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Operant Conditioning of Human Upper-Limb Stretch Reflexes

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Abstract

Operant conditioning of short-latency reflex (SLR) and H-reflex have been investigated in animals and humans. We modified previously established protocols for conditioning lower limb H-reflexes to up-condition the SLR in brachioradialis muscle and to investigate the effect of such conditioning on the long-latency response (LLR) of synergistic muscles. Our study included 12 healthy participants. Each participant took part in 3 baseline and 27 conditioning sessions in 6 weeks, followed by two additional sessions one month and two months after the last conditioning session. We found an increase in SLR magnitude in 50% of participants (by 15%$\pm$2.50). However, there was no significant effect at the population level. We could not find any statistically significant correlation between SLR in the conditioned muscle with SLR and LLR in synergistic muscles. Although our findings in healthy adults were weak, studying patients with spinal cord injury or other motor dysfunctions might show clinically significant results.

Keywords

Stretch reflex, Spinal stretch reflex, short-latency stretch reflex, long-latency response, H-reflex, operant conditioning, visual feedback, task-dependent adaptation, long-term plasticity, plasticity, spinal cord, brachioradialis, biceps brachii, pectoralis major.

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Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1

1 Introduction

Operant conditioning is a form of associative learning where a particular behaviour is reinforced by its consequences. When a child does their chores, paying them a compliment or money will make it more likely that they will do their chores in future. When a child touches a hot stove, the evoked pain will make it less likely that they touch a hot stove in future. Although operant conditioning is described at the behavioral level, operant conditioning manifests as plasticity at various levels of neural description. For example, Fetz (1969) provided monkeys with food reward when single neurons in primary motor cortex produced particularly high firing rates. He famously showed that, after some period of training, the monkeys could quickly increase the activity of an isolated neuron (Fetz, 1969). In the aplysia (a sea slug), operant conditioning of feeding behavior has been attributed to one particular neuron (B51) and the influence of dopamine (Brembs, Lorenzetti, Reyes, Baxter, & Byrne, 2002).

This thesis focuses on the plasticity of spinal circuits, specifically operant conditioning of the spinal stretch reflex. The spinal stretch reflex traverses a mono- and oligo-synaptic pathway that is purely spinal and is usually thought to support motor function by resisting unexpected muscle stretch (Liddell & Sherrington, 1924). When a physician tests a patient's reflexes by applying a tendon hammer to their patellar tendon, it is the short-latency stretch reflex that makes their leg jump (Fig.1). Historically, the spinal circuits that support the short-latency stretch reflex have been considered hardwired, contributing to the control of behavior in a stereotyped way (Hall, 1833). But we know now that the spinal circuits are plastic and extensive work suggests that this plasticity may be a major contributor to motor control and motor learning (for review, see (Grau, 2014)).

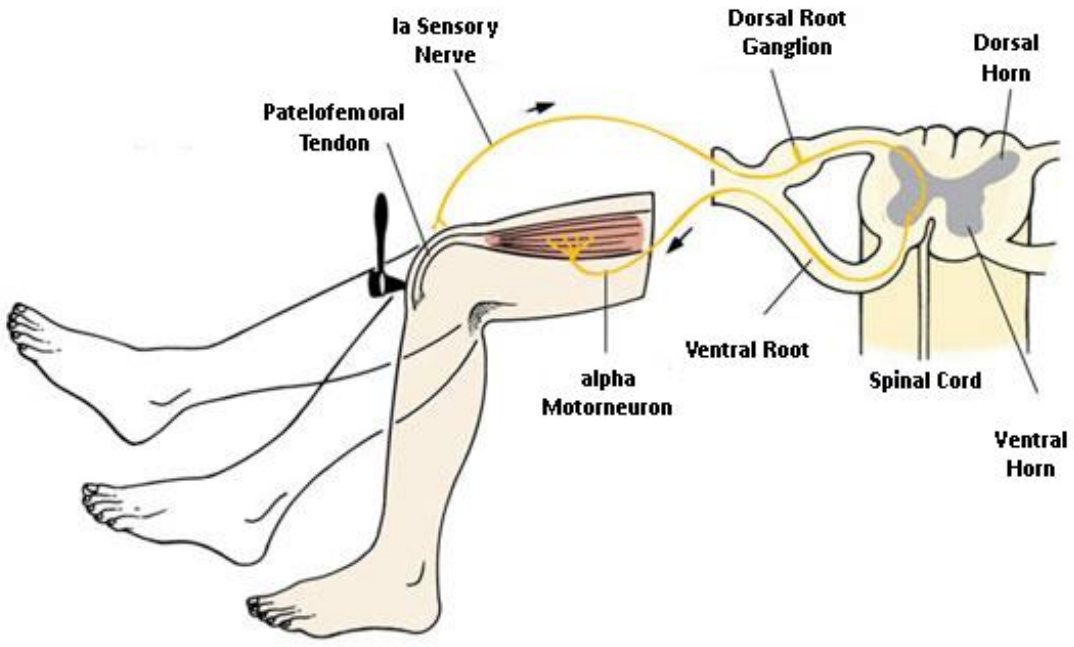

Figure 1. Simplified patellofemoral (knee jerk) reflex. Tapping on tendon stretches the muscle and therefore, intrafusal fibers within the muscle spindle are stretched. Then the muscle spindle sends signal to the spinal cord through Ia sensory nerve, which synapses with alpha motoneuron. Then alpha motoneuron goes to the stretched muscle and contracts it (downloaded and modified from:

http://somemedicalthoughts.blogspot.com/2012/02/reflexes-no-brainer.html)

One major line of research investigating the plasticity of spinal circuits, and the central motivation of this thesis, comes from the work of Jonathan Wolpaw and his colleagues who have been investigating the operant conditioning of spinal circuits, mostly Hoffman reflex (H-reflex), the electrical analog of the spinal stretch reflex, and mostly in the lower limb. Over thirty years, they have demonstrated that giving direct positive reinforcement (with a sip of water or food pellets) about the evoked magnitude of the H-reflex – on the order of thousands of trials – that humans, monkeys, rats, and mice can be operantly conditioned to up-regulate and down-regulate the sensitivity of their H-reflex (X. Y. Chen & Wolpaw, 1995, 2002, 2005; Makihara, Segal, Wolpaw, & Thompson, 2014; Segal &

Wolf, 1994; Thompson, Chen, & Wolpaw, 2009; Thompson, Pomerantz, & Wolpaw, 2013; Wolpaw, 1987; Wolpaw, Braitman, & Seegal, 1983; Wolpaw, Kieffer, Seegal, Braitman, & Sanders, 1983). Moreover, they have described the neuronal mechanisms that underlie up-regulation and down-regulation (for review, see (Thompson & Wolpaw, 2014; Wolpaw, 2010)) and have shown that the corticospinal tract (CST) and cerebellum are necessary to induce spinal cord plasticity in the context of their operant conditioning paradigm (X. Y. Chen & Wolpaw, 1997, 2002, 2005; Wolpaw & Chen, 2006). Of particular note is a series of recent studies showing that H-reflex conditioning can be done in spinal-cord injured patients and that such a procedure can be leveraged for therapeutic effects such as reducing spasticity and improving gait symmetry and speed (Segal & Wolf, 1994; Thompson et al., 2013). Thus, although spinal reflexes are very simple responses, they are also components of more complex skills and behaviors such as locomotion. This has led to the general idea that conditioning a spinal reflex can manifest as a substantial change in real-world motor behavior (Wolpaw, 2010).

This thesis is motivated by the long-term idea that, like for gait, inducing spinal cord plasticity could be useful for improving hand and arm function in various clinical groups. As a first step towards this goal, the main aim of this thesis is testing the veracity of conditioning spinal stretch reflexes of the arm in healthy adults. We focus on spinal stretch reflexes rather than H-reflexes because (1) H-reflexes are technically difficult to evoke in arm muscles, (2) the H-reflex is never evoked during real-world motor control, and (3) the stretch reflex is generated by muscle spindle afferents whose sensitivity is under active control via gamma motor neurons (Strominger, Demarest, & Laemle, 2012). Our study closely follows the most recent procedures developed by Thompson and colleagues (Thompson et al., 2013). Critically, this paradigm expands on previous work looking at stretch reflex conditioning in the upper-limb (Wolf & Segal, 1996) by including a set of non-reinforced trials (details in Chapter 2) in each training session which allows us to differentiate task-dependent adaptation, which occurs within a session and is limited to the conditioning paradigm, from the long-term plasticity that occurs over many sessions and transfers to other tasks (Thompson et al., 2009).

We had two secondary aims. First, we wanted to test whether operantly conditioning the spinal stretch reflex of a particular elbow muscle could evoke plastic changes in other synergistic elbow and shoulder muscles. Such generalization would imply that operant conditioning has a broad effect on the spinal circuitry. Second, we wanted to examine the effect of conditioning the spinal stretch reflex on longer-latency stretch reflexes that engage additional neural circuits in the brain stem and cerebral cortex (Kurtzer, 2014). Specifically, previous work has shown that long-latency stretch reflexes are generated at the shoulder when mechanical loads are applied at the shoulder and elbow such that there is no net motion of the shoulder because of interaction torques that arise between the joints (Kurtzer, Pruszynski, & Scott, 2008). Although part of this sophisticated response, which accounts for the arm's intersegmental dynamics, is the result of a transcortical processing pathway (Kurtzer, 2014; Pruszynski et al., 2011), there is also some evidence that spinal circuits contribute to the long-latency stretch response (Kurtzer, Crevecoeur, & Scott, 2014). Thus, we wanted to test whether operantly conditioning the spinal stretch reflex in an elbow muscle could evoke plastic changes in the long-latency stretch reflex of its coupled shoulder muscle. Such plasticity would be notable because it would provide the most direct evidence to date that the spinal circuit can also incorporate knowledge of the arm's intersegmental dynamics.

The sections that follow elaborate on the core concepts that underlie the thesis – specifically operant conditioning and stretch reflexes – and provide a broader account of the relevant literature.

1.1 Operant Conditioning

Operant conditioning is a form of associative learning where an animal's behaviour is modulated by its results or consequences. Rewarding favourable consequences of a behaviour can positively reinforce the behaviour while decreasing or removing the negative or unfavourable outcomes of a behaviour (avoiding negative consequences) can negatively reinforce the behaviour. Both negative and positive reinforcement increase the frequency of behaviour. On the other hand, punishment can reduce the frequency or eliminate the behavior. Perhaps the most famous experimental example comes from Skinner (Skinner,

1930), who experimentally analyzed the behavior of animals in the context of positive and negative reinforcement. For example, Skinner showed how positive reinforcement works by placing a food deprived rat in a so-called Skinner box. Inside the box was a lever. As the rat moved about the box it would at some point accidentally push the lever, which would immediately cause a food rewards to be dispensed. The rats quickly learned to press the lever when placed inside the box (Fig. 2).

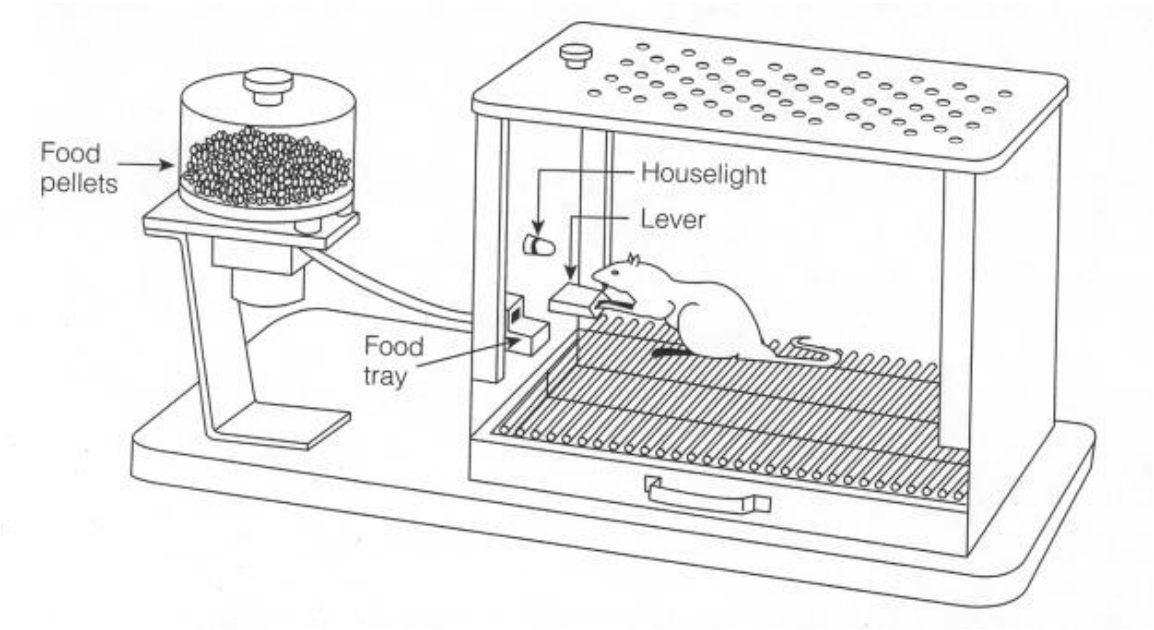

Figure 2. Skinner box or operant conditioning chamber. Pressing the lever by the rat turns on the green light and is rewarded by food pellets. (The image is download from Appalachian State University website: http://www.appstate.edu/~steelekm/classes/psy3214/images/chamber2.gif)

Although operant conditioning is inherently a behavioral phenomenon, there has been a great deal of work investigating how it manifests at various levels of the nervous system. Olds and Milner (1954) implanted permanent electrodes in different areas of rat's brain and used skinner box in combination with electrical stimulation to find rewarding centres in rat's brain. Showing desired behaviour was only rewarded by brain stimulation. They

reported that simulation of some areas (most strongly in the septal area) positively reinforces the behaviour. On the other hand, stimulation of some areas causes the opposite effect and animal would do anything to prevent the stimulation (Olds & Milner, 1954). Building on the concepts of Skinner, recent work has shown that rats can be operantly conditioned to self-administer electrical stimulation to various parts of the brain, suggesting a neural basis for reward signals (Carlezon & Chartoff, 2007; Desai et al., 2014).

Conditioning can also occur at the level of single neurons. For example. Fetz reported successfully conditioned single neuron in primary motor cortex of non-human primate (*Macaca mulatta*) (Fetz, 1969). In this work, action potentials from a single neuron were isolated and the firing rates were recorded. Anytime the firing rate of the isolated neuron reached an arbitrary level, the animals were provided food reward. During the last conditioning sessions, animals were able to readily increase the firing rate of the isolated neuron 50-500 percent above their baseline level. Thus, the animals were operantly trained to control the activation level of the recorded neuron.

Operant conditioning was also used to modulate eating behaviour in aplysia (Brembs et al., 2002). The anterior branch of esophageal nerve (En2) is both necessary and sufficient for food reinforcement in aplysia. Stimulation of En2 (as a substitute for food reinforcement) was used to operantly condition biting behaviour and it was shown that such stimulation leads to changes that last for 24 hours after conditioning abolishment, i.e., after stopping stimulating En2 Nerve. In addition, the buccal neuron (B51) pivotal for ingestion behaviour, was isolated and investigated to see whether changes in bites (because of operant conditioning) causes any change in B51, and whether B51 was the site of memory storage for operant conditioning. In vitro and in vivo investigations showed changes in cells that would increase the probability of B51 activation and developing ingestion-like behaviours due to conditioning. In the experimental group, contingent administration of dopamine led to significant decrease in burst threshold that was the same finding seen in conditioned animals. This study highlighted B51 as one of the plasticity sites in aversive behaviour of aplysia and on the role of dopamine in reward-learning and operant conditioning.

Like animals, operant conditioning can induce changes in human cortex. In a functional magnetic resonance imaging study, Puschmann and colleagues showed auditory cortex changes in participants who were trained to associate specific auditory tones with reward and such changes were not observed in non-learners. Moreover, the changes were seen in the ventral tegmental area and the nucleus accumbens, which are core dopaminergic regions. As in the animal work, these results implicate the role of dopamine in operant conditioning and plasticity in auditory cortex (Puschmann, Brechmann, & Thiel, 2013).

1.2 Reflexes

The precise definition of reflex remains debated (Prochazka, Clarac, Loeb, Rothwell, & Wolpaw, 2000) but, typically, reflexes are considered relatively simple, involuntary and automatic responses to a specific stimulus. Although reflexes occur as a consequence of sensory inputs in most, if not all, modalities (Strominger et al., 2012), here we focus on muscle stretch reflexes where sudden muscle stretch evokes a multi-phasic compensatory response that, in its simplest form, attempts to return the muscle to its pre-stretch length. The sensory input that drives the stretch reflex arises largely from muscle spindle receptors, which are transmitted to the spinal cord, brain stem, and cerebral cortex. Below, we briefly explain some of the important features of the stretch reflex.

1.2.1 Spinal Stretch Reflex

After a muscle is stretched, the earliest response (20-50 ms post-perturbation) is purely spinal and is usually called the spinal stretch reflex, but is also known as the short-latency, myotatic or deep tendon reflex. In its simplest form the SSR – first described by Sherrington and Liddel (1924) –traverses a mono-synaptic pathway that is ipsilateral and intrasegmental (i.e., synapses with the neuron at the same segment). The SSR has four key components: a muscle spindle, Ia sensory fiber, alpha motoneuron, and extrafusal muscle fibers. Muscle spindles are embedded in intrafusal muscle fibres that lie parallel to the extrafusal fibers in muscle. Intrafusal muscle fibres do not generate muscle force but they change length when extrafusal fibres contract or relax, and the muscle spindles sense these changes in length. Taping on the patellar tendon stretches spindle fibers of knee extensor

muscles. The excited spindles send their signals through Ia sensory fibers to the spinal cord. These sensory afferents directly synapse on alpha motoneuron that innervate the same muscle that houses the excited spindle which, in turn, causes the extrafusal fibers of the quadriceps muscle to contract.

While SSR seems to be a simple circuit, it is part of a larger system. For example, the SSR operates at the level of synergistic muscles. When the patellar tendon is tapped, spindles from knee extensor muscles to do not just excite motoneurons that innervate their parent muscle but, rather, make heteronymous connections to other muscles that contribute to knee extension. The SSR is also not purely mono-synaptic. Sensory inputs from the excited spindles in the stretched muscle inhibit activation of antagonistic muscle (i.e. knee flexors) through spinal interneurons. Spindles themselves are not static elements. They are innervated by gamma motoneurons that provide efferent control of their sensitivity. That is, the precise response of muscle spindles can, at least in principle, be controlled by the brain so that it optimizes the sensory inflow for the current task being performed. For example, recent work suggests that the brain controls gamma activation so that spindles signal a prediction of their future state rather than the current muscle length (Dimitriou, 2014; Dimitriou & Edin, 2008, 2010). In summary, the SSR is a monosynaptic and oligosynaptic spinal reflex but it is influenced by supra-spinal centres.

1.2.2 H-Reflex

The Hoffmann reflex or H-reflex is the electrical equivalent of SSR in which, instead of mechanically stretching a muscle, superficial or deep electrodes are used to electrically stimulate nerves (usually tibial nerve in popliteal fossa) and recruit the monosynaptic reflex pathway (Fig. 3). Stimulating the nerve in this fashion causes two muscle responses. First, the M wave is caused by direct stimulation of the axon of alpha motoneuron. Second, the H-reflex is due to stimulation of Ia sensory fibres that go to the spinal cord and synapse with alpha motoneurons. In other words, the H-reflex bypasses muscle spindle and intrafusal fibers and therefore, eliminates their effect on the activation of Ia afferent fibres (Knikou, 2008).

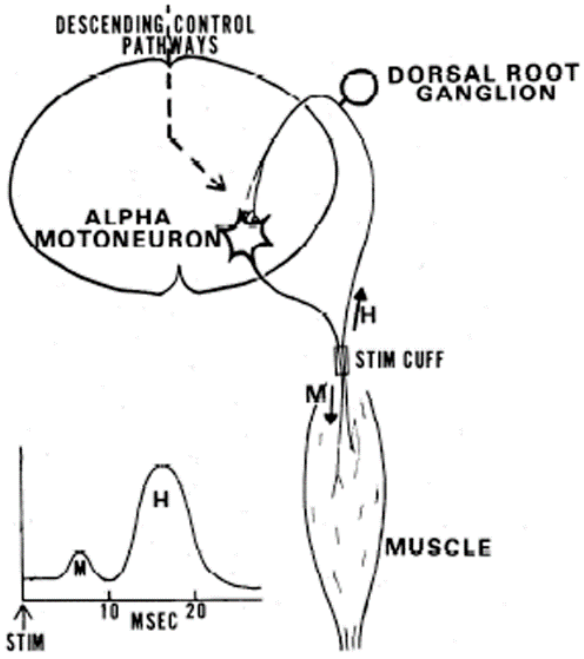

Figure 3. H-reflex and electromyographic recording of H-reflex (Wolpaw, 1987). The cuff around the nerve is used to evoke the reflex. The electrical impulse goes in two directions; one goes to the muscle through the axon of alpha motoneuron (making M wave on the EMG) and the other goes to the spinal cord through sensory nerves and synapses with alpha motor neuron. Alpha motoneuron goes to the target muscle and contracts it which is translated to H-wave on the EMG.

1.2.3 Long-Latency Stretch Reflex

As described above, the earliest response (20-50 ms post-perturbation) to mechanical stretch is called the spinal stretch reflex and is mediated purely by spinal circuits. The subsequent response to muscle stretch (50-100 ms post-perturbation) is called the long-latency stretch reflex (LLR).

The neural basis of the LLR has been an issue of substantial debate dating back to its original description by Hammond in the early 1950s. Hammond recorded biceps muscle activity after sudden elbow extension and found another stretch response after the short-latency monosynaptic stretch response that was late enough to be of non-spinal origin but

early enough not to be a voluntary reaction (Hammond, 1954). He then showed that only this later response was sensitive to how the participant intended to respond to the stimulus (Hammond, 1956). Thus, although the response was faster than typical measures of reaction time, a person's voluntary intent modulates the LLR. Based on latency, he suggested that the LLR may be caused by slowed conduction via a spinal pathway or fast conduction via a supra-spinal pathway – the latter being considered more likely because of the functional result that this reflex can be modulated by voluntary intent.

We know now that the LLR shows an incredibly broad range of functions similar to voluntary control and includes contributions from many neural generators in the spinal cord, brainstem, and cerebral cortex (for review, see (Pruszynski & Scott, 2012)). For example, spinalized cats and monkeys still exhibit a long-latency stretch reflex – indicating that supra-spinal centers are not required to generate the response (Leblond, Menard, & Gossard, 2001; Tracey, Walmsley, & Brinkman, 1980). On the other hand, patients with unwanted mirror movements caused by bifurcation of corticospinal projections at the level of primary motor cortex show bilateral long-latency reflex responses to unilateral muscle stretch (Capaday, Forget, Fraser, & Lamarre, 1991; Matthews, Farmer, & Ingram, 1990) – indicating that supra-spinal centers also contribute to the LLR.

1.3 Operant Conditioning of Spinal Reflexes

Previous studies have shown that spinal reflexes can be modulated through operant conditioning. However, operant conditioning is not successful in all participants and between-subjects variability exists. (X. Y. Chen & Wolpaw, 1995; Evatt, Wolf, & Segal, 1989; Makihara et al., 2014; Segal & Wolf, 1994; Segal et al., 2000; Thompson et al., 2013; Wolf & Segal, 1996; Wolf, Segal, Heter, & Catlin, 1995; Wolpaw, 1987; Wolpaw, Braitman, et al., 1983; Wolpaw, Kieffer, et al., 1983). The sections that follow provide a detailed account of this literature.

1.3.1 Animal Studies

The first study to operantly condition the SSR was done by Wolpaw and colleagues in 1983 (Wolpaw, Breitman, Segal, 1983). They focused on the biceps muscle in monkeys by

mechanically extending the elbow joint while recording muscle activity via chronically implanted fine wire EMG electrodes (Fig. 4). The monkeys performed 3000-6000 trials per day, their task being to maintain the hand at a set initial position. The monkeys received reward only if the magnitude of their SSR was above or below a set value (for the up- and down-conditioning groups, respectively) that changed slowly over the course of training. The SLR was successfully conditioned in 9 of 10 animals (Wolpaw, Kieffer, et al., 1983). Long-term follow-up after conditioning showed that changes in SLR sensitivity was long-lasting.

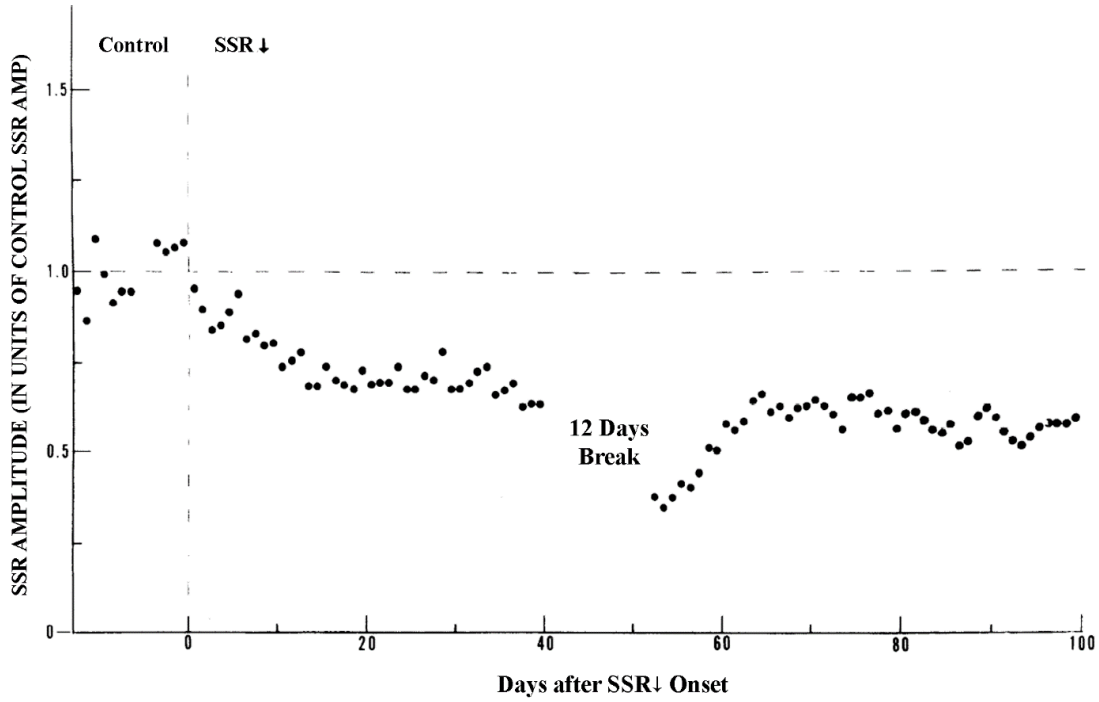

Figure 4. Daily change in SLR amplitude of a monkey under down-conditioning protocol. Twelve-day break did not wash out the effect of down conditioning Wolpaw, Braitman, et al., 1983).

In these seminal studies, Wolpaw and colleagues put forward two key ideas. First, that changes in SLR amplitude are caused by plasticity in the spinal cord as a result of supra-

spinal regulation. Second, that after long training periods, plastic changes in the spinal cord caused by supra-spinal influence could last long after supra-spinal influences were removed.

Wolpaw and colleagues quickly moved away from conditioning the upper-limb SSR, focusing instead on conditioning the lower-limb H-Reflex. There are several advantages of this approach. First, the H-reflex does not require restraining animals; that is, the animals can move freely while the stimulation is exerted and the H-reflex is recorded. The first experiment along these lines was done in monkeys, applying the same training protocol as for the biceps SSR to the triceps surae H-reflex (Wolpaw, 1987). They reported successful up- and down-conditioning of the H-reflex, suggesting that plasticity does not arise via changes in gamma motor neuron activity and changes in muscle spindle sensitivity.

Moving to the H-reflex, and the experimental freedom it allowed, prompted Wolpaw and colleagues to move towards rodent models which, in turn, permitted a more mechanistic investigation of the conditioning phenomenon. In rodents, the conditioning paradigm was essentially identical except that reward was given either via food pellet or via brain stimulation, rather than water. Indeed, rats and mice both demonstrate up- and down-conditioning of the H-reflex (X. Y. Chen & Wolpaw, 1995).

Chen and Wolpaw operantly down-conditioned and up-conditioned rats. Electromyography electrodes were implanted in the soleus muscle of 17 Sprague-Dawley rats and a nerve cuff was used to stimulate posterior tibial nerve. Rats could move freely and were rewarded by either food or medial forebrain bundle stimulation. The reflex was elicited around 3000-6000 per day per animal. Over a course of 3-4 weeks, the up-conditioning rats could increase the reflex by 158±54% and down-conditioning rats could decrease their reflex to an average (±SD) of 67±11%. The results of successful H-reflex conditioning in rats was similar to the results seen in monkey models and showed that operant conditioning of spinal reflexes and reflexes can be done in sub-primate animals and the possible similarity in results can make them suitable models for conditioning studies and understanding the mechanism of operant conditioning.

Electrophysiological studies provided information about the neuronal level plasticity and a possible mechanism of operant conditioning-induced plasticity.

Carp and Wolpaw investigated the characteristics of motoneurons of monkeys in a soleus H-reflex down-conditioning experiment. Recordings were obtained from motoneurons and were compared between successfully down-conditioned side and the contralateral side and ipsilateral side of unsuccessfully trained and non-trained monkeys. Successfully conditioned limb showed significantly more positive mean firing threshold (-52 mV vs. -55 mV) and lower mean conduct velocity (67 vs. 71 m/s) in comparison to the contralateral limb as well as naïve and unsuccessful animals (Carp & Wolpaw, 1994). Following these results and using a mathematical model, Halter et al. showed that the successful decrease in H-reflex by operant conditioning may be due to a positive shift in the voltage-dependent Na^+ channel, which would positively shift the firing threshold and decrease the excitatory postsynaptic potential (EPSP) (Halter, Carp, & Wolpaw, 1995).

Another study in rats measured the conduction velocity of tibial nerve of rats after exposing three groups of rats to triceps surae H-reflex up-conditioning, down-conditioning, and no conditioning protocols for 40 days. Conduction velocity was decreased significantly in successfully down-conditioned animals in comparison to control animals. This result was similar to the findings in monkeys. Nonetheless, no significant increase was seen in conduction velocity of successfully up-conditioned animals, which indicates different mechanisms for up-conditioning. In other words, down- and up-conditioning do not completely mirror each other at cellular level (Carp, Chen, Sheikh, & Wolpaw, 2001).

One important finding in conditioning studies is that the corticospinal tract (CST) is essential for conditioning the H-reflex. If CST is dissected before conditioning, down-conditioning does not happen, while if CTS is dissected after successful down-conditioning the effect will persist for 5 to 10 days after dissection (X. Y. Chen & Wolpaw, 1997, 2002). Longer persistence of conditioning after training in neurologically intact individuals suggests that higher centres help to maintain the effect through CST. In addition to CST, cerebellum input to brain is crucial to down-conditioning. If the output from cerebellar nuclei dentate and interpositus (DIN) is ablated before down-conditioning, H-reflex down-

conditioning will not occur. On the other hand, ablation of the output after down-conditioning leads to immediate disappearance of some conditioned decrease in reflex magnitude while most of the conditioned decrease in reflex size will remain for the next 40 days (X. Y. Chen & Wolpaw, 2005; Wolpaw & Chen, 2006).

In summary, changes in reflex magnitude through operant conditioning are the result of a complex pattern of plasticity in different parts of CNS, and the long-term effect of such plasticity and its concurrent effects on other behaviours is currently understood within the context of the negotiated equilibrium model as described in the discussion at the end of this thesis (Wolpaw, 2018).

1.3.2 Human Studies

A few human studies have investigated the operant conditioning of spinal reflexes (Evatt et al., 1989; Segal & Wolf, 1994; Wolf & Segal, 1996).

There are several important differences between human and animal studies that need to be emphasized. First, human participants cannot be restrained for a continuous experiment and their time commitment is limited. Therefore, human experiments typically involve 20-30 experimental sessions each lasting about one hour spread over approximately 1-2 months. The net result is that human conditioning protocols are based on <5% the number of conditioning trials in animal studies. Second, animals can be implanted with intramuscular electrodes to reliably stimulate the nerve and record muscle activity over long periods of time. In contrast, humans require placement of new electrodes with each experimental session – a potential source of measurement variability. Third, the nature of the reward is different. Animals have to be conditioned implicitly. They typically fasted or were water-deprived and they receive food or liquid for increasing the magnitude of their reflex response – an extremely salient reward. Other animals are conditioned via direct electrical stimulation to reward centers in the brain. In contrast, humans are conditioned explicitly, by presenting them with their reflex magnitude and asking them to increase their response above some arbitrary threshold.

Despite these differences, Evatt and colleagues (1989) first suggested that the SLR can be operantly conditioned in humans. Two additional studies using the same approach showed that up- and down-conditioning the SLR of a given muscle also yields changes in the LLR of the same muscle (Segal and Wolf, 1994; Wolf and Segal, 1996).

Most recently, Thompson et al. (2009) designed a protocol to up- and down condition the H-reflex of the soleus muscle through operant conditioning. They used superficial electrodes on the popliteal fossa to stimulate the tibial nerve. Participants stood in front of a monitor and their baseline muscle activity as well as feedback regarding H-reflex magnitude was provided on a monitor. The protocol consisted of 6 baseline sessions with no training or instruction to increase the H-reflex size, 24 training sessions with trail-by-trial feedback about H-reflex magnitude, and 4 follow-up sessions held in one-month intervals following the training sessions. Each session started by calibrating the stimulation intensity via the evoked M-wave. That is, after placing the electrodes they adjusted the stimulation so that the M-wave was the same as that in the baseline sessions – a process that attempts to account for variable electrode placement and changes in the skin-electrode interface. In baseline sessions, no feedback was provided about H-reflex size. In training sessions, an initial block was done without feedback, serving as a critical indicator of long-term plasticity that previous studies investigating the SSR did not include. Thereafter, 225 trials were performed where participants were provided trial-by-trial feedback about their H-reflex magnitude along with an explicit goal about how that magnitude should change. In their study, the H-reflex increased in 6 of 8 participants and decreased in 8 of 9 participants in up- and down-conditioning groups, respectively, with a mean percent change from baseline of 140±12% and 69±6%, respectively. They also indicated that long-term changes (indicated by differences in H-reflex magnitude between the initial control block and the baseline sessions) appeared after ~10 training days.

1.3.3 Clinical Studies

Experimenters have identified the potential of operant conditioning as a clinical intervention for conditions manifesting with changes in spinal reflexes (X. Y. Chen & Wolpaw, 1997; Y. Chen et al., 2006; Segal & Wolf, 1994; Thompson et al., 2013)

Chen et al. (2006) evaluated the effect of H-reflex up-conditioning on locomotion of rats with spinal cord lesions. The right lateral columns of 13 rats were dissected at midthoracic level (T8-T9). Treadmill locomotion was assessed after dissection and then eight rats were exposed to H-reflex up-conditioning protocol for 50 days and 5 served as controls. They used their previously established protocol to elicit H-reflex of tibial nerve and record EMG of soleus muscle. After conditioning, treadmill locomotion improved significantly. Moreover, up-conditioned rats showed symmetric step cycle on the treadmill (Fig. 5). In rats, lateral column is not essential for conditioning (X. Y. Chen & Wolpaw, 1997). Therefore, reward contingency in the brain may mediate conditioning via the dorsal column. This study showed that an appropriate protocol might help patients with spinal cord injury to restore their locomotion and step symmetry.

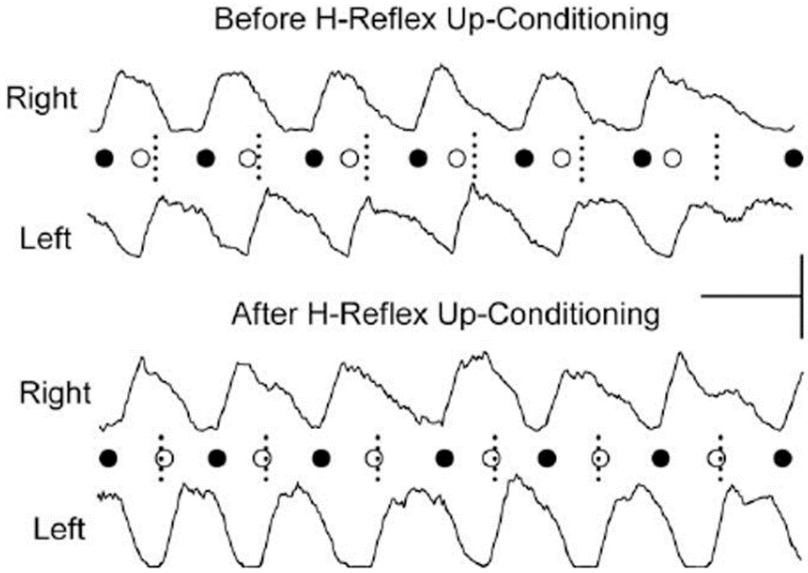

Figure 5. Improved step cycle in spinalized rat after up conditioning H-reflex (Chen et al., 2006). EMG burst of right and left hindfoot are shown. Expected times for left hindfoot step are shown by vertical dashed lines. Right and left hindfoot steps are shown by black and white circles, respectively. Before up-conditioning, left hindfoot steps occurred before expected times and caused asymmetry of step cycle.

Segal and Wolf (1994) first noted that a well-known feature of spinal cord injury is hyperreflexia and hypothesized that operant down-conditioning of the SSR in such patients could provide therapeutic benefit. They recruited seventeen chronic spinal cord injured patients, who were randomly assigned to the intervention and control group respectively. Participants performed the same number of trials over 6 baseline and 24 main sessions stretching over an 8-week period. The only difference in the groups is that the control group did not receive feedback about their reflex amplitude. By the end of the study, participants in the training group showed significantly reduced SSRs and such reductions persisted up to 4 months following the end of the training sessions. Critically, however, this work did not establish a firm link between the reduction of the SSR amplitude and a reduction in hyperreflexia. Moreover, it did not assess the effect of conditioning on restoring lost functions such as hand movement.

Thompson et al. implemented their H-reflex conditioning protocol in the lower limb (Thompson et al., 2009) to assess the behavioural effect of operantly down-conditioning the H-reflex in patients with incomplete SCI (Thompson et al., 2013). They recruited 13 ambulatory patients and assigned them to no-conditioning (4 patients) and down-conditioning (9 patients) groups. At the end of study, 67% of participants showed decreases in their H-reflex and no significant change was seen in the no-conditioning group. Interestingly, the success rate was similar to the rate of down-conditioning success in healthy individuals (Thompson et al., 2013), monkeys (Wolpaw, 1987; Wolpaw, Braitman, et al., 1983), and rats (X. Y. Chen & Wolpaw, 1995). Moreover, they showed that the H-reflex remained lower than baseline values in the first and last follow-up sessions (mean±SEM of 65±10% and 58±10%, respectively) and had a clear effect on the speed and symmetry of locomotion (Fig. 6).

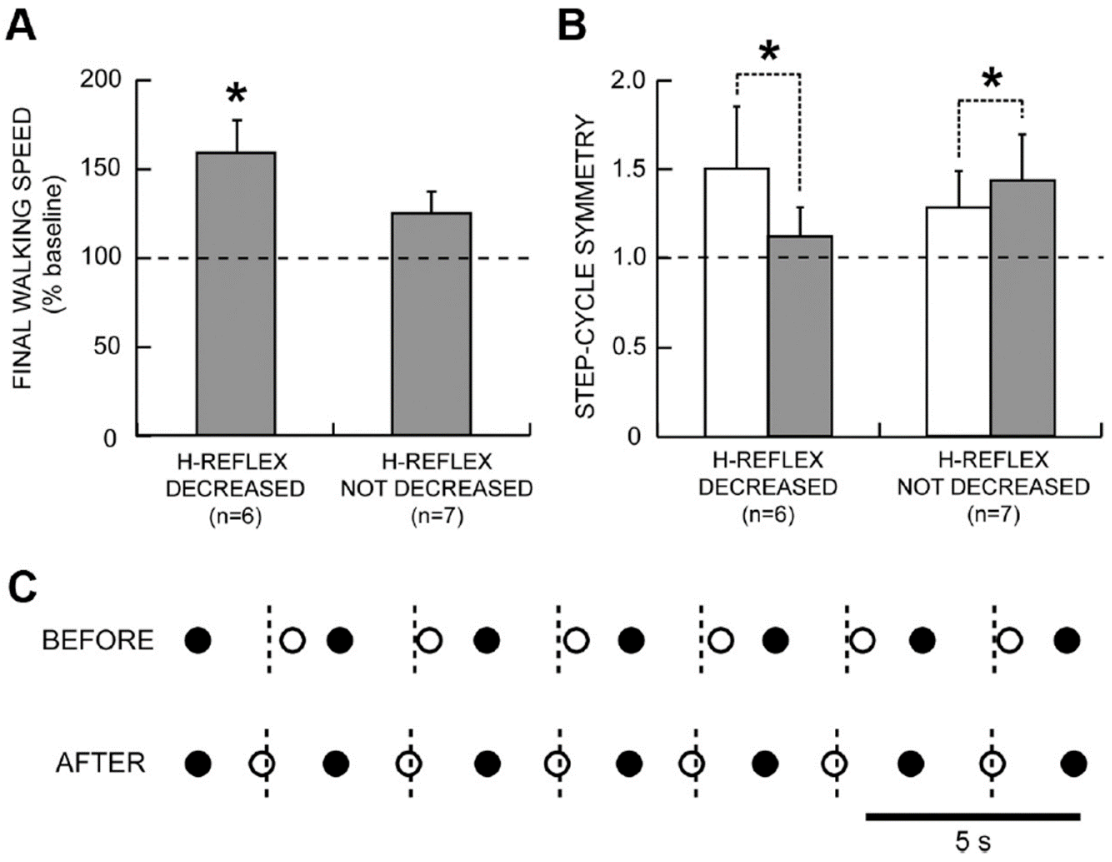

Figure 6. Effect of H-reflex down-conditioning on the step speed and symmetry (Thompson et al., 2013). A) significant increase in walking speed in patients who could down-condition their soleus H-reflex shows significant improvement in comparison to those who could not down condition their H-reflex. B) Those with down-conditioned H-reflex also have a more symmetric step cycle (ratio of left to right step time close to one); before and after experiment values are represented by white and gray boxes, respectively. C) Before conditioning, step cycle was asymmetric, and it took longer for the right foot (white circle) to step after the left one (black circle). Expected times for right foot step are shown by vertical dashed lines. After successful down conditioning of soleus H-reflex the steps become symmetric and the interval between left and right steps became almost equal.

1.3.4 Our Contribution

As described above, previous studies, mostly in animals, suggest that the SSR and H-reflex can be successfully modulated through operant conditioning though there is substantial variability across studies and participants (X. Y. Chen & Wolpaw, 1995, 2005; Evatt et al., 1989; Makihara et al., 2014; Segal & Wolf, 1994; Thompson et al., 2009; Thompson et al., 2013; Wolf & Segal, 1996; Wolpaw, 1987, 2018; Wolpaw, Braitman, et al., 1983; Wolpaw & Chen, 2006). Moreover, such modulation provides a potential mode of rehabilitation after stroke or spinal cord injury (Thompson et al., 2013; Wolpaw, 2018). Most of the work on this to date has focused on the lower-limb. We are motivated by the idea that a similar protocol may also provide therapeutic effects for the upper-limb. The goal of this thesis is to demonstrate the functionality of operantly conditioning upper-limb spinal reflexes in the healthy population – a necessary hurdle prior to implementing a clinical intervention.

We essentially adopted the protocol of Thompson and colleagues, which has recently been used to condition the H-reflex in the lower limb of healthy and spinal-cord injured people (Thompson et al., 2013). There are several key differences between their protocol and our own. First, as mentioned above, we are focused on conditioning upper-limb reflexes, specifically that of the brachioradialis muscle (an elbow flexor). Second, because we are focusing on the upper-limb, we evoked stretch reflexes rather than H-reflexes, the latter is difficult/painful to evoke in the upper-limb. Thus, we did not need to produce M-wave and H-reflex recruitment curves on a daily basis. On the other hand, we needed to ensure a constant level of pre-perturbation muscle activity which required an additional block of trials on a daily basis. Keeping a constant level of pre-perturbation helped keeping almost the same level of muscle activity before perturbation and decreasing the effect muscle spindle sensitivity on reflex size.

As described above, previous studies suggest that the SSR of elbow flexors can be modulated (Evatt et al., 1989; Segal & Wolf, 1994; Wolf & Segal, 1996; Wolf et al., 1995). There are several limitations of this previous work. First, these studies are now 20-30 years old and have never been replicated by an independent research group. Second, they are incomplete because they did not include a control block on each day where the reflex

magnitude was not reinforced. Such trials are important to ensure that changes in reflex sensitivity reflect long-term plasticity rather than short-term task-dependent shifts (Thompson et al., 2013).

As a second goal, we were interested in testing whether changes in the SLR of the brachioradialis muscle would evoke parallel changed in the LLR of the pectoralis major muscle. We have previously shown that when mechanical loads are applied at the shoulder and elbow such that there is no net motion of the shoulder, LLRs are generated at the synergistic muscles of the shoulder (Kurtzer et al., 2008). Recently it has been suggested that part of this sophisticated response is mediated by spinal circuits (Kurtzer et al., 2014). If operantly conditioning the spinal stretch reflex in an elbow muscle, brachioradialis, induces plasticity in the long-latency stretch reflex of its coupled shoulder muscle, pectoralis major, then this would provide the most direct evidence to date that knowledge of the arm's intersegmental dynamics can be incorporated by the spinal circuit.

Chapter 2

2 Materials and Methods

2.1 Setting and Participants

This study was conducted at the Brain and Mind Institute, Western University, London, Ontario, Canada. Data collection began July 2017 and ended July 2018. All experimental procedures were approved by the Western University Health Sciences Research Ethics Board (Approval #108594; Appendix 1) in accordance with the Declaration of Helsinki.

A total of 12 healthy individuals (3 males; 9 females; Age Range = 21-32) participated in a total of 384 experimental sessions. Participants were excluded if they had any history of (a) neurological or neuromuscular conditions (e.g., myasthenia gravis, muscular dystrophy, polymyalgia rheumatica) that might affect motor neurons and/or spinal reflexes, (b) metabolic conditions that affect the nervous system (e.g., thyroid disorders, diabetes mellitus, and electrolytes disorders), (c) trauma, dislocation, or fracture of the right wrist, elbow, or shoulder joints, or (d) current tendon tear or injury.

Before being enrolled in the study, participants attended an pre-enrollment session. During the pre- enrollment session, they were provided detailed information about the study task as well as the duration and scheduling of each session and the financial compensation for their time. Participants were also informed about their right to quit the study at any point without occupational or academic consequences. At the end of the pre-enrollment session, participants provided written informed consent after signing a written document containing all the information discussed during the session (Appendix 2).

Participants were compensated for their time at a rate of $10 per session. They also received a bonus whereby we doubled their total compensation upon completing the entire study.

2.2 Apparatus and Experimental Design

2.2.1 Robotic Exoskeleton

Participants performed the experiment with a two-degree-of-freedom robotic exoskeleton (KINARM, B.KIN Technologies, Kingston, Ontario, Canada). The exoskeleton allows the participant to make combined flexion and extension rotations of the shoulder and elbow to move the hand in a horizontal plane intersecting the shoulder. The exoskeleton can also independently apply mechanical perturbations at the shoulder and elbow, and record kinematic variables related to the movement of the joints and the hand (Scott, 1999). Visual stimuli and hand feedback was projected to the participant in the horizontal plane via a monitor (47-inch LCD monitor; 60 Hz; 1,920 × 1,080 pixels, LG 47LS35A, LG Inc., South Korea) and a semitransparent mirror.

2.2.2 Subject Setup

Participants were seated in the exoskeleton seat with their back straight. The height of the seat was adjusted so that the participants could comfortably see the horizontal screen in front of them and so the plane of motion intersected the shoulder joint (Fig. 7A and 7B). We then used the exoskeleton shoulder indicator laser to align the robot's shoulder axis of rotation with the participant's shoulder axis of rotation. Specifically, we used anatomical landmarks to find the coracoacromial joint of the right shoulder and the centre of the laser cross was set to be at that location. Participants put their forearm and upper arm into the exoskeleton troughs. We used one of the three different trough sizes (i.e., small, medium, and large) to keep the participants' upper arm and forearm immobile in the exoskeleton. We then adjusted the length of arm, forearm, and hand tray to make sure that the participants were comfortable and their joints were not stretched or pressed. These adjustments ensured that the applied loads selectively target the desired joints. After completing mechanical adjustments, we calibrated the robot to the visual display by having participants move their index finger to a set of visual targets presented on the screen. After calibration, a small cursor (white circle, 1 cm diameter) on the screen was aligned with the

location of the participant's right index finger tip. Lights in the experimental room were extinguished during data collection.

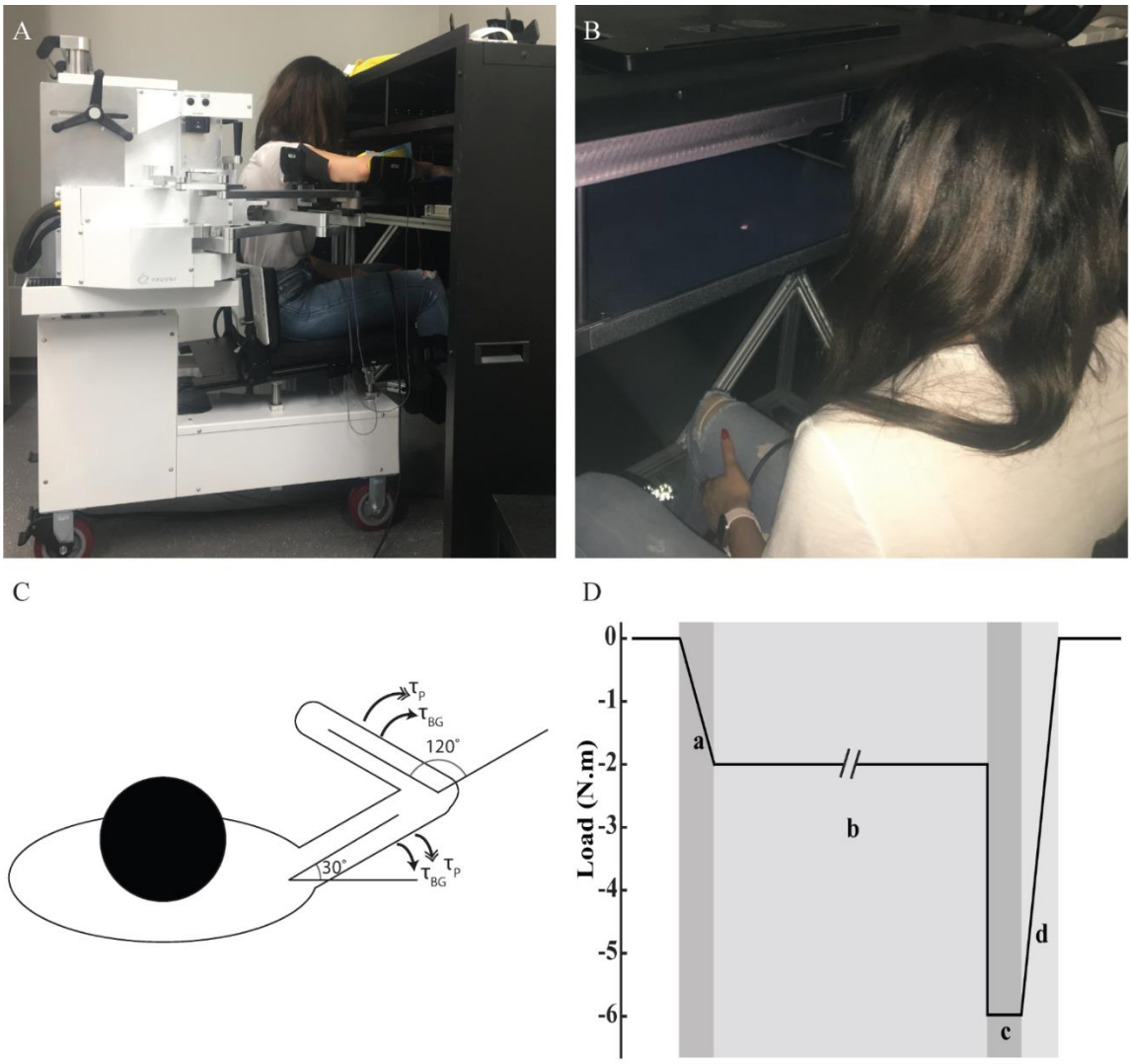

Figure 7. A) Robotic exoskeleton. A participant sat on the seat with her right arm in the exoskeleton arm and looking at the mirror that reflects the horizontal monitor parallel to it. B) the same participant and what she can see on the screen. C) Schematic presentation of the shoulder and elbow angles in the beginning of each trial. Direction of background loads (τ_{BG}) and perturbation force (τ_P) are shown by barbed-head and double-simple arrows, respectively. D) Extension forces exerted in each trial. As soon as participants reached the Home target and kept their finger cursor at the home, an extension background load was exerted and ramped up to -2 Nm (a). The background load was exerted constantly for a

random period (b) before an extension perturbation torque of -4 Nm was exerted (c). After perturbation, the extension loads quickly ramped down to zero (d) to prepare for the next trial.

2.2.3 Electromyography

We recorded muscle activity from three muscles using surface electrodes (Delsys Bagnoli-8 system with DE-2.1 sensors, Boston, MA): brachioradialis (BR, elbow flexor), biceps brachii (Bi, bi-articular flexor), and pectoralis major (PM, shoulder flexor). Electrodes were placed on the bulk of the muscle based on a specific joints positioning (neutral fingers position, hand supination at 90°, and elbow flexion at 90°) and anatomical landmarks (e.g., 2 cm distal to the antecubital fossa fold for BR muscle). The skin was abraded with rubbing alcohol and conductive gel was applied to the electrode contacts to ensure optimal contact between the skin and the electrode. Double-sided tape was used to attach the electrodes to the skin. EMG signals were amplified (gain = 10^3), band-pass filtered (20-450 Hz), and then digitally sampled at 1000 Hz.

2.2.4 Experimental Procedure

2.2.4.1 Sessions and Timeline

Participants first attended one or two preliminary sessions to see whether our protocol evoked a clear short-latency stretch reflex (SLR) in their BR muscle. This session also served to familiarize participants with the robot, the different experimental blocks, and the visual feedback that they were going to see during the study period. After confirming the presence of a clear SLR, we scheduled the experimental sessions. For each participant, the study comprised a total of 32 sessions: 3 baseline, 27 training, and 2 follow-up. The sessions were held three to four times a week within a 2-hour window due to the diurnal variability of the SLR magnitude (X. Y. Chen & Wolpaw, 1994; Lagerquist, Zehr, Baldwin, Klakowicz, & Collins, 2006; Wolpaw & Seegal, 1982). The follow-up sessions were held one-month and two months after the last training session (see Fig. 8).

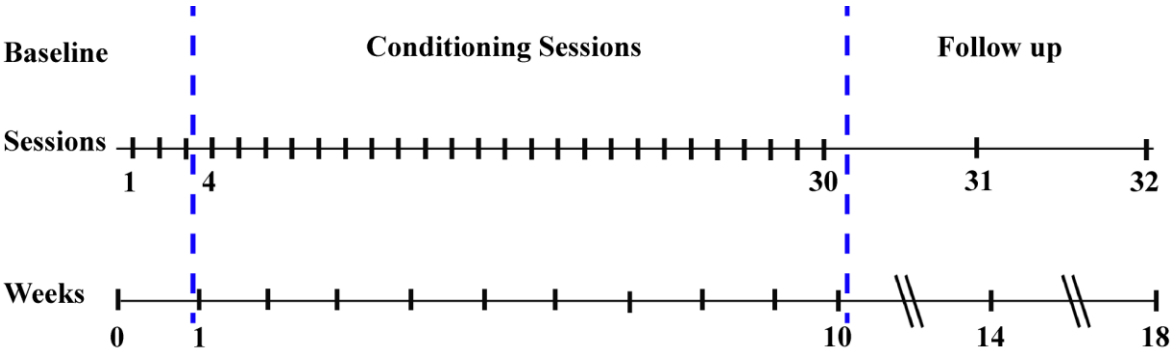

Figure 8. The Timeline of The Study Sessions

2.2.4.2 Experimental trial types

The experiment is composed of three kinds of trial types, which were always performed in blocks of 75 trials: (1) perturbation-calibration (PCAL), (2) perturbation-no-reinforcement (PNR), (3) perturbation-with-reinforcement (PWR). Each session included a PNR block that was used as within session control and for assessing long-term changes (control PNR or CPNR is the first repetition of PNR trials, see below for details). The structure and order of which trial types were done in each session is shown in Table 1.

Table 1. Blocks in Each Session

Sessions		
Baseline	**Conditioning**	**Follow-up**
1 PCAL Block 1 CPNR Block 3 PNR Blocks	1 PCAL Block 1 CPNR Block 3 PWR Blocks	1 PCAL Block 1 CPNR Block 3 PNR Blocks

PCAL trials were performed at the beginning of each session and served to calibrate the sensitivity of our EMG recordings across sessions. In PCAL trials, participants were instructed to move the hand feedback cursor to a home location placed roughly in front of their midline, corresponding to initial shoulder and elbow joint angles of 30 and 120 degrees (Fig. 7C). When the hand feedback cursor was on the home location, the color of the home target would change from red to green and a background load of –2 Nm (positive = flexion; negative = extension) was applied by the robot at both elbow and shoulder joints. These background loads served to pre-activate the Br, Bi, and PM muscles which is important for evoking clear SLRs (Bedingham & Tatton, 1984; Pruszynski, Kurtzer, Lillicrap, & Scott, 2009). The participant was required to stabilize their hand in the home target and, after a random hold period (500-1000ms), the robot applied an additional -2 Nm torque pulse lasting 100 ms (Fig. 7D). The perturbation pushed their hand out of the target and participants were trained to return to the home target within 500 ms of perturbation onset (Fig. 9). At the end of the PCAL block, we calculated a target range for pre-perturbation muscle activity of the BR muscle that was used for subsequent CPNR and PWR blocks. The target range was defined as the mean EMG activity ± 10% activity of all 75 trials over the 50 ms immediately preceding perturbation onset.

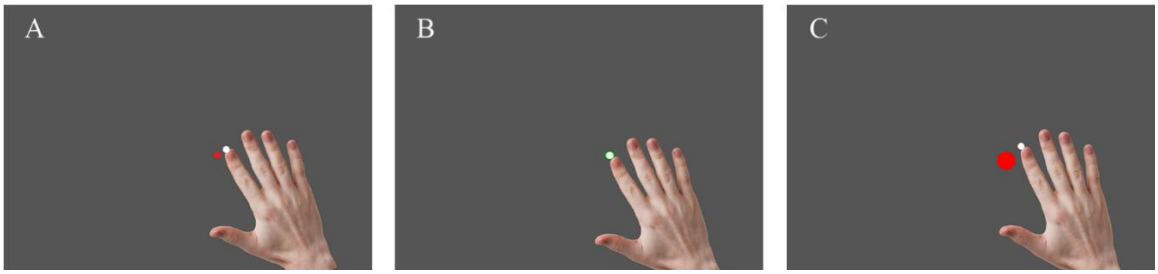

Figure 9. Perturbation-Calibration trial. A) Participant had to move his index finger tip's indicator (white circle) to the home location (red circle). B) Staying in the home location turned home location color into green. C) After a random period, an extension perturbation displaced the index finger tip's indicator from home location and the home location was turned into a larger red circle.

In PNR and PWR trials, visual feedback was provided which was adopted from previous conditioning studies (Knikou, 2008; Thompson & Wolpaw, 2014; Wolpaw, Braitman, et al., 1983) with minor changes.

PNR trials served as intersession and across session control to assess and discriminate task-dependent adaptation and long-term plasticity. In PNR blocks, participants performed the same basic task as in PCAL trials. However, in PNR trials participants were provided real-time biofeedback about the activation of their BR muscle relative to the target range defined at the end of the PCAL trials. The amplitude of BR EMG activity was mapped onto the height of a bar and the target range was displayed as a white box (Fig. 10). Mechanical perturbations were only applied if the hand was in the home target and if the BR muscle activity was kept in the target for a random period of 500 ms to 1000 ms. Note that the first PNR block was named Control PNR (CPNR) because it was used for across session comparisons (see Data Analysis).

PWR trials served to train the participants to increase their SLR magnitude through operant conditioning. PWR trials were very similar to PNR trials, the only difference being in visual feedback given to the participant after the trial had ended about the magnitude of the reflex being conditioned. Specifically, the visual feedback in PWR blocks had four components (Fig. 10). First, a bar whose height represented the magnitude of the SLR in the BR muscle on that trial. Second, a horizontal line placed at a height that represented the median SLR magnitude of all PWR trials in the previous three experimental sessions. Note that, for the first two sessions, PNR trials from the baseline session were used. Third, a white box positioned above the red line representing 105% of the median SLR magnitude of all PWR trials in the previous three experimental sessions. This box was the target for participant, encouraging them to increase their SLR magnitude. Again, for the first two sessions, PNR trials from the baseline session were used. Fourth, a counter that indicated the percentage of trials where the BR SLR was within the target box of all the PWR trials performed in that session.

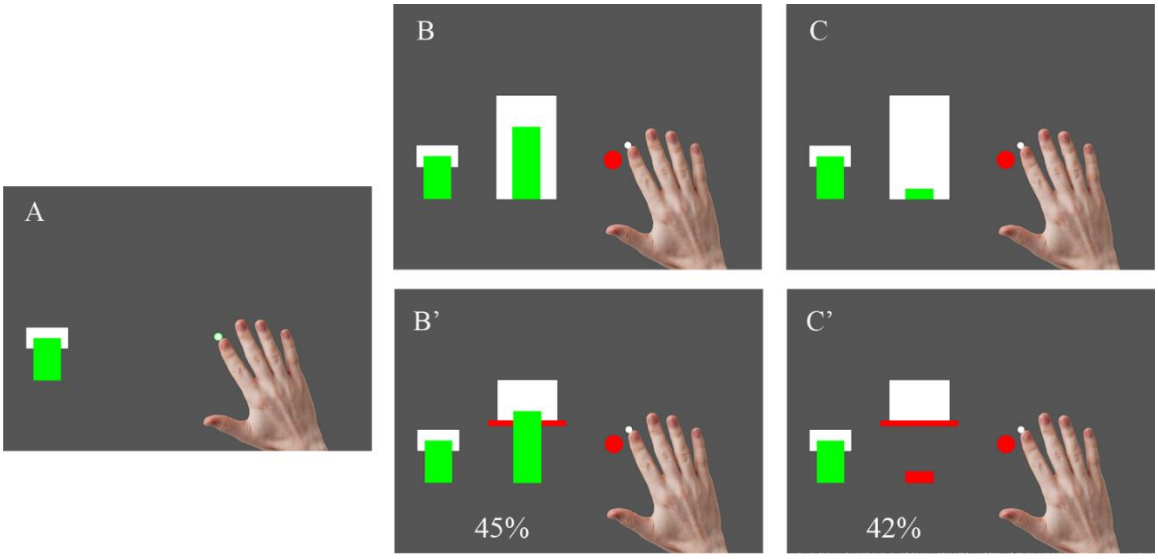

Figure 10. PNR and PWR trials. A) Muscle activity in real time; when the finger indicator is at the home location and the muscle activity is in the certain level (within the white box), the perturbation happens. B and C) In PNR trial, after perturbation, the SLR magnitude will be shown as a green bar in the large white box without any feedback about the increase or decrease of the SLR magnitude. B' and C') In PWR trials, the screen froze after perturbation exertion and the feedback on SLR size would be provided. If they increased the height of the SLR bar and made it bigger than the level of the horizontal red line and in the white box, it would become green (successful, B'), while if it did not reach that level it would be red (unsuccessful, C'). The percentage on the screen shows the success rate in the current block.

2.3 Data Analysis

Data from two participants were not analyzed because their EMG recordings were systematically noisy. All data analysis was done in MATLAB 2017b (The Mathworks, Natick, MA). Kinematic and EMG data were aligned to perturbation onset. EMG data was band-pass filtered, rectified, and normalized. SLR and LLR magnitudes were calculated by averaging EMG signal at predefined epoch. Statistical comparison between the first and last bins was performed for each of the trial types to assess the presence of task-dependent

changes and long-term plasticity. This was done within each participant and across the population of participants by using an independent samples t-test and Wilcoxon signed-rank test, respectively. A repeated measures ANOVA was used to detect any difference among bins across the population. Pearson correlation was used to assess the relationship between changes in SLR of the BR muscle and SLR and LLR of other muscles. In all tests, $p < 0.05$ was considered as significant level.

Chapter 3

3 Results

3.1 General Behavior, Kinematics, and Muscle Activity

We focus our analysis on a total of 10 individuals (2 males; 8 females; age range = 21-32), who each participated in 3 baseline sessions, 27 conditioning sessions, and two follow-up sessions. The baseline and conditioning sessions happened approximately three times per week over a span of 10 weeks and the two follow-up sessions occurred one and two months after the last conditioning session. Each session lasted approximately one hour.

There are three main components of our experimental task (see Methods). First, participants had to place their hand at a central target and counter a small background load while keeping their muscle activity within a certain range for a randomized period of time before the robotic manipulandum applied a mechanical perturbation. Second, participants had to respond to a multi-joint mechanical perturbation that caused elbow joint extension (and thus stretched their brachioradialis muscle) by bringing their hand back to the central target. Third, participants were instructed to increase the magnitude of the SLR in their brachioradialis muscle, which was presented to them at the end of each conditioning trial.

Participants had little difficulty with the first two aspects of the task. As expected, the shoulder and elbow joint perturbation applied by the robot caused substantial elbow joint rotation with minimal shoulder joint rotation (Figure 11) and, given the initial joint configuration, pushed the hand away and to the right relative to the body (Figure 12). Both the joint and hand kinematics were consistent within (Figure 11A; Figure 12A) and across sessions (Figure 11B; Figure 12B) for a given participant, as well as across participants (Figure 11C; Figure 12C). Importantly, the mechanical perturbation evoked a clear SLR and LLR in the brachioradialis (Figure 13) and biceps muscles as well as a clear LLR in the pectoralis major muscle.

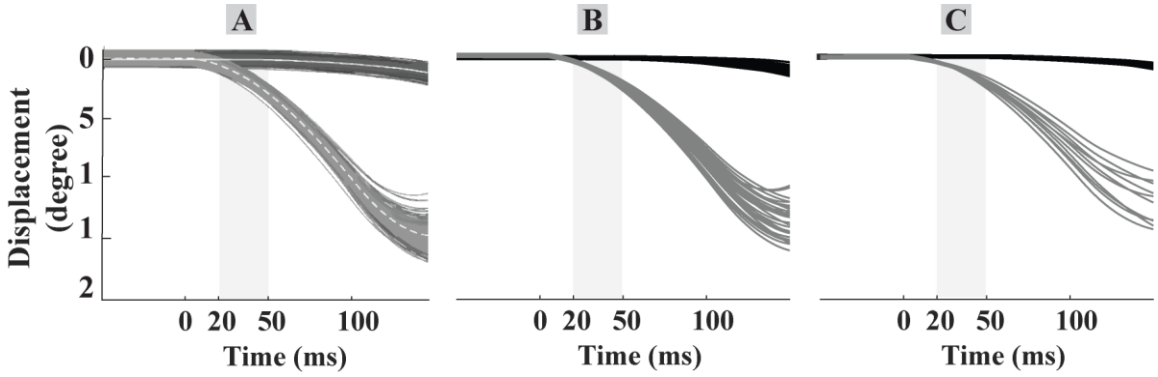

Figure 11. Changes in the angle of shoulder (black) and elbow (gray). Perturbation happens at time zero. A) Kinematics for a session in a participant (300 trials). Mean of shoulder and elbow are shown by solid and dashed white lines, respectively. B) Mean kinematics of all 30 sessions for the same participant. C) Mean of all trials in 10 participants (mean of 90,000 trials for each participant).

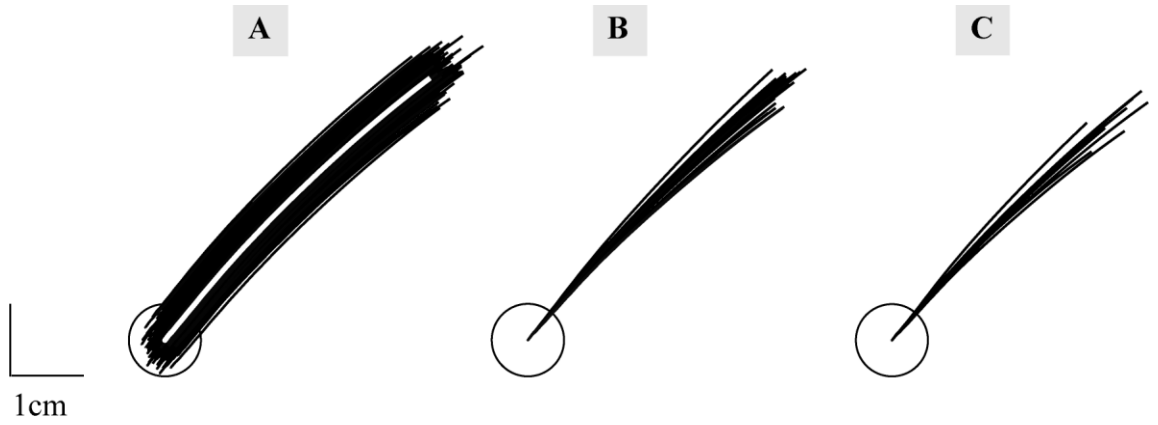

Figure 12. Index finger cursor displacement from perturbation through 100 ms after perturbation. Home position is shown by a circle. A) Cursor displacement in a session in a participant (300 trials). Mean of displacement is shown by solid white line. B) Mean displacement of all 30 sessions for the same participant. C) Mean displacement of all trials in 10 participants (mean of 90,000 trials for each participant).

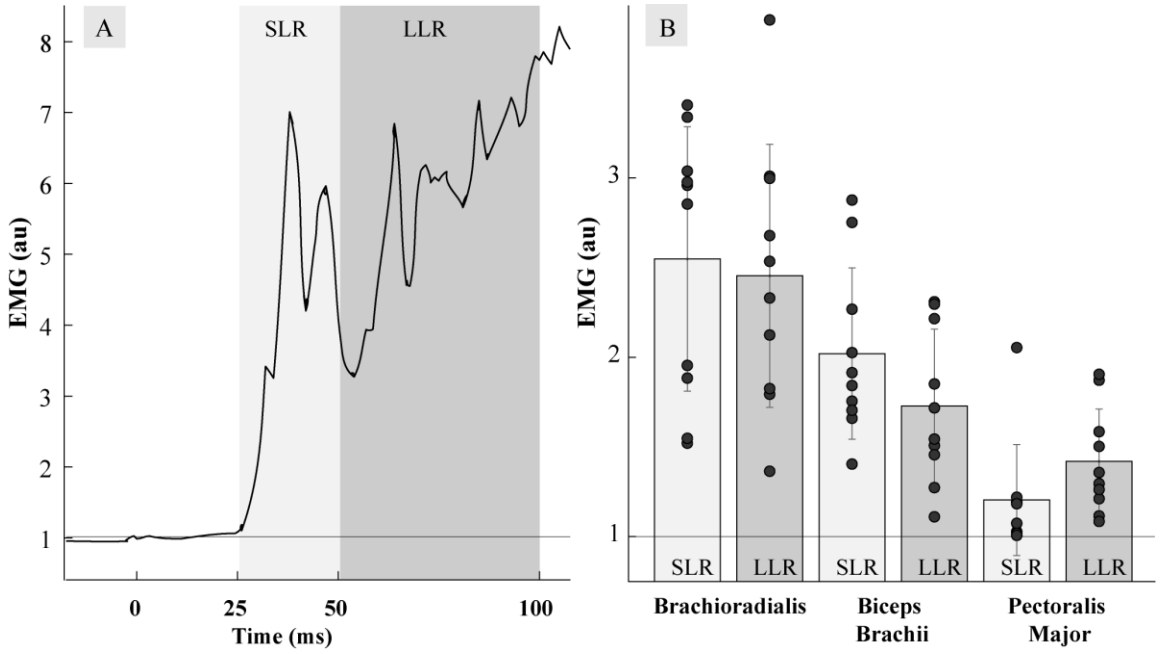

Figure 13. Mean EMG activity of brachioradialis muscle in a participant during a perturbation-no-reinforcement block (75 trials) of a baseline session (A). Mean±SD SLR and LLR magnitude of normalized control trials across all participant in C25-C27 bin (B).

Having established that our paradigm yielded the expected behavioral pattern along with robust reflex responses, we focus the remainder of our results on the two key goals of the study, both of which involve the third main component of the task: changes in the sensitivity of reflex responses over the course of many days. First, we test whether and how robustly the brachioradialis SLR can be operantly conditioned to increase its sensitivity. And second, we test whether conditioning the brachioradialis SLR yields changes in the sensitivity in unconditioned reflex epochs (i.e., the LLR) and unconditioned muscles (i.e. biceps and pectoralis major).

3.2 Brachioradialis SLR Changes

All the following analyses deal with evoked muscle activity as measured via surface electromyography (EMG). EMG signals are normalized and binned according to previously established procedures. EMG normalization is done on a per session basis by

dividing all EMG signals for a given muscle by the average muscle activity in that muscle in the 50 ms preceding perturbation onset (while the participant countered a constant load of 2Nm). Under this normalization scheme, a value of 1 represents the average EMG activity required for that muscle to counter a 2 Nm load. EMG activity was binned into two epochs, the SLR from 20-50 ms after perturbation onset and the LLR from 50-100 ms after perturbation onset (see Figure 13). These epochs reflect different neural circuits, with the SLR being generated exclusively via spinal circuits and the LLR including many neural contributors including cortical centers (for review, see (Pruszynski et al., 2011)).

Our primary goal was to test whether participants could be operantly trained to increase the sensitivity of their brachioradialis SLR. Figure 14 qualitatively shows the average changes in brachioradialis SLR magnitude across participants over the course of training. We quantified SLR changes over training by specifically comparing baseline sessions and the last three training blocks in three complementary ways.

We first tested whether participants showed a larger SLR in PWR trials from the last three conditioning sessions as compared to PNR trials from the baseline sessions. Such changes would at least partly reflect so-called task-dependent modulation since the comparison is based on PWR trials in the conditioning block, where participants are actively acting to modify their SLR sensitivity. Note that, given that all previous studies looking at conditioning the SLR in upper-limb muscles only had PWR trials, this is the only inference previous work could make (see Introduction and Discussion). Across the population, we found no significant conditioning in this respect (Wilcoxon ranked-sign test, $p > 0.05$; Figure 15A). However, five participants individually showed a significant increase (independent samples t-test, $p < 0.005$, corrected for 10 comparisons; Figure 15A and Figure 16A).

We next tested whether participants showed a larger SLR in PNR trials from the last three conditioning sessions as compared to PNR trials from the baseline sessions. As motivated by Thompson and colleagues (2009), such changes reflect long-term plasticity since the comparison is based on PNR trials in the conditioning block where participants received no feedback about SLR magnitude and thus had no reason to be actively acting to modify

their SLR sensitivity. From a clinical perspective, long-term plasticity is the most interesting type of modulation since it lasts for an extended period outside the conditioning environment and thus has the potential of influencing real-world behavior. Across the population, however, we again found no significant change in this respect, i.e., change in SLR sensitivity of control blocks (Wilcoxon ranked-sign test, $p > 0.05$; Figure 15B). In fact, there appeared to be a decrease in SLR magnitude over the course of training. Strikingly, although three participants individually showed the expected significant increase, five participants showed a significant decrease (independent samples t-test, $p < 0.005$, corrected for 10 comparisons; Figure 15B and Figure 16B).

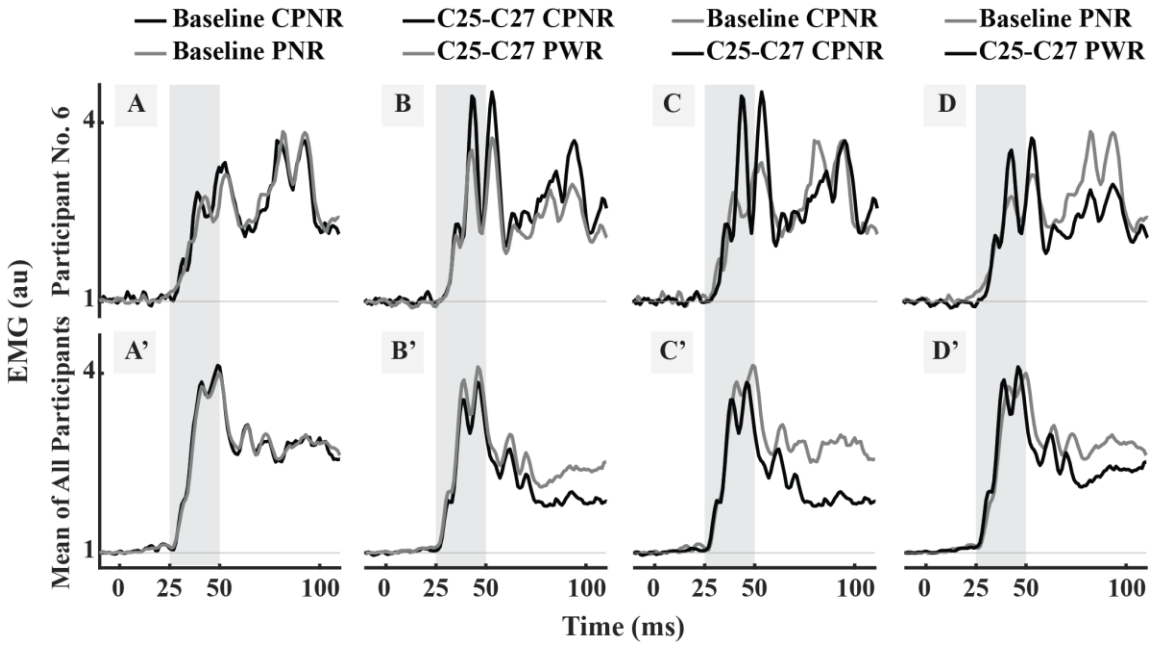

Figure 14. Mean of rectified and filter EMG of BL and C25-C27 bins in a participant who could successfully increase her SLR amplitude (Participant 5; top panel) and across all participants (bottom panel). EMG signal is shown by arbitrary unit (au) and SLR window (25-50 ms) is shown in gray. A and A') Average EMG of CPNR and PNR trials of the BL bin; B and B') average EMG CPNR and PWR trials in C25-C27 bins; C and C') average EMG of CPNR trials in the BL and C25-C27 bins; D and D') average EMG of PNR trials of BL and PWR of C25-C27 bins.

Given that SLR magnitude appeared to decrease over the course of training, our first analysis may actually underestimate the magnitude of the task-dependent changes. Therefore, we tested whether participants showed a larger difference in their SLR in PWR trials from the last three conditioning sessions as compared to PNR trials from the same sessions. This approach would provide a more direct measure of task-dependent changes since it would account for any (unexpected) long-term decrease in SLR sensitivity. Here, we found a reliable increase in SLR sensitivity at the population level (Wilcoxon ranked-sign test, $p < 0.05$; Figure 15C) with eight participants showing significant task-dependent increases in SLR sensitivity (independent samples t-test, $p < 0.005$, corrected for 10 comparisons; Figure 15C and Figure 16C).

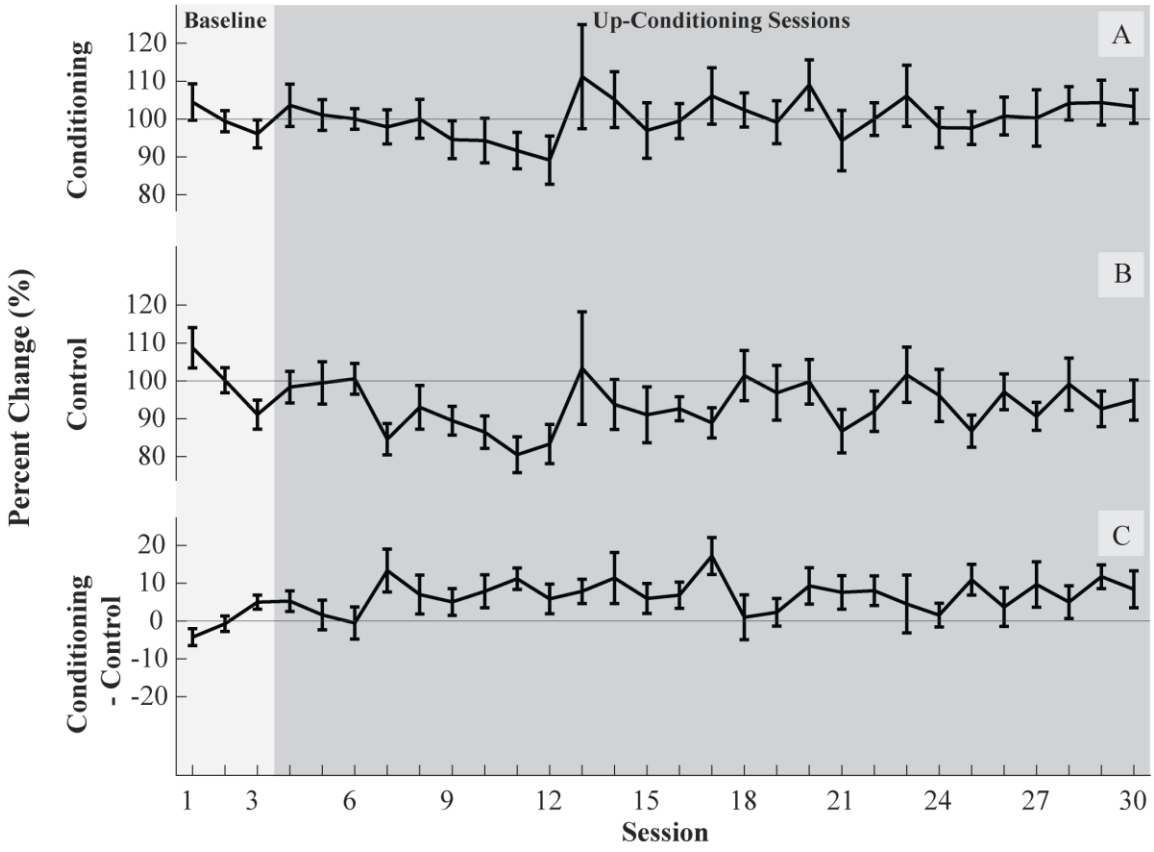

Figure 15. Mean and SEM of SLR percent change of each session in comparison to the first session for conditioning (A) and control (B) trials and the difference in the percent

change of SLR magnitude between conditioning and control trials (task dependent adaptation; C).

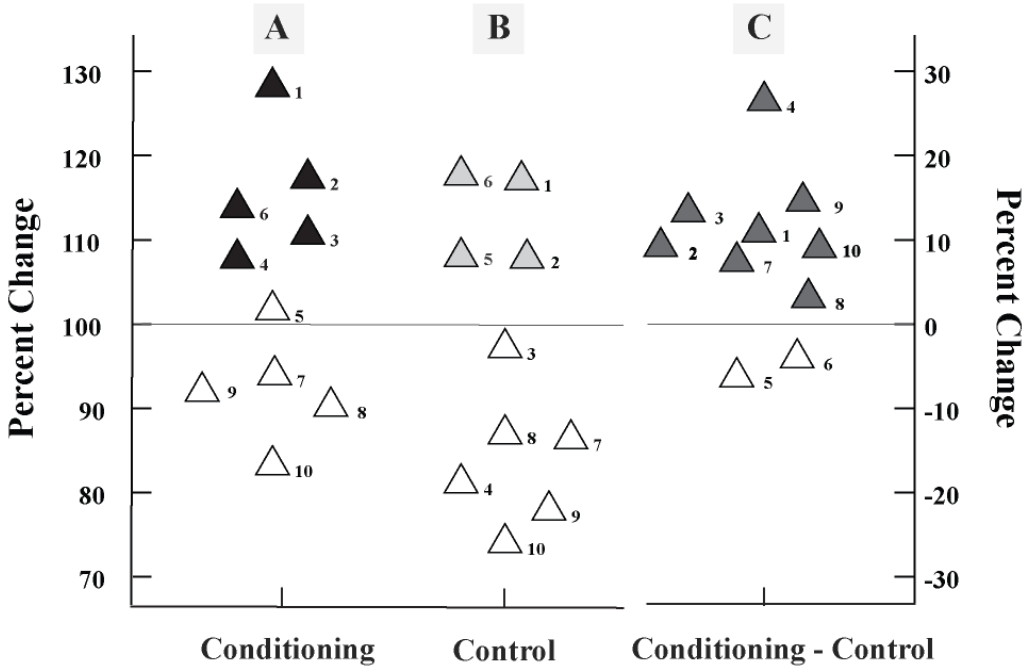

Figure 16. Percent change in SLR of Brachioradialis muscle from BL to C25-C27 bin. Each participant is tagged by an ID number at their right. Values for percent change for conditioning and control trials are illustrated on left Y axis and values for percent change of difference between conditioning and control SLR (task-dependent adaptation) are illustrated on right Y axis. Data points for insignificant changes are shown by open triangles.

3.3 Effects in Other Epochs and Muscles

Our secondary goal was to evaluate whether long-term and task-dependent changes in brachioradialis SLR influenced long-term changes in other epochs and muscles. To some degree, these analyses have to be approached with caution given the relatively weak effects we observed in terms of conditioning the brachioradialis itself. Before addressing our main

hypotheses, therefore, as a positive control we correlated task-dependent changes in brachioradialis with those in biceps and pectoralis major. Such changes are expected because it is well established that participants can readily modify these responses on a trial-by-trial basis and because all these muscles can readily contribute to the task at hand. As expected, we found statistically significant correlations in this respect both between brachioradialis and biceps ($r = 0.74$, $p = 0.01$) as well as between brachioradialis and pectoralis major ($r = 0.72$, $p = 0.02$).

We then addressed our specific hypotheses. First, we found statistically significant correlation between long-term changes in brachioradialis SLR and brachioradialis LLR ($r = 0.82$; $p = 0.003$; Figure 17A) and a weaker trend in the same direction relating task-dependent changes between these epochs ($r = 0.56$, $p = 0.09$; Figure 17B). Second, we found no statistically significant correlation between changes in brachioradialis SLR and biceps SLR (long-term: $r = 0.46$, $p = 0.18$; task-dependent: $r = 0.15$, $p = 0.68$) suggesting that modulation of spinal circuits is local to the muscle being conditioned. Third, we found no statistically significant correlation between changes in brachioradialis SLR and pectoralis major LLR (long-term: $r = 0.25$, $p = 0.49$; task-dependent: $r = 0.34$, $p = 0.34$) consistent with the idea that plasticity is local and suggesting that coordination patterns via higher level neural circuits do not readily account for this type of plasticity.

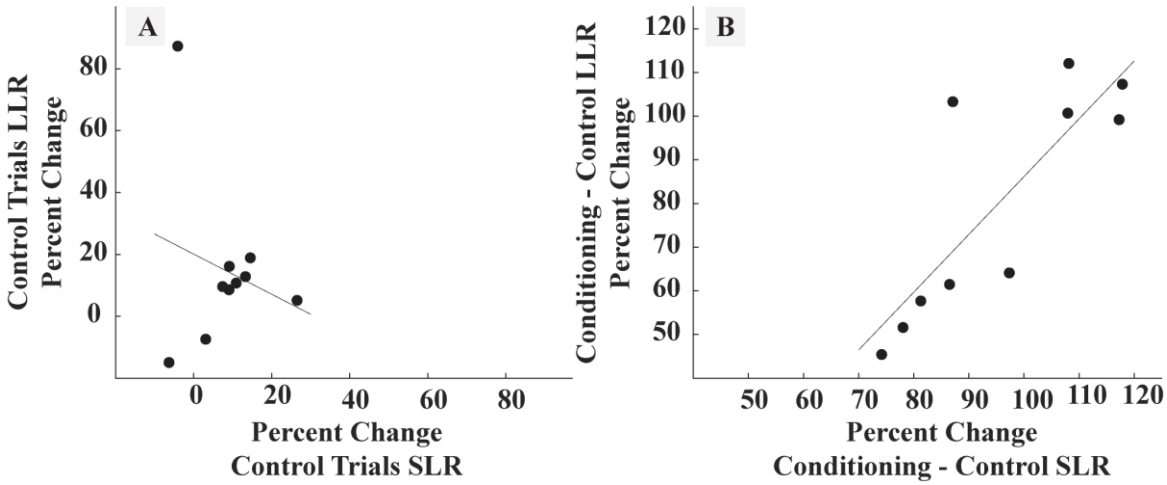

Figure 17. Correlation of brachioradialis SLR percent change with LLR percent change and fit line across participants in C25-C27 bin. Changes in control trials shows long-term plasticity (A) and changes in difference between conditioning and control trials shows task-dependent adaptation (B).

3.4 Follow-up Sessions

To assess the persistence of long-term plasticity, participants attended two follow-up sessions one and two months after the last conditioning session. This analysis has to be approached with caution given the weak conditioning effects we observed. Consistent with this weak effect, there was no statistically correlation between C25-C27 and mean control trials SLR across follow-up sessions ($r = 0.40$, $p = 0.25$; Figure 18) suggesting that whatever changes occurred in the context of training (even decreases) were not long-lasting.

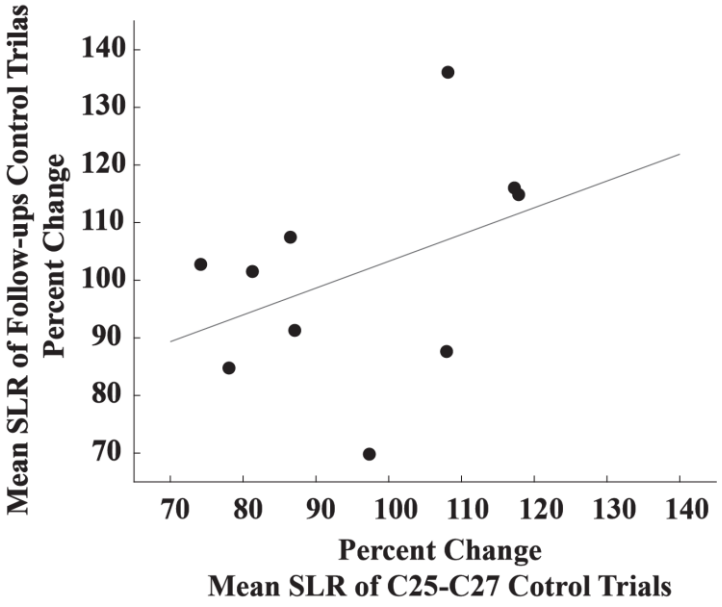

Figure 18. Correlation of mean percent change of control trials SLR in C25-C27 bin and mean percent change control trials SLR across follow-up sessions

Chapter 4

4 Discussion

Our main goal was to investigate the conditioning of the brachioradialis SLR. Overall, our results were marginal. Although 80% of participants showed task-dependent adaptation and 40% showed long-term plasticity in the desired direction, the effect size was small and considerably lower than previous reported effect size in upper limb SLR (Evatt et al., 1989; Segal & Wolf, 1994; Wolf & Segal, 1996) or lower limb H-reflex (Makihara et al., 2014; Thompson et al., 2009; Thompson et al., 2013). Nonetheless, the frequency of participants with successful task-dependent adaptation, as defined by increased difference between conditioning and controlled trials in the correct direction (Thompson et al., 2009), was similar to previous studies the used the same paradigms (Makihara et al., 2014; Thompson et al., 2009; Thompson et al., 2013).

There are few studies that have investigated operant conditioning of upper limb SLR (Evatt et al., 1989; Segal & Wolf, 1994; Wolf & Segal, 1996; Wolf et al., 1995). For technical simplicity, most studies on operant conditioning of spinal reflex have focused on lower limb H-reflex (Carp et al., 2001; X. Y. Chen & Wolpaw, 1995, 1997, 2005; Y. Chen et al., 2006; Makihara et al., 2014; Thompson et al., 2009; Thompson et al., 2013; Wolpaw, 1987; Wolpaw & Chen, 2006). Therefore, we modified and implemented the most recent protocol that was devised by Thompson et al. (2009) for operant conditioning of lower limb H-reflex, which showed promising results in both neurologically intact (Makihara et al., 2014; Thompson et al., 2009) and patients with SCI (Thompson et al., 2013).

Our study has critical differences with previous studies on operant conditioning of upper limb SLR. Operant conditioning of human upper limb SLR was first reported by Evatt and colleagues who up-conditioned and down-conditioned the SLR of biceps brachii in neurologically intact participants and reported 63.7% increase and 21.5% decrease in median SSR of up-conditioning and down-conditioning groups, respectively (Evatt et al., 1989). Thereafter, Segal and Wolf down-conditioned biceps brachii SSR in patients with SCI and reported 36% decrease in the mean SSR of down-conditioning group which was significantly lower than 12.6 % decrease seen in control patients (Segal & Wolf, 1994).

Neither of these two studies assessed other synergistic or antagonist muscles. Wolf and Segal down-conditioned SLR magnitude of biceps brachii and reported 24% decrease in comparison to 12% increase in biceps brachii SLR magnitude of control participants. In addition, SLR magnitude of brachioradialis was decreased by 18% in down-conditioning and increased by 12% in control groups. Moreover, biceps brachii LLR magnitude was decreased by 37% (Wolf & Segal, 1996).

Although our results were weak in comparison to previous studies, there are two important aspects of our study that are new to operant conditioning of upper limb SLR: 1) a more careful assessment of LLR and SLR of synergistic muscles and 2) the introduction of control trials.

4.1 SLR and LLR of Biceps Brachii and Pectoralis Major

Previous studies focused on operant conditioning of biceps brachii SLR and only one study investigated the effect of operant conditioning of SLR on LLR of biceps brachii and SLR of brachioradialis muscle (Wolf & Segal, 1996). In our study, we up-conditioned SLR magnitude of brachioradialis muscle and assessed its effects on LLR of not only brachioradialis muscle but two other synergistic muscles, namely, biceps brachii and pectoralis major. In addition, we assessed the effect of up-conditioning SLR of brachioradialis on the SLR magnitude of biceps brachii. In our setup, the shoulder joint was not moved by the perturbation because our apparatus exerted torque on both elbow and shoulder joints in a way that countered the natural intersegmental dynamics. Therefore, increased SLR or LLR magnitude of pectoralis muscle would be attributed to the changes in the common pathways shared by muscles that work synergistically to flex upper limb. However, our study could not find such a statistically significant correlation. The lack of a correlation may indicate that either conditioning effect is local to the conditioned muscle or our study had not the power to find such a correlation. However, this is conclusion is made difficult given the relatively weak conditioning of the brachioradialis muscle in the first place and small sample size of 10 participants.

4.2 Control Trials

Control trials that were first introduced by Thompson et al in operant conditioning of soleus muscle H-reflex (Thompson et al., 2009). Control trials help to discriminate task-dependent adaptation and long-term plasticity. That is, control trials enable investigators to turn conditioning on and off in humans, which is one of the main differences between operant conditioning of human and animals' reflexes. To turn the conditioning off, no visual feedback and no instruction to increase SLR is provided to participants. Because participants are not actively modulating their SLR in this condition, control trials help evaluate the baseline SLR magnitude of each session and to observe the long-term plasticity in the spinal cord which is not task-dependent. When we looked at the data across the participants, we observed that 50% of participants showed decreased control trials SLR that would be translated to long-term plasticity in the opposite direction of our expectation. This phenomenon was seen in previous studies on operant conditioning of lower limb H-reflex where direction of change in reflex was the opposite to the desired direction (Thompson et al., 2009; Thompson et al., 2013). While we have no clear explanation for such a phenomenon, different factors might have contributed to it.

First, our protocol might not be capable of explicitly inducing up-conditioning of H-reflex. This protocol and visual feedback were successfully implemented in conditioning lower limb H-reflex, but it might not have the same effect on the upper limb SLR. We had 27 conditioning sessions that might not be long enough to induce plasticity in the upper limb as upper limb has numerous functions and circuits. Unlike the lower limb, which has a relatively limited repertoire dominated by a single function (i.e. gait), upper-limb circuits are the common pathways for many motor functions and hence, changing SLR and pathways that lead to such a change might have negative effects on the other motor functions. Because the nervous system should serve both previously and recently learnt skills, it might need more time to modulate SLR so that it can serve all the repertoire that share these pathways.

Second, when we subtracted control trials SLR from conditioning trials SLR, significant task-dependent adaptation was observed in 80% of participants, some of which had shown

decreased control SLR. While participants were instructed to increase their SLR in conditioning trials, decreased control SLR might be due to the induced changes by their nervous system to serve increased SLR (task-dependent adaptation) as well as previously learnt skills (long-term plasticity). Such an effect is best explained by "negotiated equilibrium" that Wolpaw first introduced as a theory (Wolpaw, 2010) and later fully hypothesized it as a model for motor learning behaviour (Wolpaw, 2018).

4.2.1 Negotiated Equilibrium Model

Any behavioural learning encompasses plasticity in the brain and spinal cord. The sensory input from the periphery induces plasticity in the brain and brain induces changes in corticospinal tract, motoneurons' synapses and firing threshold, and interneurons. The negotiated equilibrium model, a concept recently introduced by Wolpaw (2018), proposes spinal cord and brain as the joint substrates of motor behaviour, and highlights the active maintenance of these substrates in accordance with dynamic changes of CNS. In fact, all the motor behaviours that share the common pathways undergo these changes concurrently. Any previously learnt behaviour has to induce plasticity to keep working optimally despite the changes other behaviours induce. The aggregation of all these changes is a negotiation between behaviours that share common pathways, i.e., neurons and synapses, so that each behaviour can work effectively by keeping its key features. Therefore, the spinal neurons and synapses work under an equilibrium among different behaviours that negotiate to keep themselves working properly (negotiated equilibrium) and serves all the behaviours in the repertoire. Therefore, when a new behaviour is achieved or the spinal cord or its inputs change during growth, aging, limb growth, weight changes, or pathologic events such as spinal cord trauma or stroke, a new negotiation begins to provide an equilibrium that serves previously learnt and new behaviours (Wolpaw, 2018). Most of the evidence that support negotiated equilibrium model are obtained by conditioning SSR or H-reflex in monkeys, rats, and human (Carp et al., 2001; X. Y. Chen & Wolpaw, 1995; Y. Chen et al., 2006; Evatt et al., 1989; Makihara et al., 2014; Thompson et al., 2009; Thompson et al., 2013; Wolf & Segal, 1996; Wolpaw, 1987; Wolpaw, Braitman, et al., 1983; Wolpaw, Kieffer, et al., 1983).

According to the negotiated equilibrium model, it may be that when participants of the current study tried to up-condition their conditioning trials SLR magnitude, their nervous system took different strategies so that it would not impair their other previously learnt motor behaviours. Increased difference between conditioning and control trials shows that by the end of conditioning sessions, 80% of our participants could increase their conditioning trials SLR in comparison to their control trials SLR. The control trials SLR, which can be considered as current status of negotiation between different behaviours, changed significantly in 90% of participants, which might be due to negotiation with other behaviours to serve all of the previously and recently learnt behaviours. Under current negotiation status, previously learnt behaviour are preserved but might perform under a different condition than their previous one.

After spinal cord injury, patients lose some previously learned motor functions and their nervous system works under new equilibrium to serve only the intact functions. Operant conditioning of the reflex and training patients to down-condition their reflex sensitivity brings up a new equilibrium. Under new equilibrium, spasticity of muscles would be decreased, and nervous system tries to find a way to serve previously learnt skills and recently learnt behaviour. The result is improvement in impaired motor behaviours while keeping intact behaviours working optimally under the new equilibrium.

Negotiated equilibrium can justify observed improvement in motor function of patients with spinal cord injury through operant conditioning of reflexes of lower limb (Thompson & Wolpaw, 2014). Nonetheless, our study brought up serious questions about applicability of current paradigm in neurologically intact participants and mandates more works to consider it as a therapeutic method in pathological conditions.

4.3 Clinical Application

Previous experiments in animals and humans with neurological deficits showed that SLR and H-reflex can be operantly conditioned and locomotion can be improved (Y. Chen et al., 2006; Segal & Wolf, 1994; Thompson et al., 2013). Previous studies focused on operant conditioning of H-reflex of the lower limb. Although we used the same protocol that was

developed by Thompson and Wolpaw during the last several years (Thompson et al., 2009), there are important differences between H-reflex and SLR as well as between operant conditioning of lower and upper limbs.

First, conditioning soleus muscle seems to be easy because the tibial nerve becomes superficial behind the knee (i.e., popliteal fossa) and can be excited by superficial electrodes. In contrary, nerve of upper limb lay deep and are not accessible by superficial electrodes. The exception is ulnar nerve at medial side of elbow that innervates flexors of medial fingers and wrist. Nonetheless, our apparatus was not designed to exert torque at wrist or fingers and clinical applicability necessitates focusing on gross motor movements such as mobility of elbow or shoulder rather than small joints of wrist and fingers.

Second, H-reflex bypasses muscle spindles by directly exciting Ia sensory fibers. Hence, the effect of gamma motoneuron on reflex size is bypassed. In contrast, SLR is influenced by gamma motor neuron that change the sensitivity of spindles. We minimized spindle sensitivity by applying pre-perturbation load on joints so that participants had to keep their muscle activity at certain level before torque exertion.

Third, the joints of upper limb are more complex than lower limb. For instance, main movements of knee joint are flexion and extension and ankle joint allows minimum inversion and eversion movements. In contrast, the elbow joint has flexion and extension and can supinate and pronate to change the position of wrist and hand, which in return change its musculoskeletal position during different movements. Therefore, sophisticated musculature and joints of upper limb lead to sophisticated movement in hand and fingers and ability to performed complex learnt skills ranging from grasping and writing to playing musical instruments. consequently, unlike improved locomotion and gait symmetry, which are endpoints of successful operant conditioning of lower limb, it is hard to prognosticate the final effect of conditioning on restoring motor behaviours of upper limb such as writing or grasping.

Another important issue that might limit possible clinical application of our current protocol would be the difficulty of tasks and frustrating long commitment that our study demanded. Many participants struggled to keep their muscle activity in defined range and

even those who had successfully up-conditioned their SLR had no clear idea of how they could do it and complained about inconsistency between the results and their strategy at any single trial. It might have decreased their motivation to catch up with the task and instructions. Moreover, most participants complained of the long course of study and claimed that they would leave the study if there were not enough incentives. Therefore, it will not be easy for patients with SCI who have less control over their limbs to complete the task and expect improved function by the end of study.

Nonetheless, patients might be more motivated because they would see some merit in completing the study and if it has proven effects on the lower limb (Thompson et al., 2009), there might be some effects on the upper limb as well. In addition, clinical significance might be different from statistical significance and with even a little change in reflex size, significant improvement might be observed in clinical outcome. Therefore, we cannot completely dismiss its applicability in patients unless a clinical study rejects current conditioning protocol benefits in patients with SCI.

4.4 Limitations

In addition to the above differences that can hinder achieving the same results seen with H-reflex conditioning of soleus muscle, our study had some limitations that would be due to the nature of conditioning SLR, our apparatus, participants motivations, our feedback, and small sample size.

Our exoskeleton is a general experimental device and might need a stricter design for our specific experiment. First, although the height of the seat could be adjusted for the participants' height, the exoskeleton arm and screen stayed at a constant distance from the ground and did not move up and down with the chair height. Second, participants were not kept restrained to the seat and although the angle of shoulder and elbow were kept constant (at 30°,120°, respectively), at any session they could sit a few centimeters forward/backward or to the sides, or they might change the positioning of their back and change all the angular properties of their body. Third, for fixing the shoulder, we used the acromioclavicular joint as the landmark for locking the shoulder position but any change

in body position form one session to the next would change the position of this joint. This is to say, there are setup differences across experimental sessions that may influence conditioning and/or add noise to our experimental measures.

The participants were recruited from Neuroscience students and undergraduate volunteers at Brain and Mind Institute and therefore might not represent the general population. Nonetheless, long-term commitment to study protocol mandated that we recruit people from our program as participants outside the program did not show commitment and were dropped from study. To encourage participant to be committed to the study protocol, we provide a monetary incentive that would be given to the participants at the end of study. Nevertheless, while such an incentive would enhance the commitment of our participants to the strict schedule of sessions, it might not be an enough motivation for participants to commit to increase their reflex. Participants were free to leave the study at any point as a few did after several sessions and were compensated for their participation hours without receiving the bonus incentive. Visual feedback would give participants a clue to increase their reflex from trial to trial. However, unlike animals, their success was not rewarded with food or water or brain stimulation, which were hardwired to survival instinct. In future, providing extra money based on participants success rate at each session might be effective in providing motivation and hence successful conditioning.

We recruited 12 participants and finally ran analysis for 10. We could not find statistically significant changes and correlations across our participants. However, larger sample size would have increased our statistical power and we could draw a stronger conclusion.

Of note, previous studies on operant conditioning of H-reflex in patients with incomplete SCI had better results than studies in neurologically intact participants (Thompson et al., 2013). In addition to the technical difference between operant conditioning of H-reflex of lower limb and SLR of upper limb, patients with SCI had higher motivation to modulate their reflex and improve their function than neurologically intact participants. On the other hand, according to the negotiated equilibrium model, motor functions are impaired in SCI patients and modulating spinal reflexes serves to restore impaired motor movements. Therefore, nervous system does not resist the changes in reflexes. In contrast, changing the

reflexes in neurologically intact individuals may impair previously learnt motor behaviours and therefore, nervous system attempts to prevent or even counteract the changes in the reflex magnitude to preserve the previously learnt behaviours.

4.5 Conclusion

Although the results were inconsistent and the effect size was small, our protocol could induce both task-adaptation and long-term plasticity in some fraction of participants. Studies with larger sample size, optimized experimental device and protocol, a control or down-conditioning group, and more conditioning sessions are needed to more closely examine the effect of conditioning on SLR of upper limb.

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Appendices

Appendix 1: Review Ethic Board Approval

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Delegated Initial Approval Notice

Principal Investigator: Dr. Andrew Pruszynski
Department & Institution: Schulich School of Medicine and Dentistry\Physiology & Pharmacology,Western University

Review Type: Delegated
HSREB File Number: 108594
Study Title: Training the reflexes of arm muscles
Sponsor: Natural Sciences and Engineering Research Council

HSREB Initial Approval Date: January 09, 2017
HSREB Expiry Date: January 09, 2018

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Western University Protocol	Received 2016/11/21	
Letter of Information & Consent		2016/11/21
Advertisement	Poster for Participants recruitment	2016/09/01

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer: Erika Basile ___ Nicole Kaniki ✓ Grace Kelly ___ Katelyn Harris ___ Vikki Tran ___ Karen Gopaul ___

Western University, Research, Support Services Bldg., Rm. 5150
London, ON, Canada N6G 1G9 t. 519.661.3036 f. 519.850.2466 www.uwo.ca/research/ethics

Appendix 2: Recruitment Document

PARTICIPANTS ARE NEEDED FOR OUR STUDY:

Modulating Upper Limb Spinal Stretch Reflex Through Operant Conditioning

Hello,

I am running a study to modify human upper limb spinal stretch reflex at Brain and Mind Institute, University of Western Ontario, London, ON. However, this study needs commitment and patience! We will compensate your precious time as well as your commitment. I have shortly explained the study below:

Can you change the size of your spinal reflex?

You might be familiar with knee tap that your doctor does with a reflex hammer (**Fig. 1**). In fact, it is a spinal reflex that starts with stretching the muscle through tendon tap (as you see in knee tap) or mechanical muscle stretch. The signal from muscle travels to the spinal cord and the returning signal results in muscle contraction (that's why you kick after knee tap!)

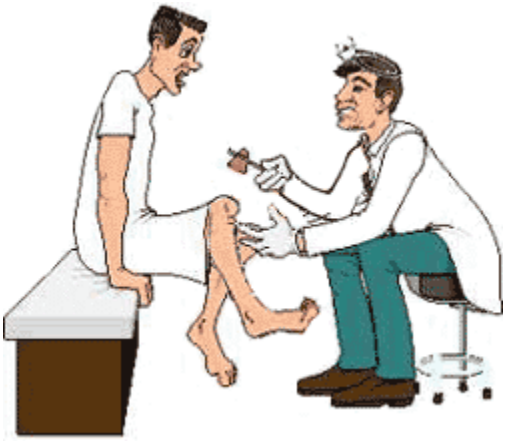

Figure 1. Knee tap or patellofemoral reflex examination.

This activity is reflected in muscle electrical changes, which is recorded through **electromyography (EMG)**. It can be seen in any muscle in human/animal body. These simple reflexes are important as they are involved in daily physical activities such as

walking and running through more complex behaviours,e.g., dancing and playing soccer. Therefore, they might be impaired in any neurological disorder such as stroke or spinal cord injury as well as metabolic disorders such as hyperthyroidism.

For example, in spinal cord injuries and strokes, the reflex size is increased and patients will suffer from spasticity and many other complications such as a deformed limb or problem in walking or grasping. Therefore, modifying these reflexes can improve these patients' overall performance and quality of life. In fact, such a benefit is shown previously in animals and in the lower limb of people with spinal cord injury. We are interested to see such effects in the upper limb as well.

Our Goal:

The main goal of this study is to train the participants to **increase the size of their reflex through a conditioning process.**

This Is How We Do It!

We will use an exoskeleton, i.e., a robot that you sit in and has a right arm that supports your right upper limb. You will see a monitor in front of you and a white circle that indicates the location of your index fingertip.

All you need to do is going to a red target (home) and stay there while resisting a small force that tries to push you away from home. As soon as you go the home circle, its colour turns green. After about 2 seconds, a perturbation will push your hand away from home and you should come back to home circle as soon as you can (**Fig. 2**). You should do it around 300 times!

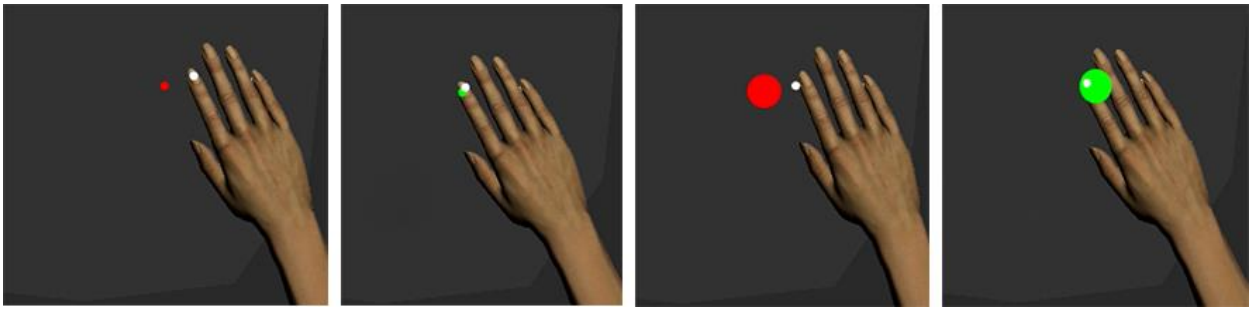

Figure 2. One trial; just keep your index figure indicator in the home target against a very small force and then come back to it after a perturbation pushes you away!

We will use **superficial electrodes** to record your muscle activity. They just attach to your skin and there is no needle or painful procedure! We will record **three muscles**: *brachioradialis* (in the top forearm), *biceps brachii* (in your arm), and *Pectoralis major* (in the top of your chest). Although we have a tank for participants**, it is better to wear a comfortable cloth so that we can access the upper chest and you feel comfortable**.

You will be randomly assigned to **Control** and **Experiment** groups; However, there is a higher chance that you end in the Experiment group as the size of it is twice as large as the Control group. The difference will be in the **visual feedback (Fig. 3)** you receive on the monitor in front of you. In experiment group, you will see a **bar representing you reflex size** and you should increase the size of the bar. In Control group, you will see the same bar but you do not need to modify it.

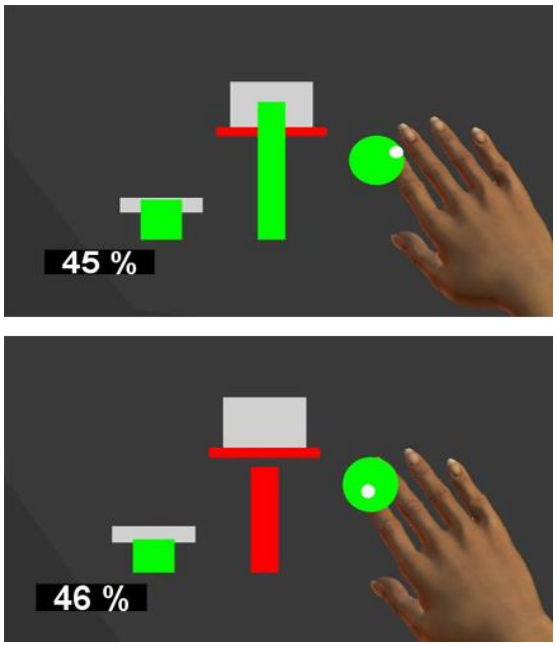

Figure 3. Visual feedback: Unsuccessful (down) and Successful (up) trials. Participants have to keep their muscle activity in a certain range (white box on the left) that makes the left vertical bar Green, then perturbation will happen and participants have to increase the size of SLR. The feedback on SLR will be shown (the vertical bar on the right). If they increase the height of the bar and make it bigger than the level of the horizontal red line and in the box, it will become green (successful), while if it does not reach that level it will become red (unsuccessful). The percentage on the screen shows the success rate in the current block.

But there is a challenge that needs your commitment!!!

Increasing the reflex size needs long-term training. Our study comprises:

2 preliminary sessions,

3 baseline sessions,

24 training sessions, and

2 follow-up sessions, which will be overall 31 sessions.

Preliminary sessions can be held anytime close to real experiment. However, baseline and training sessions must be held consecutively 3 times a week for 9 weeks (**Fig. 3**). Another issue is that your reflex size changes during the day and therefore, **you should do each session in the same daytime that you do the first session**. The follow-up sessions will be held after one and two months of last training session. Each session is planned to last **one hour**; However, some participant can finish it in 40 t 50 minutes.

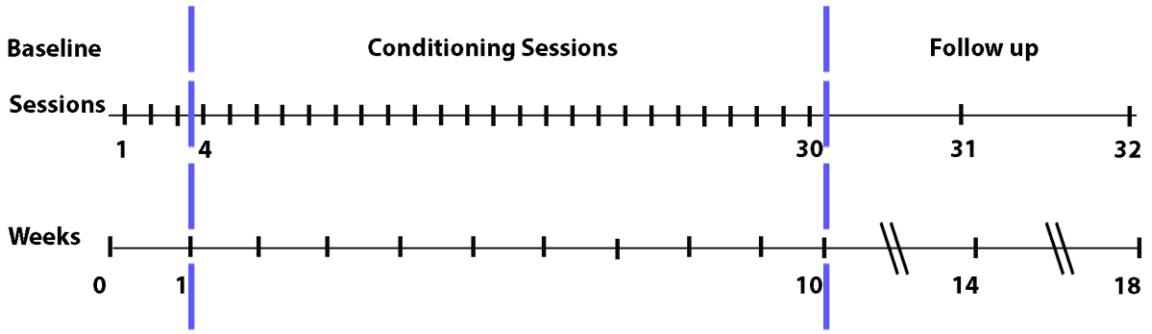

Figure 3. Study timeline and sessions

But no worries! we appreciate your time and effort and we try our best to compensate. If you attend each session (one hour), you will receive 10 CAD per session. But if you complete the study, you will receive a bonus that will be equal to whole the money you will have received by that time. In other words, will have received 20 CAD per session if you complete the study, i.e., attend the last follow-up session!

If you have no neurologic or musculoskeletal disease or problem, no history of trauma to or dislocation of your shoulder or elbow, no recent fracture of right upper limb bones, and most importantly, **if you can commit yourself to follow the study protocol for 10 weeks, YOU ARE A POTENTIAL PARTICIPANTS**.

If you are interested in participating in our study, please send me an email to arrange for a short interview and scheduling your test. If you have any question, please do not hesitate to contact me.

Curriculum Vitae

Name: **Ehsan Abolhasani**

Post-secondary Education and Degrees: Shahid Beheshti University of medical Sciences, Tehran, Iran **1999-2006 M.D.**

Related Work Experience Teaching Assistant
The University of Western Ontario
2016-2017

Publications:

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