-6 weeks period of maturation (i.e., when the animals reached 350 gm), all animals were trained on the Morris water maze (i.e., fixed, hidden platform-8 trials) for seven days and the Grid Walk task (i.e., 4 trials) for four days. Baseline scores (i.e., Morris water task latency, and Grid walk task latency and error) for spatial and behavioral tasks were recorded. Contusion lesion and bridge treatment surgeries were performed as indicated by the animals' randomly (i.e., surgeon was blind to experimental condition) assigned conditions . Commencing seven days after the surgery (i.e., the surgery rest period), all animals were assessed for their performance on the Morris water maze task (i.e., moving, hidden platform-8 trials) for 14 days post surgery and Grid walk task (4 trials) for five days. Ten days after surgery, all animals were intraperitoneally injected with Bromodeoxyuridine (BrdU-$100_{mg/kg}$) for seven consecutive days. BrdU is a thymidine analog that is incorporated into the DNA as 5-bromouracil and stains the cells during the S phase of mitosis (Kolb et al, 2000). Three, five, and ten weeks following the

BrdU administration, all animals were euthanized- receiving isoflorine ($4_{mg/kg}$) until their breathing stopped. Following the extraction, all brains were perfused.

Behavioral and Cognitive Spatial Procedures

Grid Beam Walk

The Grid Beam Walk (GBW) behavioral task is demonstrated to be sensitive to interference with somatosensory cortex function (Hovda et al., 1983; Feeney et al. 1978). Briefly, the grid beam consists of randomly spaced rods on inner sides of two parallel beams that are elevated from the table top. Placement of rods is altered between trials. Rats are motivated to traverse the grids using a bright light and white noise (i.e., negative reinforcements) and a darkened goal box (i.e., positive reinforcement) where they remain for a period of 10 seconds. On the first day of training, the rats were given four training trials using shaping (i.e., approximation of behavior to a given goal) procedures. During the first trial, rats were placed just outside the goal box, and at the second trial, they were placed midway down the beam. During the third and fourth trials, rats were placed at the start position. Prior to surgery, rats were given daily training trials for four consecutive days and until the learning curves were reached (i.e., asymptotic). Seven days after surgery, the rats were given four trials for five consecutive days.

Morris water task

Spatial cognition was examined using the Morris water task (MWT). Spatial orientation performance in the MWT has been shown to be sensitive to somatosensory cortex function (Hoane et al., 2003-04). Latencies were recorded.

Pre-surgery "Training" was conducted using the Morris water maze fixed, hidden platform procedure. Briefly, the MWT training consisted of eight trials of fixed, hidden platform tasks for seven consecutive days, in order to familiarize the rats with the task and its environment. A single trial consisted of placing a rat into the water at one of four locations (i.e., east, west, north, and south) around a circular pool's perimeter. Within the block of four trials, each rat started at one of the four locations in a random sequence. Each trial was limited to 90 seconds. If the rat did not find the platform, it was placed by hand upon the platform for 10 seconds and then returned to its cage. After the first four trials, rats were given a period of 15 minutes to rest. Following rest, the rats began the second set of four trials.

Post-surgery behavioral tasks were conducted using the Morris Water-Maze-Moving, hidden platform procedure for 8 trials, with 15 minutes of rest after the first set, with the platform moved to a new position according to a pseudorandom sequence for 14 consecutive days. The platform remained in the same position for all of the trials in a given day. The platform was never positioned in the exact center of the pool, nor was it positioned closer to the pool wall than 5 cm. Also, the center of the platform was never be positioned within 5 cm of a previously selected position (Sutherland et al., 1983; Sutherland et al., 1999).

Surgical Preparation and Procedures

Pre-Surgical Preparations:

Matrigel and growth factors solutions: Matrigel is a biodegradable and solubilized basement membrane extracted from Engelbreth-Holm-Swarm (EHS) rodent sarcoma, a tumor rich in ECM proteins (Jin et al., 2009, Jin et al., 2010). Matrigel is effective matrix for the attachment, proliferation and differentiation of both normal and transformed stem cell types, including adult neuronal stem cells (Isaji M., et al., 1997). Matrigel has been used for drug delivery and stem cell transplantation into the cortex (Jin et al. 2009). The research of Barkho et al, (2008) showed that Matrigel allows neuronal growth in normal 3 dimensional patterns, as well as migration through various matrices.

Matrigel matrix will gel rapidly at $22^{\circ C}$ to $35^{\circ C}$ room temperature and was gradually thawed at $4^{\circ C}$ temperature, 24 to 48 hours prior to preparation, (i.e., using color variations from its frozen state, yellow, to its thawed state of dark red). Matrigel was maintained at the gel state by keeping it on ice. All instruments (i.e., pipettes, tips, vials, tubes and etc.) were pre-cooled prior to mixing.

Rodent Epidermal growth factor (i.e., BD Bioscience inc. catalogue # 356010), Rodent Fibroblast growth factor -2 (i.e., FGF, BD Bioscience inc. catalogue #610871) and Rodent Vascular Epidermal growth factor (i.e., BD Bioscience catalogue # 560070) were separately reconstituted (i.e., in accordance to BD Bioscience inc. Pro # AU530121). In order to adjust the protein's concentration of saline/gel group (in comparison to growth factor groups) and avoid post surgical complications, we added 0.1 % rat serum albumen (RSA)-pyrogen free to the solution. Using pipettes, matrigel matrix and growth factors were remixed and vortexed, (i.e., a centripetal acceleration

equipment that utilizes an electric motor to rotate an object around single, fix axis) to assure for even distribution of the growth factor's concentration within the matrix medium. The mixture was divided into $500_{\mu g}$ vials via aliquot, and all vials were refrozen until the time of surgery.

Injection Preparations:

Once again, in order to assure equal distribution of growth factors within the gel matrix, and to speed the defrosting process, each vial was vortexed for approximately 45 to 60 seconds. Hamilton micro-injectors (#29 gauge) were kept cold in ice at $4^{\circ C}$ temperature and each filled with $100_{\mu g}$ GF/60_{mcL} of either, EGF/gel, FGF2/gel, VEGF/gel, GF cocktail (i.e., EGF, FGF2, VEGF)/gel, or Saline/gel (i.e., treatment control), prior to surgery. After the solution's color turned red (i.e., during the liquid phase of matrigel matrix), Hamilton injectors were filled with matrigel mixture and cautiously checked for the presence of air pockets. All instruments were kept on ice at $4^{\circ C}$, prior to mounting the injector on the stereotaxic injector apparatus.

Focal Impact Contusion and Bridge Treatment Procedures:

Focal impact contusion of the sensorimotor cortex was conducted using a focal weight-drop model under aseptic conditions (Feeney et al., 1984). Surgeries were performed on adult animals ($350\text{-}450_{gm}$) under aseptic conditions. Isofluorane (4%, $2_{lit/mi}$) was used as an anesthetic, until a surgical plane was achieved (i.e., induction), and then reduced to 2% , $2_{lit/mi}$ (i.e., maintenance). After a surgical plane was induced, the scalp was shaved and eye ointment was applied. The rats were placed in a stereotaxic frame

and their scalps were scrubbed with Betadine solution followed by alcohol.

The cortex was exposed by removing a piece of skull on right side of the midline, such that a strip of bone approximately 2_{mm} wide remained over the sagittal sinus. The scalp and periosteum were pulled back and craniotomies were performed using a dental drill over the right anterior parietal cortex, leaving the dura mater intact. The resultant bone flap was removed from the skull and placed in sterile saline. The footplate of the contusion device was positioned over the right or left anterior parietal cortex, centered 2.5_{mm} posterior and 3.5_{mm} lateral to the bregma. The footplate was lowered to the surface of the dura, and the guide tube was lowered 2.5_{mm}, limiting the depression of the area under the footplate to 2.5_{mm} below the surface of the exposed dura. After positioning of the footplate, a 20_{g} weight was dropped through the tube from a height of 20_{cm}, producing a $400_{g/cm}$ impact on the anterior parietal cortex.

With the exception of the sham, all animals received single stereotaxic injections of either gel/EGF, gel/FGF2, gel/ VEGF, gel/GF cocktail or gel/Saline. Specifically, injections were made to connect the contusion region to the rostral of corpus callosum–genu area and close to the infralimbic cortex (5.5_{mm} deep and 65 degree horizontal to oblique angle, 0.1 degree lateral to medial angle), where NCSs exit ventricle systems (i.e., bridging) at the rostral migratory stream. Each injection (containing $10_{\mu g}$ GF/60_{mcL} matrigel) was made such that a continuous stream of gel was formed, as well as three reservoirs of $20_{\mu g}$ at the infralimbic cortex (5.5_{mm} depth), dorsal cingulate cortex (3.5_{mm} depth) and secondary motor cortex (1_{mm} depth). Control sham (behavioral control) animals were anesthetized, ear punched, shaved, scrubbed, incised, and sutured as described above.

All animals received their bone flap, the cranium was sealed with bone wax, scalps were closed and sutured with clips, and the animals were removed from the ear bars. The animals were monitored for one hour to allow recovery from anesthesia before returning them to their home colony.

Histological procedures

Histological procedures involved transcardial perfusion, extraction, and sectioning. Upon completion of the BrdU regimen (i.e., daily injection of $100_{mL/Kg}$ for 10 days after the post-surgical behavioral procedure), the animals' brains were perfused with a 9% saline solution and phosphate buffer solutions, three, five and ten weeks after the surgery. All brains were embedded in tissue adhesive and kept at a frozen suspension state for stem cell counts at later times.

Results

Design and analysis

The primary analysis conducted was a repeated measures ANOVA, taking a multivariate approach, with day and trial as within-subjects factors and group as the between subjects factor. The secondary analysis conducted was Tukey HSD adjusted-pairwise comparisons among levels of the between-subjects factor of group, in order to test hypothesis in detecting the difference between the treatment and no treatment groups, (see Table 1).

		Treatment						
		Gel + Saline (treatment control)	EGF	FGF	VEGF	Combo	No treatment (Lesion only surgical control)	Sham (No Lesion-behavioral control)
Lesion	Rt	8- RtGel	9- RtE	7- RtF	6- RtV	8- RtC	6-Rt	
	Lt	6- LtGel	8- LtF	8- LtF	7- LtV	8- LtC	6-Lt	8-Sham

Table 1: Factorial design (i.e., 2 x 6 +1), number of the subject per, and group's abbreviations.

Pre-Surgery Training- Morris Water Task MWT (fixed, hidden platform), <u>Latency</u>

The repeated measures ANOVA on latency for the animal to find the fixed, hidden platform showed significant main effects of both day, $F_{(6, 77)} = 2.749$, $p < .001$, and trial, $F_{(7, 76)} = 75.577$, $p < .001$, (see Table 2).

		Treatment						
		Gel + Saline	EGF	FGF	VEGF	Combo	No treatment	
Lesion	Rt	10.353	11.075	10.071	11.196	10.841	11.341	Sham
	Lt	10.138	9.934	11.023	10.939	11.204	10.693	9.799

Table 2: Training (pre-surgery), 7 days, 8 trials grand mean latency $_{(sec)}$ of Morris water task (hidden, fix, platform).

There were also significant day by group interactions, $F_{(12, 82)} = 6.961$, $p < .001$, and trial by group interaction, $F_{(12, 82)} = 2.394$, $p = .01$. However, the test of the between-subject factor of group yield no significant main effect before lesion and treatment $F_{(12, 82)} = .894$, $p = .556$. This suggests that groups were adequately matched, (see Figure1).

Figure 1 : Training (pre-surgery)- 7 Days & 8 Trials, Grand Mean Latency $_{(sec)}$ of Morris water Task (i.e., hidden, fix platform)

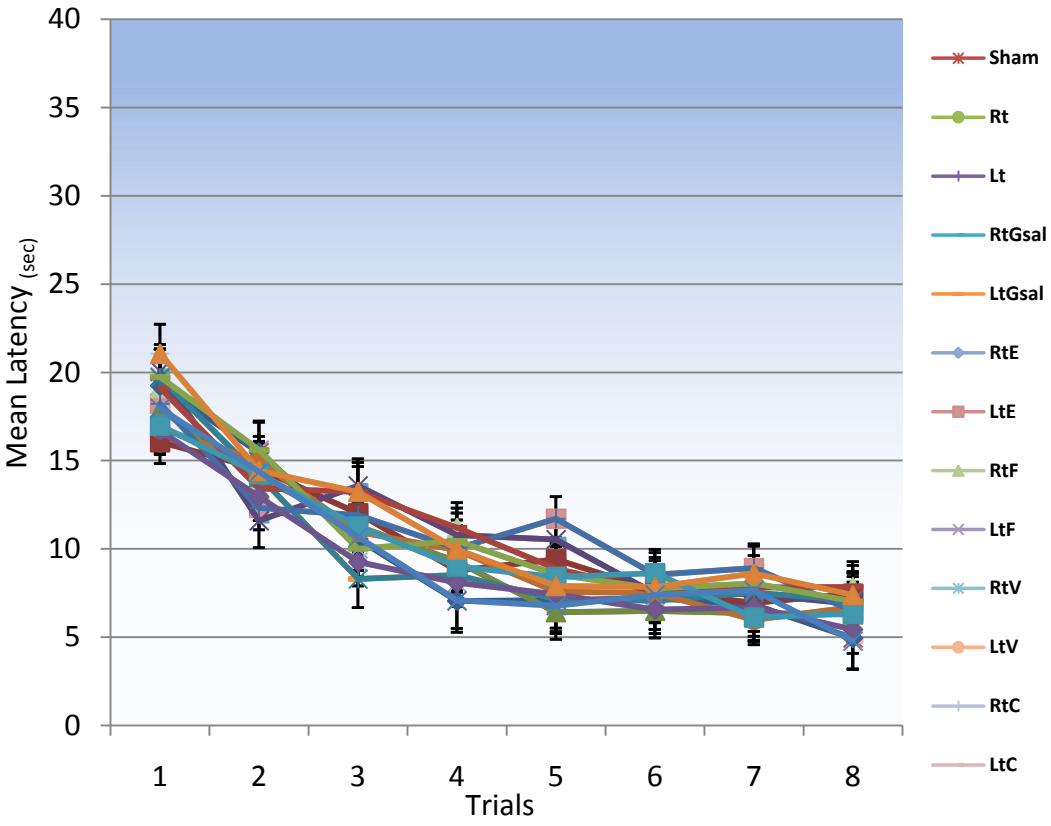

Figure 1: Training (pre-surgery), 7 days, 8 trials grand mean latency $_{(sec)}$ of Morris water task (i.e., hidden, fix, platform).

In order to measure the amount of the relation between pre and post surgery performance of Morris water task, we have conducted correlation analysis that revealed a weak correlation, r =0.02 between pre and post surgery performances

.

Pre-Surgery Training- Grid Walk Task, <u>Latency</u>

The repeated measures ANOVA on latency showed significant main effects of both day, $F_{(4, 79)} = 53.583$, $p < .0001$, and trial, $F_{(3, 80)} = 16.56$, $p < .0001$,on latency of grid walk task performance before lesion and treatment, (see Table 2).

		Treatment						
		Gel + Saline	EGF	FGF	VEGF	Combo	No Treatment	
Lesion	Rt	7.224	6.464	7.009	6.896	7.276	7.363	Sham
	Lt	7.368	6.881	6.908	6.78	6.875	7.195	7.264

Table 3: Training (pre-surgery), 4 days and 4 trials latency (sec) grand means of Grid Walk task.

The test of the between-subject factor of group did not yield any significant main effect, $F_{(12, 82)} = .348$, $p = .997$, (see Figure 2, once more showing that the groups were well matched before lesion and treatment). There were no significant interactions between day by group $F_{(12, 82)} = 1.50$, $p = .140$, and trial by group $F_{(12, 82)} = .353$, $p = .976$. However, a significant interaction of trial by day was found, $F_{(12, 71)} = 2.809$, $p = .003$, and also a three way interaction of trial by cell by group was found, $F_{(12, 82)} = 2.580$, $p = .006$.

Figure 2: Training (pre-surgery)-4 Days & 4 Trials, Grand Mean Latency $_{(sec)}$ of Grid Walk Task

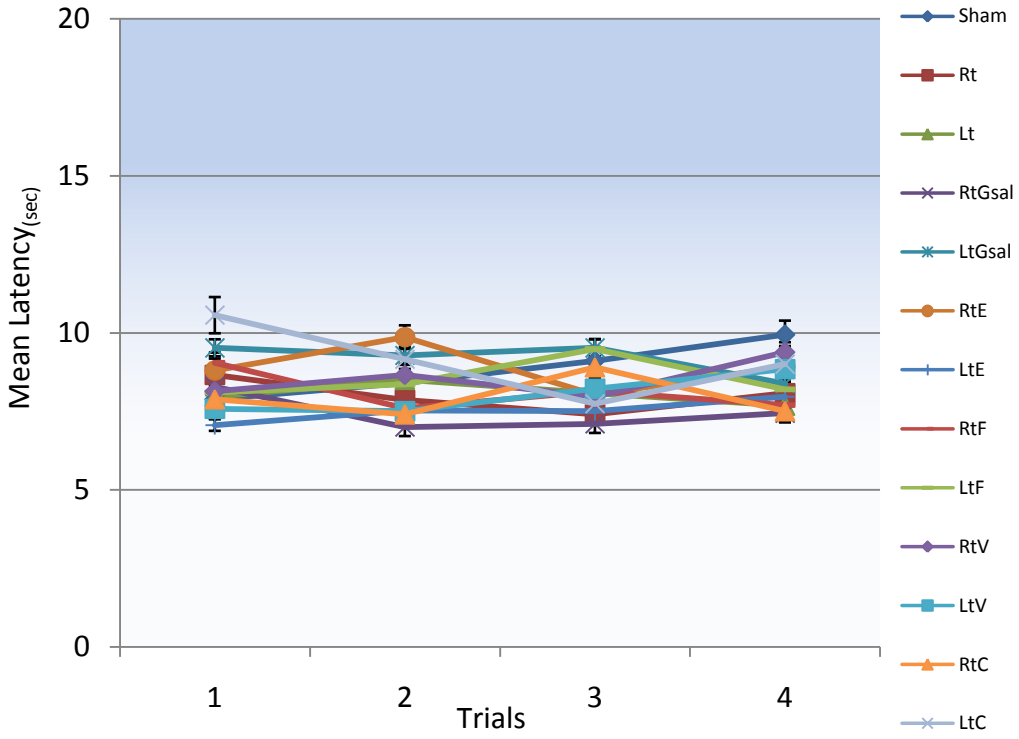

Figure 2: Training (pre-surgery), 4 days and 4 trials latency $_{(sec)}$ grand means of Grid Walk task.

Pre-Surgery Training- Grid Walk Task, <u>Error</u>

The repeated measures ANOVA on grid walk errors yield significant main effects of both day, $F_{(4, 79)} = 18.543$, $p < .001$, and trial, $F_{(3, 80)} = 47.420$, $p < .001$, (see Table 3). However, the test of the between-subject factor of group did not yield any significant main effect, $F_{(12, 82)} = .730$, $p = .998$ (see Table 4 and Figure 3).

		Treatment						
		Gel+ Saline	EGF	FGF	VEGF	Combo	No treatment	Sham.
Lesion	Rt	0.666667	0.6625	0.6562	0.633333	0.65	0.608333	
	Lt	0.642857	0.675	0.65625	0.65625	0.678571	0.658333	0.6375

Table 4: Training (pre-surgery), 4 days and 4 trials error's averaged means of Grid Walk task.

There were significant interactions of day by group $F_{(12, 82)} = 1.888$, $p = .048$, trial by group $F_{(12, 82)} = 2.156$, $p = .022$, trial by day, $F_{(12, 71)} = 6.733$, $p < .0001$, and three way interactions of trial, cell by group interaction, $F_{(12, 82)} = 4.998$, $p < .001$.

Figure 3: Training (pre-surgery)-4 Days & 4 Trials, Error's Grand Means of Grid Walk Task

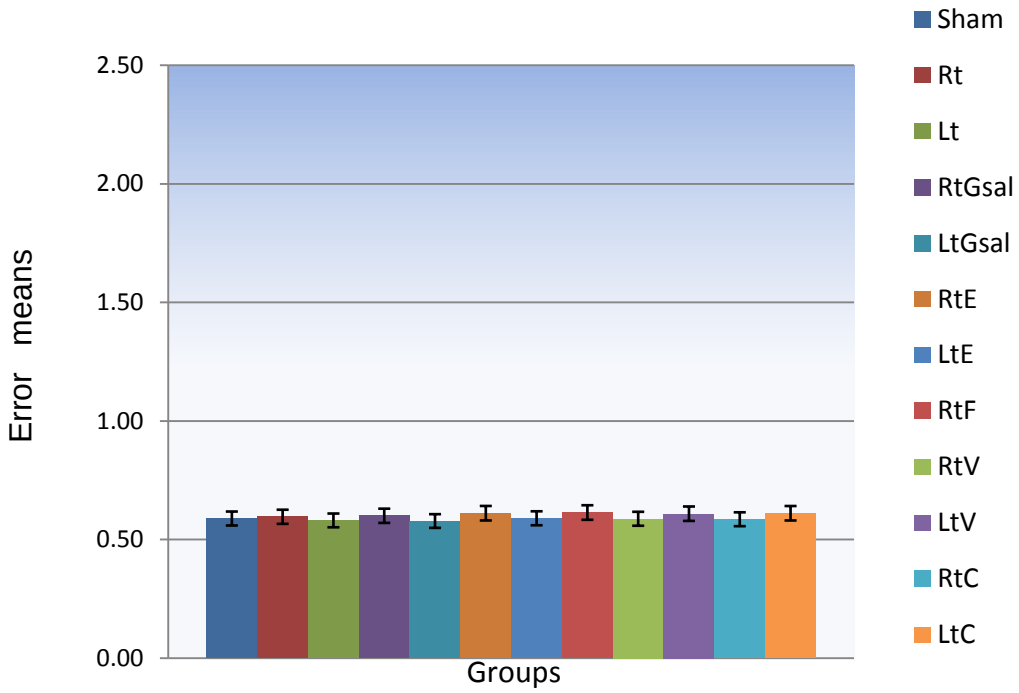

Figure 3: Training (pre-surgery), 4 days and 4 trials error's grand means of Grid Walk task. Standard errors are shown.

Post surgery- MWT (moving, hidden platform), <u>Latency</u>

The repeated measures ANOVA on latency yielded significant main effects of both day, $F_{(13, 77)} = 47.956$, $p < .0001$, and trial, $F_{(7, 76)} = 75.557$, $p < .0001$, and also both a day by group, $F_{(12, 82)} = 6.962$, $p < .0001$, trial by group interactions, $F_{(12, 82)} = 13.813$, $p < .0001$, **(s**ee Table 5).

		Treatment						
		Gel + Saline	EGF	FGF	VEGF	Combo	No treatment	Sham
Lesion	Rt	18.264	12.357	12.734	19.019	16.756	19.390	.
	Lt	16.782	11.627	11.504	18.317	15.484	18.513	10.405

Table 5: Post surgery, 14 days, & 8 trials latency$_{(sec)}$ grand means of Morris water task (hidden, moving platform).

The test of the between-subjects factor of group showed a significant main effect of group, $F_{(12,82)} = 18.115$, $p < .0001$, (see Figure 4 and 5).

Figure 4: Post Surgery- 14 Days and 8 Trials, Latency$_{(sec)}$ Grand Means of Sham, Rt, RtE, RtF, RtV, RtC, and RtGSal Performances of Morris Water Task

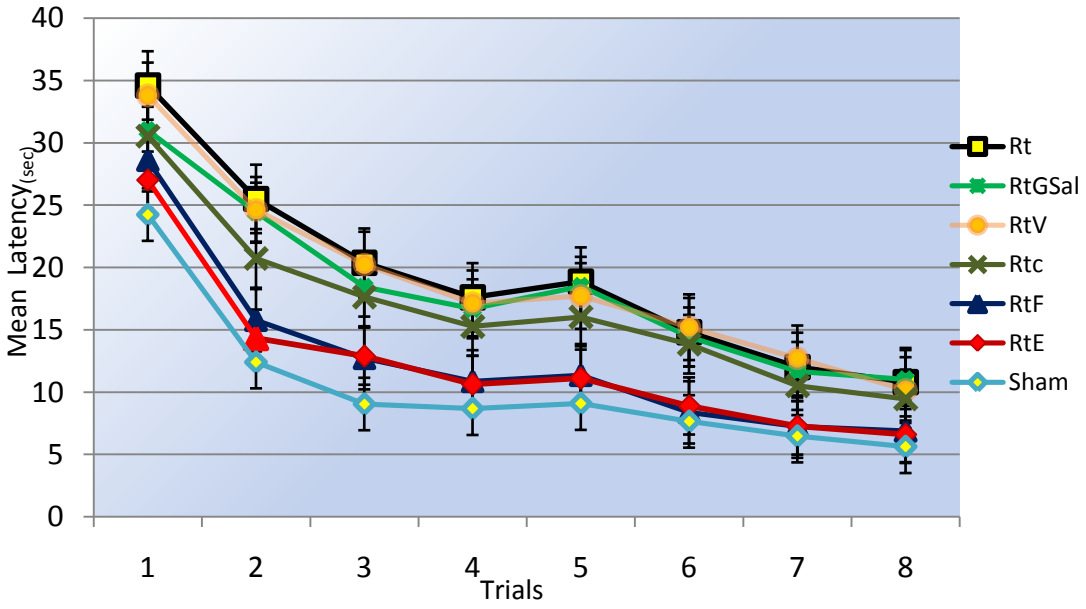

Figure 4: Post-surgery, 14 days and 8 trials- latency$_{(sec)}$ grand means of sham, right lesion and right lesion treatment group's performance of Morris water task (i.e., hidden, moving platform).

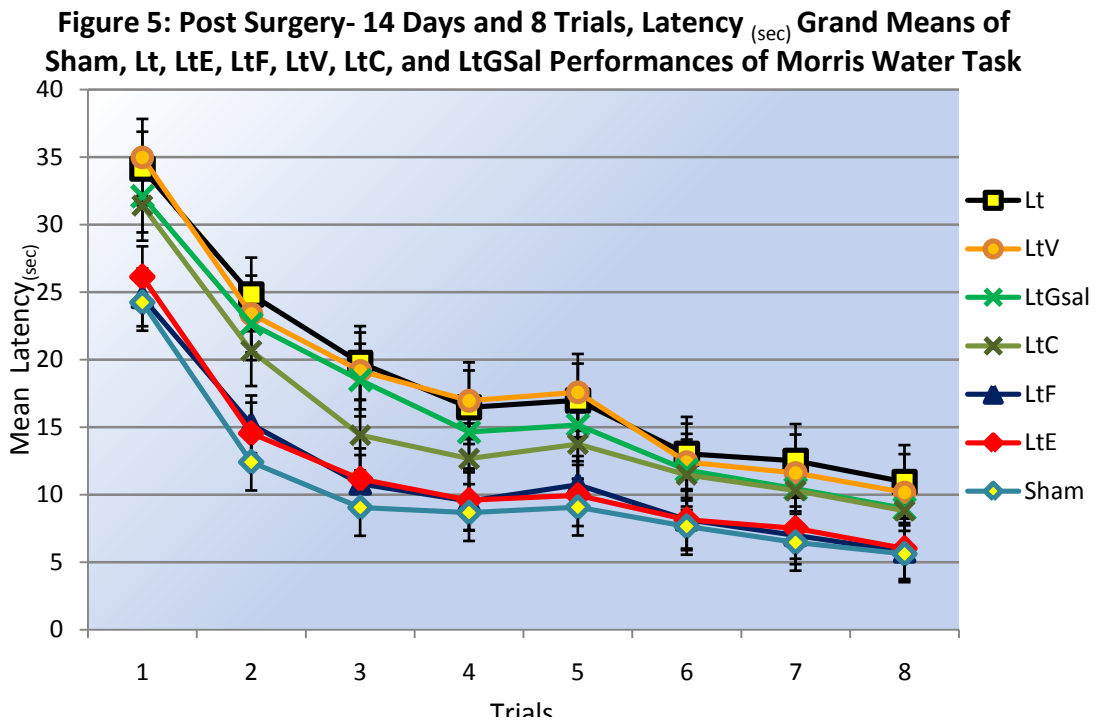

Figure 5:Post-surgery, 14 days and 8 trials- latency$_{(sec)}$ grand means of sham, Lt, LtE, LtF, LtV, LtC, and LtGSal performances of Morris water task (i.e., hidden, moving platform).

Planned pairwise comparisons reveled a significant difference between sham and right lesion untreated animals (Rt) $F_{(1, 82)} = 7.98$, $p < .0001$. On average, sham animals completed the task 9.01_{sec} faster than Rt. A significant difference was also found between sham and left lesion untreated animals (Lt) $F_{(1, 82)} = 7.2$, $p < .0001$. Sham animals completed the task 8.12_{sec} faster than left lesion untreated animals. See Figure 6 –right lesion and Figure 7 –left lesion groups.

However, planned pairwise comparisons did not yield significant differences among sham, right lesioned Epidermal GF (RtE) and left lesioned Epidermal GF (LtE) groups, right lesion Fibroblast GF (RtF) and left lesion Fibroblast GF (LtF) treated animals. Altogether, these groups performed similarly (i.e., grand mean difference ranging from 1 to 2.3_{sec}).

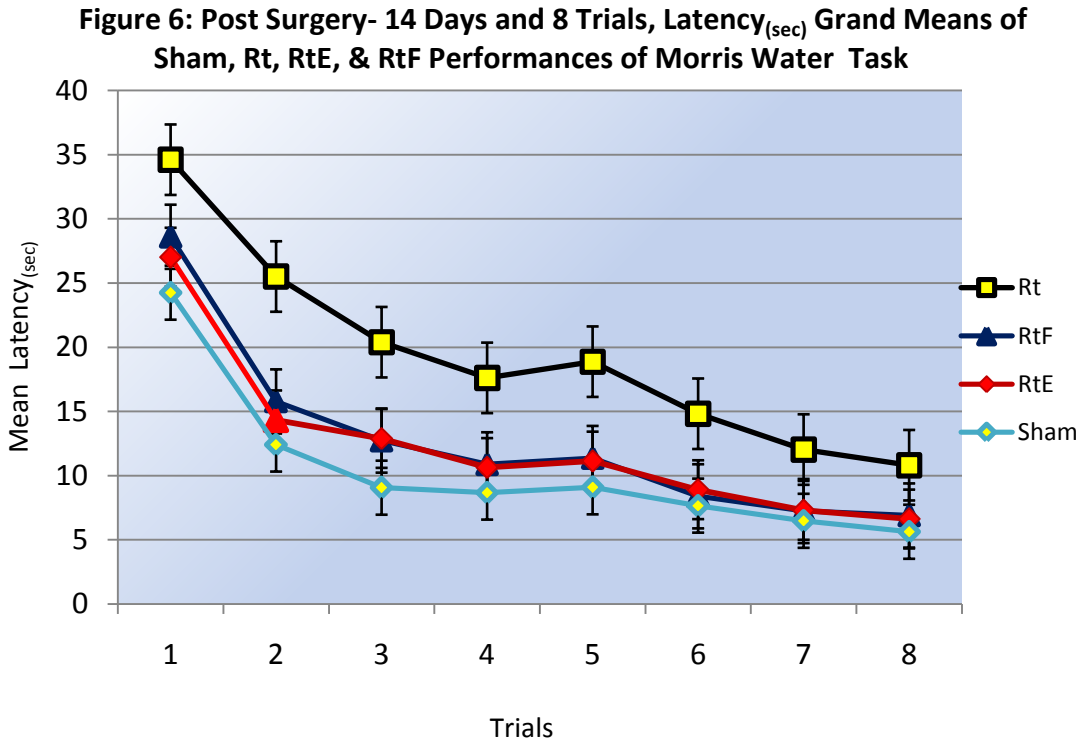

Figure 6: Post-surgery, 14 days and 8 trials – latency grand means of sham, Rt, RtE, and RtF performance of Morris water task (i.e., hidden, moving platform).

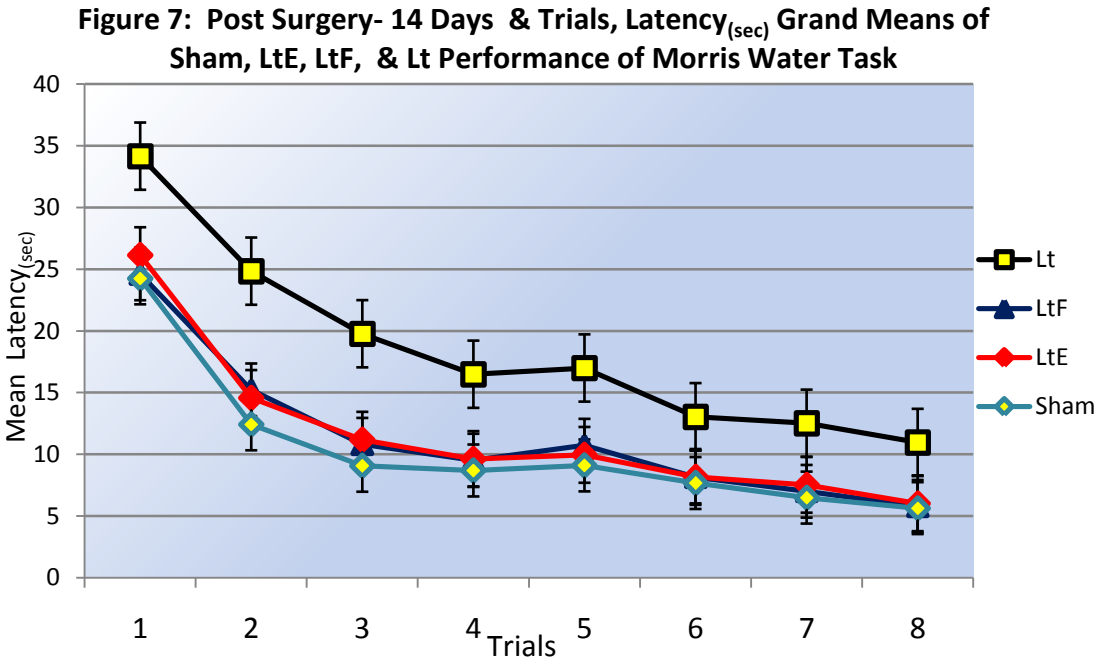

Figure 7: Post surgery, 14 days and 8 trials- latency$_{(sec)}$ grand means of sham, Lt, LtE, & LtF, performances of Morris water task (i.e., hidden, moving platform).

Furthermore, planned pairwise comparisons reveled that the RtE group performed significantly better than the Rt group $F_{(1, 82)} = 6.24$, $p < .0001$. On average, the RtE group found the hidden platform 7.03_{sec} faster than the Rt group. The RtE group also performed significantly better than the right lesion Gel/saline (RtGSal) group $F_{(1, 82)} = 5.24$, $p = .004$. On average, RtE found the hidden platform 5.9_{sec} faster than RtGSal group. The RtE group also performed significantly better than the right lesion VEGF (RtV) treated animals $F_{(1, 82)} = 5.91$, $p < .0001$. The RtE group found the hidden platform $6.67_{\mathbf{sec}}$ faster than RtV animals. The RtE group also performed significantly better than the right lesion GF cocktail (RtC) treated animals $F_{(1, 82)} = 4.22$, $p = .004$. The RtE group found the hidden platform $4.39_{\mathbf{sec}}$ faster than the RtC group, (see Figure 8).

Figure 8: Post Surgery, 14 Days and 8 Trials- Latency $_{(sec)}$ Grand Means of Sham, Rt, RtE, RtV, RtC, and RtGSal Performances of Morris Water Task.

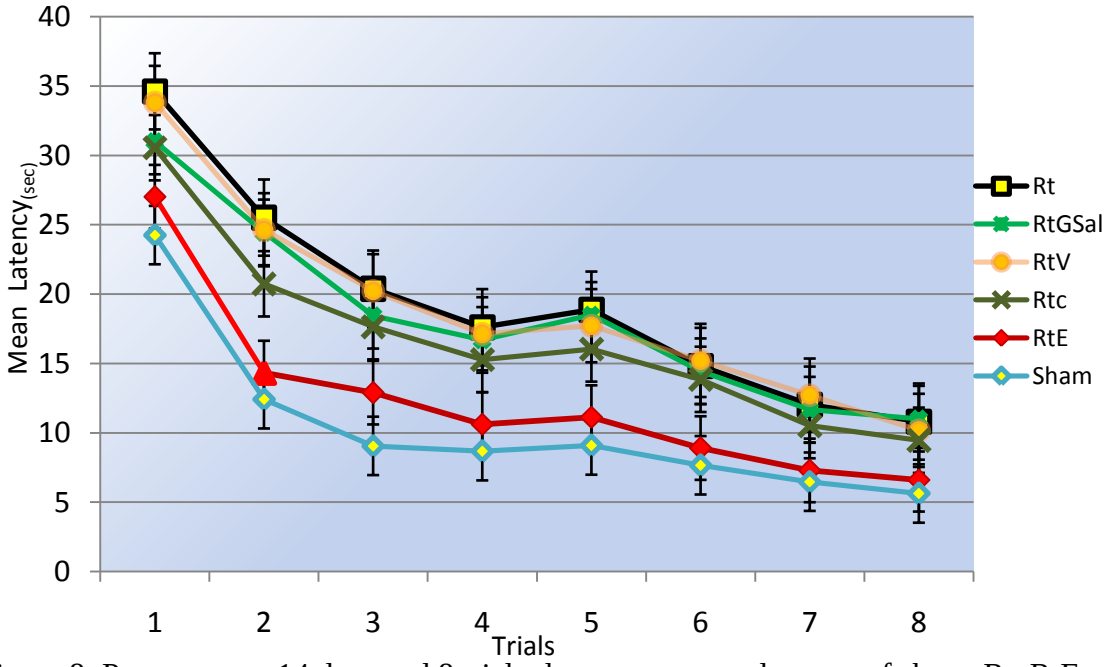

Figure 8: Post surgery, 14 days and 8 trials- latency$_{(sec)}$ grand means of sham, Rt, RtE, RtV, RtC, & RtGSal performances of Morris water task (i.e., hidden, moving platform).

Similar to RtE, planned pairwise comparisons detected significant differences between the RtF and right lesion- only Rt groups $F_{(1, 82)} = 6.06$, $p < .0001$. On average, RtF found the hidden platform 6.66_{sec} faster than Rt group. On average, Rtf also found the hidden platform 5.53_{sec} faster than right lesion Gel/saline (RtGSal) $F_{(1, 82)} = 5.03$, $p > .0001$ and RtF animals found the hidden platform 5.72_{sec} faster than right lesion VGEF (RtV) treated animals $F_{(1, 82)} = 8.28$, $p < .0001$. On Average, RtF found the hidden platform 4.02_{sec} faster than the right lesion GF cocktail treated animals (RtC) $F_{(1, 82)} = 3.97$, $p = .009$, (see Figure 9).

Figure 9: Post Surgery- 14 Days and 8 Trials, Latency $_{(sec)}$ Grand Means of Sham, Rt, RtF, RtV, RtC, and RtGSal Performances of Morris Water Task

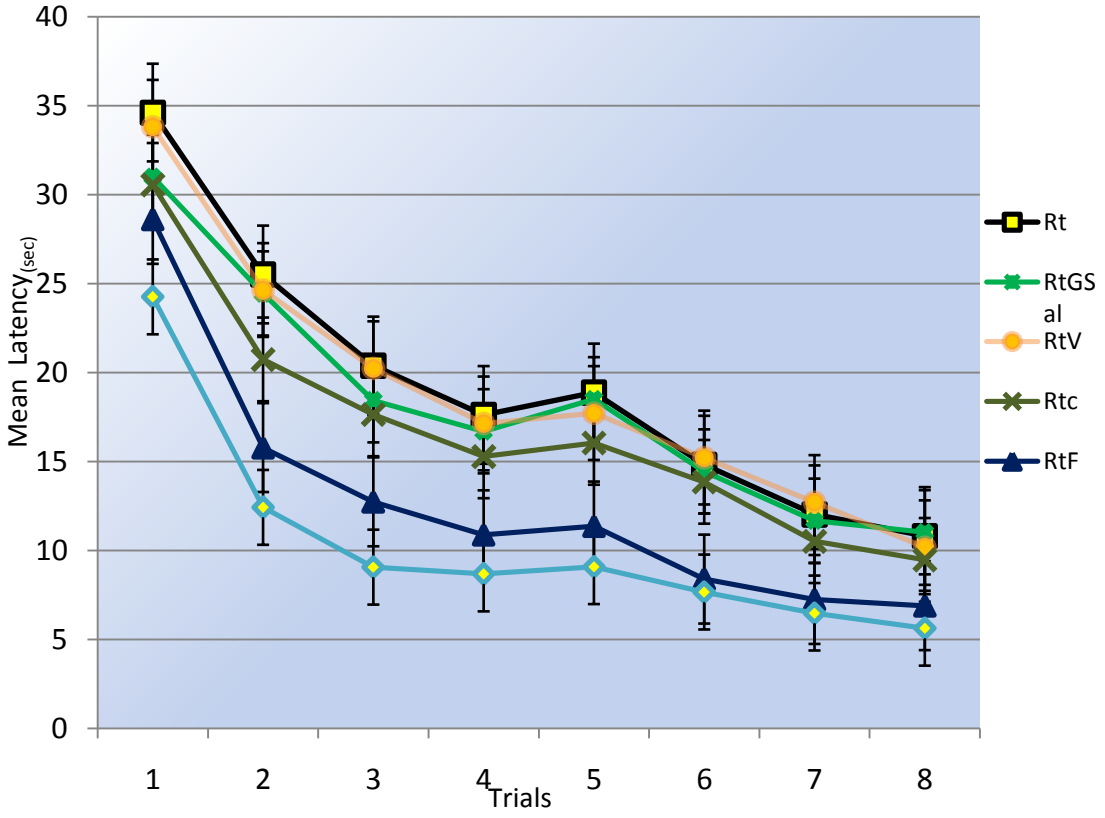

Figure 9: Post surgery, 14 days and 8 trials- latency $_{(sec)}$ grand means of sham, Rt, RtF, RtV, RtC, & RtGSal performances of Morris water task (i.e., hidden, moving platform).

Additionally, planned pairwise comparisons reveled that on average LtE found the hidden platform 6.88_{sec} faster than only lesion-Lt group $F_{(1, 82)} = 6.11$, $p < .0001$. On average, LtE found the hidden platform 5.15_{sec} faster than left lesioned Gel/saline (LtGSal) group $F_{(1, 82)} = 4.77$, $p = .001$. On average, LtE found the hidden platform $6.69_{\mathbf{sec}}$ faster left lesion VEGF (LtV) treated animals, $F_{(1, 82)} = 6.41$, $p < .0001$. On average, LtE found the hidden platform $3.98_{\mathbf{sec}}$ faster left lesion GF cocktail (LtC) treated animals $F_{(1, 82)} = 3.57$, $p = .031$, (see Figure 10).

Figure 10: Post Surgery- 14 Days and 8 Trials, Latency $_{(sec)}$ Grand Means of Sham, Lt, LtF, LtV, LtC, and LtGSal Performances of Morris Water Task

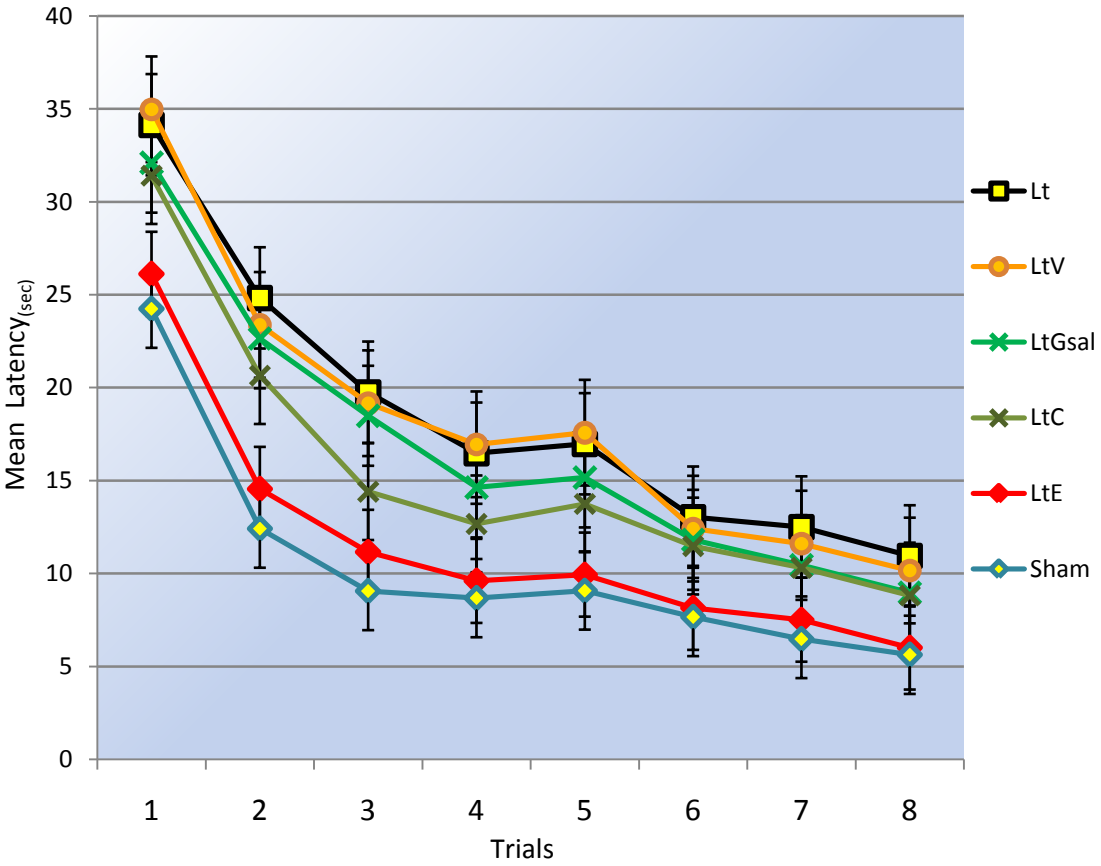

Figure 10: Post surgery, 14 days and 8 trials- latency $_{(sec)}$ grand means of sham, Lt, LtE, LtF, LtV, LtC, & LtGSal performances of Morris water Task (i.e., hidden, moving platform).

Similar to LtE, planned pairwise comparisons detected that on average, LtF found the hidden platform 6.81_{sec} faster than only lesion-Lt group $F_{(1, 82)} = 6.22$,

$p < .0001$and LtF found the hidden platform 5.27_{sec} faster than LtGSal left lesion Gel/saline (LtGSal) $F_{(1, 82)} = 4.89$, $p > .0001$. On average, LtF also found the hidden platform 6.81_{sec} faster left lesion VEGF (LtV) treated animals $F_{(1, 82)} = 6.53$, $p < .0001$ and LtF found the hidden platform 3.98_{sec} faster left lesion GF cocktail treated animals (LtC), $F_{(1, 82)} = 3.68$, $p = .022$, (see Figure 11).

Figure 11: Figure 5:Post Surgery- 14 Days and 8 Trials, Latency Grand Means of Sham, Lt, LtF, LtV, LtC, & LtGSal Performances of Morris Water Task

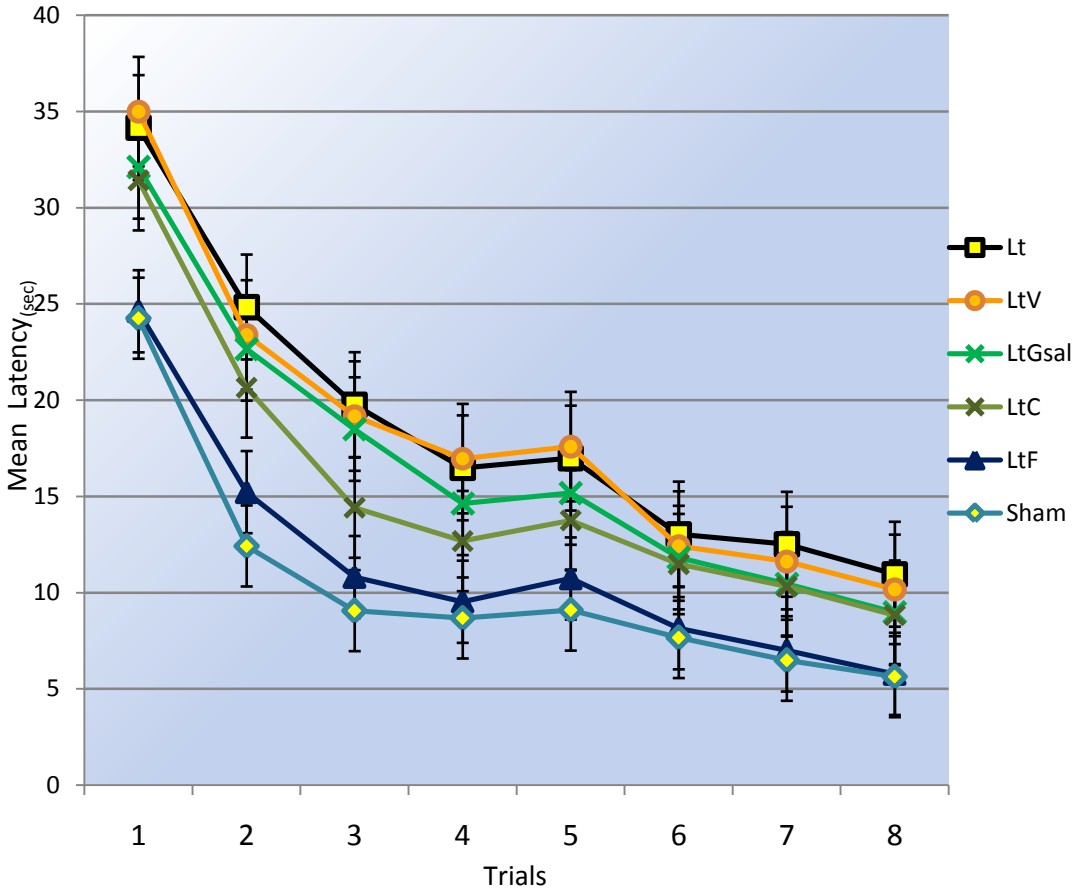

Figure 11: Post-surgery, 14 days & 8 trials grand means error, Morris water task (hidden, moving platform) of sham, Lt, LtF, and LtV,LtC, and LtGSal (i.e., hidden, moving platform).

Post surgery- Grid Walk task, Latency

The repeated measures ANOVA on latency yielded significant main effects of both day, $F_{(4,79)} = 21.558$, $p < .0001$, and trial, $F_{(3, 80)} = 12.446$, $p < .0001$, and also both

a day by group interaction, $F_{(12, 82)} = 5.592$, $p < .0001$, trial by group interaction, $F_{(12, 82)} = 8.139$, $p < .0001$, trail by day $F_{(12, 71)} = 3.929$, $p < .0001$and day by trial by group interactions, $F_{(12, 82)} = 7.152$, $p < .0001$**, (s**ee Table 6).

		Treatment						
		Gel + Saline	EGF	FGF	VEGF	Combo	No treatment	Sham .
Lesion	Rt	9.408	5.964	5.777	7.428	7.216	9.766	
	Lt	8.503	5.236	6.209	7.541	6.841	10.091	4.636

Table 6: Post surgery, 5 days, 4 trials latency $_{(sec)}$ grand mean of Grid Walk.

The test of the between-subjects factor of group showed a significant main effect of group, $F_{(12,82)} = 16.004$, $p < .0001$, (see Figures 12 and 13).

Figure 12: Post Surgery- 5 Days & 4 Trials, Latency$_{(sec)}$ Grand means of Grid Walk Performances of Sham, Rt, RtE, RtF, RtV, RtC, & RtGSal

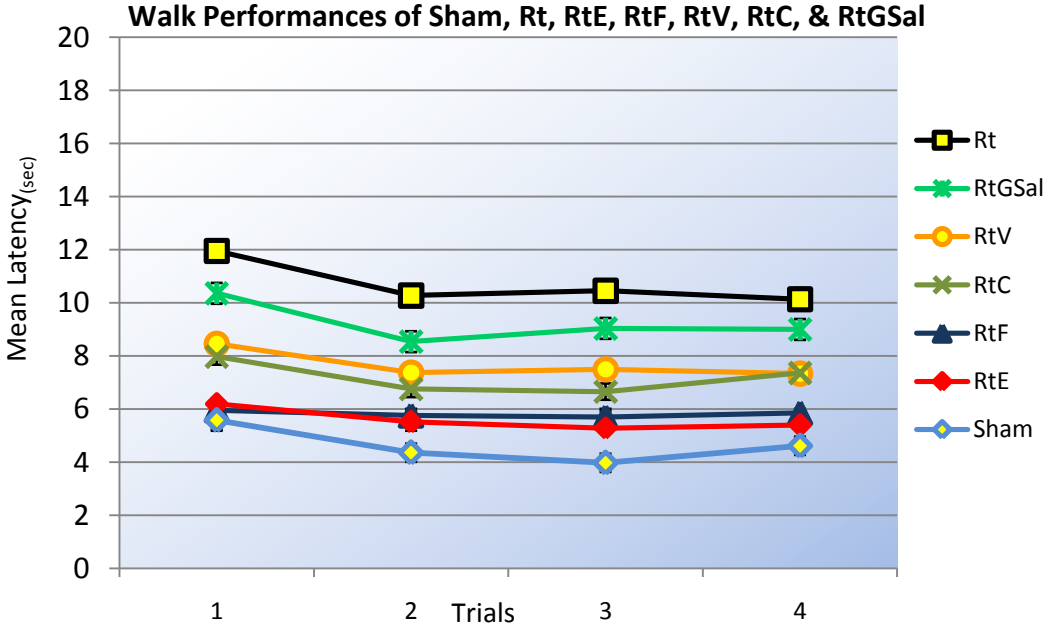

Figure 12: Post-surgery, 5 days & 4 trials latency $_{(sec)}$ grand means of Grid Walk performances of right lesion and right lesion treatment groups.

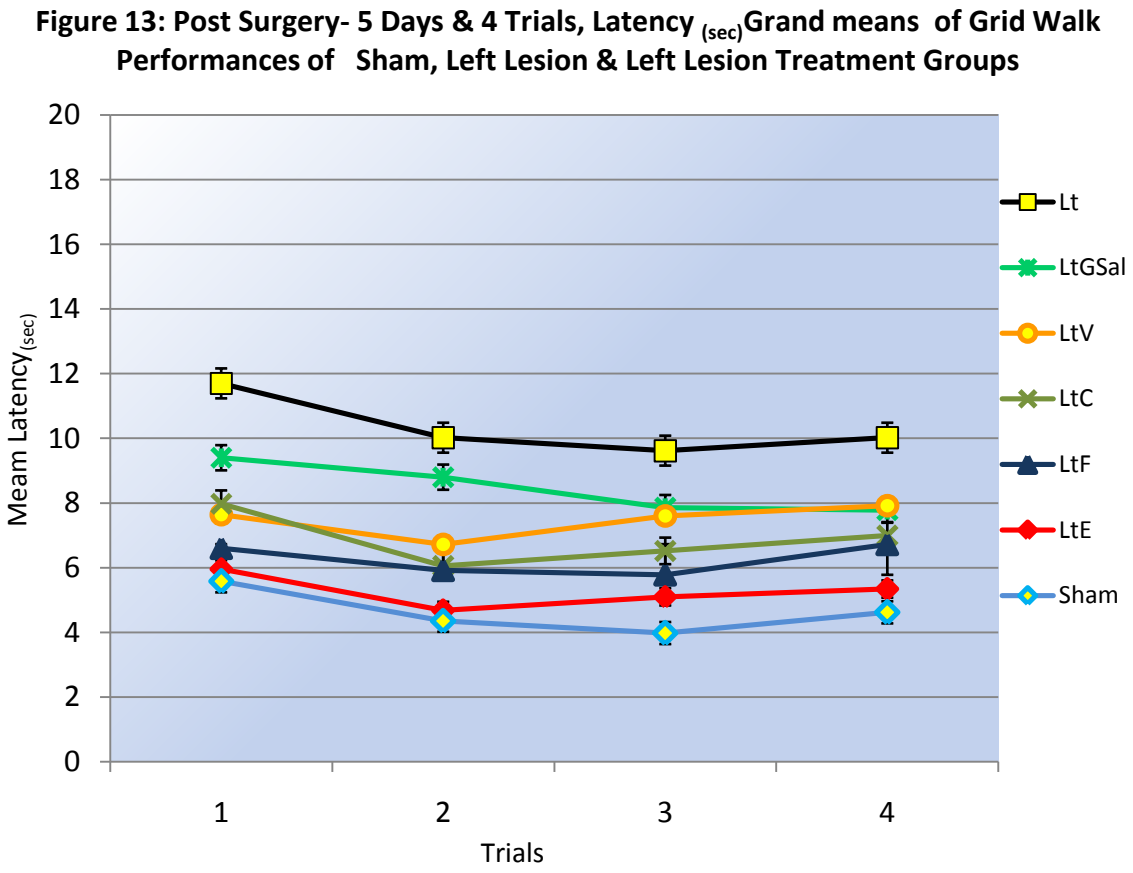

Figure 13: Post-surgery, 5 days & 4 trials latency $_{(sec)}$ grand means of Grid Walk performances of left lesion and left lesion treatment groups.

Planned pairwise comparisons reveled a significant difference between sham and right lesion untreated animals $F_{(1, 82)} = 8.27$, $p < .0001$ (i.e., on average, sham animals completed the task 5.13_{sec} faster than Rt) and between sham and left lesion untreated animals (Lt) $F_{(1, 82)} = 8.78$, $p < .0001$(i.e., sham completed the task 5.45_{sec} faster than left lesion untreated animals).

However, planned pairwise comparisons did not yield significant differences among sham, right lesioned Epidermal GF (RtE) & left lesioned/Epidermal (LtE), Right lesion Fibroblast GF (RtF) & left lesion Fibroblast GF (LtF) treated animals.

Altogether, these groups performed similarly (i.e., grand mean differences ranging from .5 to 1.5$_{sec}$), (see Figure 14 –right lesion and Figure 15 –left lesion groups).

Figure 14: Post Surgery- 5 Days & 4 Trials, Latency$_{(sec)}$ Grand Means of Grid Walk Performances of Sham, Rt, RtE, RtF, RtV, RtC, & RtGSal

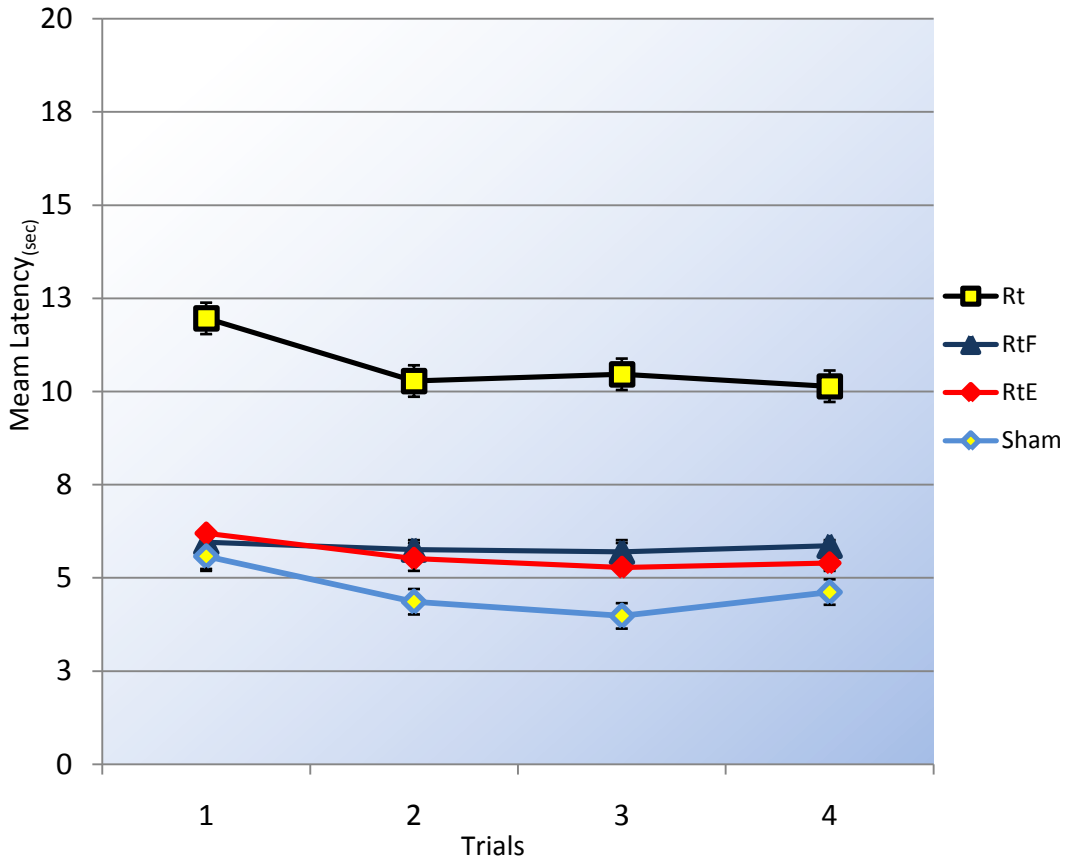

Figure 14: Post-surgery, 5 days & 4 trials latency $_{(sec)}$ grand means of Grid Walk performances of sham, Rt, RtE, and RtF.

Figure 15: Post Surgery- 5 Days & 4 Trials, Latency $_{(sec)}$ Grand means of Grid Walk Performances of Sham, Lt, LtE, & LtF

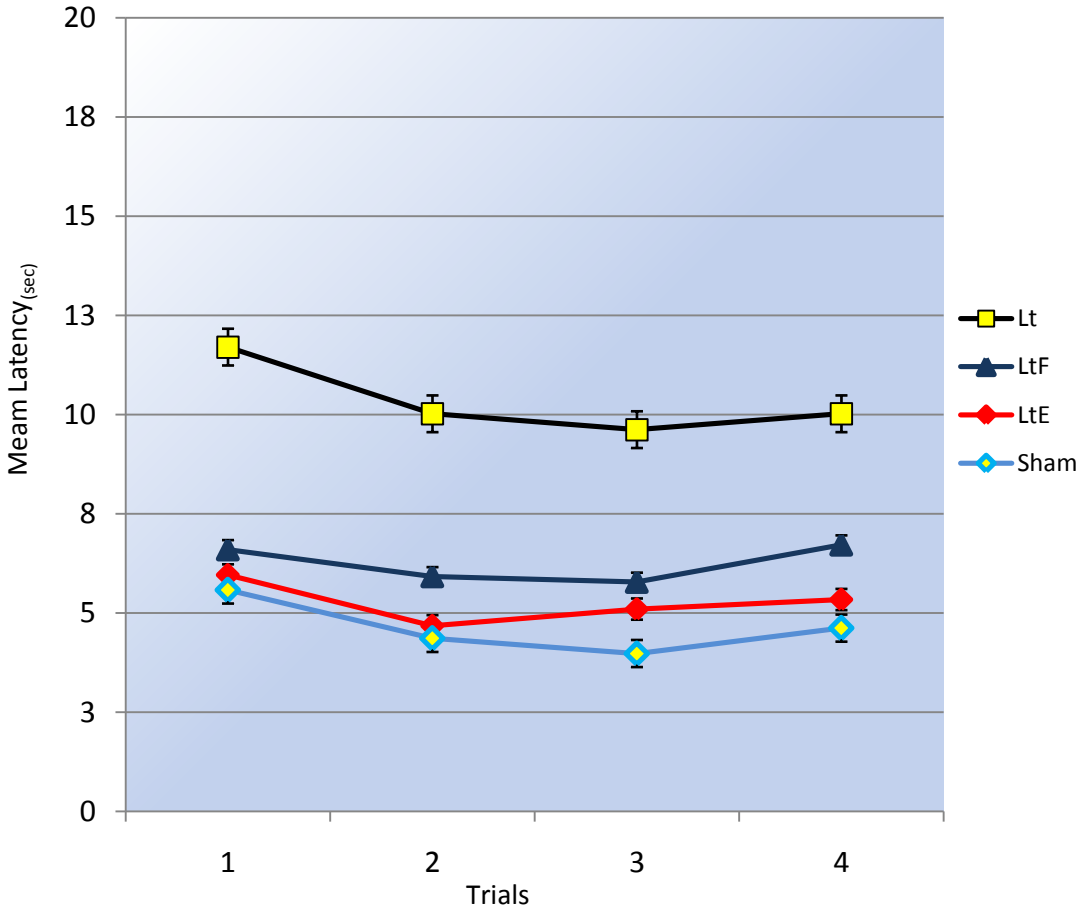

Figure 15: Post-surgery, 5 days & 4 trials latency $_{(sec)}$ grand means of Grid Walk performances of sham, Lt, LtE, and LtF.

Furthermore, planned pairwise comparisons reveled that the RtE group performed significantly better than the RT group $F_{(1, 82)} = 6.13$, $p < .0001$ (i.e., on average, RtE reached the goal box 3.81_{sec} faster than Rt group), right lesion Gel/saline $F_{(1, 82)} = 5.55$, $p < .0001$ (i.e., on average, RtE reached the goal box 3.45_{sec} faster than RtGSal). However, RtE performances did not differ significantly from right lesion

VEGF treated animals $F_{(1, 82)} = 2.36$, p=.48 and right lesion/GF cocktail treated animals $F_{(1, 82)} = 1.47$, p= .95, (see Figure 16).

Figure 16: Post Surgery- 5 Days & 4 Trials, Latency$_{(sec)}$ Grand means of Grid Walk Performances of Sham, Rt, RtE, & RtGSal

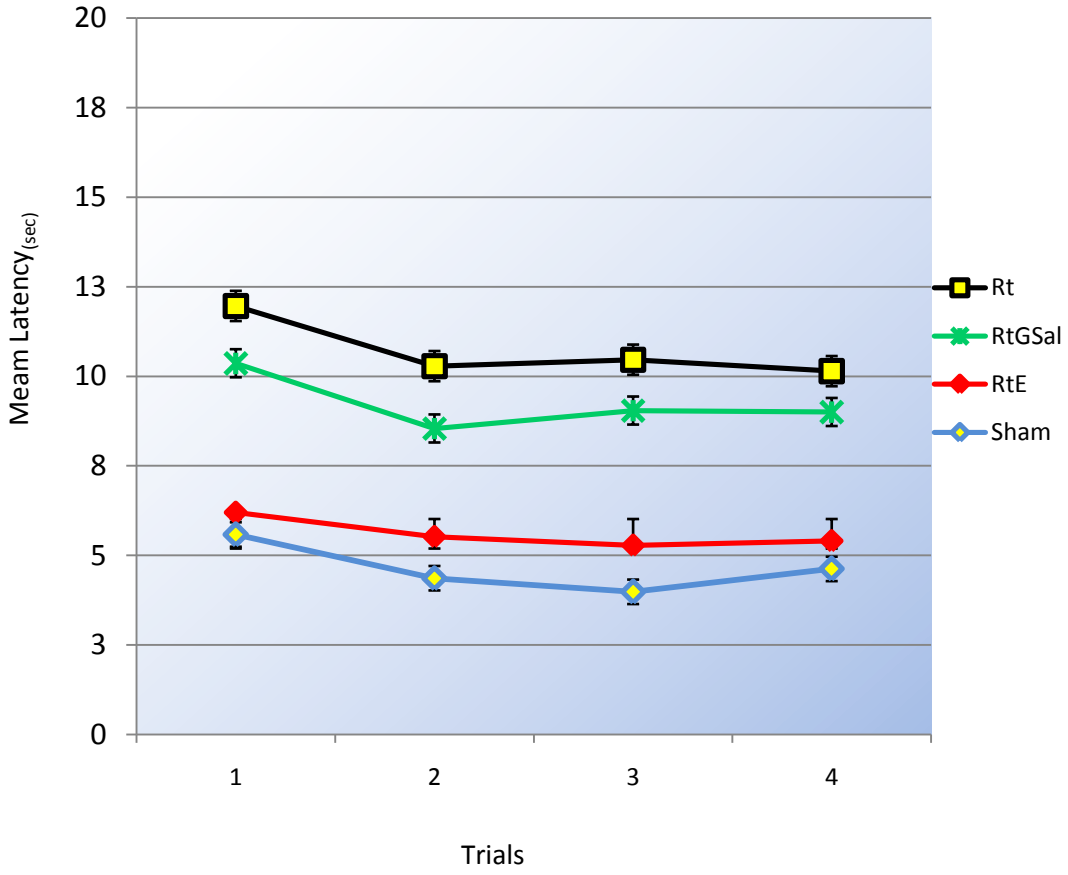

Figure 16: Post-surgery, 5 days & 4 trials latency $_{(sec)}$ grand means of Grid Walk performances of sham, RtE, Rt and RtGSal.

Similar to RtE, planned pairwise comparisons detected significant difference among between RtF and Rt $F_{(1, 82)} = 6.58$, $p < .0001$ (i.e., on average, RtF reached the goal box 3.98_{sec} faster than Rt group), right lesion Gel/saline $F_{(1, 82)} = 5.99$, $p > .0001$ (i.e., on average, RtF reached the goal box 3.63_{sec} faster than RtGSal) . However, RtF

performances did not differ from right lesion VEGF treated animals $F_{(1, 82)} = 2.73$, $p = .25$ and right lesion GF cocktail treated animals $F_{(1, 82)} = .34$, $p = .82$, (see Figure 17).

Figure 17: Post Surgey- 5 Days & 4 Trials, Latency$_{(sec)}$ Grand means of Grid Walk Performances of Sham, Rt, RtF, & RtGSal

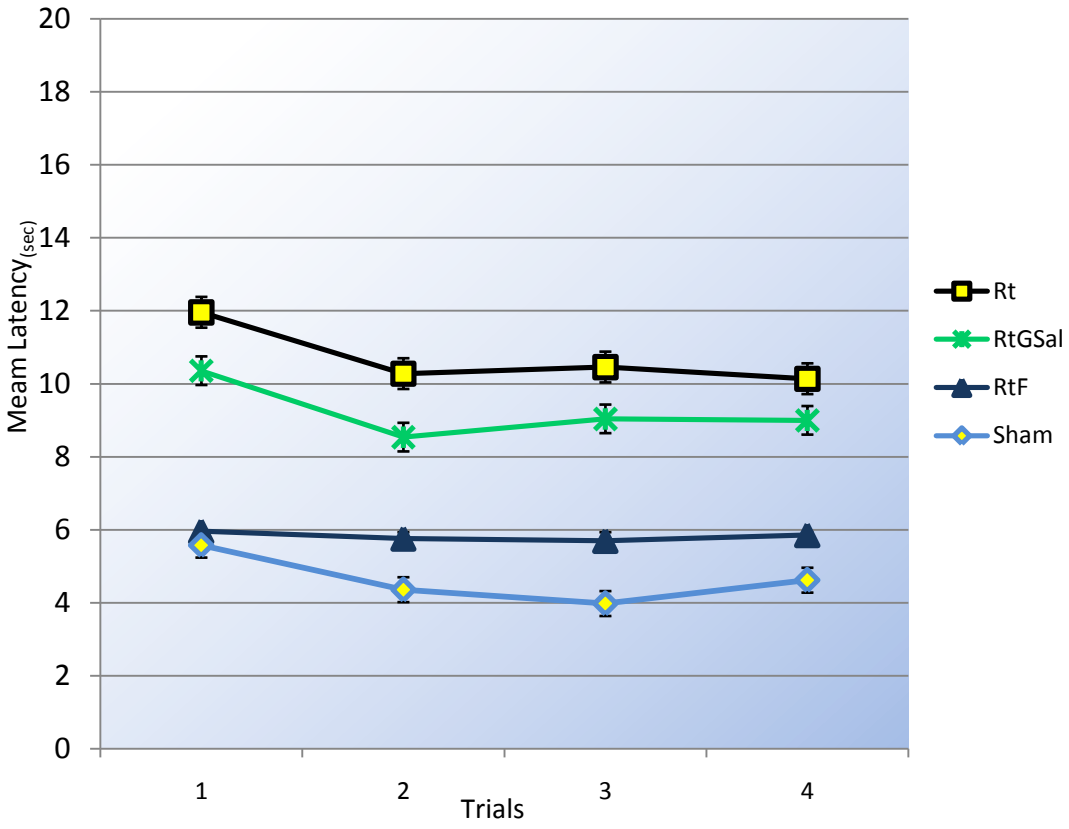

Figure 17: Post-surgery, 5 days & 4 trials latency $_{(sec)}$ grand means of Grid Walk performance for sham, Rt, RtF, and RtGSal groups.

Additionally, planned pairwise comparisons reveled that LtE performed significantly better than LT group $F_{(1, 82)} = 7.82$, $p < .0001$ (i.e., on average, LtE reach the goal box 4.86_{sec} faster than only lesion-Lt group), left lesion Gel/saline (LtGSal) $F_{(1, 82)} = 4.49$, $p < .0001$ (i.e., on average, LtE reach the goal box 3.27_{sec} faster than LtGSal), left lesion VGEF treated animals $F_{(1, 82)} = 4.02$, $p = .008$ (i.e., LtE reached the

goal box 2.31$_{sec}$ faster LtV animals). However, RtE performances did not different from right lesion GF cocktail treated animals $F_{(1, 82)} = 2.7$, $p=.28$, (see Figure 18).

Figure 18: Post Surgery- 5 Days & 4 Trials, Latency $_{(sec)}$ Grand means of Grid Walk Performances of Sham, Lt, LtE, LtV, & LtGSal

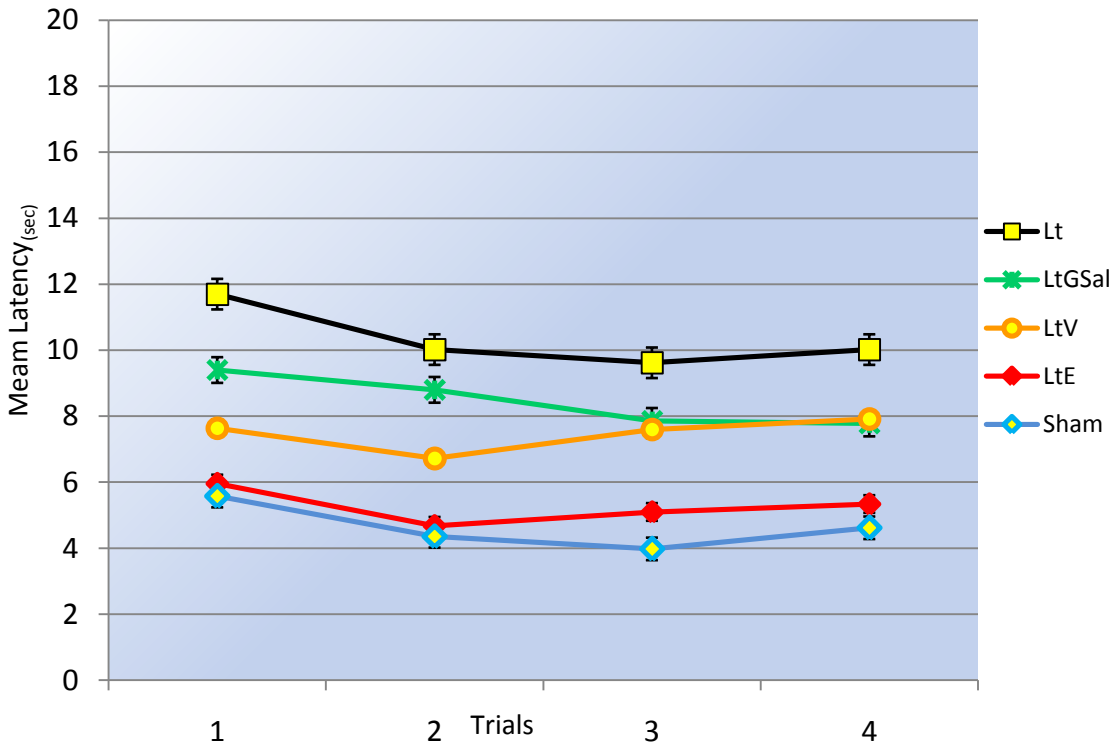

Figure 18: Post Post-surgery, 5 days & 4 trials latency $_{(sec)}$ grand means of Grid Walk performance for sham, Lt, LtE, LtV, and LtGSal groups.

Similar to the LtE group, planned pairwise comparisons detected significant difference among between LtF and Lt groups $F_{(1, 82)} = 6.25$, $p < .0001$ (i.e., on average, LtF reach the goal box 3.88$_{sec}$ faster than only lesion-Lt group), left lesion Gel/saline $F_{(1, 82)} = 3.85$, $p = .013$ (i.e., on average, LtF reach the goal box 2.29$_{sec}$ faster than LtGSal). However, left performances did not significantly differ from lesion VEGF treated animals $F_{(1, 82)} = 2.31$, $p = .51$ and left lesion GF cocktail treated animals $F_{(1, 82)} = 1.06$, $p= .97$, (see Figure 19).

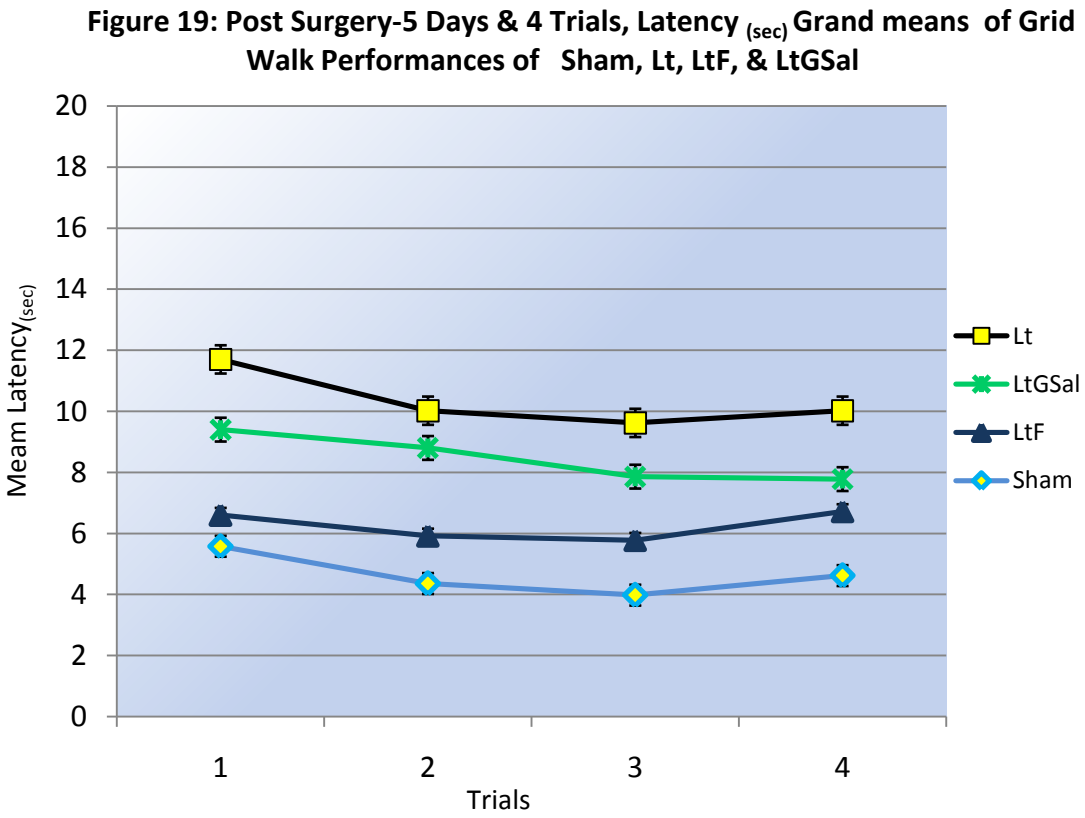

Figure 19: Post-surgery, 5 days & 4 trials latency $_{(sec)}$ grand means of Grid Walk performance for sham, Lt, LtF, and LtGSal groups.

Post surgery- Grid Walk task, <u>Error</u>

The repeated measures ANOVA on error yielded significant main effects of both day, $F_{(4, 70)}$ =17.122 , $p < .0001$, and trial, $F_{(3, 80)}$ =3.773, $p = .024$, and day by group interaction, $F_{(12, 82)} = 3.773$, $p = .014$, trial by group interaction, $F_{(12, 82)} = 7.099$, $p < .0001$and day by group by cell interaction, $F_{(12, 82)} = 6.057$, $p < .0001$**, (**see Table 7).

		Treatment						
		Gel + Saline	EGF	FGF	VEGF	Combo	No treatment	Sham.
Lesion	Rt	1.631	0.658	0.694	1.108	0.825	1.977	
	Lt	1.308	0.506	0.650	0.973	0.761	1.850	0.333

Table 7: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performances.

The test of the between-subjects factor of group showed a significant main effect of group, $F_{(12,82)} = 70.075$, $p < .0001$, (see Figure 20 and 21).

Figure 20: Post Surgery- 5 days & 4 Trials, Error 's Grand Means of Grid Walk Performances of Sham, Right Lesion & Right Lesion Treatment Groups

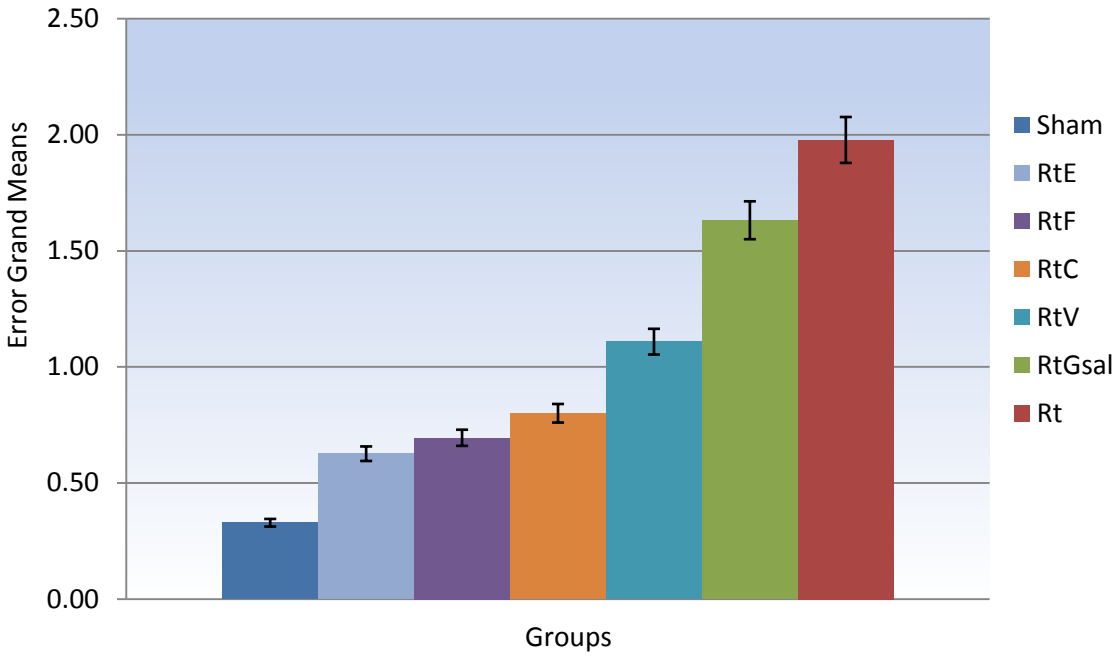

Figure 20: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performances of Sham, right lesion and right lesion treatment groups.

Figure 21: Post Surgery- 5 days & 4 Trials, Error 's Grand Means of Grid Walk Performances of Sham, Left Lesion & left Lesion Treatment Groups

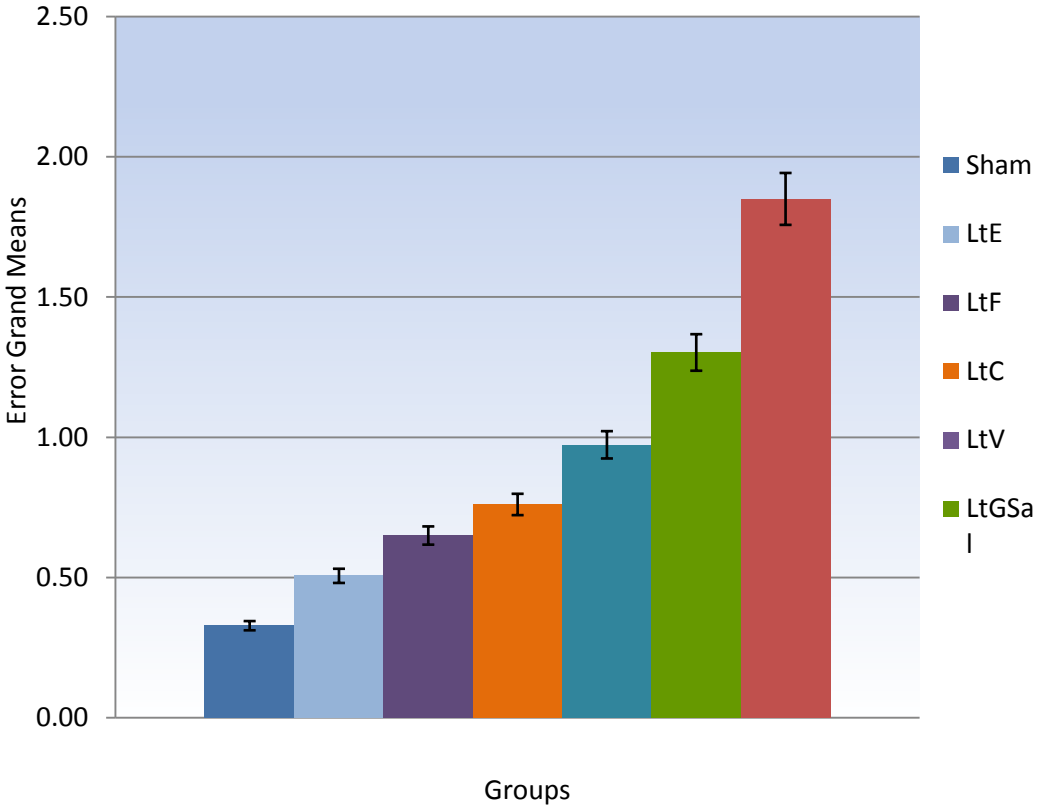

Figure 21: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performances of left lesion and left lesion treatment groups.

Planned pairwise comparisons reveled a significant difference between sham and right lesion untreated animals $F_{(1, 82)} = 18.83$, $p < .0001$ (i.e., mean difference of 1.64) and between sham and left lesion untreated animals $F_{(1, 82)} = 7.2$, $p < .0001$(i.e., sham animals completing the task 8.12_{sec} faster than left lesion untreated animals. See Figure 22 –right lesion and 23 –left lesion groups. Moreover, planned pairwise comparisons did not yield significant differences between sham and right lesioned Epidermal GF (RtE) groups.

Figure 22: Post Surgery- 5 days & 4 Trials, Error's Grand Means of Grid Walk Performances of Sham, RtE and Rt

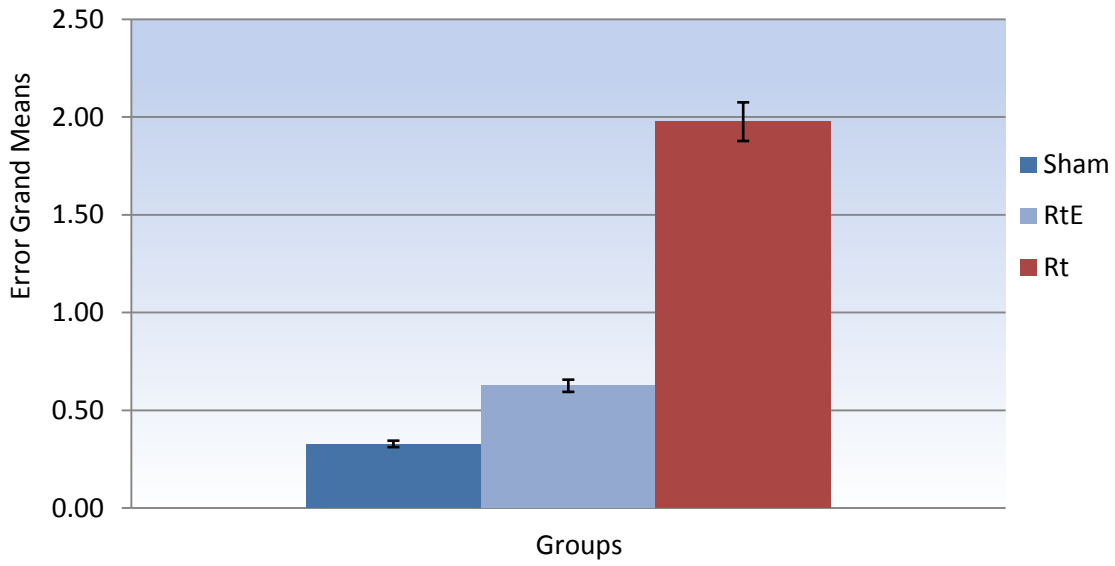

Figure 22: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performance for sham, Rt, and RtE groups.

Figure 23: Post Surgery- 5 days & 4 Trials, Error's Grand Means of Grid Walk Performances of Sham, LtE and Lt

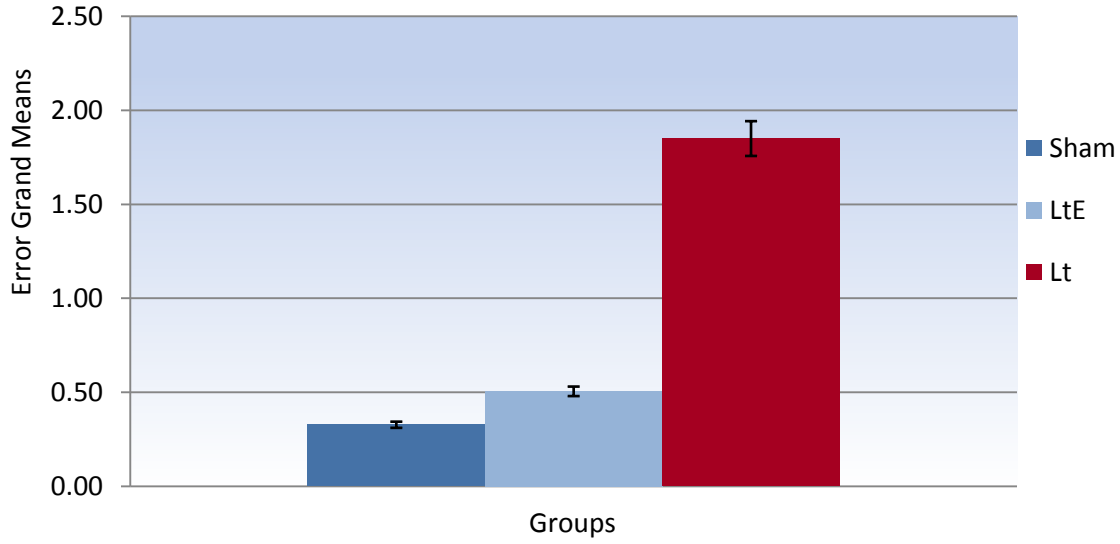

Figure 23: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performances of sham, Lt, and LtE groups.

Furthermore, planned pairwise comparisons reveled that RtE animals performed significantly better than the RT group $F_{(1, 82)} = 15.103$, $p < .0001$ (i.e., mean difference of 1.32), right lesion Gel/saline $F_{(1, 82)} = 11.15$, $p < .0001$(i.e., mean difference of 0.97), right lesion VEGF treated animals $F_{(1, 82)} = 5.16$, $p < .0001$(i.e., mean difference of 0.45). However, RtE animals' performance did not significantly differ from right lesion GF cocktail treated animals $F_{(1, 82)} = .69$, $p = .99$ (see Figure 24).

Figure 24: Post Surgery- 5 days & 4 Trials, Error's Grand Means of Grid Walk Performances of Sham,RtE, RtV, RtGSal, and Rt

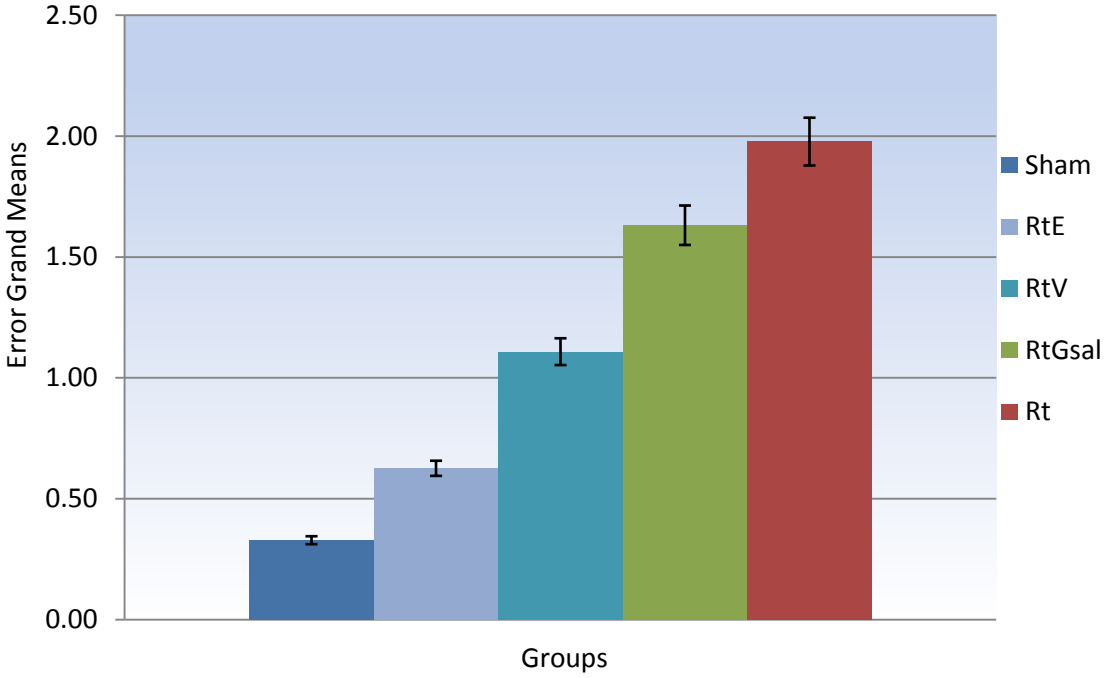

Figure 24: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performances of sham, Rt, RtE, and RtV,RtC, and RtGSal.

Similar to RtE, planned pairwise comparisons detected significant difference between RtF and Rt animals $F_{(1, 82)} = 15.04$, $p < .0001$(i.e., mean difference of 1.28), right lesion Gel/saline (RtGSal) animals $F_{(1, 82)} = 10.99$, $p < .0001$(i.e., mean difference of .94), and right lesion VEGF treated animals $F_{(1, 82)} = 4.86$, $p < .0001$ (i.e., mean difference of .41). However, RtF performance did not differ from right lesion GF cocktail treated animals $F_{(1, 82)} = .81$, $p = .89$ (see Figure 25).

Figure 25: Post Surgery- 5 days & 4 Trials, Error's Grand Means of Grid Walk Performances of Sham,RtF , RtV, RtGSal, and Rt

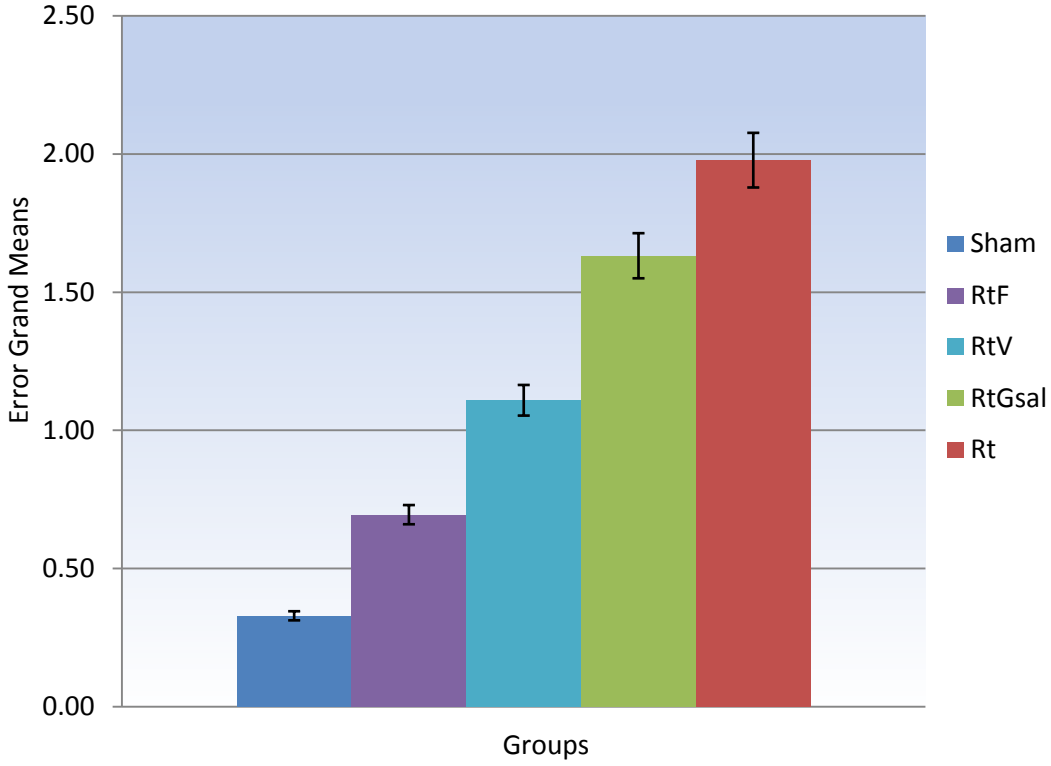

Figure25: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performance for sham, Rt, RtF, and RtV,RtC, and RtGSal groups.

Additionally, planned pairwise comparisons reveled that LtE animals performed significantly better than the LT group $F_{(1, 82)} = 15.38$, $p < .0001$ (i.e., mean difference of 1.34), left lesion Gel/saline animals $F_{(1, 82)} = 9.57$, $p < .0001$ (i.e., mean difference of .80), and left lesion VGEF treated animals $F_{(1, 82)} = 5.77$, $p < .0001$ (i.e., mean difference of .47). However, LtE group performance did not differ significantly from lesion GF cocktail treated animals $F_{(1, 82)} = 3.04$, $p = 13$ (see Figure 26).

Figure 26: Post Surgery- 5 days & 4 Trials, Error's Grand Means of Grid Walk Performances of Sham, Left Lesion & Left Lesion Treatment Groups

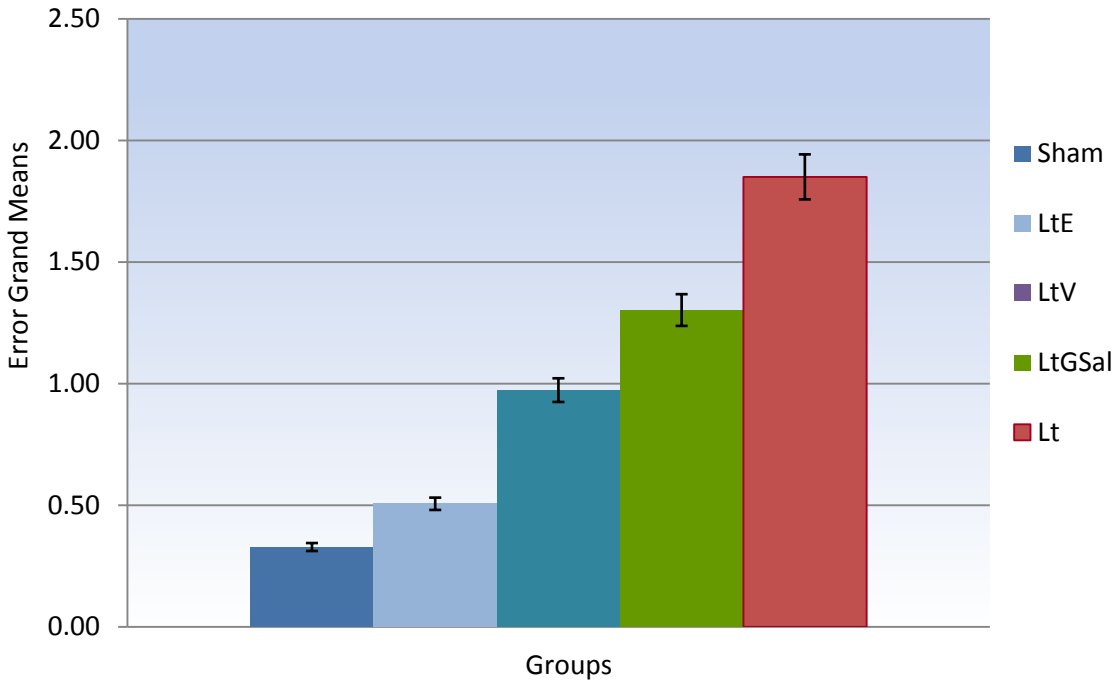

Figure 26: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performance for sham, Lt, LtE, LtV, and LtGSal groups.

Similar to LtE, planned pairwise comparisons detected significant difference among between LtF and Lt groups $F_{(1, 82)} = 13.73$, $p < .0001$ (i.e., mean difference of 1.2), left lesion Gel/saline animals $F_{(1, 82)} = 7.86$, $p > .0001$ (i.e., mean difference of .65), and left lesion VEGF treated animals $F_{(1, 82)} = 3.99$, $p = .008$. (i.e., mean difference of .32). However, LtF performances did not significantly differ from left lesion GF cocktail treated animals $F_{(1, 82)} = 1.32$, $p = 92$, (see Figure 27).

Figure 27: Post Surgery- 5 days & 4 Trials, Error's Grand Means of Grid Walk Performances of Sham, LtF, LtV, LtGsal, and Lt

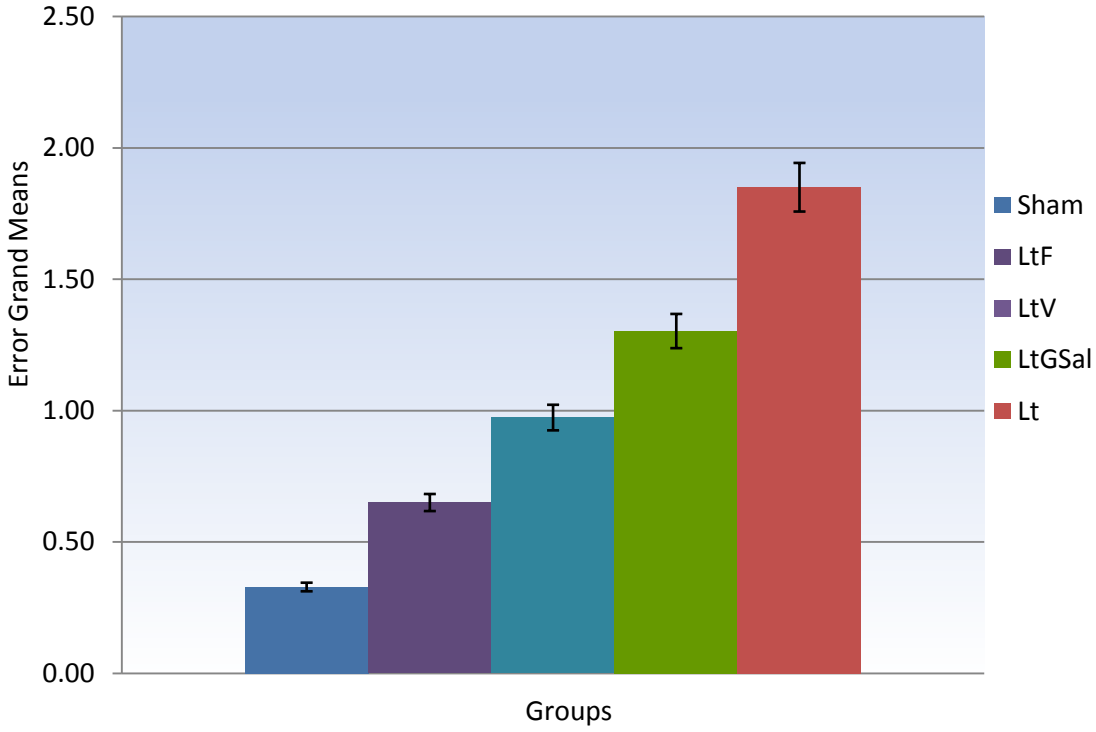

Figure 27: Post-surgery, 5 days & 4 trials error's grand means of Grid Walk performances of sham, Lt, LtF, and LtV, and LtGSal.

Discussion

The present experiment demonstrates that bridging RMS to somatosensory contusion via a single injection of matrigel containing EGF and FGF-basic growth factors, improves the recovery of behavioral and cognitive functions in adult rats. However, lesioned animals who received matrigel containing VEGF and/or the combination of three growth factors did not show functional improvements. These results are compatible with recent studies showing that with the combination of certain biological treatments and behavioral therapy, the mammalian cortex retains the ability to heal, save, and/or replace injured neurons, successfully integrate them into its system, and ultimately to restore lost functions after cortical contusion (Yamashito et al., 2006 Pressmar et al., 2001**;** Shin et al., 2000; Kolb et al. 1998, Snyder et al., 1994; Tuszynski & Gage 1992). Such studies also provide three possibilities for the improvement of cognitive and behavioral functions.

Epidermal and Fibroblast-basic growth factors are shown to have a neuroprotective function against neuronal injury during ischemic stroke (Yamashita el al., 2006; Okawa et al., 2001; Slevin et al., 2000; Naota et al., 1999) thrombic stroke (Cameron et al. 1998), spinal cord injury (Grill et al., 1997), cortical trauma (Kolb et al., 1998; Martínez-Serrano, 1996;) and Glucocorticoid induced trauma (Jeanneteau et al. 2007). After transient middle cerebral occlusion, Yamashita et al. (2006) research showed that the transplantation of a single biodegradable scaffold containing an epidermal growth factor increases dendritic arborization (i.e., an increase in dendritic field, spine density and synapse formation), axonal re-growth at synapse and down regulation of cytokine (i.e., interleukin 6, protein that is directly associated with the

increase of infract volume/contusion cyst and induction of neuronal apoptosis (i.e., genetically-programmed cell death) after ischemic stroke and cortical contusion). Similarly, fibroblast growth factor-2 has also been shown to promote axon branching of cortical neurons by influencing morphology of the primary growth cone (Szebenyi et al., 2000). Altogether, these studies support the argument that the behavioral improvement in this investigation is due to beneficial neuroprotective and mitogenic effects of the growth factors on surrounding tissue. Kolb et al. (1997) histological result showed that EGF and FGF-2 growth factors have been shown to increase dendritic arborization after aspiration model of injury. Their result also demonstrated that treatment with growth factors salvaged and reorganized the circuitries associated with the site of injury (Kolb et al., 1998a-1997). They further maintained that in order to achieve recovery of the previous functions, the circuits formed by existing neurons were rewired and took over the lost function. Therefore, the simplest interpretation of our results is that the beneficial behavioral and cognitive effects we observed were due to the action of growth factors on surrounding damaged, but recovering, tissue.

A second possibility involves the endogenous neuronal and glial precursors from RMS (i.e., that are normally migrate through the basal forebrain to anterior olfactory structures) were rerouted to the injured somatosensory via the route of bridge treatment. An example of directed migration to the injured site is the research of Fallon et al., (2000), that involved the Parkinson model of brain injury (i.e., 6-hydroxydopamine lesion of the substantia nigra dopaminergic neurons) and implantation of an osmotic mini pump that allowed for continuous intracortical infusion of growth factor a (i.e., TGFa) into forebrain structures and striatum. Their histological results clearly

demonstrated a rapid proliferation of forebrain NCSs followed by a timed migration of a ridge of neuronal and glial progenitors directed toward the region of the TGFa infusion site three to nine days post injury. Furthermore, their behavioral result also showed significant improvement seven to nine days after the treatment. Such results support the idea that the mammalian brain contains NSCs that are capable of proliferation, directed migration, and differentiation in response to cortical injury and exogenous treatments of growth factor. Our results provide future behavioral evidence for the positive effect of growth factor treatment and directed migration.

Another example of such investigation is Gould et al. (1999), showing that in normal physiological condition, adult macaques have new neurons scattered in brain regions that are associated with the behavioral plasticity (e.g., prefrontal, posterior parietal and inferior temporal cortex). The new neurons are spread into several regions, where they extend their axons (Gould et al., 1999-2002; Magavi et al., 2000). These results also suggest that originally, these stem cells of the subventricular zone are the source of an additional population of new neurons that migrate through fiber tracts to neocortical regions. Moreover, several studies also show that the mechanisms inhibiting the migration of the stem cells into certain parts of the neocortex (i.e., olfactory bulb) could be interrupted (Alvarez-Buylla et al., 2002; Heather et al., 2001), especially during brain trauma. Such interruptions could allow for more of the stem cells to migrate out of the rostral migratory stream and into the cortex. Therefore, the probability of migrating NCSs originating from the rostral migratory stream is higher than previously thought. However, further histological analyses (i.e., profiling the stem

cell on the filament via specific cell markers) are vital to the investigation of the sources and the types of cells in this study.

Several studies have shown evidence for stem cells in scattered sites within the cortex (Altman., 1963 & 1965; Bedard et al., 2001; Kornack et al., 2000; Gould et al., 1999 & 2002; Pencea et al., 2001; jiang et al., 2001). The Jiang et al. (2001) anatomical-photothrombotic stroke study identified the newborn cortical stem cells in the morphologically restored cortical region at risk (i.e. somatosensory cortex that underwent severe morphological damage) in adult rats. Their result showed that neurogenesis accounted for 3 to 6% of the newly-generated cortical cells at 7 and 10 days after stroke (Jiang et al., 2001). The newborn cortical neurons were randomly scattered throughout the cortex, but were more concentrated in regions near the injured cortex. The latter finding suggests that there are more sources and routes for neuronal and glial stem cells allowing them to pass through into the cortex, which can contribute to plasticity or to the rewiring of the damaged circuitry after the brain trauma. The behavioral functionality of these new neurons, however is at the center of debate (Kaplan et al., 2001; Gross et al., 2000), especially after pathological brain injury (Johansson et al., 2000; Gage, 2000). Therefore, another possibility for the improvement in behavioral performance during this study may involve the multiple sources for in-migrating cells.

To the best of our knowledge, there are no scientific investigations that utilize the bridge treatment and rerouting of the rostral migratory stream for recovery of function after brain trauma. There are many studies that use different transplantation techniques to transport the stem cells to the site of the injury, and they have successfully

demonstrated the behavioral and cognitive recoveries of functions in various regions of the mammalian brain.

Ignoring the ethical issues (i.e., fetal or cross-species transplantation), most stem cell transplantation requires elaborate preparation, such as splicing, grafting and culturing; and eventually, it also involves transporting the tissue to the specific damage sites (Johansen et al., 2000; Fredrick et al, 1998). The bridge treatment utilizes endogenous stem cells; therefore it reduces the risk of the multiple steps associated with transplantation surgeries and at the same time may provide answers to some of the ethical issues concerned with stem cell transplantation.

Altogether, the identification of stem cells near the site of injury after the stroke (Sun et al., 2009; Jiang et. al., 2001; Jin et al., 2001), and the *in vitro* ability of the stem cells to produce a highly differentiated neuron (Gage et al., 2002; Kempermann et al., 2002; Shamblott, et al., 1998) reveals that during brain trauma, the failure of the mammalian central nervous system to regenerate is not an intrinsic deficit of the stem cells, but rather a distinctive feature of the damaged environment that can't support the regeneration process (Gage et al., 2002; Khun et al., 2002).

In conclusion, our result shows that bridging RMS to somatosensory contusion via a single injection of matrigel containing EGF and FGF-basic growth factors can accelerate recovery of behavioral and cognitive functions. Although we cannot, as yet, definitively reach a conclusion about the basis for the observed accelerated recovery, the results of this investigation are consistent with the notion that endogenous adult stem cells can be assisted to migrate to the site of injury. Notably, our results may suggest that the sprouting of new functional neurons lowers the total cognitive and

behavioral functional losses that are associated with the lesion of rodent somatosensory cortex. However, it must be mentioned that further scientific investigation is necessary to validate the future functionality and fate of these new neurons.

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