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TIME COURSE QUANTIFICATION OF SPASTIC HYPERTONIA FOLLOWING SPINAL HEMISECTION IN RATS

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Abstract—Progressive changes in the muscle tone and stretch reflex after spinal cord injury (SCI) provide insight into the time-course development of spasticity. This study quantified the time-course changes of hypertonia for rats following SCI of T8 hemisection. A miniature manual stretching device measured the reactive torque via a pair of pressure sensing balloons; the angular displacement was measured via an optoelectronic device. Various stretching frequencies were tested, specifically 1/3, 1/2, 1, 3/2 and 2 Hz. The reactive torque and angular displacement were used to derive the viscous and elastic components representing the viscosity and stiffness of the rat's ankle joint. The enhanced velocity-dependent properties of spasticity were observed in the SCI hemisection rats (*n*=9) but not in the controls (*n*=9). Time-course measurements from pre-surgery to 56 days following SCI showed that the muscle tone of the hemisection rats dropped immediately after spinal shock and then gradually increased to reach a peak around 21 days postinjury (*P*<0.01). The muscle tone remained at least 75% of the peak value up to the end of an 8 week observation period (*P*<0.05). The changes of muscle tone can also be verified from the electrophysiological evaluations of electromyography (EMG) (*P*<0.05). In addition to conventional BBB motor behavior score, our results provided time-course quantification of the biomechanical and electrophysiological properties of muscle tone from the onset of SCI. Such data are useful for investigating progressive recovery of spinal damage in animal model and for future objective assessment of improved treatment for SCI human subjects.

Key words: muscle tone, spasticity, stretch reflex, hemisection, spinal cord injury.

Spasticity is a common clinical symptom presenting as a form of increased muscle tone or hypertonia. Spasticity has been characterized as a velocity-dependent increase in the tonic stretch reflex resulting from CNS lesions such as spinal cord injury (SCI) (Lance, 1980; Sheean, 2002). However, many aspects of the specific neurophysiologic mechanism on the increase of muscle tone or the time-course changes after the onset of CNS lesion remain unclear (Elbasiouny et al., 2009; Hiersemenzel et al., 2000; Olsson et al., 2006). Thus, several upper motor neuron (UMN) injured animal models such as stroke and SCI models have been established for investigating the pathophysiological changes (Bose et al., 2002; Jinks et al., 2005; Kwon et al., 2002) as well as for exploring novel treatment for spasticity (Antri et al., 2002; Hefferan et al., 2006). SCI experimental animal models also have been developed for observation of spasticity occurring in the hindlimbs of animals (Bose et al., 2002).

A variety of SCI lesion techniques have been established for different purposes (Basso et al., 1996). One of the most common methods is contusion of the spinal cord using the NYU weight-drop device (NYU impactor) (Basso et al., 1996), which closely mimics the incomplete SCI condition in humans (Gale et al., 1985; Kwon et al., 2002). Other research has alternatively suggested that spinal cord transection techniques, either full or partial, can be carried out by sectioning the spinal cord at specific locations with standard and repeatable lesions (Kwon et al., 2002). Although complete spinal cord transection ensures absolute and complete injury, the spontaneous recovery of behavior and locomotion is less evident and sometimes does not occur. In contrast, partial transection models lend insight into the importance of different neuroanatomical structures for regulating and modifying behavior, providing useful information for studying the development of spasticity after SCI. Furthermore, it has been suggested that bilateral lesions may produce symmetrical changes whereas unilateral lesions may be more sensitive for detecting differences in locomotor function (Muir and Whishaw, 2000).

Since spinal cord hemisection provides a useful animal model for investigating neuronal plasticity and spontaneous recovery of the locomotor function (Gulino et al., 2007; Qin et al., 2006), methods have been developed to assess the integrity of spinal pathways related to hindlimb functions such as locomotor capacity and reflexes (Bose et al., 2002; Webb and Muir, 2002). After hemisection, rats not only become hemiplegic on the hemisected side but also show some contralateral weakness (Arvanian et al., 2009; Kato et al., 1985; Saruhashi et al., 1996). Most rats recover their locomotor capability over a period of 3–4 weeks, usually verified from a scale-based score, but little quantitative data on reflex components are available about the underlying recovery mechanisms. For example, severity of SCI in locomotor capacity is frequently assessed by scaling approaches such as the Basso, Beattie and Bresnahan (BBB) 21-point scale (Basso et al., 1996). The BBB test

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Abbreviations: BBB, Basso, Beattie and Bresnahan; EMG, electromyography; LE, linear envelope; RMS, root mean squares; SCI, spinal cord injury.

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has been used for quantifying hindlimb locomotor abilities of contusion injury rats at the thoracic level (Basso et al., 1996) which has been modified for observing long-term hindlimb movement of spinal hemisection rats (Arvanian et al., 2009; Gwak et al., 2004; Hains et al., 2001).

For reflex assessment, neurophysiologic and biomechanical approaches have been developed for muscle tone in human subjects (Chen et al., 2005; Katz and Rymer, 1989; Lee et al., 2002, 2004; Zhang et al., 2002). However, the typically large size of suitable devices makes it difficult to apply them to animal models. Accordingly, various miniature assessment devices have been developed for quantifying muscle tone in rats (Bose et al., 2002; Kakinohana et al., 2006). One approach utilized force transducers to record reactive torque from the rat's hindlimbs (Kakinohana et al., 2006; Kolasiewicz et al., 1987; Marsala et al., 2005; Thompson et al., 1996). Others measured the reactive force indirectly from a strain-gauge system attached to an electromechanical manipulator (Johnels and Steg, 1982). Other workers have used syringe plungers to detect pressure change (Dickinson et al., 1982) or spring balance devices for quick measurement of the reactive torque of an immobilized rat (Fischer et al., 2002). In our previous studies, the displacement and reactive torque recorded during sinusoidal movements were used to quantify the viscoelastic components of rigidity in raclopride-treated parkinsonian and adenosine A2A agonist-treated rats (Wu et al., 2007, 2009).

Electromyography (EMG) is the most commonly used methodology for electrophysiological assessment of spasticity in the field of clinical neurophysiology. In most animal studies, wire electrodes were surgically implanted or needle electrodes are inserted to record EMG signals. More recently, noninvasive multi-electrode surface EMG (MSEMG) using miniature pin electrodes has overcome the poor spatial resolution of conventional surface EMG electrodes, allowing easy application without surgery and preventing the infections that often accompany invasive techniques (Sun et al., 1999; Wu et al., 2007). Moreover, MSEMG can monitor long-term time-course alterations, providing improved quantitative assessment for small animal models.

After SCI, the progressive changes in muscle tone and stretch reflex provide insight into the development of spasticity and also into functional behavior recovery following SCI. The aims of this study were to quantify the time course of spasticity changes for 56 days following midthoracic spinal hemisection using biomechanical and electrophysiological approaches modified from our previous study (Wu et al., 2007, 2009). A conventional BBB locomotor rating scale was also recorded as a reference.

EXPERIMENTAL PROCEDURES

Miniature muscle tone assessment device

The portable miniature muscle tone measurement device developed in our previous study was modified for simultaneous recording of EMG, reactive torque and angular displacement (Wu et al., 2007, 2009). Both photo and schematic drawing of the complete muscle tone assessment device can be seen in Fig. 1. Rats were secured in a restrainer for the testing after prior habituation. To provide consistent alignment during testing, the rat's hindlimb of lesion side was allowed to protrude from a bottom slot of tube (Fig. 1A). In addition, the specific fixture for the rat was used to constrain thigh and knee of rat during passive stretching of the tested ankle to ensure the ankle was centered on the frictionless link. The human researcher manually moved the rat's foot back and forth in a reciprocal pseudo-sinusoid movement within a set of angular limiter (100° range of motion from rat's ankle plantarflexion 80° to dorsiflexion 20°) while the rat was relaxed the Plexiglas tube (Fig. 1B).

To detect the change of joint angle during sinusoidal stretch of the rat's ankle joint, an optical angle sensor (S720 Miniature Joint Angle Shape Sensor, Measurand, Inc., Canada) was mounted over the ankle joint. The optic sensor was fixed to 2 bars which make the optic sensor parallel with the rigid linkages, which were connected by a frictionless joint.

The measurement of reactive torque using the pressure difference between two balloons (Patrick et al., 2001; Prochazka et al., 1997) was adopted in this study. The balloons were mounted on the dorsal and ventral surface of the foot (Fig. 1B). Reactive resistance was measured by the pressure change of the two balloons as sensed by a differential pressure sensor (DP45-32, Validyne, USA). Calibration of sensing balloon was performed with a process similar to the enlarged air-bag sensing device designed for our previous human study (Lee et al., 2004). By attaching the balloon to the pressure sensors, we calibrated the sensing balloon by placing the weights ranging from 10 to 160 g on an acrylic plate above the balloon. The measured balloon pressure was linearly correlated to the applied weight with correlation coefficients of 0.974 which can be used for converting the pressure to Newton (N) value.

Balloon pressure was maintained at 140–180 mm Hg, which ensured suitable sensitivity and was sufficient to prevent underestimation of the measured value during reciprocal stretching (Wu et al., 2007). A schematic close-up of the measurement set-up is shown in Fig. 1B. The pressure difference and angular displacement signals were recorded simultaneously via an analogue-to-digital converter (ADC) with 16 bit resolution (DAQPad-6015, National Instruments, USA). The data were sampled at 1000 Hz and displayed in real-time by a LabVIEW program for monitoring purposes and also stored in a conventional personal computer for further signal processing by Matlab (MathWorks, Natick, MA, USA). During stretch experiment, the rat's ankle was manually stretched in the frequency paced by a metronome. Five stretching frequencies were employed, namely 1/3, 1/2, 1, 3/2 and 2 Hz. At each frequency, at least 15 cycles were recorded and the central 10 successive signals were chosen for subsequent analysis. The rat was allowed to rest for at least 30 s between trials.

For electrophysiological measurement of muscle activity, an EMG multi-electrode was placed on the gastrocnemius muscle with a reference electrode attached to base of the rat's tail. The multi-electrode device was modified from that used in human subjects (Sun et al., 1999) by minimizing the inter-electrode distance of 3 mm and lowering the contact tension of the spring-loaded recording pins. Each contact pin (0.2 mm diameter contact surface) was gold-plated and spring-loaded to ensure good contact over the uneven surfaces of the hindlimb skin during sinusoidal movement. The recorded signals from each pin were sent to a pre-amplifier (10×) (MPA-8-I, MCS, Germany) then amplified (1000×) prior to digitization at 10 kHz (DAQPad-6015, National Instruments, USA) and stored on computer for further processing. A spatial filter was then used to increase the resolution of the local EMG pickup area (Wu et al., 2007). In order to measure the stretch reflex, EMG bursts and ankle angular displacement were recorded during sinusoidal stretches of the rat's ankle.