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BIOMECHANICAL AND ELECTROMYOGRAM CHARACTERIZATION OF NEUROLEPTIC-INDUCED RIGIDITY IN THE RAT

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Abstract—Rodent models of Parkinson's disease (PD) are usually assessed using measures of akinesia, but other important parkinsonian symptoms such as rigidity are only rarely quantified. This is in part due to technical difficulties in obtaining such measures in small animals. In the present study we developed quantitative methods to provide time-course assessment of the alternations of muscle tone of parkinsonian rats. A portable and miniature biomechanical stretching device was established to manually stretch the hindlimb of awake rats with muscle rigidity induced by dopamine D2-receptor antagonist raclopride (5 mg/kg, i.p.). From the measured angular displacement angle and reactive torque of sinusoidal stretches at five varied frequencies, viscoelastic components of the muscle tone can be derived. In addition, non-invasive multielectrode was applied to record the tonic and phasic components of the gastrocnemius muscle electromyogram (EMG). Our biomechanical measurements showed not only increase in stiffness ($P<0.05$) but also increase in viscous components ($P<0.05$) that matched the time course of increased amplitude of EMG activity ($P<0.05$). There was a significant positive correlation between all of these measures and akinesia, as measured by the conventional bar-test for catalepsy (with a correlation coefficient of 0.87 at stiffness, 0.92 at viscosity and 0.96 at amplitude of EMG). Phasic contraction counts (PCC) of voluntary EMG exhibited a significantly negative correlation with the bar test scores (correlation coefficient=−0.78). These results confirm that akinesia induced by D2-receptor blockade also induces a rigidity that shares many features with human PD. These novel techniques for quantifying biomechanical and electromyographic parameters provide objective assessment methods for investigating the time-course changes of abnormal muscle tone in rat models of PD that will be useful for evaluating novel treatments.

Key words: raclopride, rigidity, catalepsy, quantitative assessment.

Parkinson's disease (PD) symptoms include difficulty in initiating movement (akinesia), tremor, and a characteristic form of rigidity, which affect most patients (Marsden, 1982). Animal models of PD are widely used to investigate pathophysiological mechanisms of PD and for exploring potential treatments (Dawson, 2000; Rodriquez et al., 2001; Cenci et al., 2002). Typically, models of PD are characterized by measures of akinesia, such as bar and cling tests for immobility, or tests of paw usage such as the stepping and cylinder tests (Wolfarth et al., 1985; Fischer et al., 2002). In contrast, little attention has been focused on quantification of rigidity in these animal models. Immobility can be a result of many factors, including motivation, and the extent to which rodent models mimic the classic PD rigidity remains uncertain. This is important, since in human cases the mix of symptoms can vary from patient to patient, suggesting the possibility of different underlying mechanisms and that different treatment regimens may have different efficacy with regard to specific symptoms.

In humans, muscle tone, rigidity or spasticity has been assessed directly from biomechanical measurements of reactive torque during stretching of the limb as well as indirectly from electrophysiological measurement of muscle activity (Lee et al., 2002, 2004; Chen et al., 2005). There are however several difficulties with applying analogous techniques to widely used rat models of PD. Motor-driven stretch devices, developed for measuring the muscle tone on human subjects (Wiegner and Watts, 1986; Caligiuri, 1994; Lee et al., 2002), cannot easily be adopted for small animals due to the relatively tiny reactive resistance and the small size of the limb, and are particularly constrained for measurements in awake, un-anesthetized animals. Of the available previous studies on movement resistance, some measured reactive resistance only at a single position, failed to obtain position data, or only measured a single direction of reactive resistance preventing quantification of stiffness (Dickinson et al., 1982; Johnels and Steg, 1982). These techniques would also not be applicable to studies of other forms of hypertonicity, as the length-dependent and velocity-dependent aspects of muscle tone cannot be measured (Latash, 1998a,b). In some cases, the rats have to be anesthetized before measuring (Cutlip et al., 1997) which would affect non-reflex as well as reflex measures. In a study which most closely matched the kind of data that can be obtained in humans (Kamper et al., 2001), Wolfarth et al. (1997) measured reactive torque in a selected angle range by stretching the rat's ankle in a ramp-and-hold mode using a motor-driven apparatus (Kolasiewicz et al., 1987). Although motor-driven apparatus could move the ankle joints in precisely controlled pattern, the limited range of movement they provided at the rat ankle and the inevitable inertia of the motor

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Abbreviations: B, viscosity; Bω, viscous component; EMG, electromyogram; H1, 1 h after raclopride injection; H2, 2 h after raclopride injection; H3, 3 h after raclopride injection; H4, 4 h after raclopride injection; H5, 5 h after raclopride injection; MDF, median frequency; PCC, phasic contraction counts; PD, Parkinson's disease; RMS, root mean square.

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and the limb allowed only a short stretching period which may not reach constant stretching velocity. These limitations prevent quantifying the muscle tone in terms of viscous and elastic components. It is important to separate these components, which can be measured in human subjects (Lee et al., 2002), as they may be differentially affected by pathological processes. Besides, motor-driven apparatus is rather bulky and complicated to set up in a limited space for animal study.

Electromyogram (EMG) recordings, which monitor the level of muscle activation, are widely accepted as the indirect method for monitoring the increased muscle tone in humans caused by problems with the neuromotor control system (Hwang and Abraham, 2001). Whereas in humans surface electrodes are easily applied, these are generally too large for animal use. Although small wire electrodes have been commonly used to measure the muscle activities (Lorenc-Koci et al., 1996; Hemsley and Crocker, 1999), these EMG measurements in awake animals generally required the surgical implantation of wire electrodes to the small muscles of animals. Such implanted electrodes not only increase the risks of infection, but can also fail to record a broad enough sample of motor units to completely characterize activity of measured muscle.

To address these issues, we developed a miniature muscle tone assessment system which could be used conveniently and manually for quantitatively evaluating the muscle tone of awake rats, and a novel non-invasive multielectrode for surface EMG measurement. In the present study we used these devices to quantify the stiffness, including viscous component ($B\omega$), of rats treated with dopamine D2 receptor antagonist raclopride, a common acute model for parkinsonian akinesia. We asked whether this animal model also showed changes in joint stiffness characteristic of parkinsonian rigidity, over the same time course as akinesia (as assessed with the classic bar test for catalepsy) and whether these stiffness changes were associated with appropriate changes in EMG activity.

EXPERIMENTAL PROCEDURES

Clinical evaluation of catalepsy score

Parkinsonism in rat models is traditionally quantified using tests for immobility. To compare the time course of biomechanical and EMG changes with this behavioral effect we used the bar-test catalepsy score. During the bar test, both of the rat's forepaws were put on a horizontal acrylic bar, which was 9 cm high. We recorded the time from placing of forepaws to the first complete removal of both of them from the support bar. The maximal testing duration was set at 180 s. For analysis, the time the rat remained with forepaws on the bar was rescaled using square root transformation, where times from 0–0.08 min were scored as 0, 0.09–0.35 min=1, 0.36–0.80 min=2, 0.81–1.42 min=3, 1.43–2.45=4, and ≥2.25 min=5 (Ahlenius and Hillegaart 1986).

Biomechanical measurement of rigidity

We developed a miniature muscle tone assessment system capable of providing precise measurement of the effects of sinusoidal stretching on two major biomechanical parameters, reactive resistance and joint angle, in the rat. Instead of conventional utilization of torque sensors, the reactive resistance of the ankle joint was determined by recording the pressure difference between two balloons (Prochazka et al., 1997; Lee et al., 2004) mounted on the dorsal and ventral surface of the foot (Fig. 1A). The balloons were mounted in two isolated chambers by ball valve and connected to the opposite openings of a differential pressure sensor (DP45, Validyne Engineering, Northridge, CA, USA). Each chamber was connected to a 20-ml syringe by rigid pipes and airtight terminals for pumping air in to inflate the balloons. The 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 (Lee et al., 2004). Joint angle during movement was measured by using a miniature optic angle sensor (S720 Miniature Joint Angle Shape Sensor, Measurand Inc., Fredericton, NB, Canada). The sensitivity of this optic sensor is ±1 V for ±90° of the range at a resolution of 0.5°.

In order to provide consistent alignment during testing, we designed a specific fixture for the rat (Fig. 1B). The rat was placed in a cylindrical restrainer with two slots at the rear that allowed both of the rat's hindlimbs to protrude. The left hindlimb was positioned and a limiter constrained the displacement of thigh and knee during passive stretching of the tested ankle so that the ankle was centered on the frictionless link between two rigid rods, the upper rod attached to the frame and the lower running parallel with the foot. The balloon assemblies were then positioned to ventral and dorsal sides of rat's foot respectively for recording the reactive resistance. The curve center of the optic position sensor was centered on the frictionless joint for recording the ankle's angular rotation. A curtain was hung over the restrainer covering the front part of the rat to provide a darkened environment to keep rats quiet and to eliminate visual disturbance. The lower fixation rod extended beyond the foot and was used to manipulate the limb to provide the sinusoidal stretching movement (described later).

The pressure difference between the two balloons during imposed (passive) movement and data of ankle joint angle were sampled at 500 Hz via an analog-to-digital converter (ADC) with 12-bit resolution (DAQPad-6020E, National Instruments [NI], Austin, TX, USA). The data were displayed in real-time controlled by LabView (NI) program for monitoring purpose and then stored in a laptop computer for further signal processing use Matlab (The MathWorks, Natick, MA, USA).

EMG recording

For EMG recording, we used a novel four-pin multielectrode (Fig. 1C). This electrode is a miniaturized version of a device we have previously reported for human use (Sun et al., 1999). Each contact pin (0.2 mm diameter contact surface) is gold-plated and spring-loaded to maximize constancy of contact over uneven surfaces and during movement. These design features enable good quality EMG signals to be obtained without the need for conductive gel. The pins can be sterilized and reused, or replaced. The total external dimension is 13×12 mm with inter-pin distance of 3 mm, as recommended in previous studies of rat EMG (Scholle et al., 2001; Schumann et al., 2002). During the recording, the multielectrode was placed on the gastrocnemius of the left hind limb and held in place by taping (Fig. 1D) with a reference electrode attached the base of the rat's tail. To improve signal to noise ratios, voltage-follower buffers (TL064, SMD package) with a high input impedance ($>10^{12}$ Ω) were mounted directly on the back of the multielectrode. Signals from each pin were then amplified (5000×) and filtered (60 Hz notch, 10 Hz-1.6 kHz bandpass) (Cyberamp 380, Axon Instruments, Foster City, CA, USA) prior to digitization at 10 kHz (DAQ 6281, NI) and storage on computer for further processing. A spatial filter was then used to increase the resolution for localizing the EMG pickup area. The localized area activity was derived from three channels and the weighting factors for channels 1, 2 and 3 were 1, −1 and 1, respectively, as shown in Fig. 1C.

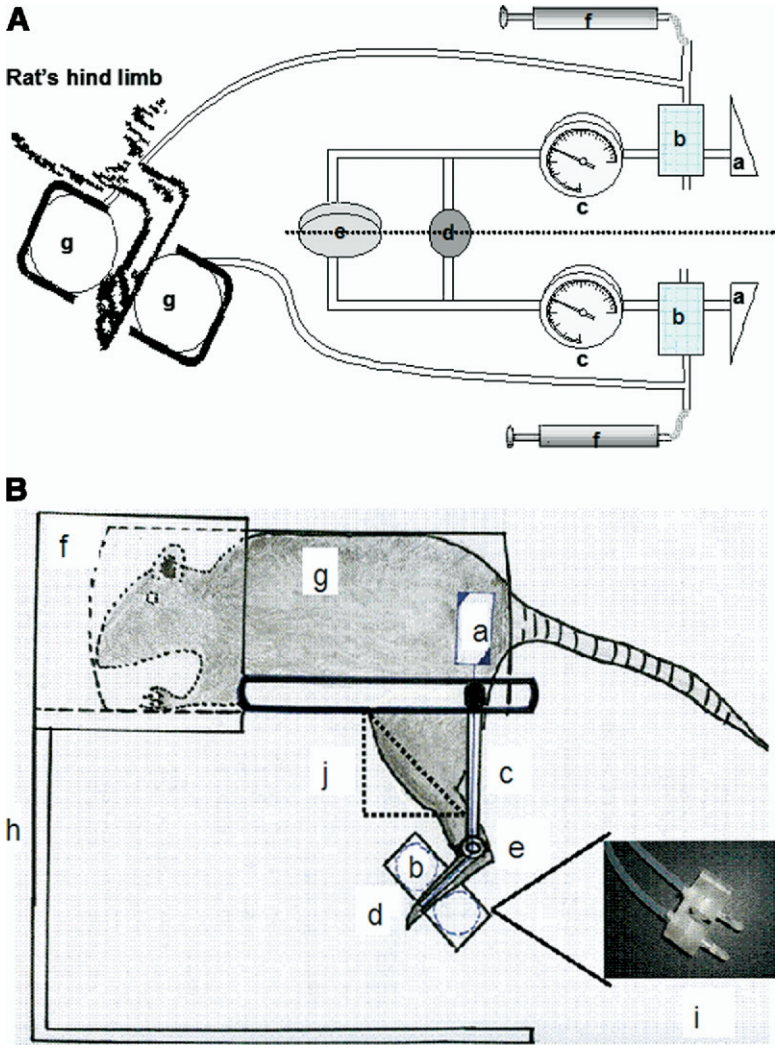

Fig. 1. (A) Illustration of quantitative muscle tone assessment system for recording reactive resistance, trajectory, and multielectrode EMG. The vents (a) control air flow via needle valves (b). The gauges (c) monitor the pressure of two isolated compartments separated by ball valves (d); pressure is then continuously detected by the differential pressure sensor (e). The syringes (f) pump air into compartments to inflate balloons (g) situated in the balloon set. (B) Optic angle sensor (a) is attached on the rigid linkages (c) and (d), which are connected by a frictionless joint (e). Two balloons are located in the balloon set (b), which is anchored on the foot firmly. The zoom-in picture of the paired balloon is shown (i). The frictionless joint is aimed at the ankle axis and the ankle is fixed by metal stops (j). The rat is put in the restrainer (g), which is placed in the specially designed fixture (h). In the fixture, a curtain (f) is used to cover the front of the rat's body for calming the rat down. (C) Configuration of multi-electrode and the weights for spatial filter. The inside springs are used to provide good contact. (D) Zoom-in photo of the multielectrode mounted on the rat's leg held in our device.

Animals and procedures

Eight Male Wistar rats (300–400 g) were used in the current study. The animals were housed in pairs in the cages in colony room maintained on a 12-h light/dark cycle, with dark onset at 20:00 h. All animals had continuous access to food and water throughout the experiments. Experimental procedures were approved by the National Cheng Kung University Medical Collage Animal Use Committee. All procedures were also performed in accordance with the guidelines of the institutional animal care and use committee and international guidelines on the ethical use of animals. Every effort was made to minimize the number of animals used and their suffering. Rats were familiarized with the restraint apparatus for 30 min, twice a day for 1 week before the experiment.

Parkinsonian rigidity was induced by injection of raclopride (Sigma, St. Louis, MO, USA) dissolved in the saline and injected intraperitoneally in a volume of 1 ml/kg, at dosage of 5 mg/kg (Hemsley and Crocker, 1999). In control experiments, rats were injected with equivalent volumes of vehicle alone. Biomechanical, EMG and bar test recording were conducted before and after raclopride or saline injection in three separate experiments. Biomechanical measures and the bar test were performed before and then every 20 min after injection for 5 h, for a total of 16 tests per experiment. For better representation of data, the bar test scores and biomechanical values were averaged for each hour. During each biomechanical test, we applied 15 successive cycles of passive sinusoidal movements of 100° at five different frequencies (1/3, 1/2, 1, 3/2 and 2 Hz) manually. The different stretching frequencies were guided audibly by the metronome. The rat's foot was stretched back and forth in a reciprocal pseudo-sinusoid movement within a set of limiters. To maintain the consistency, we could monitor the stretch ankle range from the real-time display of LabView program and observe the stretch frequency from off-line frequency analysis of stretch movement. The data would be discarded if the range of movement as well as the dominant stretch

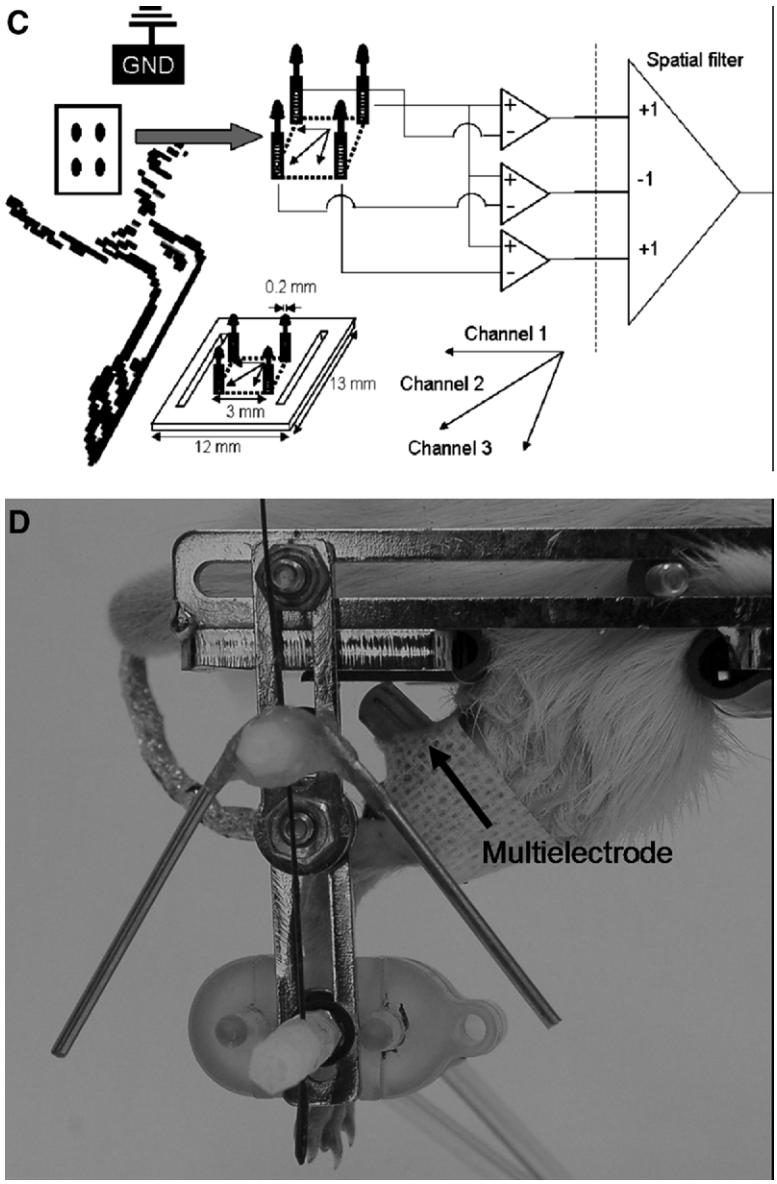

Fig. 1. (*Continued*).

frequency did not meet the designed experiment. In EMG recording experiments, the EMG was continuously recorded from 0.5 h before injection to 5 h after injection. Fig. 2 shows the time schedule for all measures.

Data analysis

Biomechanical measurement. Fig. 3A shows example raw data for the pressure difference between the two balloons during

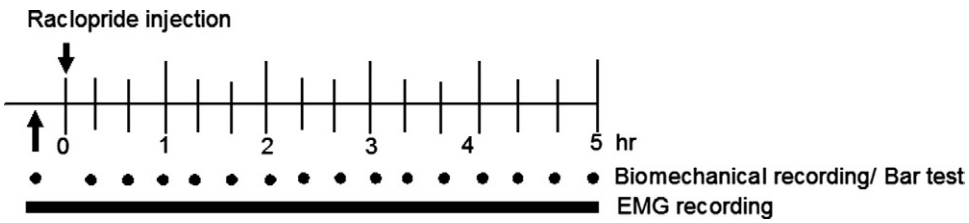

Fig. 2. Scheme of time schedule for all measures. Downward arrow is the time point for raclopride injection. Upward arrow is the start for recording. Dots are the times for recordings of biomechanical data and bar scores, every 20 min within 5 h. Continuous horizontal line is the EMG recording period which started 30 min before the injection.

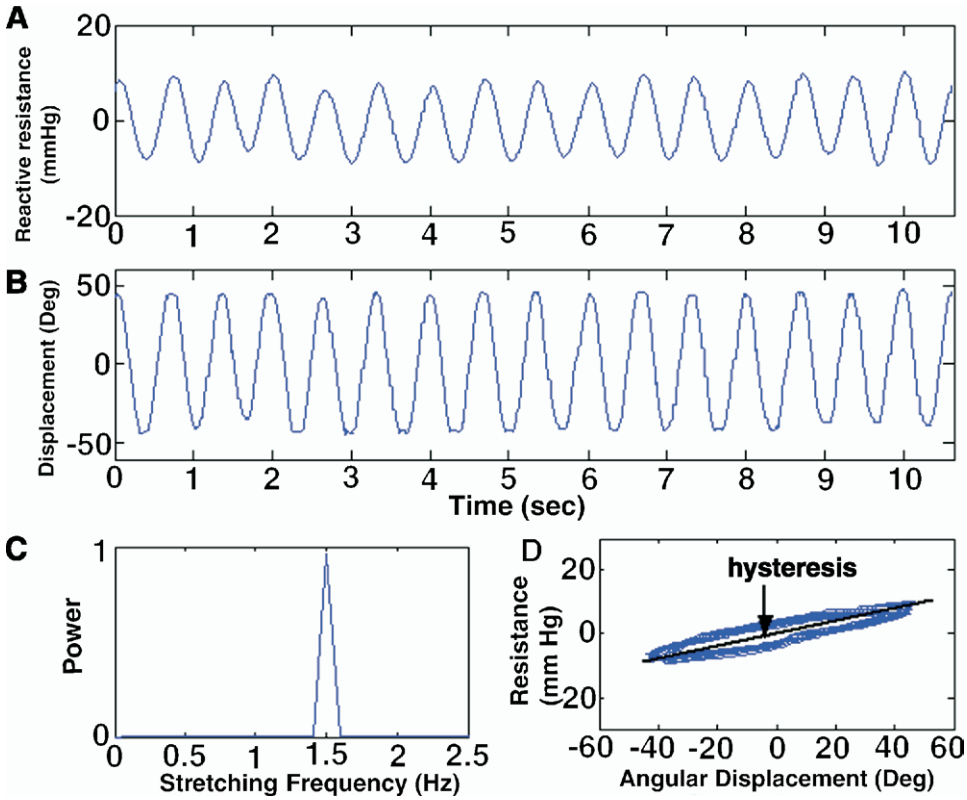

Fig. 3. Example of recorded reactive resistance (A) and angular displacement (B) during manual stretching at a stretching frequency around 1.5 Hz, as shown in the frequency spectrum (C). The hysteresis loop (D) is a plot of reactive resistance versus angular displacement, from which the stiffness (dotted line) can be derived from resistance over displacement and B can be obtained from the width of the circular loop.

imposed (passive) movement of the joint (Fig. 3B), which represents the reactive resistance provided by the joint for responding the sinusoid movement. As a function of displacement, a measure of "stiffness" which reflects the spring-like action can be derived from a linear regression calculated for these two variables (Fig. 3D). In addition, we have developed techniques (Lee et al., 2004; Chen et al., 2005) to estimate the Bω and derived viscosity (B) which reflect the damping effect of the joint. Details of the algorithm can be found in our previous studies (Lee et al., 2004; Chen et al., 2005). The estimation of the Bω is based on the relationship between the externally imposed joint displacements and the corresponding joint resistance, which is generally modeled as a second-order system. From the biomechanical model, the Bω, measured at five stretching frequencies, one B can be derived by linear fitting process for each rat. In brief, the Bω could reflect the width of hysteresis derived from the reactive resistance and displacement for each stretching frequency (Fig. 3D). B is then defined as the slope of the regression line for Bω measured at five different frequencies as a function of frequency (ω). Frequency analysis of angular displacement data (Fig. 3C) was used to determine the actual dominant frequency achieved by our paced manual system, over the data acquisition period, and showed that the achieved frequencies matched well with the paced values.

EMG signal processing. The EMG signal after spatial filtering of three differential outputs was processed to determine the resting (tonic activity) and phasic (voluntary contraction) EMG. First, the raw EMG was fed through notch and band-pass (10 Hz–1000 Hz) filters for eliminating power line interference and motion artifact, respectively. The amplitude of resting EMG is presented in terms of root mean square (RMS) value and the frequency distribution is characterized by median frequency (MDF). It is noted that the phasic component of EMG was excluded from the calculation of the RMS and MDF of tonic EMG activities. To determine the phasic EMG, the filtered EMG was processed in a linear envelope (LE) representation after rectification and low-pass filtering at 10 Hz. The phasic EMG was defined as periods of EMG signal with amplitude greater than the mean plus 3 S.D. and lasting less than 1 min (Ellenbroek et al., 1985) as shown in Fig. 4. The numbers of phasic EMG found during one session of experiment is represented as phasic contraction counts (PCC) which is expressed as counts/min for each session.

Statistics

Non-parametric statistics (Wilcoxon signed-rank test) were used for multiple comparisons with significance level of $P<0.05$. All data after injection at each hour were compared with the data of pre-injection in the raclopride-treated group. Besides, the data of two adjacent time points were also compared in both groups. To investigate the differences between the raclopride-treated group and the control group along the time course, the statistics (Mann-Whitney U test) with significance level of $P<0.05$ was also conducted on each hour. Pearson correlation was used for correlating catalepsy scores (bar test scores) versus all biomechanical, EMG parameters as well as PCC. Correlation was significant at significance level of $P<0.05$. The SPSS software (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis.

RESULTS

Biomechanical and electrophysiological measurements of muscle tone and bar test were compared according to the time course, before injection (H0), 1 h (H1), 2 h (H2), 3 h (H3), 4 h (H4) and 5 h (H5) after raclopride injection. Fig. 5 shows

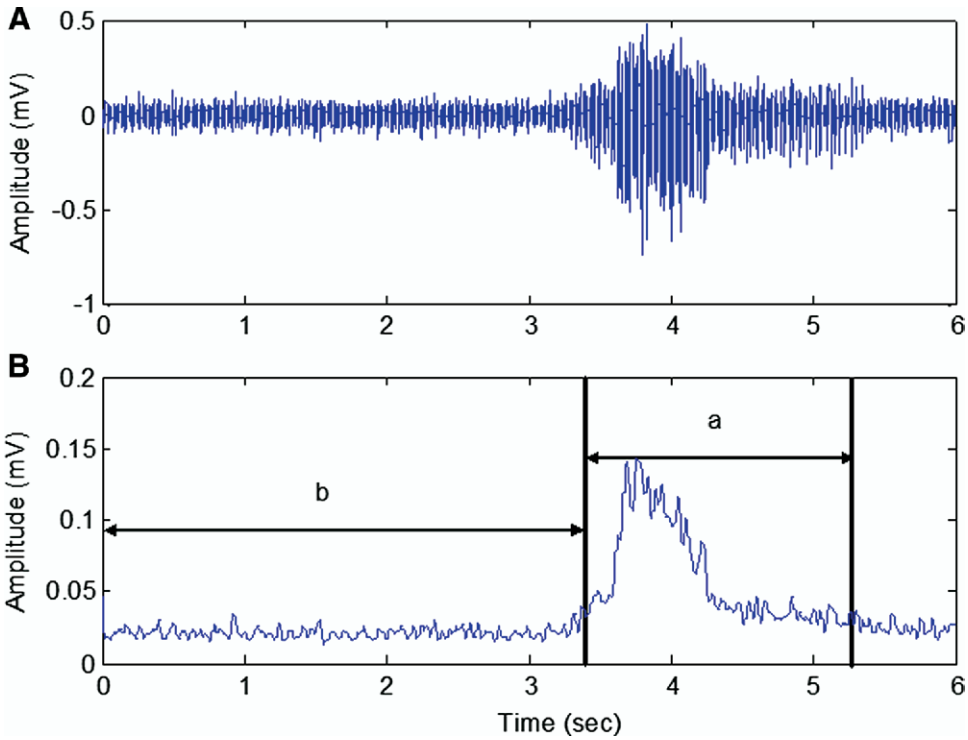

Fig. 4. The raw EMG (A) and its derived linear envelope form (B), which is used for determining the phasic EMG activity (a) and tonic activity (b).

the average bar test times for the eight tested rats. Compared with H0, bar test scores increased significantly ($P<0.05$) after the injection at all recorded time points (H1 to H5). In time-course comparison, the scores increased significantly between H0 and H1 as well as H1 and H2 and then started to decrease at H4 in comparison with H3 ($P<0.05$).

Biomechanical measurements

Fig. 6 shows example data for the reactive torque versus angular displacement at different applied frequencies in control and raclopride-treated groups. The stiffness (the slope of curve) and Bωs (obtained from the encircled hysteresis) increased after raclopride injection. Comparing raclopride-treated data (2 h after injection as the example) to control baseline data, stiffness increased at each stretching frequency (from 0.11–0.27 at 1/3 Hz, from 0.16–0.21 at 1/2 Hz, from 0.13–0.2 at 1 Hz, from 0.12–0.19 at 3/2 Hz and from 0.08–0.16 Hz at 2 Hz). Fig. 7 shows that in this example the B derived from fitting regression line of Bω of each five stretch frequencies in-

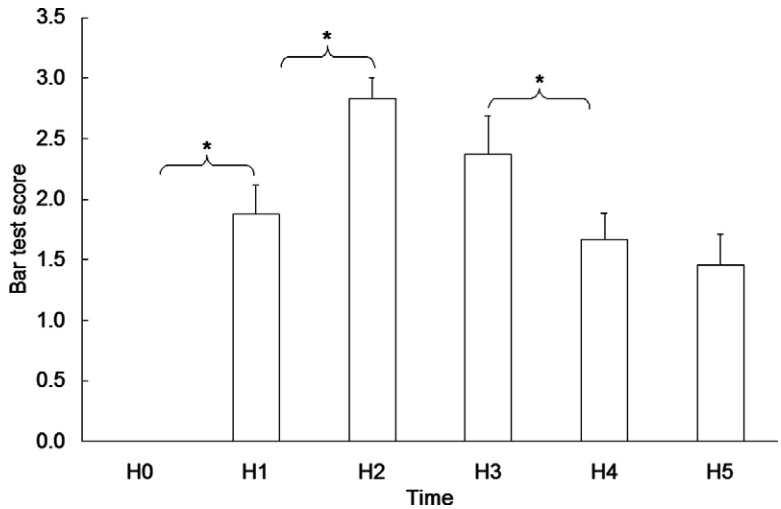

Fig. 5. Time-course changes of catalepsy observed from bar test scores of raclopride-treated rats. Asterisks (*) indicate a significant difference ($P<0.05$) between two adjacent time points in the raclopride-treated group; analysis was conducted by means of a Wilcoxon signed-rank test.

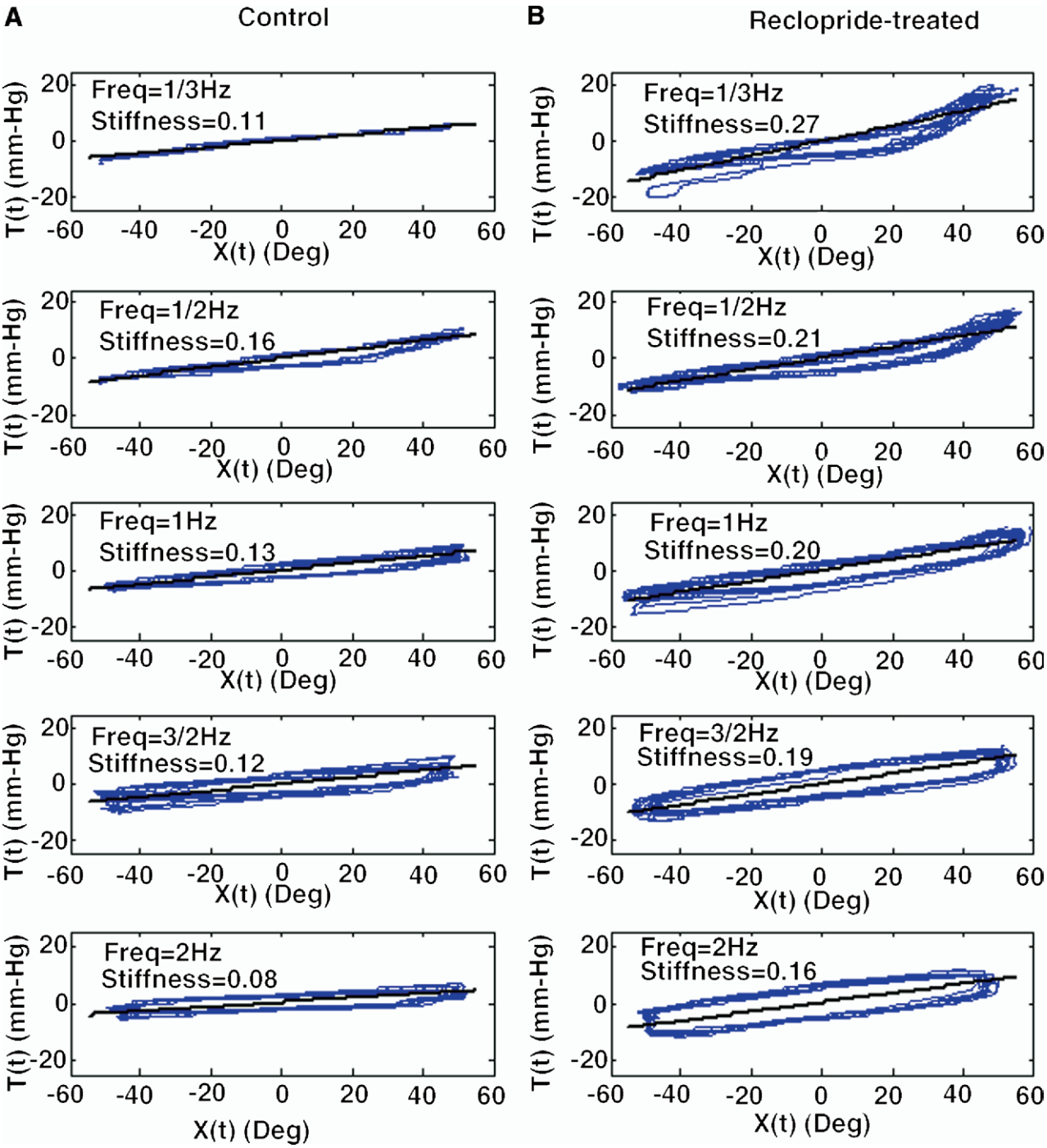

Fig. 6. Examples of hysteresis changes for (A) control group and (B) raclopride-treated rats stretched at five different frequencies, 1/3, 1/2, 1, 3/2, and 2 Hz (from top to bottom). The stiffness (unit: mm Hg/degree) can be derived from the linear regression line of the hysteresis loop which increased significantly after raclopride injection in all stretch frequencies. The width of the hysteresis loop represents the viscous contribution which is smaller at lower stretch frequencies and became larger after injection.

creased after raclopride injection, returning to approximately baseline level after 5 h.

Fig. 8 shows the group data for stiffness and B obtained in eight rats with raclopride-induced rigidity and after saline injections. Compared with pre-injection values, raclopride significantly increased ($P<0.05$) both stiffness (Fig. 8A) and average B (Fig. 8B) at H1, H2, H3 and H4 which decreased to pre-injection levels at H5 ($P>0.05$). However, control injections did not affect either measure at any time point ($P>0.05$). Compared with controls at varied test points, significant differences ($P<0.05$) in both biomechanical measurements can be found from H1 to H4 but not at H5. Table 1 shows that no significant difference was found between two adjacent time points after the raclo-

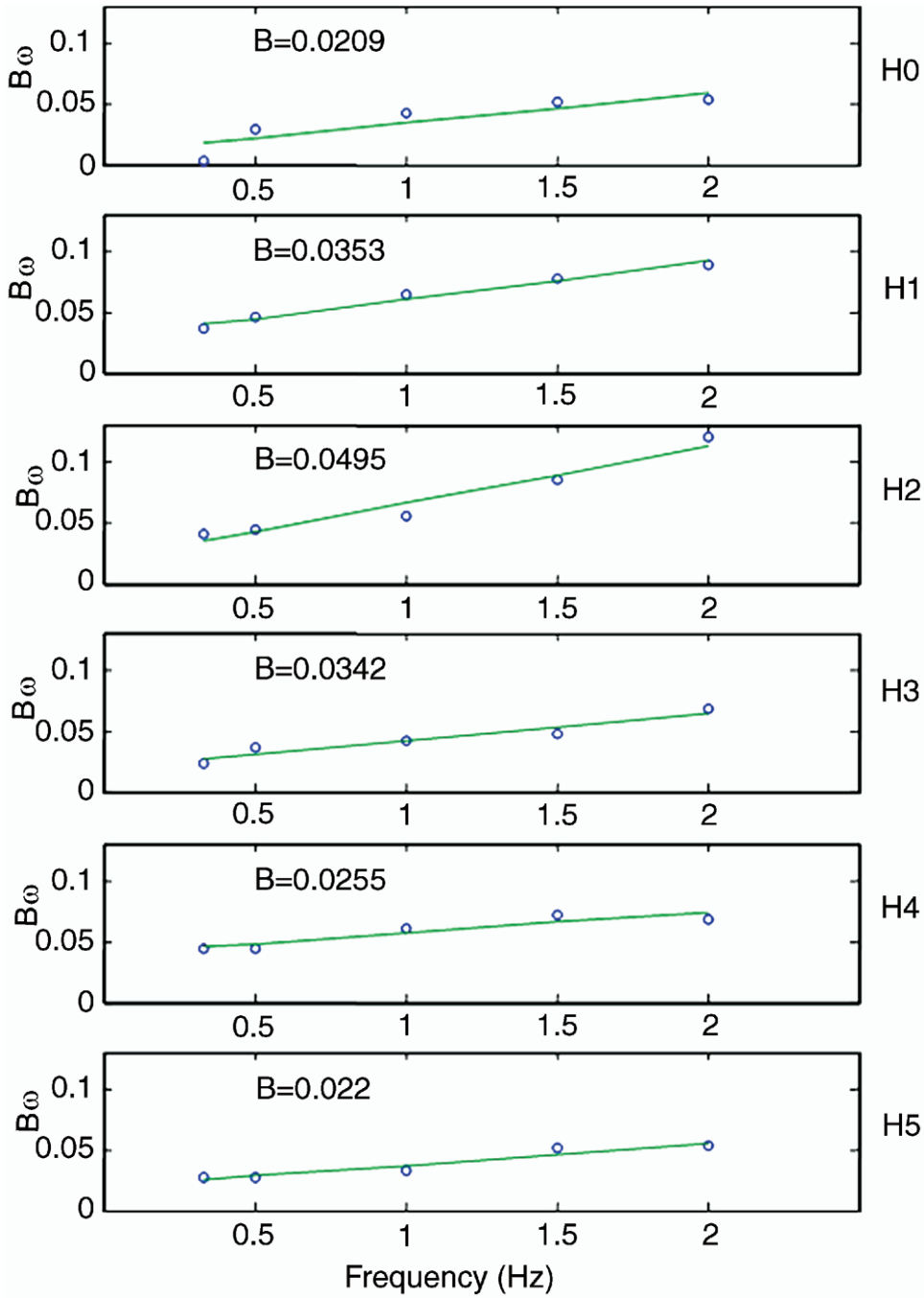

Fig. 7. The viscosities (B) were derived from Bωs of pre-injection (H0), and 1–5 h after injection (H1∼H5).

pride injection in the biomechanical properties including stiffness and B after H1. We can observe the time-course changes from Fig. 8 that both biomechanical measurements increased significantly in H1 from H0 then maintained at least for 4 h.

EMG measures

Examples of typical 20-s periods of tonic EMG data from six sequential samples (H0–H5) in a raclopride experiment are shown in Fig. 9A. There is a clear increase in RMS values of tonic EMG which peaked at H3 and then decreased toward pre-injection control value. Fig. 9B shows an example of the MDF change over the same six periods The MDF shifted toward low frequency component after raclopride injection with lowest MDF at H3 and maintained at a relatively low value.

Fig. 10 shows the time-course changes of RMS and MDF for eight rats in the control and raclopride-treated studies. Compared with H0, the RMS amplitude of all time periods except for H5 increased significantly

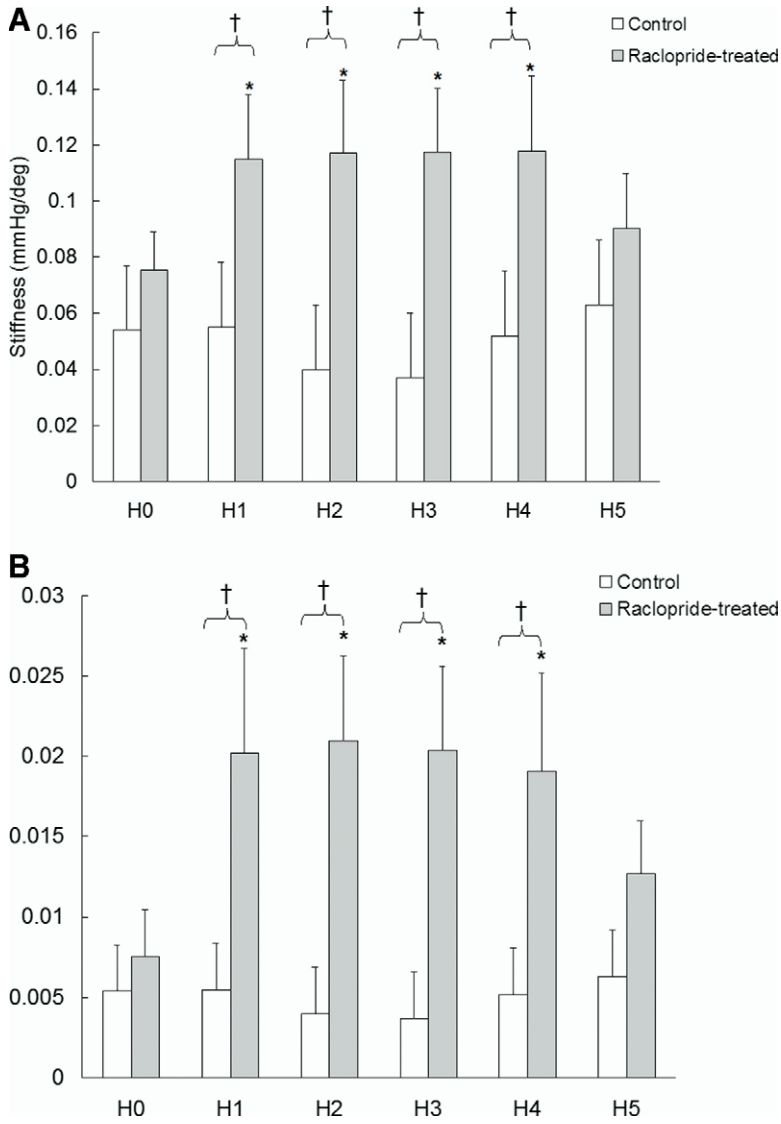

Fig. 8. Time-course changes of (A) stiffness and (B) B values, represented as mean±S.E.M. in control and raclopride-treated groups. Asterisks (*) indicate a significant difference ($P<0.05$) between different periods and H0 in raclopride-treated group; analysis was conducted by means of a Wilcoxon signed-rank test. Daggers (†) indicate significant difference ($P<0.05$) between two groups at varied time points by means of a Mann-Whitney *U* test.

($P<0.05$) in the raclopride-treated group but there was no change in the control group ($P>0.05$) (Fig. 10A). Compared with the control group, the amplitude was significantly higher ($P<0.05$) at raclopride-treated group after the injection. MDF (Fig. 10B) declined following raclopride, i.e. there was a shift toward lower frequency after raclopride injection. However, the time course appeared somewhat different than for other measures, reaching significance only at H3 ($P<0.05$) and remaining low for the remainder of the experiment. The signif-

Table 1. Changes between adjective 2 h from pre- (H0) to 5 h (H5) post-raclopride injection in varied variables

Variable	*P* value				
	H0 vs. H1	H1 vs. H2	H2 vs. H3	H3 vs. H4	H4 vs. H5
Stiffness	0.01*	1	0.67	0.67	0.16
Viscosity	0.01*	0.78	0.88	0.67	0.40
RMS	0.01*	0.88	0.30	0.78	0.08
MDF	0.78	0.40	0.20	0.67	1

Wilcoxon signed-rank test was used to test the significance of the differences. Asterisks (*) indicate significant difference.

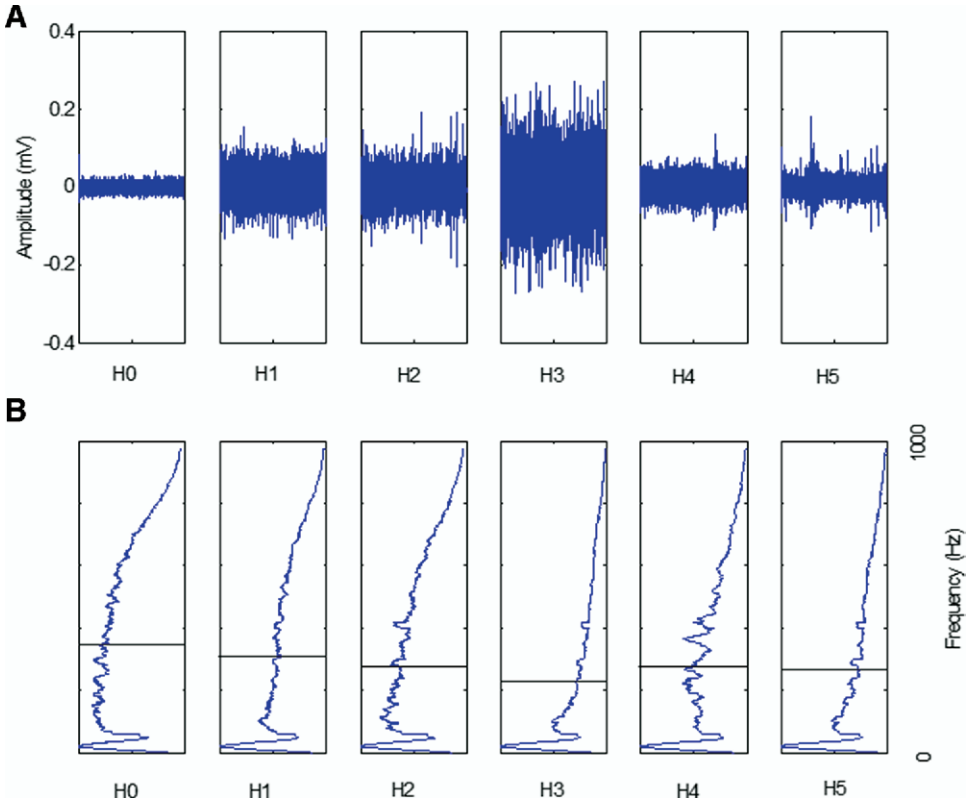

Fig. 9. Examples of time-course changes in tonic EMG (A) and its derived MDF (B), denoted as a bar in B before injection (H0) and five periods (H1–H5) after injection.

icant differences of MDF between two groups could be found from H3 to H5 ($P<0.05$). The comparisons between two adjacent time points indicate that significant change in EMG amplitude only occurred at H0 versus H1 ($P<0.05$) but no significant difference was found in the adjacent time points of MDF (Table 1).

Fig. 11 shows an example of PCC data in continuous 10 min at H0 (Fig. 11A) and H1 (Fig. 11B) in a raclopride-treated rat. In this example, and in the group data (Fig. 11C), compared with pre-injection values there is a decrease in PCC at H1. In other words, the average interval between phasic contractions increased after raclopride injection ($P<0.05$). Fig. 11C shows the time-course changes of PCC of the group data. The PCC decreased from H0 to H3 and then gradually increased.

Correlational analysis

To investigate the correlation between catalepsy, measured by the bar test, the stiffness and EMG measures, we calculated Pearson correlation coefficients. There were significant positive correlations between bar test scores and biomechanical measures of stiffness and B, and the RMS measure of the EMG and a significant negative correlation to PCC as listed in Table 2. The correlation coefficient for MDF did not reach significance (Cohen et al., 2003).

DISCUSSION

There were two main outcomes from this study. First, we demonstrate the feasibility of quantifying muscle tone and EMG in the un-anesthetized rat. This has the major advantage of eliminating the influence of anesthetics to muscle tone (Lee et al., 1994). Our miniaturized system enables stretching trajectories and reactive resistances to be measured, and thus stiffness and B to be derived as quantitative indices to evaluate the muscle tone. These have been the major advantages of our system compared with previous methods (Dickinson et al., 1982; Kolasiewicz et al., 1987; Wolfarth et al., 1997; Hemsley and Crocker, 1999, 2002).

The second outcome pertains to the objective assessment of muscle tone during neuroleptic-induced catalepsy. Neuroleptics such as raclopride produce a sustained but reversible akinesia, due to blockade of dopamine D2 receptors, and this neuroleptic-induced Parkinsonism is a major side-effect of their use in treatment of schizophrenia. Neuroleptics have thus been used as an acute model of Parkinsonism (Hillegaart and Ahlenius, 1987; Marin et al., 1993; Fowler and Liou, 1998; Hemsley and Crocker, 1999, 2002; Wadenberg and Seeman, 1999; Alcock et al., 2001). In animal studies Parkinsonism is usually assessed using tests for akinesia alone, whereas rigidity is infrequently assessed and then only semi-quantitatively (Fischer et al., 2002). However the pathways involved in rigidity and cat-

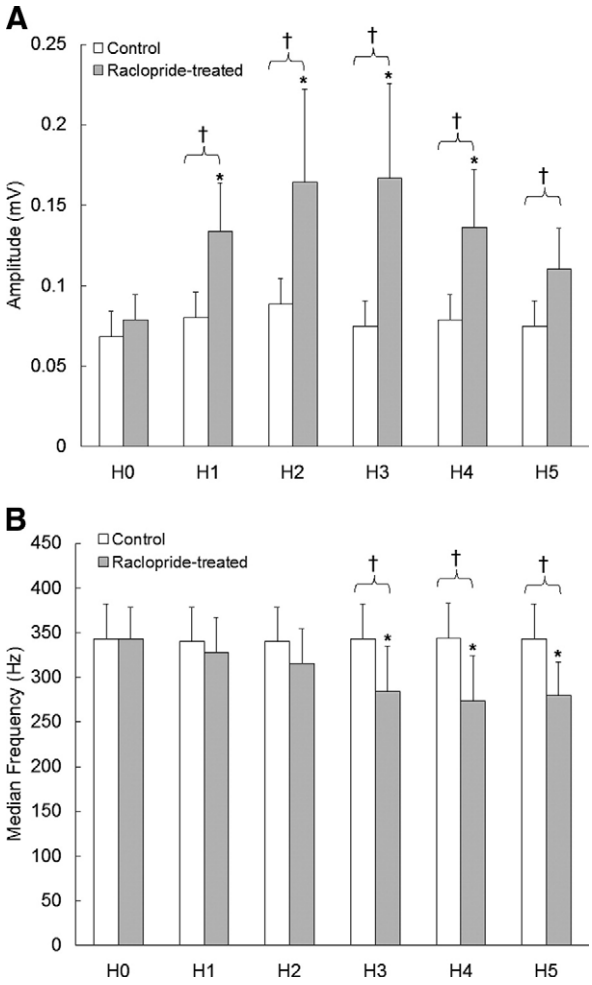

Fig. 10. Time-course changes of (A) RMS and (B) MDF, represented as mean±S.E.M. in control and raclopride-treated groups. The individual comparison of different time with H0 in the raclopride-treated group was conducted by means of a Wilcoxon signed-rank test. Significant difference is denoted by * $P<0.05$. Daggers (†) indicate significant difference ($P<0.05$) between two groups at varied time points by means of a Mann-Whitney *U* test.

alepsy are different and cannot be differentiated by the semi-quantitative scoring scale generally used in behavior tests (Wolfarth et al., 1985). Clinically, rigidity is separable from immobility; patients may be more or less rigid as scored on physical examination, separate from any difficulties they may have with akinesia/bradykinesia. It is thus very important to be able to objectively quantify rigidity in animal models, and to be able to compare the properties of the change in limb flexibility with what is observed in PD.

Previous studies on human subjects have reported the contribution of elastic stiffness in parkinsonian rigidity to be greater than in normal muscle (Watts et al., 1986; Wiegner and Watts, 1986). Hence the changes of stiffness could be used as quantifying index for assessing rigidity (Watts et al., 1986; Caligiuri, 1994). In the present study we found that both stiffness and B were increased by raclopride injection, and that the time course of the effect matched very closely with that of akinesia as measured by the bar test. These data suggest that acute blockade of dopamine receptors produces not only reduced mobility similar to parkinsonian akinesia, but also a parallel change in joint stiffness with characteristics similar to the lead-pipe rigidity seen in Parkinson's patient. However, these increases in joint stiffness and B could be considered as the secondary change caused by alterations in the stretch reflex evoked by neuroleptics interventions. Importantly, these changes differ from what we have previously quantified in spasticity produced by upper motor neuron lesion, in which there are higher velocity-dependent values of B (Lee et al., 2002).

The EMG data provide another index for rigidity in muscle, and also potential insights into the cause of the rigidity. Of the EMG parameters we measured, RMS and PCCs correlated highly with the bar test scores and shared similar time course with the behavior and with the measurements of muscle stiffness. The other measure, median EMG frequency, did not correlate well with the behavior. Increased magnitude of the RMS of EMG signal is probably due to increased recruitment of motor units (Nigg et al., 2000). This is consistent with a previous study in neuroleptic Parkinsonism in rat (Hemsley and Crocker, 1999) and has also been seen in fentanyl-induced muscular rigidity (Lee et al., 1994). Increased tonic EMG activity is also seen in human PD (Cantello et al., 1995) and may reflect disordered differential activation of motor pathways to agonist and antagonist muscle. It is known that stretch reflex plays an important role in regulating the muscle tone which has been widely studied in humans (Voerman et al., 2005) and in rats (Ossowska et al., 1996). Stretch reflex is generally assumed as the primary physiological mechanism to regulate the muscle tone which would reflect on the EMG amplitude of short-latency component resulting from the monosynaptic pathway as well as the long-latency component resulting from polysynaptic pathways. Researchers found the increased amplitude of the long-latency component of EMG in PD patients or after neuroleptics intervention (Berardelli et al., 1983; Ossowska et al., 1996). Furthermore, muscle rigidity was accompanied with the increases in resting EMG amplitude and the stretch-induced long-latency EMG activity after haloperidol administration in the rats (Lorenc-Koci et al., 1996). Hence, it has been postulated that not only the monosynaptic reflex but also supraspinal reflex loops may contribute to the muscle resistance regulation (Ossowska et al., 1996).

In contrast to the positive correlation of tonic EMG amplitude with bar-test scores, PCC was negatively correlated with akinesia. This is consistent with the findings of Ellenbroek et al. (1985), who also found the phasic muscle contraction decreased in the rats with catalepsy. The phasic EMG bursts quantified by the PCC score reflect the occurrence of phasic descending volleys from higher centers to the spinal motor neuron pools that under normal circumstances are concerned with initiation of voluntary movements. The reduced PCC scores thus suggest reduced attempts by the central motor apparatus to generate discrete movements of the limb. One possibility is that the rigidity itself makes movement more difficult, and so animals adapt by making less attempts to move. Alternatively,

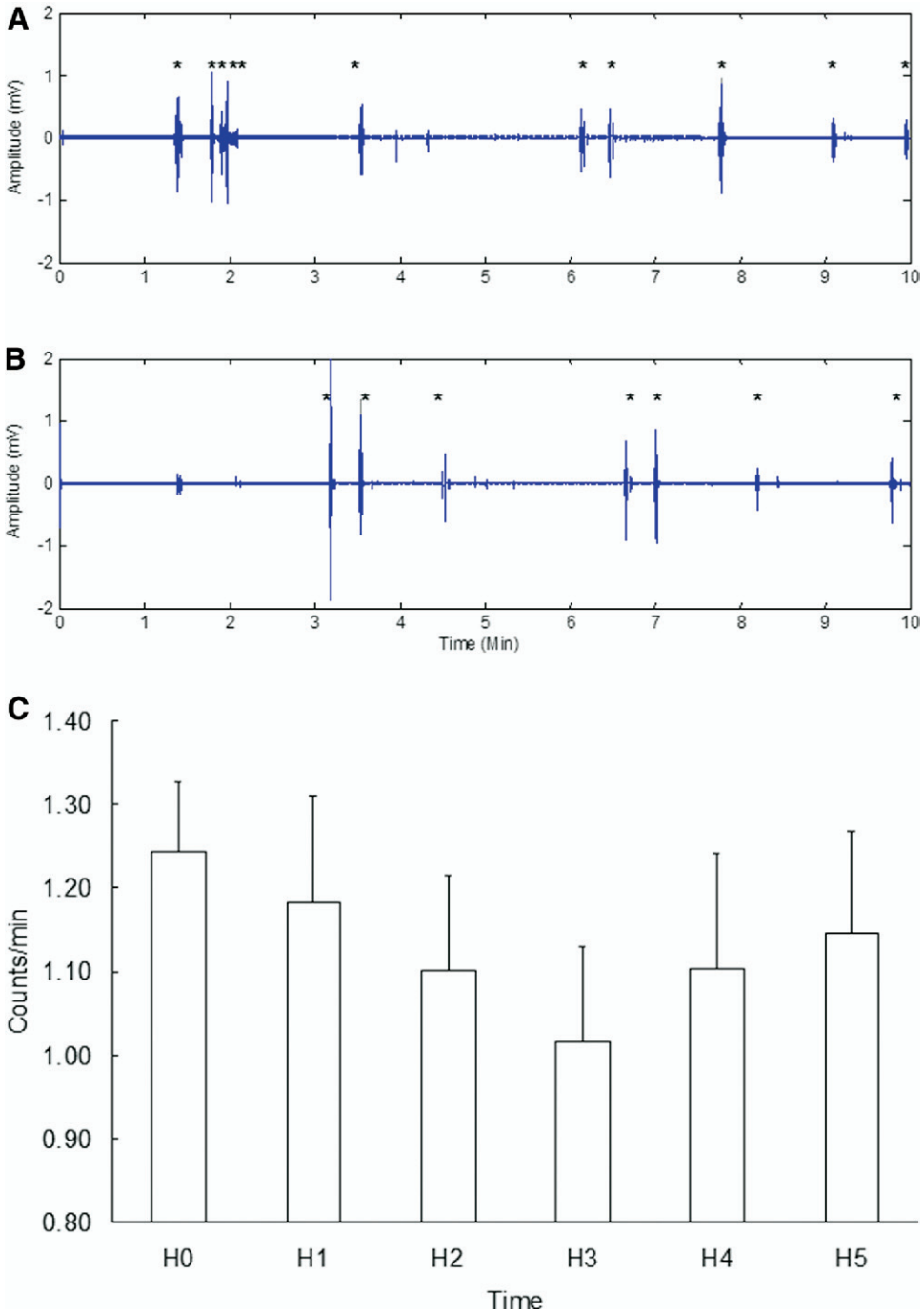

Fig. 11. Examples of PCCs detected in continuous 10-min duration before (A) and after (B) raclopride injection. Asterisk indicated the identified phasic contraction component. (C) The phasic contraction, represented as PCC, decreased from H0 to H3 and then increased.

or in addition, D2 antagonists may act directly to reduce the ability of cortical and basal ganglia motor pathways to generate descending commands. Indeed, reduced phasic activation of motor cortex neurons during bradykinetic movement and during rest in this animal model has recently been demonstrated (Parr-Brownlie and Hyland, 2005). PCC measurements are relatively simple to obtain and quantify, and so provide a relatively straightforward index for monitoring central changes in descending voluntary command in animal models.

In contrast to high correlations between the EMG amplitude and phasic activity to behavior bar tests, the EMG MDF exhibited a rather different time-course pattern, a steady and gradual decrease toward lower frequency. One factor that can decrease MDF is increased synchronization of motor units firing which has been reported in animal

Table 2. Correlation coefficients between bar test score and all other parameters

	Stiffness	B	RMS	MDF	PCC
Bar test score	0.87	0.92	0.96	−0.40	−0.78

Pearson correlation was used for correlating each parameter versus bar test score.

studies (Lee et al., 1994). However, D2 antagonist administration does not lead to increased synchrony of motor cortical neurons in the rat (Parr-Brownlie and Hyland, 2005), although such increases have been reported in the parkinsonian monkey (Goldberg et al., 2002). Furthermore, MDF can be affected by other biophysical changes, such as muscle action potential conduction velocity not related to recruitment pattern (Nigg et al., 2000). Whatever the cause of the change in this measure, the time-course change was different from the other measures, in particular the onset time was delayed. The observed changes in MDF are thus likely to be secondary to the rigidity, rather than causative. For instance, they may reflect some kind of compensatory strategy, or a change in dynamics of motor unit control secondary to disturbed properioceptive feedback from the rigid muscles. Our data suggest that the initial event in raclopride-induced akinesia was increased tonic muscle activation through enhanced recruitment numbers. This matches with findings that in human PD subjects with rigidity results from increasing recruitment numbers rather than firing rate modulation (Cantello et al., 1995).

CONCLUSION

In conclusion, the miniaturized devices we have developed provide a method for objective quantitative biomechanical and electrophysiological assessment of muscle tone and joint stiffness in the rat. The present results confirm that akinesia induced by dopamine D2 antagonist is accompanied by development of limb rigidity similar to that seen in PD changes, and showing that this acute model therefore captures both the akinesia and rigidity components of Parkinsonism. Such quantification of the time course of biomechanical and EMG markers of Parkinsonism has the potential to be very useful for the assessment of the effectiveness of novel treatments for PD.

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