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THERAPEUTIC APPROACHES FOR SPINAL CORD INJURY INDUCED SPASTICITY

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Abstract

Spasticity is evident in both humans and animals following spinal cord injury (SCI) and can contribute to significant functional limitation and disruption in quality of life of patients with this disorder. This mini-review describes a number of preclinical and clinical studies that promise to improve outcomes for, especially in terms of spasticity and hyper-reflexia, patients with SCI. A gold standard for the quantification of spasticity has proved elusive, but the combination of H-reflex frequency dependent depression and a novel stretch reflex (SR) windup protocol have the potential to provide new insights. As the pathophysiology of hyper-reflexia and spasticity continue to be investigated, the documented onset in the animal model of SCI provides critical time points for further study into these complex mechanisms. The positive effects of a passive exercise protocol and several potential pharmacological interventions are reviewed as well as a novel potential mechanism of action. Further work is needed to determine additional mechanisms that are involved in SCI, and how to optimize multiple therapies to overcome some of the deficits induced by SCI.

Keywords

Spinal Cord injury • Spasticity • Hyper-reflexia • H-reflex • Stretch reflex • Windup • Modafinil • L-Dopa • Exercise • Review

1. Introduction

At least 250,000 Americans currently suffer from spinal cord injury (SCI), and each year 10,000 new cases occur in the U.S.A. (NSCIA, 2006). Many of these individuals will require lifetime care due to motor deficits suffered as a result of the injury. Spasticity is prevalent in both humans and animals following SCI and can contribute to significant functional limitations affecting the ability of a patient to transfer between surfaces and in the case of an incomplete injury, regain locomotion [1] . At the same time, some individuals with SCI report benefits of spasticity with a need for improved management rather than complete suppression of all motor control that often occurs with current management techniques [1,2]. This mini-review describes a number of preclinical and clinical studies that promise to improve outcomes, especially in terms of spasticity and hyper-reflexia, in patients with SCI.

One of the major components of spasticity is hyper-reflexia [3] that develops over time following SCI [4-6]. While the pathophysiology of hyper-reflexia is not completely defined, numerous components have been identified [5,7]. The components that have been identified to contribute to the development of hyper-reflexia include decreased pre-synaptic inhibition of Ia afferents [8-12], upregulation of postsynaptic receptors [5], terminal sprouting [5] changes in intrinsic properties of motor neurons [13-16] and recently, changes in gap junctions between spinal cord neurons [17,18] (see Figure 1). One measure used to quantify hyper-reflexia is the electrical analogue of the classic tendon jerk reflex, the Hoffman or H-reflex [11,19-23]. The H-reflex is a compound electromyographic (EMG) response that can be recorded from the muscle following activation of motor neurons via muscle afferents that are stimulated by applying an electrical current to the nerve (see Figure 1). The H-reflex is rate sensitive in spinally-intact individuals and demonstrates depressed amplitude, due to marked habituation, once stimulus frequencies reach or exceed 1 Hz [24,25]. In humans or animals with chronic SCI, frequency dependent depression (FDD) of the H-reflex is markedly less evident [6,8,24,26].

Spasticity is classically defined as resistance to passive limb movement in proportion to the velocity of movement [27]. This velocity-dependent resistance is thought to be due to increased stretch reflex (SR) responses in the lengthened muscle [28-30], although increased stiffness in limb compliance is also a factor [31,32]. The noninvasive measurement of SR using electromyography (EMG) and torque response to a movement perturbation has been reported in the human [33,34]. Thompson et al developed a device to quantify the SR in rats and documented the velocity dependent response in normal rats [35], and in rats with a contusion injury of the spinal cord [36]. However, the SR response in a transection (Tx) injury has not been reported, and evidence

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from human studies indicates that SR responses differ based on completeness of injury [37,38]. From a methodological standpoint, single session quantification of the EMG response to imposed stretch is possible, but longitudinal comparison of EMG responses in rodents is problematic because of the inability to normalize the EMG signal. Validity of non-normalized comparisons can be compromised because measured differences could be due to altered electrode location or spacing rather than a true treatment effect [39].

One paradigm that overcomes this limitation is the study of the windup of repeated stretches. In a windup protocol, the EMG response to the first stretch is used to normalize subsequent responses. Windup behavior is characterized by temporal facilitation that results in increased amplitude and duration of the reflex responses. This has been demonstrated for flexor reflexes [40], and more recently in SRs in humans post SCI [41]. It has been suggested that the mechanism for this prolonged SR response is due to alterations in intrinsic motoneuron properties, namely persistent inward currents (PICs). In reduced preparations, PICs demonstrate the ability to amplify and prolong the response to brief inputs [42,43], and their emergence is linked to the onset of hyper-reflexia [16,44].

Therefore, the use of both H-reflex and SR would yield assessments of hyper-reflexia and spasticity that may shed light on the mechanisms underlying the pathology of altered motor reflexes post SCI.

Over the past several years, we have used a model of complete spinal cord transection in the rat, and studied humans with SCI, to focus on the hyper-reflexia that occurs following SCI in an effort to determine 1) the precise onset of hyper-reflexia following SCI; 2) whether passive exercise can alleviate the hyper-reflexia that occurs following SCI in animals with both acute and chronic SCI; 3) whether passive exercise can alleviate the hyper-reflexia that occurs in humans after SCI; 4) what are the effects of pharmacological agents, either alone, or in combination with passive exercise, on hyper-reflexia in animals with both acute and chronic SCI; and 5) possible mechanisms underlying the changes in FDD of the H-reflex that occur following SCI. In addition, we include 1) data from studies in which we used a new percutaneous, longitudinal method of recording H-reflex in awake rats that more closely mimics H-reflex recording methods in humans; 2) onset data for windup of the SR; and 3) molecular data supporting the regional changes in gap junction protein expression and the effects of various treatments.

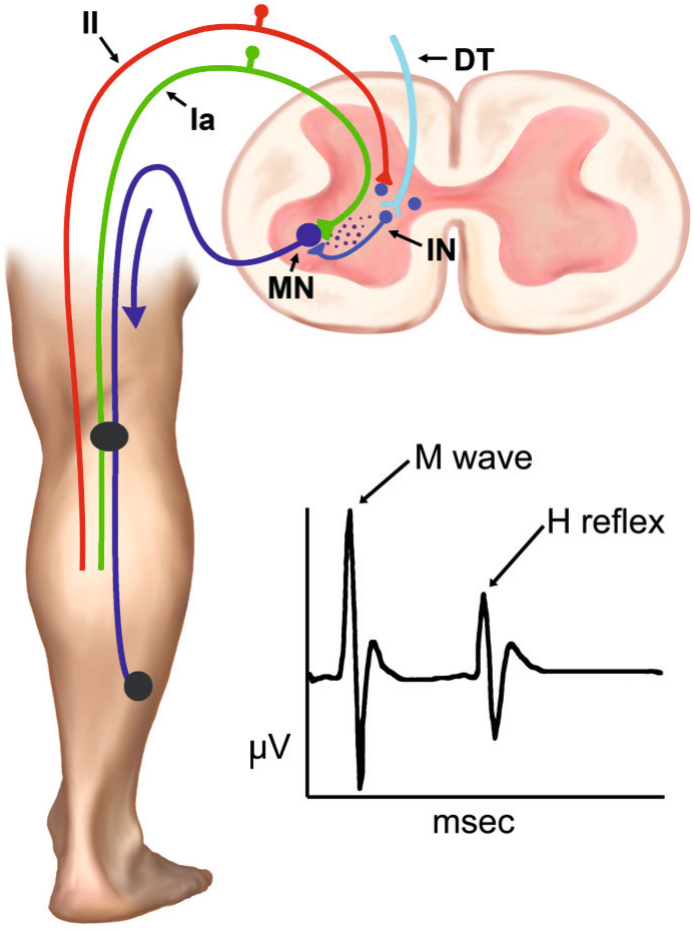

Figure 1. Representation of a human H-reflex recording and contributing pathways. DT= descending tract, IN= inhibitory interneuron, MN= motoneuron, Ia= group Ia afferent fibers, II= group II afferent fibers, small dots in the medial ventral spinal cord represent Renshaw cells. A stimulus delivered to the popliteal fossa (large black oval, stimulating electrode) first produces an orthodromic (descending dark blue pathway) volley recorded over the soleus muscle (small black circle, recording electrode), as the initial motor (M wave) response. The same stimulus will activate ascending Ia fibers (green pathway) antidromically that results in a monosynaptic connection with the motoneuron (MN), inducing a response that descends (dark blue pathway), and is recorded over the soleus muscle in the human as the longer latency H-reflex (H reflex). Mechanisms that have been proposed to contribute to spasticity include a) loss of presynaptic inhibition of group Ia and II afferent (red pathway) fibers due to loss of descending inhibition from descending tracts (DT, light blue pathway), b) intrinsic changes of the motoneurons, c) upregulation of postsynaptic receptors, and d) terminal sprouting. The present results also suggest that changes in electrical coupling in the area of the interneurons (IN, medium blue pathway) occur after SCI. Interventions outlined in the text include oral modafinil that might influence changes in electrical coupling at the level of the interneurons and motoneurons, passive exercise resulting in possible changes to the motoneuron, and L-dopa, which has been suggested to influence the group II afferent pathways.

1.1 Onset of Hyper-reflexia Following SCI

The timing of the onset of hyper-reflexia following SCI has been investigated in both animals and humans [6,18,26,45]. Schindler-Ivens and Shields [6] examined changes in the human H-reflex in a longitudinal study over 44 weeks and concluded that attenuation of FDD occurs gradually. They reported gradual changes from high-rate sensitivity to low-rate sensitivity between 6-18 weeks in the human that correlated with the transition from a flaccid to a spastic state. Studies of SCI in the rat have reported the loss of FDD at somewhere between 6 and 28 days in the contusion model [45].

Our initial studies examined FDD of the H-reflex in terminal experiments at various time points, following complete spinal cord transection (Tx) in adult rats. Three groups of animals underwent complete Tx at T10, and each group was tested for H-reflex FDD at 7, 14, and 30 days following Tx. A fourth group served as non-Tx controls. Statistically significant differences were found at 10 Hz beginning at 14 days (Figure 2). Our results suggested that the decrease in H-reflex FDD occurred between 7 and 14 days after Tx [18] and the time course observed in the Tx model was similar to that observed by others using the contusion model [42].

Recently, we developed a method of testing the H-reflex in rats longitudinally in the same animals using percutaneous electrodes [46]. This method allows us to perform testing in unanesthetized animals using the same animals repeatedly throughout the course of the study, rather than in terminal experiments. Testing the H-reflex using this method in awake animals more closely mimics the testing conditions employed with human subjects, and allows us to track the development of hyper-reflexia in the same animal over time.

Adult rats were tested for FDD of the H-reflex prior to (control), and weekly following T10 spinal cord Tx. During the 30 day post-surgical testing period, H-reflex FDD decreased significantly over time. At 10 Hz, a significant increase in H-reflex excitability was seen at 7 days post surgery compared to control. This change in excitability (loss of FDD) became progressively more marked at 14, 21, and 30 days [46] (Figure 2). These results demonstrated a slightly earlier onset of hyper-reflexia than found in studies in which animals were tested using the terminal, direct nerve stimulation method. In addition, data on the onset of SR windup is included as a comparison. Animals did not demonstrate significant windup until 49 days after Tx (Figure 2). The difference in the onset in H-reflex compared to SR suggests different mechanisms, and emphasizes the unique role of both measures in exploring the mechanisms and treatment of spasticity.

1.2 Effects of Passive Exercise on Hyper-reflexia Following SCI

We developed a method of providing passive exercise for spinalized rats over sixteen years ago and reported our first results on the effects of motorized bicycle exercise training (MBET) on H-reflex FDD in rats with complete Tx in 1994 [47]. Since that time, we have focused on determining the optimal parameters of exercise capable of alleviating the hyper-reflexia following complete spinal cord Tx in adult rats with both acute and chronic injuries. Experiments involving separate groups of animals exercised for 15, 30, 45, and 60 days demonstrated FDD of the H-reflex in a MBET duration-dependent pattern [26]. MBET for only 15 days did not induce a statistically significant effect on H-reflex habituation, but did have a numerical effect, increasing FDD (decreasing percent inhibition). However, after 30 days of MBET, there were statistically significant increases in habituation at all frequencies tested, suggesting that passive exercise prevented the loss of H-reflex FDD. The effects of longer durations of MBET produced decreases in habituation that were linearly decrementing at 10 Hz. In all cases, the initiation of the exercise intervention occurred at 7 days post Tx that, according to our studies, coincided with the first appearance of hyper-reflexia. Therefore, our data indicated the ability of MBET to prevent the onset of hyper-reflexia after SCI [17].

We then became interested in whether the same intervention could restore H-reflex FDD

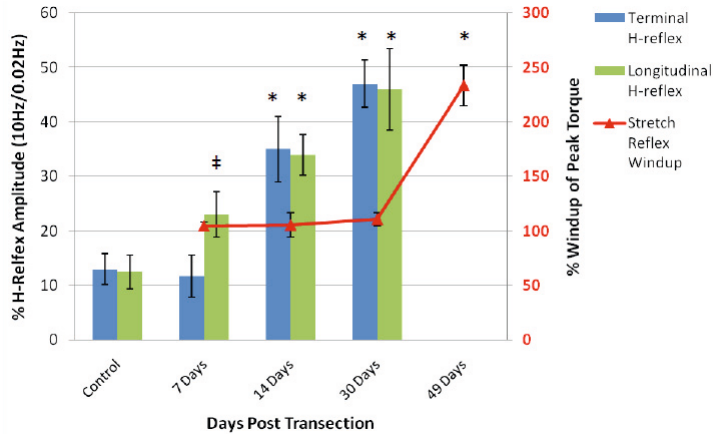

Figure 2. The emergence of spasticity and hyper-reflexia in rats post transection (Tx) injury. Frequency dependent depression (FDD) of the H-reflex is shown as column plots using two different techniques. FDD is a measure of hyper-reflexia and is expressed as %H-Reflex amplitude (10Hz/0.02Hz; left axis). Stretch reflex (SR) windup of the peak plantarflexion torque is shown as the line plot. This novel method of spasticity measurement is expressed as percentage increase in torque to a series of 10 imposed stretches (right axis). Control animals show strong FDD in H-reflex amplitude with both terminal and longitudinal techniques showing good agreement. At 7 days post-Tx, terminal H-reflex data show no difference over controls, however, the longitudinal technique in the awake animal shows a significant increase in amplitude (‡p<0.05). Although there are EMG responses to movement perturbations, the SR does not windup at this time point. At 14 days, both H-reflex measures are significantly elevated, establishing the presence of hyper-reflexia (*p<0.01). In contrast, SR windup does not occur. By 30 days, hyper-reflexia reaches a plateau but the SR windup remains absent. At 49 days spasticity is evidenced by the presence of significant windup of the stretch reflex.

in animals in which hyper-reflexia was well established. An initial experiment examining the effects of passive exercise in animals that demonstrated hyper-reflexia (chronic injury) revealed that 30 days of MBET did not restore FDD. These results are consistent with the work of Norrie et al. [48], who reported that animals with a contusion SCI that received a three week training session after a delay, were less responsive to rehabilitation training than those immediately trained after injury. Therefore, a delay in training can impact potential functional recovery and reflex modulation. However, if animals were exercised for 60 days of MBET after they had developed hyper-reflexia, restoration of the H-reflex FDD occurred at 5 and 10 Hz. Therefore, this work suggests that passive exercise can be used to rescue the spinal cord circuitry once hyper-reflexia has been established, but requires a longer duration of therapy [17].

To confirm this finding, exercise MBET was initiated 30 days post-Tx, once hyper-reflexia was well established in a group of rats, and continued for 60 days (until 90 days post-Tx). H-reflex was tested using the longitudinal, surface electrode method in each animal before surgery (control) and on days 7, 14, 30, 45, 60, 75, and 90 following Tx. Results indicated that MBET alone was able to restore H-reflex FDD to near control levels by 75 days post-Tx (45 days MBET) when tested at 10Hz. While H-reflex amplitudes tested in the early days after Tx were significantly higher than control levels (loss of FDD), H-reflex amplitudes tested at 75- and 90-days were not significantly different from the pre-Tx levels (restoration of FDD). These findings provided a clear indication that MBET was effective in reversing the hyper-reflexia that developed after spinal cord Tx, and that as little as 45 days of passive exercise was enough to restore H-reflex FDD in animals with established hyper-reflexia (chronic SCI) [46].

One additional group of animals was used to determine the effects on the H-reflex after exercise was discontinued. The group of animals (n=7) began exercise 7 days post transection, exercised per MBET protocol for 30 days and then did not exercise for 30 days (Figure 3). The H-reflex was tested and this group of animals demonstrated significant **savings** (maintained H-reflex FDD) after therapy was discontinued when hyper-reflexia was prevented by early passive exercise [15]. Further studies are needed to determine the effects on the SR and the duration of savings that can be gained if exercise is initiated in the chronic stage of injury.

Our lab has also studied the effects of passive exercise in patients with SCI (ASIA B) and the use of a motorized bicycle exercise trainer MBET (Figure 4). This patient showed habituation of the H-reflex after 8-10 weeks of training [49]. The H-reflex gains observed in a chronic patient (>1 year after injury) with a SCI

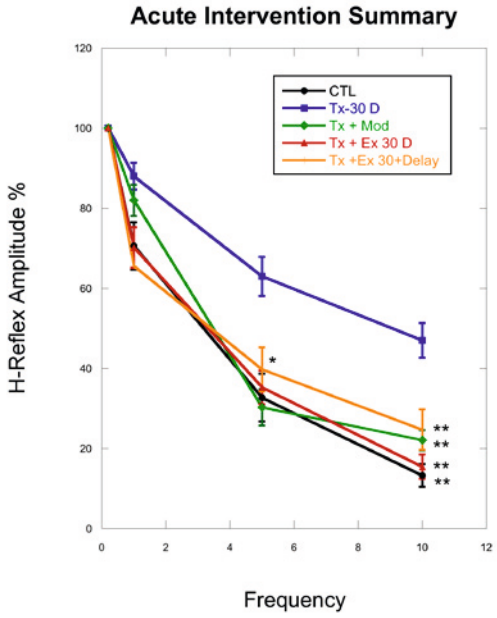

Figure 3. Summary graph for interventions used in the acute stage of SCI. Interventions were initiated 7 days following complete SC Tx. H-reflex amplitude at 0.2, 1, 5 and 10 Hz for intact animals (Control, black circles), MBET 30 days (Tx+ Ex 30D, red triangle), acute exercise for 30 days and then delay 30 days (Tx+ Ex 30D+ Delay, orange dash), an oral modafinil (Tx+Mod, green diamond). Frequency-dependent depression of the H-reflex at 0.2 Hz was designated 100%, and statistical comparisons made against the Tx 30 D group (blue squares) . At 10 Hz, the Tx 30 D group (**) differed from the Tx+Ex 30 D + Delay, the acute exercise group (Tx+Ex 30 D), the Tx+ Mod group and the control group ($p<0.01$).

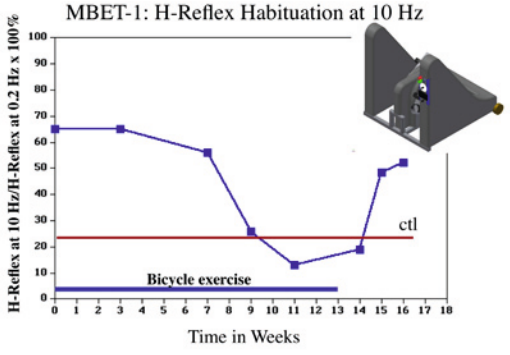

Figure 4. Effect of MBET on the H-reflex for a patient with SCI. Frequency-dependent depression of the H-reflex measured at 10 Hz over the course of 13 weeks of MBET sessions in a patient with a chronic SCI. Bicycle exercise consisted of 60 min sessions 5 days per week indicated by the blue bar. Following cessation of exercise at 13 weeks, the H-reflex trended back toward pre-exercise levels indicating minimal savings.

who regained normalization of the H-reflex after a thirteen week training program only showed savings over a two week period after cessation of passive exercise [47]. That is, there was minimal savings after rescue in a human chronic patient with a SCI.

In addition, bicycle training (single bout with a tandem bicycle setup) has recently been shown to be effective in reducing hyper-reflexia in patients with an incomplete SCI [50]. These translational studies suggest that bicycle training can play a role in the rehabilitation of individuals post SCI and that the optimization of training protocols and possible combination with drug therapies needs to be explored further.

1.3 Effects of Pharmacological Agents and Non-pharmacological Agents on Hyper-reflexia Following SCI

Hyper-reflexia and spasticity are present following a multitude of upper motor neuron disorders. Pharmacological agents such as Baclofen, Diazepam, Tizanidine, Dantrolene, and others have been used in human subjects in an attempt to decrease spasticity following SCI. However, all of these drugs have some undesirable side effects on patients and some have no effects on hyper-reflexia [51].

Interventions for spasticity after SCI range from conservative physical therapy treatments to more invasive surgical approaches and pharmacologic regimens. Physical therapy interventions tend to focus on the impairment and include the use of modalities as well as standing programs, passive stretching, and serial casting [52]. Surgical interventions such as selective dorsal rhizotomy and the surgical implantation of an Intrathecal baclofen pump have been used to treat spasticity with success. Selective dorsal rhizotomy has been used more commonly in the case of children with spasticity of cerebral origin and has associated risks and side effects [53]. The surgical intervention of ITB placement has risks and side effects as well including surgical risks, establishing and maintaining an optimal dose, headache, nausea/vomiting, sedation, pump complications, constipation, drowsiness, dizziness and muscular hypotonia (especially of the trunk), and tolerance [54,55]. Non-surgical interventions used for spasticity include the use of botulinum toxin injections [56]. Botulinum toxin is limited to treating a focal spasticity and has been effective in post stroke patients although side effects include the potential of decreased muscle function and weakness in addition to the potential requirement for repeated injections.

Due to the limitations of the available current therapies, we have investigated the effects of two pharmacological agents, L-dopa and modafinil, on the hyper-reflexia that occurs following SCI. Dopaminergic agonists have been implicated in the initiation and continuation of locomotion. The dopamine precursor, L-dopa, is capable of inducing locomotor activity of long duration in decerebrate neonatal rats [57]. In mid-thoracic spinalized adult cats, L-dopa initiated hind limb locomotion when the hind limbs were placed on a moving treadmill [58,59]. In addition, dopaminergic agonists have been found to ameliorate motor deficits in animals and humans following brain injury. After cerebral frontal cortex injuries, amphetamine increased the rate of recovery of beam-walking ability in rats and in cats [60,61], an effect that was blocked by haloperidol [62]. Also in cats, both amphetamine and apomorphine improved the rate of recovery of tactile placing following motor cortex injury [63]. While the recovery rate was accelerated with treatment, the end result, however, was about the same.

In a study of short duration, a single dose of amphetamine or placebo was given to stroke patients followed within 3 hrs by a single physical therapy session. The next day, motor scores were significantly improved in patients receiving amphetamine [64]. Also in a single dose study, L-dopa given to patients with SCI suppressed all muscle reflexes induced by large muscle afferents with the peak reduction occurring at 1 hour [65]. In stroke rehabilitation in human patients over a 6-week period, motor recovery improved significantly beginning at 2 weeks when L-dopa was administered 30 minutes prior to physical therapy [66]. This effect was maintained for the duration of the test period, i.e. 3 weeks after treatment ceased.

The effects of L-dopa on hyper-reflexia and spasticity have not been investigated extensively, despite the fact that it is effective with minimal side effects in treating motor disorders associated with Parkinson's disease and has been reported to decrease spasticity in subjects with complete and incomplete SCI [65]. Therefore, we investigated the ability of L-dopa, both alone and in combination with exercise, to alleviate the hyper-reflexia that occurs following SCI. Once hyper-reflexia was well established (by 30 days post-Tx) as evidenced by loss of FDD of the H-reflex, animals were then randomly divided into four groups. Each group received a different intervention: Tx only, MBET, L-dopa, and MBET plus L-dopa. Interventions were administered for 60 days (5 days per week), and H-reflex was tested every two weeks during the intervention period. Testing of the H-reflex at 10 Hz revealed that by 90 days post-Tx, H-reflex FDD was restored to pre-Tx levels in the MBET, L-dopa, and MBET plus L-dopa groups. In addition, there appeared to be an additive benefit of L-dopa when added to MBET compared to exercise alone in restoring H-reflex FDD in these animals with chronic SCI [46]. Additional work is needed to investigate the benefits of combined passive exercise and L-dopa in the human.

1.4 Modafinil

The literature revealed limited studies of modafinil as a treatment for hyper-reflexia or spasticity induced by SCI. Mukai and Costa [67] reported positive effects from modafinil on self-esteem in 2 patients with SCI. Hurst et al [68] described a retrospective study of the use of modafinil in a population of children diagnosed with cerebral palsy. They reported 76% of the patients studied reported decreased spasticity after treatment with modafinil, and showed decreased tone after physical examination. Hurst and Lajara-Nanson [69] conducted a pilot study to examine the benefits of modafinil on spasticity and went on to hypothesize that modafinil reduces spasticity via central descending effects. An additional study in 2006 by Hurst et al [70] reported that 29/59 pediatric patients with spastic cerebral palsy who were treated with modafinil demonstrated improvements in gait during the treatment.

We investigated if modafinil, administered orally, would normalize the loss of FDD of the

H-reflex that is observed in spinally Tx rats. The results of these studies revealed that modafinil, when given orally for 30 days, can be used to restore FDD of the H-reflex [71]. Results of the interventions outlined above can be seen in the summary graph (Figure 3).

1.5 Potential Mechanisms Underlying H-reflex Changes After SCI

The mechanism of action of modafinil was unknown until recently, but was credited with increasing glutamate, acetylcholine, noradrenaline and serotonin release, and decreasing GABA release [72]. However, modafinil was recently found to increase electrical coupling between nerve cells in the inferior olivary nucleus, cortical interneurons, and thalamic reticular neurons [73]. Following pharmacological blockade of connexin permeability, modafinil restored electrotonic coupling within 30 minutes. The effects of modafinil were counteracted by the gap junction blocker mefloquine (MEF). Urbano et al [74] proposed that modafinil may be acting in a wide variety of cerebral areas by increasing electrotonic coupling in such a way that the high input resistance typical of GABAergic neurons is reduced. Studies in our lab that investigated the use of daily oral administration of modafinil being used to prevent the loss of FDD of the H-reflex in acutely spinalized rats, raise a number of intriguing questions regarding the mechanisms behind hyper-reflexia and spasticity.

The role of electrotonic coupling in modulating motor behavior has not been extensively studied. Interestingly, if the spinal cord of the rat is transected before 15 days of age, the animal regained the ability to elicit reflexive locomotor movements on a treadmill, but if the Tx is performed after 15 days of age, the animal did not regain the ability to generate reflexive locomotion on a treadmill [75]. The period in which the spinal cord is optimal for plastic changes in the rat has been determined to be 18 days of age [76]. What is occurring in development that could contribute to the changes in plasticity at the end of the critical period? Electrical coupling of neurons via gap junctions have been shown to exist in early postnatal development of spinal motoneurons [77]. Motoneuron electrotonic coupling has been shown to decrease with postnatal age in the rat from postnatal day 0 through postnatal day 14 [78]. Such coupling may allow stronger synchronized contractions in weak muscles during development, for example, when chicks must break the eggshell at hatching [79]. Does a correlation exist between the end of critical period of developmental plasticity in the rat spinal cord and the decreased gap junction coupling observed? The decrease in coupling has been attributed to the development of fine motor control by independent recruitment of motor units [35]. An important study showed that there are populations of locomotion-related interneurons in the ventromdedial gray matter that are electrically coupled [80], suggesting that not only motoneuron pools may be coupled. In general, electrical synapses allow the reciprocal flow of ionic currents and small molecules between neurons, often providing synchronization of subthreshold and spiking activity [81]. Connexins form clusters of channels that allow direct cell to cell communication. Electrical communication between neurons has been attributed to gap junctions made up of Connexin 36 (Cx-36). In mammals, Cx-36 is specifically expressed in neurons [82]. Deans et al studied the knockout mouse of Cx-36 and determined that many physiologic properties of the knockout were similar to the wild-type with the exception that electrical coupling in the knockout mouse was rare and weak compared with the wild-type supporting the theory that gap junctions comprised of Cx-36 are responsible for electrical coupling [83].

So how do spinal cord gap junctions influence motor coordination? Electrical synapses may contribute to the generation and maintenance of synchronized neuronal bursting firing patterns [44]. Tresh and Kiehn demonstrated that motor patterns in the neonatal rat spinal cord were observed during blockade of chemical synapses, probably through the synchronization of bursting through gap junctions [16,84]. They suggested, "Gap junction-mediated neuronal coordination contributes to the basic function and organization of spinal motor systems." These authors also suggested the existence of numerous independent rhythms in distinct motor pools. However, the modulation of gap junction communication in the adult spinal system and the adult system after injury are poorly understood.

In an effort to explore the possible role of gap junctions in hyper-reflexia and spasticity, our lab has documented the changes Cx-36 protein levels post-Tx. In whole spinal cord samples taken from the lumbar enlargement, there was a transient decrease in Cx-36 protein levels after Tx but this returned to near normal levels by 30 days [18]. A separate study demonstrated the normalizing effect on hyper-reflexia by modafinil treatment, but our molecular data have failed to show a significant change in Cx-36 protein levels with administration of modafinil [71]. It is thought that examination of the whole cord may result in a masking of regional changes in gap junction proteins. As an initial step to explore this idea, our lab recently investigated if regional decreases in Cx-36 mRNA expression are present post-Tx and whether or not interventions including modafinil and MBET might restore these levels.

The spinal cord from below the Tx level was removed intact and immediately frozen in solid CO2. 1mm transverse slices were made throughout the lumbar enlargement. 0.5 and 0.7mm tissue punches were used to take cores from the dorsal and ventral horns of the lumbar gray matter. Samples were homogenized, and total RNA extracted. cDNA was made as previously described [85,86]. Primers and standards were developed specifically to quantify Cx-36 transcripts by real-time qRT-PCR following procedures described previously [87]. qRT-PCR was conducted as previously described [86]. Fluorescence was measured at the end of each cycle during amplification. A melting-curve cycle was used to monitor specificity of amplification. To further ensure accuracy, each reaction was repeated at least twice. The mean value of the repeat was used for each gene per sample to calculate the ratio between the mean value of the target gene (Cx-36) and the two rat housekeeping genes (Gapdh and Rpl). The results of the regional mRNA expression can be seen in Figure 5A-B. In contrast to the whole cord data, Cx-36

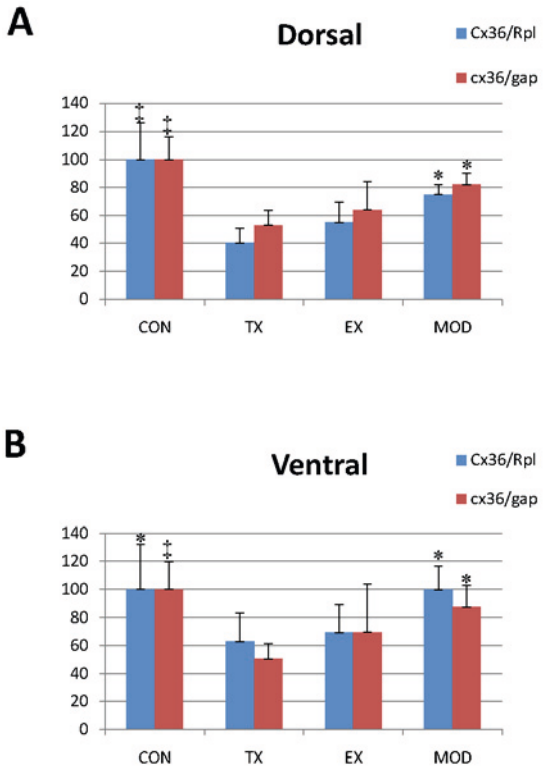

Figure 5. Regional Cx-36 expression in the spinal cord. RT-PCR results for two different housekeeping genes (Rpl and gap) in rats before and after spinal transection (Tx). Results are normalized to the control (CON) values (n=6). Group means ± SD are shown for 7 weeks post-Tx (n=4), following 6 weeks of exercise (Ex) (n=7), and 6 weeks of oral modafinil (MOD) (n=4). Cx-36 expression was significantly decreased post-Tx in the dorsal (A) and ventral horns (B); Exercise failed to restore Cx-36 to control levels. Treatment with modafinil, however, showed an increase in Cx-36 over Tx levels with no significant difference from control animals. While both MOD and EX animals show normalization of Hyper-reflexia and spasticity, the molecular data support differing mechanisms of action. (Statistical comparison to Tx values, * $p<0.05$, ‡$p<0.01$).

mRNA expression was significantly decreased compared to control levels at 7 weeks post-Tx. This might reflect changes in the regulation of mRNA translation so that normal (control) levels of protein are synthesized from less mRNA, i.e. an increase in mRNA translation control. Translational control of Cx-36 mRNA has been reported after amphetamine withdrawl [88] and after ischemia [89].

Animals given 30 days of MBET failed to show significant increases in Cx-36 levels as compared to Tx. However, those treated with modafinil, showed a return toward baseline levels with a significant increase over the Tx group. These results support the notion of gap junctions playing a role in hyper-reflexia and point to a contributing mechanism to abnormal motor reflexes. Interpretation of changes in protein and mRNA levels must be made with care since it is not clear if these changes are further modulated by changes in exteriorization, trafficking, alignment or opening/closing processes of Cx-36 hemi-channels. Future work is needed in exploring the potential mechanism involving gap junctions following SCI, as well as functional changes in gap junctions post SCI.

2. Summary

Our Center has investigated H-reflex FDD as a valuable research outcome measure to quantify hyper-reflexia in the animal model and the human patient. We have modified this technique to also include measuring the H-reflex FDD in the awake animal longitudinally. We have measured the SR and developed the windup protocol as an outcome measure to quantify the development of spasticity in the Tx rat.

We have investigated several interventions including passive MBET, developed a MBET for human use, and tested pharmacologic interventions including the use of oral modafinil and L-dopa to determine whether these interventions are useful in both the acute and chronic stages of injury to reduce or normalize hyper-reflexia and spasticity. We are currently investigating the role of gap junctions in the spinalized animal and working to determine the regional differences that exist in Cx-36 mRNA. Further work is needed to determine additional mechanisms that are involved in SCI, and how to optimize multiple therapies to overcome some of the deficits induced by SCI.

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References

[1] Little J.W., Micklesen P., Umlauf R., and Britell C., Lower extremity manifestations of spasticity in chronic spinal cord injury, American Journal of Physical Medicine & Rehabilitation, 1989, 68(1), 32-6

[2] Cook K.F., Teal C.R., Engebretson J.C., Hart K.A., Mahoney J.S., Robinson-Whelen S., et al., Development and validation of Patient Reported Impact of Spasticity Measure (PRISM), J Rehabil Res Dev, 2007, 44(3), 363-71

[3] Adams M.M. and Hicks A.L., Spasticity after spinal cord injury, Spinal Cord, 2005, 43(10), 577-86

[4] Malmsten J., Time course of segmental reflex changes after chronic spinal cord hemisection in the rat, Acta Physiol Scand, 1983, 119(4), 435-43

[5] Little J.W., Ditunno J.F., Jr., Stiens S.A., and Harris R.M., Incomplete spinal cord injury: neuronal mechanisms of motor recovery and hyperreflexia, Archives of Physical Medicine & Rehabilitation., 1999, 80(5), 587-99

[6] Schindler-Ivens S. and Shields R.K., Low frequency depression of H-reflexes in humans with acute and chronic spinal-cord injury, Exp Brain Res, 2000, 133(2), 233-41

[7] Nielsen J.B., Crone C., and Hultborn H., The spinal pathophysiology of spasticity--from a basic science point of view, Acta Physiol (Oxf), 2007, 189(2), 171-80

[8] Calancie B., Broton J.G., Klose K.J., Traad M., Difini J., and Ayyar D.R., Evidence that alterations in presynaptic inhibition contribute to segmental hypo- and hyperexcitability after spinal cord injury in man, Electroencephalogr Clin Neurophysiol, 1993, 89(3), 177-86

[9] Nielsen J., Petersen N., and Crone C., Changes in transmission across synapses of Ia afferents in spastic patients, Brain, 1995, 118 (Pt 4), 995-1004

[10] Pierrot-Deseilligny E., Electrophysiological assessment of the spinal mechanisms underlying spasticity, Electroencephalogr Clin Neurophysiol Suppl, 1990, 41, 264-73

[11] Faist M., Mazevet D., Dietz V., and Pierrot-Deseilligny E., A quantitative assessment of presynaptic inhibition of Ia afferents in spastics. Differences in hemiplegics and paraplegics, Brain, 1994, 117 (Pt 6), 1449-55

[12] Delwaide P.J., Human monosynaptic reflexes and presynaptic inhibition: an interpretation of spastic hyperreflexia New developments in Electromyography and Clinical Neurophysiology, 1973, 508-522

[13] Eken T., Hultborn H., and Kiehn O., Possible functions of transmitter-controlled plateau potentials in alpha motoneurones, Progress in Brain Research, 1989, 80, 257-67

[14] Li Y. and Bennett D.J., Persistent sodium and calcium currents cause plateau potentials in motoneurons of chronic spinal rats, J Neurophysiol, 2003, 90(2), 857-69

[15] Li Y., Gorassini M.A., and Bennett D.J., Role of persistent sodium and calcium currents in motoneuron firing and spasticity in chronic spinal rats, J Neurophysiol, 2004, 91(2), 767-83

[16] Bennett D.J., Li Y., Harvey P.J., and Gorassini M., Evidence for plateau potentials in tail motoneurons of awake chronic spinal rats with spasticity, J Neurophysiol, 2001, 86(4), 1972-82

[17] Yates C.C., Charlesworth A., Reese N.B., Skinner R.D., and Garcia-Rill E., The effects of passive exercise therapy initiated prior to or after the development of hyperreflexia following spinal transection, Exp Neurol, 2008, 213(2), 405-9

[18] Yates C., Charlesworth A., Allen S.R., Reese N.B., Skinner R.D., and Garcia-Rill E., The onset of hyperreflexia in the rat following complete spinal cord transection, Spinal Cord, 2008,

[19] Milanov I., Examination of the segmental pathophysiological mechanisms of spasticity, Electromyogr Clin Neurophysiol, 1994, 34(2), 73-9

[20] Angel R.W. and Hofmann W.W., The H Reflex in Normal, Spastic, and Rigid Subjects, Arch Neurol, 1963, 9, 591-6

[21] Little J.W. and Halar E.M., H-reflex changes following spinal cord injury, Archives of Physical Medicine & Rehabilitation, 1985, 66(1), 19-22

[22] Olsen P.Z. and Diamantopoulos E., Excitability of spinal motor neurones in normal subjects and patients with spasticity, Parkinsonian rigidity, and cerebellar hypotonia, J Neurol Neurosurg Psychiatry, 1967, 30(4), 325-31

[23] Yablon S.A. and Stokic D.S., Neurophysiologic evaluation of spastic hypertonia: implications for management of the patient with the intrathecal baclofen pump, Am J Phys Med Rehabil, 2004, 83(10 Suppl), S10-8

[24] Ishikawa K., Ott K., Porter R.W., and Stuart D., Low frequency depression of the H wave in normal and spinal man, Exp Neurol, 1966, 15(1), 140-56

[25] Curtis D.R. and Eccles J.C., Synaptic action during and after repetitive stimulation, J Physiol, 1960, 150, 374-98

[26] Reese N.B., Skinner R.D., Mitchell D., Yates C., Barnes C.N., Kiser T.S., et al., Restoration of frequency-dependent depression of the H-reflex by passive exercise in spinal rats, Spinal Cord, 2006, 44(1), 28-34

[27] Lance J.W., Pathophysiology of Spasticity and Clinical Experience with Baclofen., in Spasticity: Disordered Motor Control., R.G. Feldman, R.R. Young, and W.P. Koella., Editors. 1980, Year Book: Chicago. p. 185-203.

[28] Kuhn R.A., Functional capacity of the isolated human spinal cord, Brain, 1950, 73(1), 1-51

[29] Powers R.K. and Rymer W.Z., Effects of acute dorsal spinal hemisection on motoneuron discharge in the medial gastrocnemius of the decerebrate cat, J Neurophysiol, 1988, 59(5), 1540-56

[30] Ju M.S., Chen J.J., Lee H.M., Lin T.S., Lin C.C., and Huang Y.Z., Time-course analysis of stretch reflexes in hemiparetic subjects using an on-line spasticity measurement system, J Electromyogr Kinesiol, 2000, 10(1), 1-14

[31] Dietz V., Quintern J., and Berger W., Electrophysiological studies of gait in spasticity and rigidity. Evidence that altered mechanical properties of muscle contribute to hypertonia, Brain, 1981, 104(3), 431-49

[32] Sinkjaer T., Toft E., Larsen K., Andreassen S., and Hansen H.J., Non-reflex and reflex mediated ankle joint stiffness in multiple sclerosis patients with spasticity, Muscle Nerve, 1993, 16(1), 69-76

[33] Schmit B.D., Dhaher Y., Dewald J.P., and Rymer W.Z., Reflex torque response to movement of the spastic elbow: theoretical analyses and implications for quantification of spasticity, Annals of Biomedical Engineering, 1999, 27(6), 815-29

[34] Schmit B.D., Benz E.N., and Rymer W.Z., Afferent mechanisms for the reflex response to imposed ankle movement in chronic spinal cord injury, Exp Brain Res, 2002, 145(1), 40-9

[35] Thompson F.J., Browd C.R., Carvalho P.M., and Hsiao J., Velocity-dependent ankle torque in the normal rat, Neuroreport, 1996, 7(14), 2273-6

[36] Bose P., Parmer R., and Thompson F.J., Velocity-dependent ankle torque in rats after contusion injury of the midthoracic spinal cord: time course, J Neurotrauma, 2002, 19(10), 1231-49

[37] Calancie B., Molano M.R., and Broton J.G., Interlimb reflexes and synaptic plasticity become evident months after human spinal cord injury, Brain, 2002, 125(Pt 5), 1150-61

[38] Nakazawa K., Kawashima N., and Akai M., Enhanced stretch reflex excitability of the soleus muscle in persons with incomplete rather than complete chronic spinal cord injury, Arch Phys Med Rehabil, 2006, 87(1), 71-5

[39] Soderberg G.L. and Knutson L.M., A guide for use and interpretation of kinesiologic electromyographic data, Phys Ther, 2000, 80(5), 485-98

[40] Hornby T.G., Rymer W.Z., Benz E.N., and Schmit B.D., Windup of Flexion Reflexes in Chronic Human Spinal Cord Injury: A Marker for Neuronal Plateau Potentials?, J Neurophysiol, 2003, 89(1), 416-426

[41] Hornby T.G., Kahn J.H., Wu M., and Schmit B.D., Temporal facilitation of spastic stretch reflexes following human spinal cord injury, J Physiol, 2006, 571(Pt 3), 593-604

[42] Crone C., Hultborn H., Kiehn O., Mazieres L., and Wigstrom H., Maintained changes in motoneuronal excitability by short-lasting synaptic inputs in the decerebrate cat, J Physiol, 1988, 405, 321-43

[43] Hounsgaard J., Hultborn H., Jespersen B., and Kiehn O., Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan, Journal of Physiology, 1988, 405, 345-67

[44] Bennett D.J., Gorassini M., Fouad K., Sanelli L., Han Y., and Cheng J., Spasticity in rats with sacral spinal cord injury, J Neurotrauma, 1999, 16(1), 69-84

[45] Thompson F.J., Reier P.J., Lucas C.C., and Parmer R., Altered patterns of reflex excitability subsequent to contusion injury of the rat spinal cord, J Neurophysiol, 1992, 68(5), 1473-86

[46] A. Arafaj R.D.S., C. Yates, K. Garrison, N.B. Reese, E. Garcia-Rill. Reversal of H-reflex Hyperactivity by L-dopa and Exercise in Alert, Chronically Spinalized Rats. in Neurosci Abstr. 2009.

[47] Reese N.B., Houlé, J.D., Peterson, C.A., Gurley, C.M., Berry, C.L., Skinner, R.D. and Garcia-Rill, E Effects of fetal spinal cord implants and exercise on muscle atrophy in chronic spinal rats. in Neurosci. Abstr. 1994.

[48] Norrie B.A., Nevett-Duchcherer J.M., and Gorassini M.A., Reduced functional recovery by delaying motor training after spinal cord injury, J Neurophysiol, 2005, 94(1), 255-64

[49] Kiser T.S., Reese N.B., Maresh T., Hearn S., Yates C., Skinner R.D., et al., Use of a motorized bicycle exercise trainer to normalize frequency-dependent habituation of the H-reflex in spinal cord injury, J Spinal Cord Med, 2005, 28(3), 241-5

[50] Phadke C.P., Flynn S.M., Thompson F.J., Behrman A.L., Trimble M.H., and Kukulka C.G., Comparison of single bout effects of bicycle training versus locomotor training on paired reflex depression of the soleus H-reflex after motor incomplete spinal cord injury, Arch Phys Med Rehabil, 2009, 90(7), 1218-28

[51] Kita M. and Goodkin D.E., Drugs used to treat spasticity, Drugs, 2000, 59(3), 487-95

[52] Watanabe T., The role of therapy in spasticity management, Am J Phys Med Rehabil, 2004, 83(10 Suppl), S45-9

[53] Park T.S. and Johnston J.M., Surgical techniques of selective dorsal rhizotomy for spastic cerebral palsy. Technical note, Neurosurg Focus, 2006, 21(2), e7

[54] Stempien L. and Tsai T., Intrathecal baclofen pump use for spasticity: a clinical survey, Am J Phys Med Rehabil, 2000, 79(6), 536-41

[55] Hsieh J.C. and Penn R.D., Intrathecal baclofen in the treatment of adult spasticity, Neurosurg Focus, 2006, 21(2), e5

[56] Marciniak C., Rader L., and Gagnon C., The use of botulinum toxin for spasticity after spinal cord injury, Am J Phys Med Rehabil, 2008, 87(4), 312-7; quiz 318-20, 329

[57] Iwahara T., Van Hartesveldt C., Garcia-Rill E., and Skinner R.D., L-dopa-induced air-stepping in decerebrate developing rats, Brain Res Dev Brain Res, 1991, 58(2), 257-64

[58] Barbeau H. and Rossignol S., Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs, Brain Res, 1991, 546(2), 250-60

[59] Pearson K.G. and Rossignol S., Fictive motor patterns in chronic spinal cats, J Neurophysiol, 1991, 66(6), 1874-87

[60] Hovda D.A. and Fenney D.M., Amphetamine with experience promotes recovery of locomotor function after unilateral frontal cortex injury in the cat, Brain Res, 1984, 298(2), 358-61

[61] Sutton R.L., Hovda D.A., and Feeney D.M., Amphetamine accelerates recovery of locomotor function following bilateral frontal cortex ablation in cats, Behav Neurosci, 1989, 103(4), 837-41

[62] Feeney D.M., Gonzalez A., and Law W.A., Amphetamine, haloperidol, and experience interact to affect rate of recovery after motor cortex injury, Science, 1982, 217(4562), 855-7

[63] Feeney D.M. and Hovda D.A., Amphetamine and apomorphine restore tactile placing after motor cortex injury in the cat, Psychopharmacology (Berl), 1983, 79(1), 67-71

[64] Crisostomo E.A., Duncan P.W., Propst M., Dawson D.V., and Davis J.N., Evidence that amphetamine with physical therapy promotes recovery of motor function in stroke patients, Ann Neurol, 1988, 23(1), 94-7

[65] Eriksson J., Olausson B., and Jankowska E., Antispastic effects of L-dopa, Exp Brain Res, 1996, 111(2), 296-304
[66] Scheidtmann K., Fries W., Muller F., and Koenig E., Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: a prospective, randomised, double-blind study, Lancet, 2001, 358(9284), 787-90
[67] Mukai A. and Costa J.L., The effect of modafinil on self-esteem in spinal cord injury patients: a report of 2 cases and review of the literature, Arch Phys Med Rehabil, 2005, 86(9), 1887-9
[68] Hurst D.L., Lajara-Nanson W.A., Dinakar P., and Schiffer R.B., Retrospective review of modafinil use for cerebral palsy, J Child Neurol, 2004, 19(12), 948-51
[69] Hurst D.L. and Lajara-Nanson W., Use of modafinil in spastic cerebral palsy, J Child Neurol, 2002, 17(3), 169-72
[70] Hurst D.L., Lajara-Nanson W.A., and Lance-Fish M.E., Walking with modafinil and its use in diplegic cerebral palsy: retrospective review, J Child Neurol, 2006, 21(4), 294-7
[71] Yates C.C., Charlesworth A., Reese N.B., Ishida K., Skinner R.D., and Garcia-Rill E., Modafinil normalized hyperreflexia after spinal transection in adult rats, Spinal Cord, 2009, 47(6), 481-5
[72] Kistler W.M., De Jeu M.T., Elgersma Y., Van Der Giessen R.S., Hensbroek R., Luo C., et al., Analysis of Cx36 knockout does not support tenet that olivary gap junctions are required for complex spike synchronization and normal motor performance, Ann N Y Acad Sci, 2002, 978, 391-404
[73] Skinner F.K., Zhang L., Velazquez J.L., and Carlen P.L., Bursting in inhibitory interneuronal networks: A role for gap-junctional coupling, J Neurophysiol, 1999, 81(3), 1274-83
[74] Urbano F.J., Leznik E., and Llinas R.R., Modafinil enhances thalamocortical activity by increasing neuronal electrotonic coupling, Proc Natl Acad Sci U S A, 2007, 104(30), 12554-9
[75] Stelzner D.J., Ershler W.B., and Weber E.D., Effects of spinal transection in neonatal and weanling rats: survival of function, Exp Neurol, 1975, 46(1), 156-77
[76] Fawcett J.W., Overcoming inhibition in the damaged spinal cord, J Neurotrauma, 2006, 23(3-4), 371-83
[77] Fulton B.P., Miledi R., and Takahashi T., Electrical synapses between motoneurons in the spinal cord of the newborn rat, Proc R Soc Lond B Biol Sci, 1980, 208(1170), 115-20
[78] Walton K.D. and Navarrete R., Postnatal changes in motoneurone electrotonic coupling studied in the in vitro rat lumbar spinal cord, J Physiol, 1991, 433, 283-305
[79] Wenner P. and O'Donovan M.J., Mechanisms that initiate spontaneous network activity in the developing chick spinal cord, J Neurophysiol, 2001, 86(3), 1481-98
[80] Hinckley C.A. and Ziskind-Conhaim L., Electrical coupling between locomotor-related excitatory interneurons in the mammalian spinal cord, J Neurosci, 2006, 26(33), 8477-83
[81] Connors B.W. and Long M.A., Electrical synapses in the mammalian brain, Annu Rev Neurosci, 2004, 27, 393-418
[82] Condorelli D.F., Parenti R., Spinella F., Trovato Salinaro A., Belluardo N., Cardile V., et al., Cloning of a new gap junction gene (Cx36) highly expressed in mammalian brain neurons, Eur J Neurosci, 1998, 10(3), 1202-8
[83] Deans M.R., Gibson J.R., Sellitto C., Connors B.W., and Paul D.L., Synchronous activity of inhibitory networks in neocortex requires electrical synapses containing connexin36, Neuron, 2001, 31(3), 477-85
[84] Tresch M.C. and Kiehn O., Motor coordination without action potentials in the mammalian spinal cord, Nat Neurosci, 2000, 3(6), 593-9
[85] Laird D.W., Life cycle of connexins in health and disease, Biochem J, 2006, 394(Pt 3), 527-43
[86] Heister D.S., Hayar A., Charlesworth A., Yates C., Zhou Y.H., and Garcia-Rill E., Evidence for Electrical Coupling in the SubCoeruleus (SubC) Nucleus, J Neurophysiol, 2007, 97(4), 3142-7
[87] Galarreta M. and Hestrin S., Electrical synapses between GABA-releasing interneurons, Nat Rev Neurosci, 2001, 2(6), 425-33
[88] McCracken C.B., Patel K.M., Vrana K.E., Paul D.L., and Roberts D.C., Amphetamine withdrawal produces region-specific and time-dependent changes in connexin36 expression in rat brain, Synapse, 2005, 56(1), 39-44
[89] Oguro K., Jover T., Tanaka H., Lin Y., Kojima T., Oguro N., et al., Global ischemia-induced increases in the gap junctional proteins connexin 32 (Cx32) and Cx36 in hippocampus and enhanced vulnerability of Cx32 knock-out mice, J Neurosci, 2001, 21(19), 7534-42