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Abstract

Stroke is a leading cause of long-term disability with most patients suffering from persistent unilateral upper-limb impairments. These impairments impede daily living and independence as well as impose many other social and financial burdens. Current rehabilitation methods focus on compensatory movements relying largely on the nonimpaired limb. Unfortunately, the current methods of rehabilitation do not effectively promote full recovery of motor skills on the impaired body side. Rehabilitation in a mouse model of stroke has shown promising results, however, the training regimen used is much more intensive than the rehabilitation that human survivors receive, and the minimum amount of intensity required to promote functional benefits is unknown. The current study aims to investigate the effects of intensity of rehabilitation on motor function in the mouse model. Mice were trained preoperatively on a skilled reaching task then given a unilateral photothrombotic stroke. Postoperatively, mice received either traditional training (15 minutes or 100 reaches daily), low intensity training (10 minutes or 50 reaches daily), or modified traditional training (10 minutes or 50 reaches, twice daily). All groups were assessed on the original skilled reaching task after 28 training sessions. The results of this pilot study were inconclusive. Further research should be done to determine if the rehabilitation groups in this study are effective at promoting full recovery of function, with the intent to close the gap between the animal model and human outcomes.

Keywords: stroke rehabilitation, intensity, motor recovery

Introduction

There are about 795,000 Americans affected by stroke every year, with a new stroke occurring about every 40 seconds (Virani et al., 2020). In fact, stroke is the leading cause of long-term disability in the United States, with unilateral upper-limb impairments being one of the most common and persistent disabilities in survivors (Krakauer et al., 2012; Bell et al., 2014). These long-term motor disabilities are burdensome for the individual and their families' due to a loss of independence. With improvements in technology and treatment interventions, stroke has become a less fatal injury, resulting in a larger population of people living with long-term disability (Rimmele et al., 2021). As a result, a majority of stroke survivors are left with motor deficits that impede their ability to perform tasks of independent daily living. Chronic disability after stroke also has large economic and societal implications (Mascaro et al., 2014). In 2014-2015, the United States spent $45.5 billion for the direct and indirect costs of stroke survivors (Virani et al., 2020). These values are predicted to more than double by 2035 with an estimated cost of $94 billion as the population ages. Long-term healthcare for stroke survivors requires a great allocation of resources, making it very important to improve the current rehabilitation strategies to allow patients better functional outcomes and greater independence.

Following a stroke, the brain experiences a period of naturally occurring plastic changes known as spontaneous recovery that allows for minimal recovery of motor function. These improvements usually occur within the first few months following a stroke. Additionally, the improvement rate of motor function in the first month following stroke is a strong predictor of overall motor recovery (Stanescu et al., 2019). Experimental models using rodents show that motor function can be further recovered through rehabilitative training (Bell et al., 2014; Kerr et al., 2016), however, in human subjects motor function recovery is often incomplete with patients averaging only 70% of their prestroke ability after rehabilitative training (Krakauer et al., 2012). The rehabilitation effects in experimental models are not replicated to the same degree in clinical practice, and it has been hypothesized that differences in rehabilitation intensity between experimental models and clinical practice are responsible for the discrepancy in recovery outcomes.

Stroke usually affects one hemisphere of the brain (i.e., is unilateral), leaving the contralateral side (the opposite side) impaired. Since only one side of the body is affected, there is an impaired body side and a non-impaired (or perhaps more accurately, less-impaired) body side following injury. Rehabilitation commonly focuses on compensatory movements utilizing the non-impaired limb to allow patients to complete independent tasks of daily living and hopefully allow them to return home faster. However, it has been shown that nonuse of the impaired limb (which occurs with compensatory training of the non-impaired limb) can have detrimental effects for motor recovery (Kerr et al., 2016; Taub et al., 2006). Additionally, experimental results indicate that higher intensity rehabilitation of the paretic limb results in faster recovery in rodent models (Bell et al., 2014). Unfortunately, the lower boundary of intensity has not yet been determined. This study aims to investigate the effects of lower intensity training and modified intensity training to help close the gaps seen in recovery between experimental models and clinical application.

Mechanisms of Stroke

Ischemic stroke is the most common type of stroke making up 87% of all stroke injuries (Salvador et al., 2021). Ischemic strokes are the result of blocked cerebral blood flow, cutting off oxygen to the brain. Brain tissue is extremely sensitive to ischemia, with even brief periods of occlusion leading to cellular death (Woodruff et al., 2011). Within a few minutes after the onset of cerebral ischemia, the cells located in the core of the ischemic infarct suffer necrotic cell death, and in the following hours or days after injury a complex chain of neurological changes, known collectively as diaschisis, occur in the brain tissue, expanding the injury. The cells surrounding the core injury are functionally impaired, but not yet dead and remain susceptible to neuronal death-processes including glutamate-mediated excitotoxicity and apoptosis (Filippo et al., 2008).

After cells experience deprivation of oxygen and glucose, they are unable to produce ATP to maintain their normal cellular functions. As a result, the ionic gradients are lost and glutamate is released from the cell. This glutamate over activates the NMDA glutamate receptors, allowing an influx of calcium ions (Filippo et al., 2008). Excess intracellular calcium activation ultimately leads to the activation of caspases and other apoptotic molecules, the end result of glutamate-mediated excitotoxicity. These processes that occur as a result of ischemic injury also play a role in the induction and maintenance of synaptic plasticity and long-term potentiation in the brain (Filippo et al., 2008). For example, the influx of calcium ions is an essential step in long-term potentiation, which results in a strengthening of synaptic connections. Additionally, these pathways modulate the cAMP-response-element-binding protein and extracellular signal-related kinase pathway, resulting in activation of proteins responsible for gene expression and synaptic plasticity (Filippo et al., 2008). The pathways activated as a result of ischemic injury act to remove the cells that have been damaged beyond repair, as well as activating mechanisms to allow for re-strengthening of the synapses that were damaged in the ischemic event, but remain viable. Following a stroke there is also a growth permissive period that is described in further detail below.

Early restoration of blood flow is best for optimal outcome in terms of limiting the size of injury. With improvements in education and treatment techniques, there has been a decrease in morbidity following ischemic stroke, leaving an increasing number of survivors with long term disabilities (Woodruff et al., 2011). Among survivors of ischemic stroke, 50% have some hemiparesis and 26% are dependent on others for activities of daily living 6 months after stroke (Mohaptra et al., 2016).

Deficit after stroke/long-term disabilities

Overall, the extent of damage following a stroke is highly variable depending on the location and extent of brain damage, but it can leave the individual with severely disabling impairments in motor, sensorimotor, and cognitive function (Klein et al., 2012). The most common, chronic disability in stroke survivors is upper limb impairments, including hemiparesis and weakness (Virani et al., 2020; Cortes et al., 2017). Of the seven million stroke survivors in the U.S., up to 88% live with upper extremity motor deficits (Barth et al., 2020) and 50-80% of survivors suffer arm paresis persisting up to six months following stroke (Cortes et al., 2017). Weakness and paralysis most commonly affect one side of the body, the side contralateral to, or opposite, the side of brain damage. Deficits are commonly seen in both strength and motor control defined as the ability to make coordinated, accurate, goal-directed movements (Cortes et al., 2017). Skilled arm movements in stroke patients are often described as slow, inaccurate, and fragmented (Kantak et al., 2018). Without the ability to make goal-directed movements with accuracy and control, the patient's ability to complete activities of daily life such as cooking, eating, bathing, and dressing are greatly impeded, often requiring them to be dependent on caregivers or family members. Additionally, other aspects of motor control can be impacted following stroke, such as walking. While a majority of patients regain the ability to walk, they often experience deficits in gait and postural control, rendering these activities unsafe to be completed without assistance (Kal et al., 2016).

The annual stroke death rate has declined by 35.8% over the last decade, resulting in more patients living with long term disabilities (Mohaptra et al., 2016). This number is only expected to rise with the aging population and current life expectancy. The global lifetime risk of stroke has had a relative increase of 8.9% from 1990 to 2016 (Virani et al., 2020). Such factors as high blood pressure, smoking, diabetes, and lack of physical activity increase the risk of strokes, especially in an aging population. Additionally, stroke survivors are at an increased risk of suffering additional strokes or other complications. After the initial high-risk period immediately following initial injury, those who have suffered a stroke have a 10-year stroke risk of 19% and combined risk of stroke or vascular death of 43% (Virani et al., 2020). With growing incidence rates of stroke, the need for improving the current rehabilitation efforts is increasingly important.

Spontaneous recovery

During the first month after a stroke, the brain undergoes an intense period of plasticity resulting in synaptic and structural changes, allowing dynamic change in neuronal connectivity.

As a result of ischemia, there is a sprouting of new neuronal dendrites and axons in both the perilesional cortex and in regions not related to the lesion (Coleman et al., 2017). There is also an increase in growth factor signals that promote synaptogenesis. These changes in neuronal structure likely account for the restoration of activity and motor improvements seen in the first month after stroke (Krakauer et al., 2012). The widespread changes in connectivity occur in both the contralateral and ipsilateral regions of the brain (Krakauer et al., 2012; Lee et al., 2020). One example of this is cortical reorganization which occurs within the first three days following a stroke in mice. During this time, stimulation of the impaired limb leads to activation of the ipsilateral brain hemisphere, showing a reorganization of sensory inputs to the intact hemisphere (Coleman et al., 2017). This shifting of brain activity is also shown in humans and while it provides an adaptive advantage in the moment, eventually, the activity must shift back to the impaired hemisphere and the degree to which function shifts back to the injured hemisphere is correlated with behavioral recovery (Krakauer et al., 2012).

Early initiation of rehabilitation resulted in enhanced functional motor outcome in animal studies, providing support for the increased plasticity of the brain following a stroke (Faralli et al., 2013). It has been said that spontaneous neurobiological recovery is the most significant predictor in recovery during the first 8 to 10 weeks post stroke (Zandvliet et al., 2020). In order to take advantage of the brain's natural response to stroke through spontaneous recovery, there are ways to promote brain plasticity through pharmacological agents and physical training (Mascaro et al., 2014).

Animal Models of Rehabilitation

Most of what we know about ways to promote neural plasticity after stroke comes from the exploration of animal models. Rodents serve as a promising model for gaining an understanding of rehabilitation outcomes for stroke patients. Rats and mice are capable of learning skilled reaching tasks that humans carry out daily, such as reaching and grasping objects, making them a commonly utilized behavioral model in stroke research (Bell et al., 2014). In fact, rodents' reaching behavior is very similar to that of humans, and rodents possess anatomical similarities to the human forelimb in musculature and skeletal structure, as well as the neural control necessary to control movement (Klein et al., 2012). The movements of lifting, reaching, and advancing the arm to a target object are controlled mainly by the upper arm with assistance from the elbow. They also have dexterity of their digits that allow them to grasp in a way very similar to that of humans (Klein et al., 2012).

Additionally, rodents experience deficits in forelimb function after a stroke that resemble the impairments seen in humans (Hsu & Jones, 2006). Analysis of reaching ability in rats and mice post stroke shows permanent impairments of finger flexion, wrist rotation, sensory abnormalities and compensatory movements similar to those displayed by humans including trunk rotation to assist limb movement (Klein et al., 2012). Rodent models have also demonstrated a window of spontaneous recovery and response to rehabilitation, much like that in humans following stroke.

Gaps in Rehabilitation

As stated previously, the brain is subject to neuronal reorganization following ischemic injury, indicating an optimal time window for rehabilitation to occur. Additionally, neural plastic changes are influenced by experience, with one of the major drivers of neuroplastic change being meaningful behavior, indicating that plasticity can be positively impacted by rehabilitation (Carey et al., 2019). It has been shown that high-intensity, repetitive, task-oriented training results in the greatest improvement in motor recovery after a stroke in humans (Connell et al., 2014; Vive et al., 2020). However, the current rehabilitative efforts in practice do not achieve the required intensity to maximize recovery.

Changing the intensity of training during rehabilitation can influence the recovery performance, however most of the research has investigated the effects of increasing intensity in animal studies. Bell et al. (2014) showed that increasing training intensity leads to greater functional recovery in mice, with animals trained twice a day returning to pre-injury performance levels faster than those trained only once a day. In this study however, the low intensity group received training for 15 minutes or 100 reaches, whichever occurred first, which has previously been identified as more intensive training than what human patients receive during their rehabilitation (Krakauer et al., 2012). To investigate the effects of lower intensity training, Nemchek et al. (2021) implemented a lower intensity-intermittent training where animals were trained every other day on the impaired limb. Their results showed that intermittent training resulted in better functional recovery compared to control animals who did not receive training, but they performed worse than the traditional training animals which were trained daily. This suggests that training every other day is not optimal for maximum recovery. These studies indicate that there needs to be further investigation into an optimal rehabilitative strategy that more accurately depicts the intensity seen in human trials.

Current Study

The current study serves to further investigate the effects of training intensity to reach optimal rehabilitation. It is already known that daily skilled reach training following a stroke in rodents results in improved motor ability. However, the appropriate amount of rehabilitation required for optimal motor function is still questionable. It has previously been found that higher intensity rehabilitation with two daily training sessions of the pasta matrix reaching task (PMRT) results in faster acquisition and more persistent performance (Bell et al., 2014). The intensity level in the mentioned study allowed for 100 reaches or 15 minutes of training per trial. Unfortunately, human stroke patients do not usually receive this level of intensity with their rehabilitation. The goal to find a training paradigm that can be reasonably applied to humans is still unresolved. Research investigating the lower boundary of intensity in mice models found intermittent training of every other day to still be less effective than traditional daily training on the PMRT (Nemchek et al., 2021). The current extends on these two findings by training mice with a lower intensity of training, 50 reaches or 10 minutes per trial, two times a day. The reasoning behind this design is that human rehabilitation relies on compensatory movements and as a result is less intense than training done with mice. However, if the intensity during a session can be decreased, then the patients could potentially replicate their rehabilitation in their own time to allow for optimal motor control improvements.

Methods

Subjects

The study began with 40, well handled, 4-month old male C57BL/6J mice. The mice were group housed with three or four animals per cage and standardized housing supplementation including cardboard roll and PVC pipe (Tennant & Jones, 2009). Animals were on a 12:12 light-dark cycle to maintain normal circadian rhythms. Animals were mildly food deprived throughout the study to encourage reaching behavior, with each animal receiving 2.5-3g of standard rodent chow daily. Animals were weighed daily with food allotments adjusted to maintain at least 85% of free-feeding body weight. Animal use was in accordance with a protocol approved by Illinois Wesleyan University's Institutional Animal Care and Use Committee.

Preoperative Behavioral Methods

All animals were trained on the Pasta Matrix Reaching Task (PMRT) to assess skilled motor function (Tennant & Jones, 2009). The PMRT took place in a Plexiglass chamber (8.5 cm wide x 15 cm long x 20 cm tall) where mice were trained to reach through a small (0.5 cm) slit in the center wall of the chamber to break 3.2 cm pieces of vertically oriented, uncooked capellini pasta. The pasta pieces were placed 2 mm apart in a 10 x 10 heavy-duty plastic block located outside the reaching chamber with half of the pasta piece located down inside the plastic block and the other half protruding above. Prior to the beginning of pre-operative training, mice were shaped to allow them to designate their preferred limb and introduce them to the reaching chamber. Shaping procedures consisted of placing animals individually in the reaching chamber with the pasta matrix full of pasta, allowing animals to reach with either limb. Each shaping session lasted either 10 minutes or until the mouse reached 10 times. Limb preference was determined when a minimum of 70% reaches were made with the same forelimb.

Upon determination of limb preference, all mice were trained on the PMRT to establish skilled motor performance. During training sessions, the pasta matrix was filled on the side contralateral to the preferred limb, forcing the animal to reach only with the desired forelimb. Animals were trained 5 days a week for a minimum of 20 and a maximum of 24 training sessions. Each training session lasted for 15 minutes or until the animal made 100 reaches, whichever happened first. The number of completed reaches and number of pasta pieces broken were recorded. The number of pasta pieces broken during the final three training sessions were averaged together to determine the pre-operative performance level. In order for the animal to reach criterion and continue in the study, they must have an average minimum of nine broken pieces. Those who successfully met criterion (n = 23) were given a photothrombotic surgery to induce ischemic stroke contralateral to the preferred reaching limb.

Photothrombotic Stroke Induction

Upon the completion of preoperative training on the PMRT, the mice who met criteria (breaking an average of at least nine pieces) received a unilateral photothrombotic stroke affecting the preferred reaching limb (n=23). Mice were first given an intraperitoneal (i.p.) injection of photosynthetic dye Rose Bengal (100 mg/kg; Sigma Aldrich) at least 10 minutes prior to laser illumination. Immediately following the Rose Bengal injection, mice were anesthetized with ketamine (100 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.). They were then placed in a stereotaxic frame and an incision was made midline in the scalp to expose the skull. A 532 nm, 20 mW green laser (1 mm in diameter; Beta electronics) was illuminated for 20 minutes over the exposed skull, directly above (5 mm) the brain region responsible for motor movement of the preferred limb (0.3 mm anterior to Bregma; 1.5 mm from midline, i.e., forelimb reaching area contralateral to preferred limb). The incision was then sutured and treated with antibiotic ointment. Animals were allowed to recover in a heated chamber and given buprenorphine (3 mg/kg at 0.015 mg/mL in sterile saline, subcutaneously) as an analgesic before returning to their home cages. A total of 6 mice were excluded from the study due to surgical complications: one mouse died during stroke induction, the remaining five died or were anesthetized within the 48-hour window for failure to thrive after surgery.

Post-operative Behavioral Methods

All mice were given three days to recover from the photothrombotic insult before receiving an assessment of their impaired forelimb on postoperative day 4 (POD 4). The remaining 17 mice were divided up into 4 postoperative training groups: The traditional group (n=4) who received training for 15 minutes or 100 reaches once daily for 28 days, the modified traditional group (n=4) who received training for 10 minutes or 50 reaches, two times daily for 14 days (28 total trials), low intensity (n=5) who received training for 10 minutes or 50 reaches, once daily for 28 days, and control animals (n=4) who did not receive postoperative training. Groups were matched based on preoperative performance. This difference in training days accommodated the different training modalities, such that all animals received the same total trials of rehabilitative training.

Mice began rehabilitative training on POD 5, with the animals trained to reach with their impaired limb on the same PMRT used in the preoperative procedure. Control animals were placed in the reaching chambers, but were not given a pasta matrix to reach for. Control animals were paired with a trained animal and an equal number of pasta pieces broken by the trained animal were dropped into the control animals' chambers. Control animals remained in the Plexiglass chamber for the same amount of time as their training partner. All animals were assessed on their impaired limb after 14 and 28 days of rehabilitative training. Assessments consisted of 15 minute trials or 100 reaches, whichever occurred first. After all animals completed their rehabilitative training, probe assessments were given every 7 days to assess the retention of the improvements made in performance from rehabilitative training.

Tissue Processing and Lesion Analysis

After all of the postoperative training and assessments were completed, mice were euthanized by 0.2mL of Pentobarbital and transcardially perfused with 50 mL 0.1M phosphate buffer, followed by 100 mL 4% paraformaldehyde. Brain tissue was extracted and stored in 4% paraformaldehyde. Lesion verification will be performed at a later date.

Results

A one-way ANOVA revealed no statistical differences between groups on the preoperative reaching data ($F_{(13,3)}$ = .490, p = .695), indicating that all groups had similar preoperative reaching abilities.

Statistical analyses were performed using repeated measures ANOVAs looking at the success of reaches across assessments, post-operative day, and rehabilitative trials. Results can be viewed graphically in Figure 1, Figure 2, and Figure 3. Within subjects effects reveal a significant main effect of Assessment ($F_{(5,65)}$ = 8.703, $p < .001$). The main effect of Assessment by Day interaction was not significant ($F_{(15,65)}$ = 1.556, p = .112). Additionally, there was no main effect of Group in the between subjects analysis ($F_{(3,13)}$ = 2.425, p = .112), indicating no difference in assessment performance among the different treatment groups.

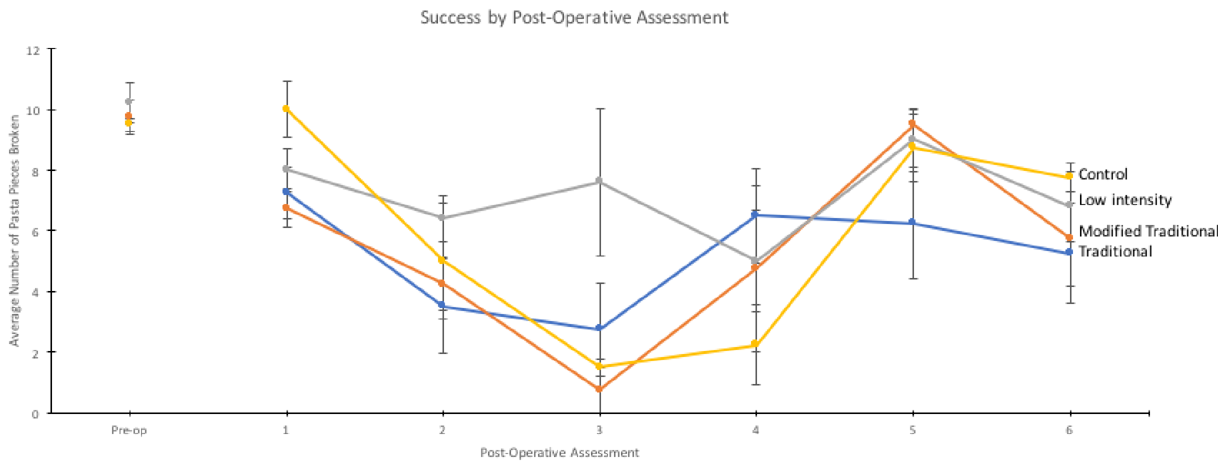

Figure 1. All animals performed similarly on preoperative assessments. Following the completion of rehabilitative training, animals across all groups were still not performing at their preoperative levels and there was not a significant difference in reaching success among the groups.

When looking at reaching success by trial (depicted in Figure 2), within subjects analyses revealed a significant main effect of Trial ($F_{(27,270)} = 3.098$, $p < .001$) and a Trial by Group interaction ($F_{(54,270)} = 2.293$, $p < .001$), indicating that all animals exhibited changes in reaching success across rehabilitative trial. However, there was not a significant main effect of Group in the between-subjects analysis, ($F_{(2,10)} = 3.00$, $p = .095$), suggesting no difference in performance between treatment groups.

Figure 2. Reaching success by trial shows that animals showed differences in their performance on individual training trials. However, there were not significant differences in the performance among the different treatment groups.

Finally, within subjects analyses revealed a significant main effect of Postoperative Day ($F_{(13,130)}$ = 4.795, p < .001; Figure 3) and a significant Postoperative Day by Group interaction, ($F_{(26,130)}$ = 2.204, p = .002), indicating all animals exhibited changes in reaching performance across postoperative day. Similar to the previous analyses, there was not a main effect of Group in the between-subjects analysis, ($F_{(2,10)}$ = 2.173, p = .165), revealing no difference in reaching success between treatment groups.

Figure 3. Reaching success depicted by training day showed no significant differences among the treatment groups. All animals appeared to be improving until around day 10 or 11 when performance begins to decline across all groups and persist throughout the completion of training.

The means, standard deviations and standard errors of means for postoperative assessments are depicted in Table 1 below. Means and standard errors for individual training sessions can be provided upon request.

Post-Operative Reaching Success

	Pre-Op Average	Mean	St. Dev	SEM
Pre-Op	Traditional	9.50	.431	.216
	Modified Traditional	9.75	1.100	.550
	Low intensity	10.20	1.466	.656
	Control	9.50	.431	.216
Assessment 1	Traditional	7.25	1.708	.854
	Modified Traditional	6.75	1.258	.629
	Low intensity	8	1.581	.707
	Control	10	1.826	.913
Assessment 2	Traditional	3.50	3.109	1.554
	Modified Traditional	4.25	1.708	.854
	Low intensity	6.4	1.673	.748
	Control	5	3.830	1.915
Assessment 3	Traditional	2.75	3.096	1.548
	Modified Traditional	0.75	1.500	.750
	Low intensity	7.6	5.413	2.421
	Control	1.5	.577	.289
Assessment 4	Traditional	6.5	3.109	1.554
	Modified Traditional	4.75	5.500	2.75
	Low intensity	5	3.742	1.673
	Control	2.25	2.630	1.315
Assessment 5	Traditional	6.25	3.686	1.843
	Modified Traditional	9.50	1.000	0.500
	Low intensity	9	2,345	1.049
	Control	8.75	2.217	1.109
Assessment 6	Traditional	5.25	3.304	1.652
	Modified Traditional	5.75	3.100	1.548
	Low intensity	6.8	2.588	1.157
	Control	7.75	.957	.479

Table 1

Table showing means, and standard errors among all groups for all assessments

Discussion

This study is a pilot study and, as such, concrete conclusions cannot be drawn from the data. However, for the purposes of this thesis project, I would like to speculate what the current data might indicate and how it could be improved upon to find more conclusive results.

Analysis of brain tissue and lesion verification will occur at a later date and will be very important for this study, especially since the behavior did not follow the typical pattern expected in this type of study. Across all groups, the animals performed surprisingly well on their first assessment on POD 4. This might indicate that the lesion was not as large as we originally thought, and thus did not have as much of an impact on their reaching ability. Interestingly, there was a large drop in all animals' reaching success after assessment 1, which does suggest that they received strokes. In fact, the data recorded on POD 5 actually resembles what is typically seen in the first assessment on POD 4. The photothrombotic stroke model is a progressive lesion (Labat-Gest & Tomasi, 2013), but data have consistently reported deficits after four days (Nemchek et al. 2020a and b; Bell et al., 2014). The delay in reaching impairment is an interesting finding, and tissue analysis will give further insight into possible reasons this may have happened.

Additionally, the rate of spontaneous recovery seems to be delayed in these animals as well. Typically, the control animals, who do not receive any rehabilitative training, do show some improvements on their reaching abilities after their initial post-stroke assessment, although they do not always reach preoperative performance levels (Bell et al., 2014; Kerr et al., 2016; Nemchek et al., 2021). The control animals in this study show a decrease in reaching success until after assessment 4, on POD 41, at which point they begin to show improvements that persist for the remainder of the study. This is atypical, as the animal rehabilitation literature has shown that spontaneous recovery usually happens in the first month after a stroke, when the brain is the most plastic. Human stroke survivors also exhibit some level of spontaneous recovery, again with most of the gains demonstrated in the first three months after stroke (Wahl & Schwab, 2014).

One of the major complications in the data is that there does not appear to be rehabilitation with traditional postoperative training. During training and immediately after, animals across all groups were performing at their worst, even worse than immediately following their strokes. This is inconsistent with all of the data that has previously been collected in the lab, as well as the literature consensus (Tennant et al., 2009; Kerr et al., 2016; Bell et al., 2014). Since we did not replicate the rehabilitation effect in the traditional training group, we cannot make any claims about what is happening in the modified traditional and low intensity groups. There may be some suggestion that the low intensity training group is a sufficient treatment based on their late assessment data, however we cannot be sure about this without a definite rehabilitation effect control group to compare to. What is interesting is that beginning at assessment 4, one week after training ended for the low intensity and traditional training groups, animals did exhibit improved performance. This suggests that assessment 3 was an anomaly which will be explained in greater detail below.

Another important consideration in this study is how we tested rehabilitation improvement. When the animals performed their assessments, they all consisted of 100 reaches or 15 minutes, whichever occurred first. The modified traditional rehabilitation group and low intensity group were used to only performing 50 reaches during their training sessions.

It is possible that they learned that they did not have to reach as many times during their sessions than they previously were during the preoperative training. It is also possible that these animals did not have the physical stamina to reach 100 times in a session and became fatigued during their assessments. This could contribute to their lower levels of reaching performance during assessments, especially assessment 3, since many animals did not perform 100 reaches during that trial. However, the low intensity training group seemed able to perform 100 reaches without trouble during most assessments, so it seems like 100 reaches for an assessment is suitable and something the animals are capable of performing. As mentioned, it is also what all animals were trained to do preoperatively, so while it was not immediately familiar to some animals reaching less postoperatively, it was not novel. Perhaps, performing two sessions in one day made the modified intensity group more fatigued than the lower intensity group, but they are ultimately performing the same total number of reaches that the traditional rehabilitation group received, so there is no reason to believe that the modified traditional group is too intense of training that would hinder their performance. Additionally, the animals in the modified traditional training group completed their rehabilitation before the other groups since they were training twice a day, compared to only once a day. The modified group would have then had 14 days without training before assessment 3, while the other animals were still training daily. This could also contribute to the decreased reaching attempts seen in assessment 3, since they had not been performing the task in a while. However, the control animals did not have a pasta matrix in front of them and did not perform reaches during any of the training sessions, and they still were capable of completing the assessments and performing 100 reaches, indicating that the rest period the modified traditional group received should not have disrupted their reaching abilities.

For argument's sake, it seems as though the third assessment was an anomaly, and it is likely that there were abnormalities in the environment that we were not aware of while conducting assessments. This speculation is based on the lack of reaching behavior widespread across groups, as well as a couple animals who were overweight. If assessment day 3 data is excluded, and assessments 2, 4, 5, and 6 are analyzed, it appears as though we have replicated the rehabilitation effect. It also appears as though modified traditional and low intensity training methods are effective treatment options. This is a very interesting finding and should be investigated further, with a larger sample size to distinguish between minor differences between the low intensity and modified traditional groups that may not be present in the current study.

Looking at the rehabilitation success by day also shows interesting results. First, it appears as though there was an external change around day 10 or 11, as all of the animal groups seemed to be rehabilitating until this point, in which the performance levels of all groups dramatically decreased. Reaching success picks back up again in the later probe assessments, specifically assessment 5 and 6, suggesting that the animals did rehabilitate their impaired limb, they just were not utilizing it during the late postoperative training days. This could have occurred as a result of fatigue or a lack of motivation. Additionally, during those later assessments, all groups were performing better than the control animals, indicating that the rehabilitative training was effective at improving motor function of the impaired limb.

Future Directions

The most obvious limitation to this study is the sample size. It is very difficult to analyze the effects of different treatment groups with only four or five animals per group, and especially difficult to perform statistical analyses on small groups. With a small sample size, any outliers have a significant impact on the overall group averages, which normally flushes out in a larger group size or can be systematically identified and eliminated. To improve on this study, and be able to draw conclusions on the effects of different rehabilitation manipulations, the sample size should be at least 7-10 animals per group.

Another suggestion for replication of this study in the future should ensure that animals are weighed every day, and maintained at 85% of free feeding body weight to increase their motivation for reaching performance. During the current study, animals were weighed at least 5 times a week to determine food allotments, with the assumption that the amount of food provided over the weekend could be kept consistent. As it turns out, this is not as reliable as expected. As a result, there were instances of mice being over their 90% free-feeding weight during rehabilitation training or assessments, negatively impacting their total reaching performance. Additionally, maintaining the animals closer to their 85% free-feeding weight instead of 90% should increase their motivation to reach for pasta. It also allows for more leniency in weight shifts from day to day, as they would be more likely to remain below their 90% weight, and motivated to reach for pasta.

Lastly, consideration of the training and testing parameters in future studies is incredibly important. This study aims to shed light on the effects of different intensity levels of rehabilitation training on functional motor recovery. We know that experimental models of stroke rehabilitation are more intense than what human patients receive in clinical settings. In order for patients to reach the higher level of intensity that is required for optimal recovery, it is possible for the patients to break up their rehabilitation into multiple sessions, while still allowing for them to reach the total intensity in a daily session that is necessary for recovery, modeled by the modified intensity training group. Additionally, the lower boundary of intensity has yet to be determined, which was investigated in this study by the lower intensity training group. Both modified intensity training and lower intensity training groups were expected to perform 50 reaches, but were limited by a maximum time allowance of 10 minutes. It is possible that removing that time allowance and just focusing on the total number of reaches performed, would give more insight into the success of the rehabilitation training. The main goal of the rehabilitation training is for the animals to perform a specific number of reaches that is correlated with different intensity levels. It is possible that when these two groups did not perform 50 reaches, it was due to a lack of time, and if they had performed the maximum number of reaches during each training session, the results of their reaching success and total recovery may have been higher. In future studies, providing a more gracious time allotment that does not interfere with their ability to achieve the goal number of reaches per trial, might allow for greater recovery and shed more light on the effectiveness of these different training groups.

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