Sympathetic activation by the cold pressor test does not increase the muscle force generation capacity

Roatta S, Farina D. Sympathetic activation by the cold pressor test does not increase the muscle force generation capacity. JOURNAL OF APPLIED PHYSIOLOGY. 110 pp: 1526-1533.
DOI: 10.1152/japplphysiol.00039.2011

Submitted to: *Journal of Applied Physiology*

SYMPATHETIC ACTIVATION BY THE COLD PRESSOR TEST DOES NOT INCREASE THE MUSCLE FORCE GENERATION CAPACITY

Silvestro Roatta[1,]*, Dario Farina[2]

[1]Dipartimento di Neuroscienze, Sezione di Fisiologia, Università di Torino, Torino, Italy

[2]Department of Neurorehabilitation Engineering, Bernstein Center for Computational Neuroscience, University Medical Center Göttingen, Georg-August University, 37075 Göttingen, Germany

Running title: Sympathetic activation and force generation capacity

Corresponding author:

*Silvestro Roatta, Ph.D.

Dept. Neuroscience, Physiology Div.

Università di Torino

c.so Raffaello 30, 10125 Torino, Italy

tel. +39 011 6708485; fax + 39 011 6708174

e-mail: silvestro.roatta@unito.it

ABSTRACT

A positive inotropic action by the sympathetic nervous system on skeletal muscles has been observed and investigated in animal and in-vitro studies. This action provided a theoretical basis for the putative ergogenic action of catecholamines and adrenergic agonists, although there is no clear evidence of this effect in humans. The aim of this study was to investigate the occurrence of inotropic effects associated to physiological sympathetic activation in healthy subjects. The muscle force capacity was investigated in the tibialis anterior (n = 9 subjects) and in the soleus (n = 9) muscles electrically stimulated with single pulses, double pulses with variable inter-spike interval (ISI: 4-1000 ms) and short pulse trains (frequency: 5-14 Hz) before, during and after sympathetic activation by the cold pressor test (CPT). CPT significantly decreased by 10.4±7.2 % and 10.6 ± 4.4 % the force produced by single and double pulse stimulation, respectively, and produced smaller decreases in the force obtained by train stimulation in the tibialis anterior while no significant changes were observed in either type of contraction in the soleus muscle. CPT failed to induce any increase in the force capacity of the investigated muscles. The prevalent decrease in force evidenced in this study support the concept that the weakening sympathetic action on type-I fiber, already shown to occur in humans, prevails over the putative potentiating action.

Keywords: inotropic effect, electrical stimulation, twitch force, catecholamines, adrenaline

INTRODUCTION

Sympathetic activity is known to support motor function by acting at different levels, including the cardiovascular, respiratory, and motor systems. The release of catecholamines in the blood accompanies physical exercise depending on its extent and duration (39), and catecholamine outflow was found to be correlated with motor performance (11). In addition, administration of sympathomimetics, particularly beta2 adrenergic agonists, such as salbutamol, have been shown to improve motor performance in different types of tasks (8, 20, 32, 34). To explain this *ergogenic* effect, a specific potentiating action on skeletal muscle contractility is often invoked (36). Indeed, a positive inotropic effect of epinephrine (EPI) and adrenergic agonists on skeletal muscles has been well documented in anesthetized animals as well as in isolated muscles and fiber bundles. This effect has been found to be mediated by beta2 adrenergic receptors leading to increased Ca release from the sarcoplasmatic reticulum (1, 3, 5, 12, 35). There are, however, studies in which administration of EPI or beta2 agonists failed to induce contractility potentiation (21) or improvement in motor performance (7, 19).

One possible factor behind these conflicting results may be the complexity of the adrenergic action, differentially affecting the contractile machinery of type-I and type-II muscle fibers. A positive inotropic effect is indeed mainly exhibited by type-II fibers whereas the prevailing effect in type-I fibers is a positive *lusitropic* effect, i.e., a shortening of the duration of the twitch force due to increased relaxation rate (3, 29). Notably, while the former effect mediates a potentiation of the contraction, the latter corresponds to a *weakening* action because shorter-lasting twitches result in diminished twitch summation and therefore in a lower average force level in sustained subtetanic contractions (3, 28, 29).

In addition, the weakening effect (shortening of the twitch force) in type-I fibers could be experimentally elicited at “low” concentrations of the adrenergic agonist while the potentiating effect on type-II fibers required concentrations 4-12 times greater (1, 3), casting doubts on its physiological relevance (3).

Although several animal experiments and in-vitro studies have investigated the modulation of muscle contractility by sympathomimetics, little evidence exists on the adrenergic modulation of muscle contractility in humans, in physiological conditions. Only very recently, evidence of a weakening effect on type-I fibers, as reported in animal studies, was provided for low-threshold (presumably composed of type-I fibers) motor units during physiological sympathetic activation by the cold pressor test (CPT) in healthy subjects (28). Conversely, whether endogenouos catecholamines effectively mediate an inotropic effect in skeletal muscles (specifically type-II fibers) in humans under physiological conditions remains to be demonstrated and the possible implication in motor control and performance remains a matter of debate.

The aim of this study was to investigate the occurrence of a positive inotropic effect in skeletal muscles of healthy subjects during physiological sympathetic activation by the CPT. In our previous study (28), the weakening effect on type-I fibers was investigated during low-level voluntary contractions in which only few low-threshold, presumably type-I, motor units are recruited. This condition allows the twitch force of single motor units to be estimated by means of the spike-triggering averaging technique. This approach is however technically inapplicable to high-threshold type-II motor units. The current study was thus based on electrically-elicited contractions. The putative sympathetic-induced increase in muscle force capacity should be related to the fraction of type-II fibers in the

muscle. In order to test this hypothesis the study was conducted on two muscles with a different fiber type composition, the tibialis anterior muscle (about 70% type-I, 30% type-II) and the soleus muscle (85% type-I, 15% type-II) (16). Due to its larger fraction of type-II fibers, a larger positive inotropic effect was expected in the tibialis anterior muscle.

MATERIALS & METHODS

The study consisted of two experiments in which electrically-elicited isometric contractions were measured in the tibialis anterior (experiment 1) and in the soleus (experiment 2) muscles, before, during and after activation of the sympathetic nervous system by the cold pressor test (CPT). Unless otherwise specified, the descriptions of the methods refer to both experimental conditions.

Subjects

Eleven (age: 27.3 ± 4.1 yrs; height: 173 ± 9 cm; weight 67 ± 9 kg) and 12 (age: 28.3 ± 3.8 yrs; height: 174 ± 9 cm; weight 70 ± 12 kg;) healthy men participated in experiment 1 and 2, respectively. They were recruited among the student population and the research staff at the University Campus, none of them practicing sport at agonistic level. The experimental protocols, approved by the local ethic committee (N-20070017), were in accordance with the Declaration of Helsinki. All subjects gave their informed consent before participation in the experiments.

Experimental set-up

Subjects were asked to refrain from meals and coffee in the hour before the beginning of the experiment. The subject was seated on a dental chair of adjustable height with his right foot fixed to a foot plate. The positions of chair and foot plate were adjusted

115 so that the knee and ankle joint angles were approximately 100° and 70°, respectively
116 (study 2) and both 90° (study 1). The leg was stabilized by Velcro straps and by a vacuum-
117 packed kapok-filled pillow (Ambu, Kristianstad, Sweden) that prevented side movements
118 of the leg. Care was taken in tightly fixing the foot to the foot plate. For this purpose, no
119 padding was used in order to avoid damping of the torque measurement at the ankle joint.
120 *Torque and EMG*
121 The footplate was equipped with a strain gauge providing a signal proportional to
122 the elastic deformation. This signal was amplified (Amplifier Unit LAU 73.1, Soemer,
123 Lennestadt, Germany) and used to measure the absolute torque level produced at the ankle
124 (1.05 Nm/V; bandwidth 0 - 50 Hz).
125 Surface EMG signals were recorded using bipolar circular electrodes (1 cm
126 diameter, 2-cm apart) placed along the direction of the muscle fibres on the tibialis anterior
127 muscle, about 2-cm lateral to the tibial bone and 5-cm distal to the tibia tuberosity (study
128 1), and on the soleus muscle below the gastocnemius muscle, 2-cm lateral to the tendon
129 (study 2). The ground electrode was placed at the ankle.
130 *Blood pressure and subjective pain ratings*
131 Systolic and diastolic blood pressures were measured with a digital blood pressure
132 meter (UA-751, Simonsen & Weel). The manometer cuff was released after each measure
133 and the arm raised up a few seconds for quick recovery of perfusion regimen in the arm.
134 The pain intensity was continuously scored by the subjects on an electronic 10-cm
135 visual analog scale (VAS) with the lower extreme labelled “no pain” and the upper extreme
136 labelled “most pain imaginable”.

EMG, torque, and VAS were concurrently sampled (12-bit A/D conversion, 2 kHz sampling frequency) and stored on a PC.

Electrical stimulation

Electrical stimulation was provided by a voltage-controlled current source stimulator (NoxiSTIM; JNI Biomedical A/S, Aalborg, Denmark). In study 1, the stimulation of the tibialis anterior muscle was obtained by stimulating the peroneal nerve, the cathode (electrode diameter: 2 cm) being placed just above the fibula neck and the anode (3 x 3 cm) at the patella. In study 2, the calf muscle was stimulated by a cathode electrode (diameter 2 cm) placed on the tibial nerve at the popliteal fossa. In order to reduce the contribution of the gastrocnemius muscle, the knee was flexed at about 100°. However, in this position the electrode nerve coupling is impaired, as compared to the knee-extended position. Therefore, a custom device was fixed to the thigh and exerted an adjustable pressure on the cathode electrode at the popliteal fossa in order to improve the effectiveness and reliability of the nerve stimulation. The anode electrode (3×3 cm) was placed at the patella. The anode position was adjusted in order to avoid unwanted contractions of antagonist muscles during the stimulation, which was detected by monitoring EMG activity on the tibialis anterior muscle.

For each subject the stimulation intensity evoking the maximum compound muscle action potential was determined. However, in some cases, a supramaximal intensity of stimulation was reported to be painful. Because it was important to avoid preventive pain-induced sympathetic activation related to the stimulation, in those cases we adopted the maximum stimulation intensity which was non-painful.

Procedures

The types of stimulation performed were single pulses, doublets, and pulse trains. The stimulation pattern for single stimuli and doublets consisted of a sequence of 22 alternated single and paired pulses separated by 1-s interval. The paired pulses (doublets) had an inter-spike interval ranging between 4 and 1000 ms (4, 8, 12, 15, 20, 30, 50, 75, 100, 125, 150, 175, 200, 225, 250, 300, 400, 500, 750, 1000 ms) according to a protocol adopted in previous studies (17, 18). This sequence of pulses was followed by 4 pulse trains of 5 s in duration, separated by 5-s intervals. The pulse trains had frequencies 5, 8, 10, and 12 Hz (Fig. 1B).

The set of stimulations was repeated seven times, corresponding to three control conditions (C1, C2, C3), one condition of sympathetic activation (CPT), and three recovery conditions (P1, P2, P3) (Fig. 1A). Each recording condition was separated by 5-min intervals.

In the CPT condition, the left hand was immersed in iced water (3-4°C), stirred by a peristaltic pump, for 4 min. The subjects could withdraw the hand from the water if the pain became unbearable, in which case the data were excluded from the analysis. In study 2 only, in one of the control conditions the left hand was immersed in water at 32-35 °C (neutral condition) and the sequence of the control and neutral conditions was randomized.

Systolic and diastolic blood pressures were measured during the control condition just after C2, immediately after the CPT and during the recovery just after P2 (see black dots in Fig 1A).

Signal analysis

Values of systolic and diastolic blood pressure and VAS ratings were averaged over each condition.

Time-to-peak (TTP), half-relaxation time (HRT), and peak amplitude (PA) were computed from the average of 21 single twitch torques, following the first stimulus. The twitch torque elicited by the first stimulus was excluded because it was systematically smaller than all the others. The same parameters were extracted from the doublet stimulation for the second stimulus in each pair of stimuli. The PA value was identified as the maximum torque increase (with respect to the pre-stimulation level) reached after the stimulating pulse. Since no changes in nerve conduction velocity were expected, TTP was more conveniently computed as the interval between the stimulation pulse and the time instant corresponding to the torque peak, rather than between the onset of torque development and the torque peak. HRT was computed as the interval between the torque peak and the instant in which the torque was reduced to half its peak value.

From the pulse trains, the average torque and the amplitude of torque oscillations were extracted. The average torque was computed by averaging the torque signal over the last 1-s of stimulation during the pulse train. The amplitude of torque oscillation was obtained as the peak-to-peak amplitude of the torque signal, as average value over the last 1-s of stimulation. These values were normalized with respect to the average of all conditions before averaging over subjects.

Statistical analysis

For both experiments, non-parametric statistical analysis was adopted because the normality tests failed for some of the analyzed variables (diastolic blood pressure in control condition, experiment 1 and 2). The Kruskal-Wallis analysis of variance (ANOVA) and Mann-Whitney U-test were used to compare blood pressure changes and VAS score in the two studies. One way ANOVA for repeated measures was used to assess an effect of

condition (*C1, C2, C3, CPT, P1, P2, P3*) on the measured variables. When ANOVA was significant ($P < 0.05$), pair-wise comparisons were tested by the Newman-Keuls post-hoc test. Values are presented as mean and SD in the text and as mean and standard error of the mean (SE) in the figures.

RESULTS

Experiment 1 – stimulation of peroneal nerve

Two out of the eleven recruited subjects had to be discarded because of unstable force recording due to involuntary contractions during the CPT. The results are collected from the remaining 9 subjects.

On average, the intensity of stimulation of the peroneal nerve was 113.0 ± 9.3 % of the intensity producing the maximum amplitude in the EMG response to single stimuli (M-wave) in the tibialis anterior muscle (range: 95 – 120%).

CPT evoked a persistent painful sensation that outlasted the duration of the test. The peak VAS score was 4.5 ± 1.9 (range: 2.7-8.7). The painful sensation vanished in all subjects before P2.

CPT produced an increase in diastolic blood pressure from 73.8 ± 6.0 to 89.0 ± 9.2 mmHg ($P < 0.01$) and systolic blood pressure from 109.0 ± 9.7 to 127.6 ± 11.0 mmHg ($P < 0.01$). Both variables returned to control values when reassessed, after P2 condition (diastolic: 75.7 ± 5.6 mmHg; systolic: 113.2 ± 8.2 mmHg)

The effect of CPT on the torque twitch evoked by single electrical stimuli is exemplified by the recordings from a representative subject (Fig. 1A). Group effects across all subjects on AMP, TTP and HRT are shown in the bar diagrams in Fig. 2B-D. We

observed a slight increase in AMP during the three control conditions: C3 being higher then C1 by 8.2 ± 9.1 % ($P<0.01$), possibly due to post contraction potentiation mechanisms. However, AMP was significantly reduced by 10.4±7.2 % ($P<0.01$) during CPT with respect C3. A gradual recovery of twitch amplitude was observed in the recovery conditions, with P3 being significantly different from CPT and matching the value of C3. Conversely, no significant changes were instead observed in the time course of TTP (116±13 ms in C1, Fig. 2C) and HRT (73±17 ms, in C1, Fig. 2D).

The analysis of the twitch torque produced by the second of two spikes administered with variable ISI is shown in Figure 3. For ISI smaller than 20 ms, the two twitches are fused together and the amplitude is almost independent of the ISI. With increasing ISI above 20 ms, the two twitches begin to split and the amplitude of the second one starts to fall (Fig. 3A). Above 300-400 ms the second twitch is completely separated from the first one and its characteristics tend to approach the characteristics of the single twitch described in Fig. 2. With ISI >30ms the two twitches are only partly fused and the peak amplitude (detected after the second pulse) starts to decrease. The TTP and HRT also exhibit a clear dependency on ISI.

The CPT influenced the amplitude of the peak torque produced by the second pulse at all ISIs tested (thick line in Fig. 3B). The differences were significant for the average peak amplitude of the first three doublets (ISI= 4, 6, 8 ms) which was reduced by 10.6 ± 4.4 % during the CPT with respect to C3 and was significantly different from all other conditions ($P<0.01$, except vs. C1: $P<0.05$). TTP and HRT were not influenced by CPT (Fig 3C,D).

The contractions evoked by burst stimulations were analyzed in terms of the torque reached at the end of the burst and of the amplitude of torque oscillations. On average, the torque developed during CPT was lower than that developed in all other conditions for each of the stimulation frequencies employed, however the significance level was not reached. The amplitude of torque oscillation during burst stimulation at 5 Hz exhibited a similar time course as the torque twitch amplitude, i.e., a slight increasing trend between C1 and C3 (10 ± 12 %) and between P1 and P3 (9 ± 13 %) but with a decrease between C3 and CPT (6 ± 13 %). Similar but less marked changes were observed at 8, 10 and 12 Hz although none of these changes reached statistical significance.

Experiment 2 – Stimulation of the tibial nerve

One subject had to be excluded because of instability of the force recording and two other subjects were discarded because of the presence of a H-reflex in response to the electrical stimulation. The results are described for the 9 remaining subjects.

Electrical stimulation of the tibial nerve appeared to be relatively more painful than stimulation of the peroneal nerve and, in order to avoid pain sensations associated to the electrical stimulation, the intensity often had to be reduced below the one producing the maximum M-wave (89 ± 10.3 %, range: 76 - 106 %).

Hand immersion in water at neutral temperature did not evoke a pain sensation (VAS= 0 in all subjects) while CPT evoked similar effects to those described for experiment 1. The VAS score peaked at 5.6 ± 3.1 (range: 2.3-9.2) during the test and returned to 0 in all subjects at P2. Diastolic blood pressure rose from 73.8 ± 6.0 to 89.0 ± 9.2 mmHg ($P < 0.01$) and systolic blood pressure from 109.0 ± 9.7 to 127.6 ± 11.0 mmHg ($P < 0.01$). Both variables returned to control values when reassessed, after P2 condition

(diastolic: 75.7 ± 5.6 mmHg; systolic: 113.2 ± 8.2 mmHg). VAS and blood pressure changes were not significantly different in Experiment 2 as compared to Experiment1.

A representative example of the single twitch of calf muscles evoked by stimulation of the tibial nerve is reported in Fig. 4A. The time course of the twitch torque in the control condition (average of C1-C3) was slower than in Experiment 1, as observed both for TTP (124 ± 19 ms vs. 105 ± 11 ms; $P<0.01$) and for HRT (99 ± 23 ms vs. 71 ± 18 ms; $P< 0.05$). In one of the control conditions the left hand was immersed in water at neutral temperature and on average, the twitch parameters did not depend on the control condition. CPT did not influence the twitch amplitude (Fig. 5B), nor TTP (Fig. 5C) nor HRT (Fig. 5C), although the latter was reduced in 7 of the 9 subjects during CPT (4% reduction with respect to the average of C1-C3).

An example of the evoked contractions during the stimulation with double pulses is shown in Fig. 5A, while Figs. 5B-C report the mean curves for the different conditions. Also for this stimulation paradigm the parameters did not depend on the condition. As observed for the single twitch, HRT was slightly but not significantly reduced at ISI < 50 ms (Fig. 5C). Absence of systematic changes in the time course of the contraction is also confirmed by the absence of changes in the pattern of summation of the double-twitches, as indicated by the curves in Fig. 5B.

The torque developed by burst stimulation was also unaffected by CPT both in terms of average torque developed and of amplitude of torque oscillation.

DISCUSSION

296 Physiological sympathetic activation by CPT did not produce a potentiation of the
297 contraction in any of the tested muscles. Conversely a significant decrease in twitch
298 amplitude was observed in TA while only a trend towards twitch shortening was observed
299 in the calf muscles. This set of results indicates that a weakening rather than a potentiating
300 effect has been induced by sympathetic activation.

301 *Potentiation vs. weakening*

302 In-vitro studies (5, 12) have elucidated that adrenergic agonists may modulate the
303 contractile machinery of skeletal muscles in two ways: i) by increasing the reuptake of
304 Ca++ in the sarcoplasmatic reticulum, thus shortening the twitch duration (positive
305 lusitropism) and resulting in a weakening effect – this mechanisms being present in type-I
306 fibers only - and ii) by augmenting the release of Ca++ from the SR, thus producing a
307 twitch of bigger amplitude, which is a potentiation of the contraction – this mechanisms is
308 present in both fiber types although it has been observed mostly in fast-twitch muscles (1,
309 3, 4). These classic studies, performed on animal models, already pointed out that higher
310 doses of EPI or β_2-agonist had to be administered to elicit a potentiating effect in fast-
311 twitch muscles with respect to the dose required to elicit the weakening effect in slow-
312 twitch muscles (1, 3, 4). This difference can be partly attributed to the fact that type-I fibers
313 have a higher density of adrenergic receptor than type-II (15, 22). On the other hand, it is a
314 widely held view that the sympathetic nervous system potentiates the contraction of skeletal
315 muscles (9, 11, 33, 36). This idea fits well with the other actions that the sympathetic
316 nervous system exerts, particularly on the cardiovascular system, to support intense muscle
317 work, and is appropriate in a context of *fight or flight*. However, it must be emphasized that
318 no human study currently evidenced the occurrence of sympathetic-mediated potentiation

of skeletal muscles. Moreover many animal and in-vitro studies that report catecholamine-mediated potentiation refer to muscles that were previously fatigued (3, 23) or to muscle fibers immersed in a iperkalemic medium (13). The force potentiation of fatigued muscles, also called “anti-fatigue” effect, is based on the recovery of cell excitability by EPI-induced potentiation of the Na/K pump (3, 13, 29), and does not mediate the positive inotropic effect observed in resting fast-twitch muscles (1, 3, 4).

In the present study, CPT failed to induce any potentiation in either TA or calf muscles, although the same stressor was adequate to induce the weakening effect in low threshold, presumably type-I, motor units of the TA (28). This supports the concept that the positive inotropic effect has a higher threshold of activation than the weakening effect. These results also support and integrate the only investigation in humans about the effects of exogenous (not spontaneously released, as in the present study) EPI on muscle contractility by Marsden & Meadows (21). Although their interest was mostly focused on the tremor-genic action of EPI, the authors evidenced a weakening adrenergic effect in both the calf muscles (5 subjects) and the adductor pollicis (3 subjects). In particular, they showed a reduction in HRT of the twitch force in the calf muscles (~15%), no significant effect on the twitch force of adductor pollicis but a decrease in the force of subtetanic contraction (10Hz stimulation), as we did observe for TA.

The protocol adopted in the present study included electrical stimulation by paired stimuli at varying inter-spike interval within the range 4-1000 ms. This stimulation pattern was previously employed for the investigation of the velocity recovery function of muscle fibers (18) and of twitch summation (17). It was adopted in the present study for two reasons: 1) the response to the doublet at short ISI is stronger than the single twitch and

thus provides an improved signal-to-noise ratio for the detection of changes in muscle contractility; 2) possible increase/decrease in twitch duration, resulting in increased/decreased twitch fusion, would have been evidenced by rightward/leftward shift of the torque amplitude vs. ISI curve in this stimulation pattern.

The reduction in single twitch amplitude in TA was confirmed by the reduction in the response to paired stimuli ($4<ISI<30$, Fig. 3B) as well as by a reduction in the torque developed by burst stimulation at the different frequencies. This supports the interpretation that sympathetic activation by CPT produced a weakening effect. In fact, in many animal studies a marked decrease of the twitch amplitude was observed in response to EPI injection, as a consequence of the lusitropic effect occurring in type-I fibers (1, 3, 4). It is possible that, this latter effect was masked in the present study, due to the co-activation of unresponsive or differently-responding type-II fibers in TA. A decrease in HRT was instead observed in response to CPT-induced sympathetic activation in our previous study where single, low-threshold, presumably type-I motor units were investigated (28) while an increased HRT was observed in the TA of healthy subjects in response to blockade of β-adrenergic receptors (2).

In the soleus muscle, the reduction in HRT observed on the single twitch was also observed in response to close paired stimuli ($4\leq ISI\geq 30$, Fig. 5D), although the effect was probably too weak to reach statistical significance and to produce appreciable changes in the amplitude-vs-ISI curve as well as in the burst contractions.

Electrical stimulation does not allow selective recruitment of type II muscle fibers. Therefore the possibility exists that a potentiating effect occurring in type-II fibers was canceled by concomitant weakening effects in type-I fibers when the muscle is composed

of a balanced proportion of the two types of fibers. In fact, Bowman and Zaimis (4) observed clear cut potentiation in the fast-twitch tibialis muscle and marked weakening in the slow-twitch soleus muscle of the cat, intravenously injected with EPI, while minor effects were observed in plantaris and gastrocnemius muscles characterized by a more balanced fiber-type composition. In humans, both TA and soleus muscles have a preponderance of type-I fibers, so the possibility cannot be excluded that potentiation effects have been canceled by weakening effects occurring in these fibers. On the other hand, selective activation of type II fibers is also unlikely to occur in voluntary contractions since the orderly recruitment of motor units according to the size principle predicts that type-I motor units are recruited first (14). On this basis, the possible ergogenic action of catecholamines would anyway hardly become functionally meaningful, given that most human skeletal muscles have a large percentage of type-I fibers.

A possible complication in the interpretation of our results is that greater sympathetic activation may be required for observing potentiating effects on muscle fiber contractility than that provided by CPT. This opens for a potential functional role of potentiation of contractility at higher activation levels of the sympathetic system. However, CPT, which provokes a consistent increase in arterial blood pressure and in plasma catecholamines (31), is a stimulus which is already quite difficult to sustain: VAS pain scores up to 9.2 were reported in the present study while in previous studies some subject could not tolerate the pain level and interrupted the test before completion (28). Nevertheless, the involvement of the sympathoadrenal axis in the stress response is stressor-dependent (27, 29, 31) and it cannot be excluded that the ergogenic effect can be better detected in response to other experimental stimuli.

CPT is also reported to increase muscle sympathetic activity (10) and to reduce blood flow to resting limb muscles (37). Reduction in the blood supply was shown to decrease the muscle force capacity in fatigued and resting muscles (25, 38). Although it is unlikely that a small reduction in blood flow (20% in the study of Wray et al (37)) for a duration of few minutes might have affected the force capacity of a resting muscle, this possibility cannot be completely excluded. On the other hand, the increase of vascular resistances and limitation of blood flow to different organs, including skeletal muscles, are part of sympathetic activation patterns, so this indirect weakening effect of sympathetic activation on muscle force capacity should anyway also be taken into account when considering functional effects.

Limitations

In a context of generalized sympathetic activation, motor control may be affected at different central and peripheral levels. In order to focus the investigation on the sympathetic effects on muscle contractility, this study was based on electrically stimulated contractions which provide a standardized model to reproduce muscle contraction with high repeatability and independence from the central motor command. This choice was motivated by the observation that the motor command adapted and compensated for changes occurring at the effector level, during sympathetic activation (28). However, limitations of this approach need to be taken into account.

Supramaximal percutaneous nerve stimulation, which is generally adopted to obtain full muscle activation, may be rather painful. The pain produced by the electric shocks is a powerful stimulus for sympathetic activation (6, 26), just like the cold-induced pain at the immersed hand during the CPT (26). Providing pain stimuli throughout all sessions would

411 have raised basal sympathetic outflow, thus attenuating or masking the effect of CPT under
412 investigation. For this reason the intensity of stimulation was not increased beyond
413 low/moderate pain levels. This, however, resulted in submaximal stimulation of the tibial
414 nerve in most subjects which may have introduced some additional variability in the data
415 and decreased the sensitivity in the technique, especially in experiment 2.

416 In addition, nerve stimulation does not allow to selectively stimulate a single
417 muscle. This is particularly true for stimulation of the tibial nerve which leads to the
418 contraction of other muscle groups in addition to the soleus, including the gastrocnemius
419 muscle. The 100 deg knee flexion position was indeed adopted to disengage the
420 gastrocnemius muscle, thus limiting its contribution. However, some extra force
421 contribution might have remained and be possibly responsible for the non-smooth shape of
422 the twitch in some subjects (21). These two limitations may partly account for the lack of
423 effects observed in Experiment 2.

424 *Functional implications*

425 The present results do not support the presence of a sympathetically-mediated
426 ergogenic effect on muscle contractility. Conversely they confirm the presence of a
427 weakening effect even in the relatively fast-twitch tibialis anterior muscle. How does a
428 weakening effect comply with the needs of a fight-or-flight response and with the
429 numerous studies reporting increased muscle performance after administration of beta2
430 adrenergic agonists?

431 First of all the maximum force capacity of the muscle is not impaired by the
432 lusitropic effect (3), although an increased driving frequency would be necessary to attain
433 the same force (28). Secondly, the possibility to produce rapid alternating movements, as

434 those required in fight and flight, should be improved by faster muscle relaxation (29). On
435 this basis it is not surprising that significant increases in performance after administration
436 of beta2-agonists has been reported almost only for short-lasting and rapid tasks, such as
437 the wingate test (8, 20, 32, 34).

438 Besides the generalized sympathetic activation that characterizes the fight-or-flight
439 response, sympathetic outflow is known to be highly differentiated to different organs and
440 tissues depending on the context or stimulus according to the so-called autonomic
441 "signature", which also concerns the balance between sympatho-neural and sympatho-
442 adrenal pathways (24, 27, 30). Thus, sympathetic modulation of muscle contractility should
443 also be expected to occur in other situations activating the sympatho-adrenal axis. In this
444 respect and in support of the current view, it is interesting to mention the anecdotal reports
445 of back and leg muscle weakness during states of fear and anxiety as well as in response to
446 adrenaline infusion (3).

447 *Conclusion*

448 In conclusion, for the first time the occurrence of an adrenergic-mediated positive
449 inotropic action has been sought during physiological sympathetic activation. The CPT
450 failed to induce any ergogenic effect while producing instead some decrease in the
451 electrically-stimulated muscle force. Peripheral effects of either direct (on the muscle
452 fibers) or indirect (secondary to circulatory changes) adrenergic actions are presumed to
453 underlie the weakening sympathetic action.

454

455 ACKNOWLEDGEMENTS

456 We are grateful to Mara Rolando, Fabrizio Picariello, Corrado Cescon and Ernest N.
457 Kamavuako for their help with experimental procedures and data analysis. This work was
458 supported by grants from Regione Piemonte (Ricerca sanitaria finalizzata 2007-2009).

459

460 REFERENCES

461 1. **Al-Jeboory AA, and Marshall RJ**. Correlation between the effects of salbutamol
462 on contractions and cyclic AMP content of isolated fast-and slow-contracting muscles of
463 the guinea pig. *Naunyn Schmiedebergs Arch Pharmacol* 305: 201-206, 1978.
464 2. **Alway SE, Hughson RL, Green HJ, and Patla AE**. Human tibialis anterior
465 contractile responses following fatiguing exercise with and without beta-adrenoceptor
466 blockade. *Clin Physiol* 8: 215-225., 1988.
467 3. **Bowman WC**. Effects of adrenergic activators and inhibitors on the skeletal
468 muscles. In: *Handbook of experimental pharmacology, Adrenergic activators and inhibitor*,
469 edited by Szekeres L. Berlin, Heidelberg, New York: Springer, 1980, p. 47-128.
470 4. **Bowman WC, and Zaimis E**. The effects of adrenaline, noradrenaline and
471 isoprenaline on skeletal muscle contractions in the cat. *J Physiol* 144: 92-107., 1958.
472 5. **Cairns SP, and Dulhunty AF**. The effects of beta-adrenoceptor activation on
473 contraction in isolated fast- and slow-twitch skeletal muscle fibres of the rat. *Br J*
474 *Pharmacol* 110: 1133-1141., 1993.
475 6. **Christou EA, Jakobi JM, Critchlow A, Fleshner M, and Enoka RM**. The 1- to
476 2-Hz oscillations in muscle force are exacerbated by stress, especially in older adults. *J*
477 *Appl Physiol* 97: 225-235., 2004.
478 7. **Collomp K, Candau R, Millet G, Mucci P, Borrani F, Prefaut C, and De**
479 **Ceaurriz J**. Effects of salbutamol and caffeine ingestion on exercise metabolism and
480 performance. *Int J Sports Med* 23: 549-554, 2002.

8. **Collomp K, Le Panse B, Portier H, Lecoq AM, Jaffre C, Beaupied H, Richard O, Benhamou L, Courteix D, and De Ceaurriz J**. Effects of acute salbutamol intake during a Wingate test. *Int J Sports Med* 26: 513-517, 2005.

9. **Dienstbier RA**. Behavioral correlates of sympathoadrenal reactivity: the toughness model. *Med Sci Sports Exerc* 23: 846-852, 1991.

10. **Fagius J, Karhuvaara S, and Sundlof G**. The cold pressor test: effects on sympathetic nerve activity in human muscle and skin nerve fascicles. *Acta Physiol Scand* 137: 325-334., 1989.

11. **French DN, Kraemer WJ, Volek JS, Spiering BA, Judelson DA, Hoffman JR, and Maresh CM**. Anticipatory responses of catecholamines on muscle force production. *J Appl Physiol* 102: 94-102, 2007.

12. **Ha TN, Posterino GS, and Fryer MW**. Effects of terbutaline on force and intracellular calcium in slow-twitch skeletal muscle fibres of the rat. *Br J Pharmacol* 126: 1717-1724., 1999.

13. **Hansen AK, Clausen T, and Nielsen OB**. Effects of lactic acid and catecholamines on contractility in fast-twitch muscles exposed to hyperkalemia. *Am J Physiol Cell Physiol* 289: C104-112, 2005.

14. **Henneman E, Somjen G, and Carpenter DO**. FUNCTIONAL SIGNIFICANCE OF CELL SIZE IN SPINAL MOTONEURONS. *J Neurophysiol* 28: 560-580, 1965.

15. **Jensen J, Brennesvik EO, Bergersen H, Oseland H, Jebens E, and Brors O**. Quantitative determination of cell surface beta-adrenoceptors in different rat skeletal muscles. *Pflugers Arch* 444: 213-219, 2002.

16. **Johnson MA, Polgar J, Weightman D, and Appleton D**. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci* 18: 111-129, 1973.

17. **Kamavuako EN, and Farina D**. Time-dependent effects of pre-conditioning activation on muscle fiber conduction velocity and twitch torque. *Muscle Nerve* 42: 547-555, 2010.

18. **Kamavuako EN, Hennings K, and Farina D**. Velocity recovery function of the compound muscle action potential assessed with doublet and triplet stimulation. *Muscle Nerve* 36: 190-196, 2007.

19. **Kindermann W**. Do inhaled beta(2)-agonists have an ergogenic potential in non-asthmatic competitive athletes? *Sports Med* 37: 95-102, 2007.

20. **Le Panse B, Arlettaz A, Portier H, Lecoq AM, De Ceaurriz J, and Collomp K**. Effects of acute salbutamol intake during supramaximal exercise in women. *Br J Sports Med* 41: 430-434, 2007.

21. **Marsden CD, and Meadows JC**. The effect of adrenaline on the contraction of human muscle. *J Physiol* 207: 429-448., 1970.

22. **Martin WH, 3rd, Coggan AR, Spina RJ, and Saffitz JE**. Effects of fiber type and training on beta-adrenoceptor density in human skeletal muscle. *Am J Physiol* 257: E736-742, 1989.

23. **Mikkelsen UR, Gissel H, Fredsted A, and Clausen T**. Excitation-induced cell damage and beta2-adrenoceptor agonist stimulated force recovery in rat skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 290: R265-272, 2006.

24. **Morrison SF**. Differential control of sympathetic outflow. *Am J Physiol Regul Integr Comp Physiol* 281: R683-R698, 2001.

25. **Murthy G, Hargens AR, Lehman S, and Rempel DM**. Ischemia causes muscle fatigue. *J Orthop Res* 19: 436-440, 2001.

26. **Nordin M, and Fagius J**. Effect of noxious stimulation on sympathetic vasoconstrictor outflow to human muscles. *J Physiol* 489 (Pt 3): 885-894, 1995.

27. **Pacak K, and Palkovits M**. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocr Rev* 22: 502-548, 2001.

28. **Roatta S, Arendt-Nielsen L, and Farina D**. Sympathetic-induced changes in discharge rate and spike-triggered average twitch torque of low-threshold motor units in humans. *J Physiol* 586: 5561-5574, 2008.

29. **Roatta S, and Farina D**. Sympathetic actions on the skeletal muscle. *Exerc Sport Sci Rev* 38: 31-35, 2010.

30. **Roatta S, Mohammed M, and Passatore M**. Detecting activation of the sympatho-adrenal axis from haemodynamic recordings, in conscious rabbits exposed to acute stress. *Acta Physiol (Oxf)* 201: 323-337, 2011.

31. **Robertson D, Johnson GA, Robertson RM, Nies AS, Shand DG, and Oates JA**. Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. *Circulation* 59: 637-643., 1979.

32. **Signorile JF, Kaplan TA, Applegate B, and Perry AC**. Effects of acute inhalation of the bronchodilator, albuterol, on power output. *Med Sci Sports Exerc* 24: 638-642, 1992.

33. **Tod D, Iredale F, and Gill N**. 'Psyching-up' and muscular force production. *Sports Med* 33: 47-58, 2003.

548 34. **van Baak MA, Mayer LH, Kempinski RE, and Hartgens F**. Effect of salbutamol
549 on muscle strength and endurance performance in nonasthmatic men. *Med Sci Sports Exerc*
550 32: 1300-1306, 2000.
551 35. **Van Der Heijden HF, Zhan WZ, Prakash YS, Dekhuijzen PN, and Sieck GC**.
552 Salbutamol enhances isotonic contractile properties of rat diaphragm muscle. *J Appl*
553 *Physiol* 85: 525-529, 1998.
554 36. **Williams JH, and Barnes WS**. The positive inotropic effect of epinephrine on
555 skeletal muscle: a brief review. *Muscle Nerve* 12: 968-975., 1989.
556 37. **Wray DW, Donato AJ, Nishiyama SK, and Richardson RS**. Acute sympathetic
557 vasoconstriction at rest and during dynamic exercise in cyclists and sedentary humans. *J*
558 *Appl Physiol* 102: 704-712, 2007.
559 38. **Wright JR, McCloskey DI, and Fitzpatrick RC**. Effects of systemic arterial
560 blood pressure on the contractile force of a human hand muscle. *J Appl Physiol* 88: 1390-
561 1396., 2000.
562 39. **Zouhal H, Jacob C, Delamarche P, and Gratas-Delamarche A**. Catecholamines
563 and the effects of exercise, training and gender. *Sports Med* 38: 401-423, 2008.
564
565
566
567

568

569 Fig. 1. Experimental Protocol. A) The same protocol was applied for the stimulation of the
570 peroneal nerve (Experiment 1) and the posterior tibialis nerve (Experiment 2). The same
571 stimulation pattern was repeated seven consecutive times: before (C1, C2, C3), during
572 (CPT) and after (P1, P2, P3) administration of the cold pressor test (CPT, left hand
573 immersed in icy water for 4 min). (*) In a randomized control condition (C1, C2 or
574 C3) the left hand was also immersed in warm water (neutral) (Experiment 2, only). Black
575 dots indicate measurement of arterial blood pressure. B) torque developed by the
576 stimulation pattern in a control stimulation in one subject. The stimulation pattern consists
577 of a sequence of 22 single pulses interleaved with 22 double pulses (doublets) with
578 interspike-interval increasing from 4-to 1000 ms, followed by 4 bursts at constant
579 frequency of 5, 8, 10, 12 Hz, lasting 5 s.

580

581 Fig. 2. Stimulation of the peroneal nerve with single pulses (Experiment 1). A) tracing of
582 the twitch torque from a representative subject in three conditions (C1, CPT and P3). Each
583 trace is the average of 20 single twitches. B, C, D) effect of CPT on twitch amplitude
584 (AMP, B), time to peak (TTP, C) and half relaxation time (HRT, D). AMP values were
585 normalized with respect to the average over all conditions. (*) Significantly different from
586 C3, P2 and P3, $p<0.01$.(n=9)

587

588 Fig. 3. Analysis of torque developed by stimulation with double pulses of the peroneal
589 nerve (Experiment 1). A) superimposition of the torque produced by the 21 doublets at
590 increasing ISI, in one subject in a control condition. B, C, D) effect on amplitude, time to

peak and half relaxation time of the torque produced by the second pulse in the doublet is displayed vs. the ISI (ms), for 3 conditions. Each trace is the average of all individual traces (n=9) and ctrl is the average of the three control condition. Abbreviations as in Fig. 2.

Fig. 4. Stimulation of the tibial nerve with single pulses (Experiment 2). A) tracing of the twitch torque from a representative subject in three conditions (C1, CPT and P3). Each trace is the average of 20 single twitches. Effect of CPT on twitch amplitude (B), time to peak (C) and half relaxation time (D). Amplitude values were normalized with respect to the average over all conditions. Abbreviations as in Fig. 2. (n=9)

Fig. 5. Analysis of torque developed by stimulation with double pulses of the tibial nerve (Experiment 2). A) superimposition of the torque produced by the 21 doublets at increasing ISI, in one subject in a control condition. B, C, D) effect on amplitude, time to peak and half relaxation time of the torque produced by the second pulse in the doublet is displayed vs. the ISI (ms), for 3 conditions. Each trace is the average of all individual traces (n=9) and ctrl is the average of the three control condition. Abbreviations as in Fig. 3.

Fig. 1

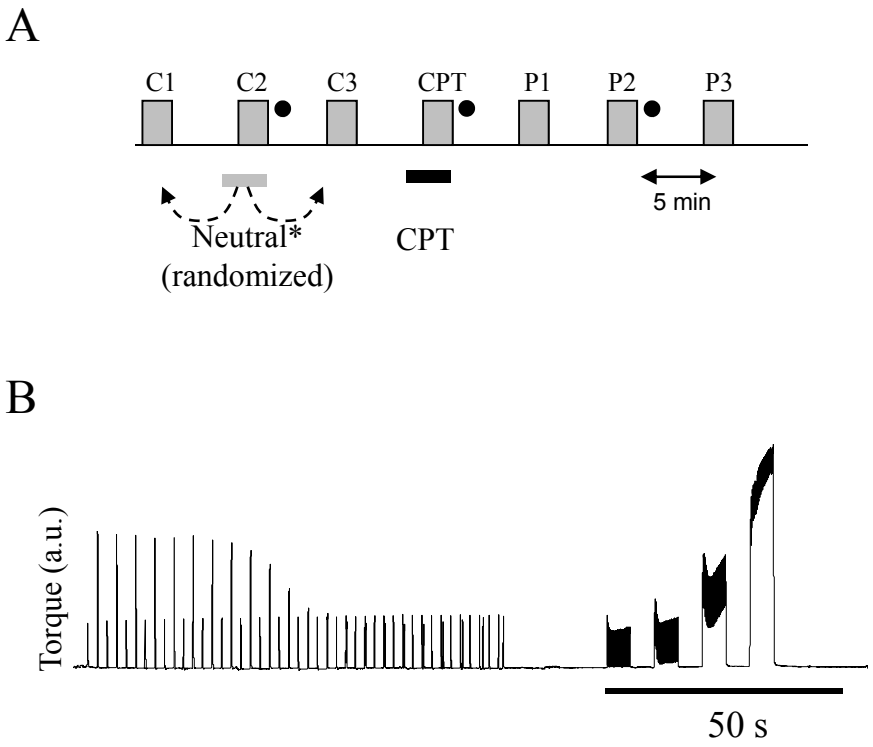

A
C1
C2
C3
CPT
P1
P2
P3
5 min
Neutral*
(randomized)
CPT
B
Torque (a.u.)
50 s

Fig. 2

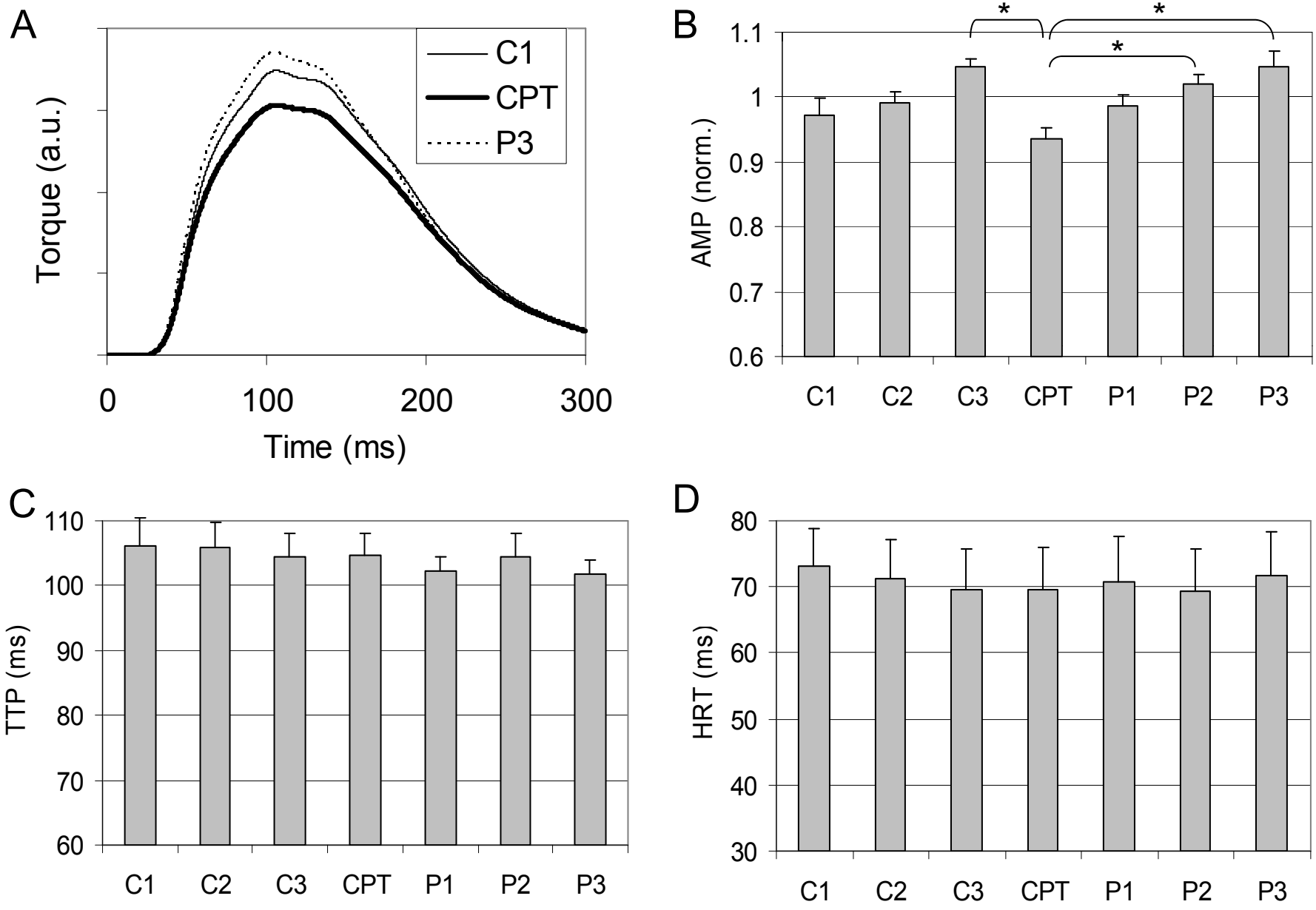

A
Torque (a.u.)
C1
CPT
P3
0
100
200
300
Time (ms)
B
AMP (norm.)
1.1
1
0.9
0.8
0.7
0.6
*
*
*
C1
C2
C3
CPT
P1
P2
P3
C
TTP (ms)
110
100
90
80
70
60
C1
C2
C3
CPT
P1
P2
P3
D
HRT (ms)
80
70
60
50
40
30
C1
C2
C3
CPT
P1
P2
P3

Fig. 3

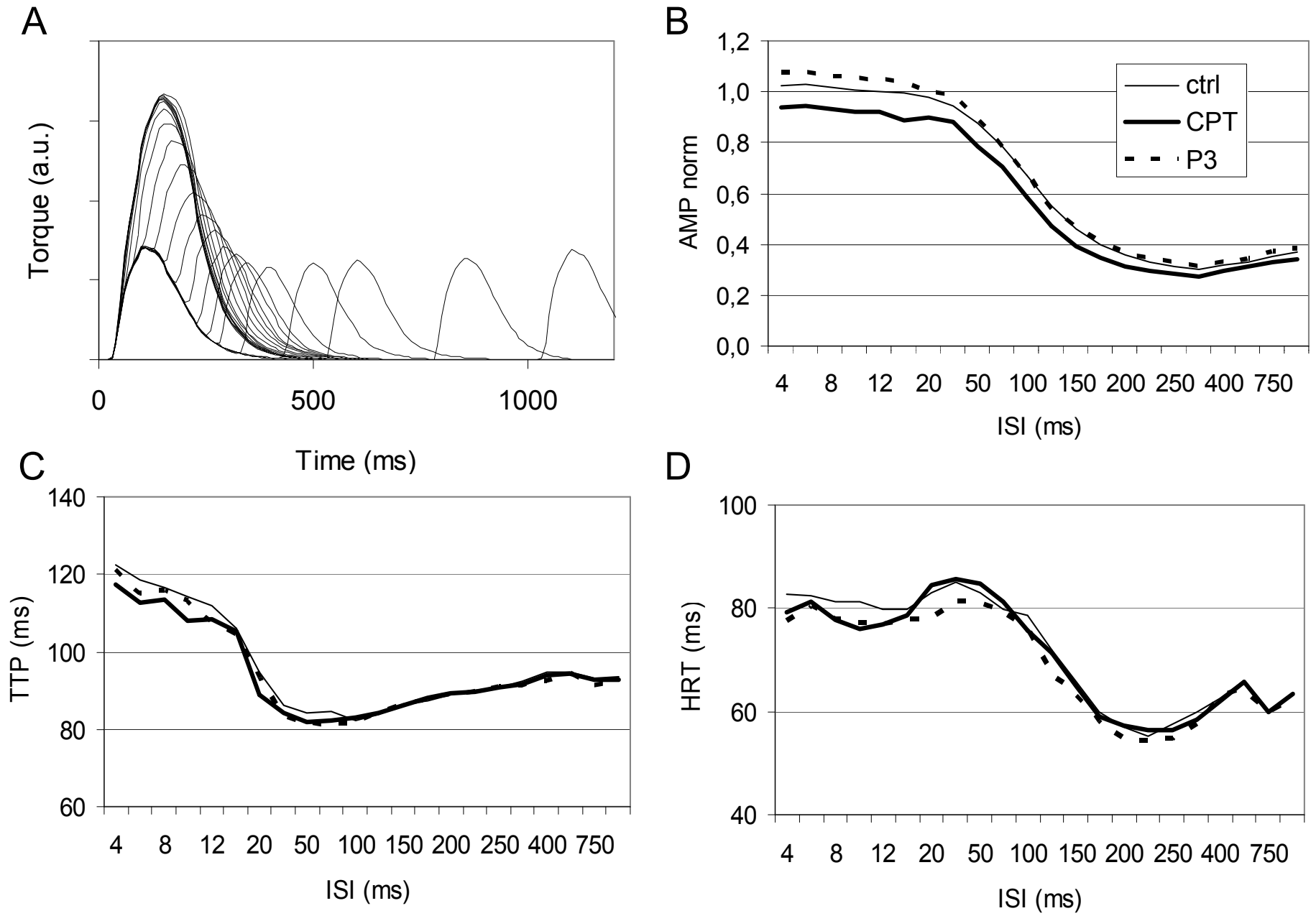

A
Torque (a.u.)
0
500
1000
Time (ms)
B
AMP norm
1,2
1,0
0,8
0,6
0,4
0,2
0,0
ctrl
CPT
P3
4
8
12
20
50
100
150
200
250
400
750
ISI (ms)
C
TTP (ms)
140
120
100
80
60
4
8
12
20
50
100
150
200
250
400
750
ISI (ms)
D
HRT (ms)
100
80
60
40
4
8
12
20
50
100
150
200
250
400
750
ISI (ms)

Fig. 4

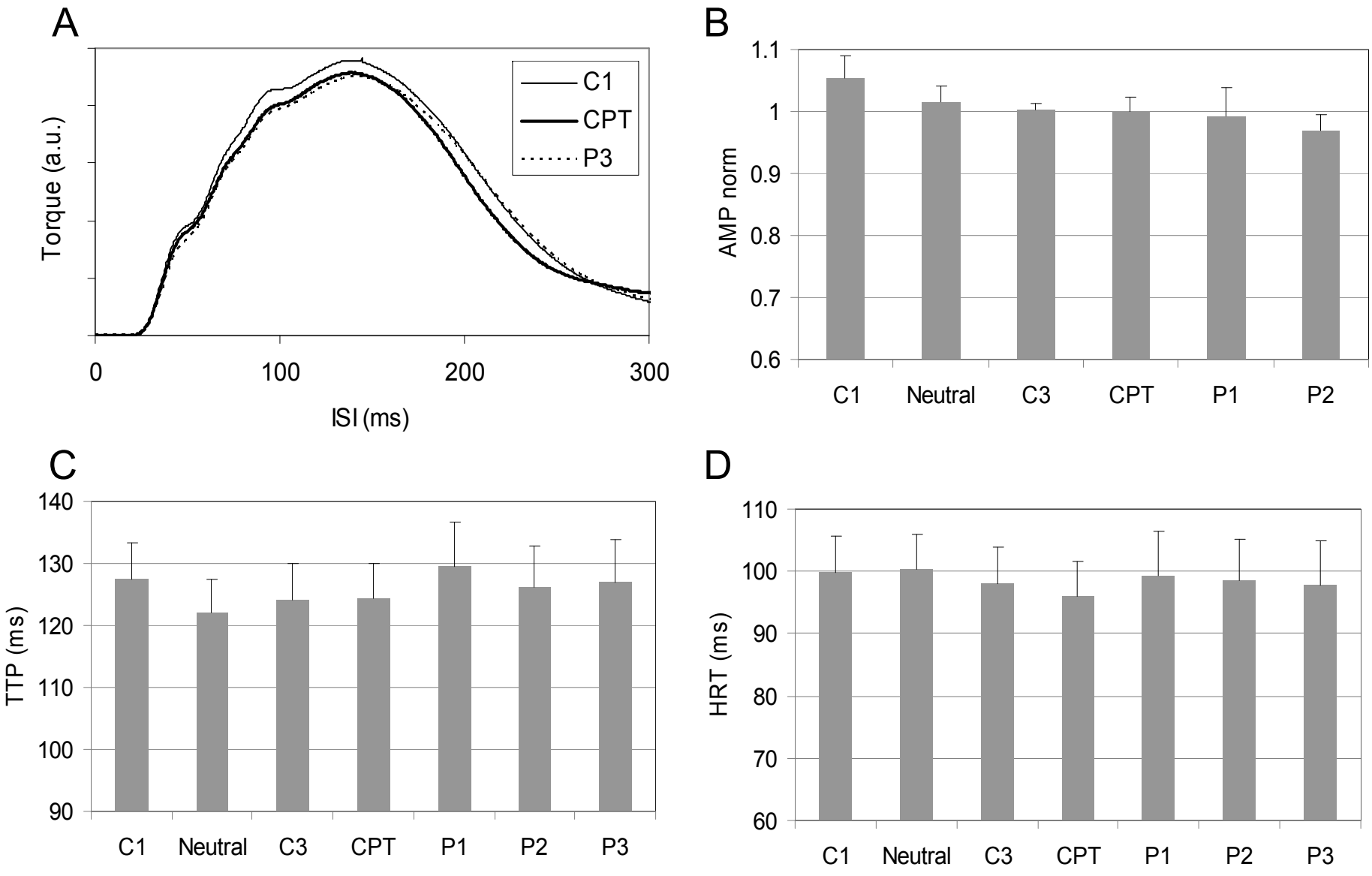
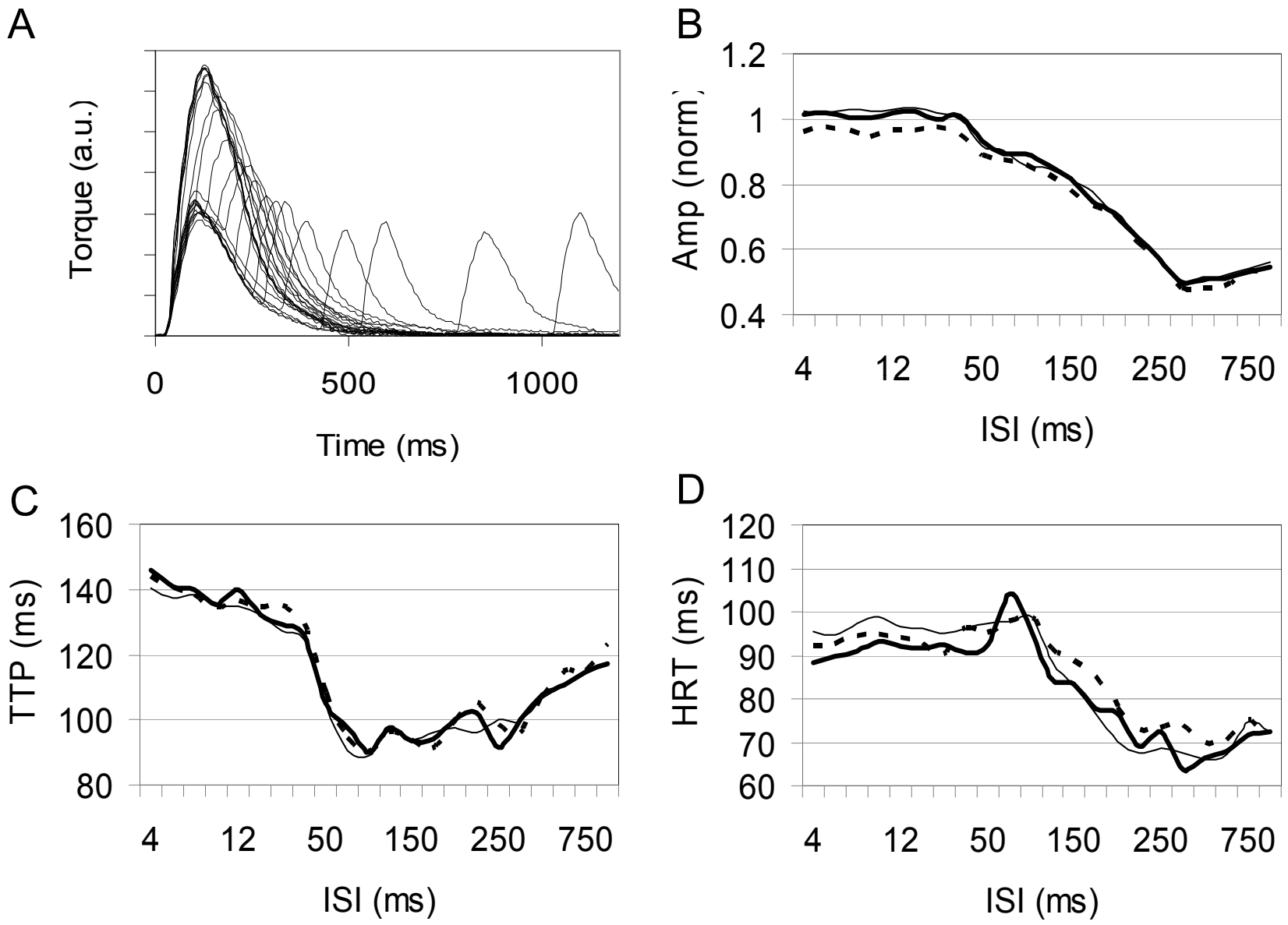

A
Torque (a.u.)
C1
CPT
P3
0
100
200
300
ISI (ms)
B
AMP norm
1.1
1
0.9
0.8
0.7
0.6
C1
Neutral
C3
CPT
P1
P2
C
TTP (ms)
140
130
120
110
100
90
C1
Neutral
C3
CPT
P1
P2
P3
D
HRT (ms)
110
100
90
80
70
60
C1
Neutral
C3
CPT
P1
P2
P3

Fig. 5


A
Torque (a.u.)
0
500
1000
Time (ms)
B
Amp (norm)
1.2
1
0.8
0.6
0.4
4
12
50
150
250
750
ISI (ms)
C
TTP (ms)
160
140
120
100
80
4
12
50
150
250
750
ISI (ms)
D
HRT (ms)
120
110
100
90
80
70
60
4
12
50
150
250
750
ISI (ms)