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Preparation of Anticipatory Postural Adjustments Prior to Stepping

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MacKinnon CD, Bissig D, Chiusano J, Miller E, Rudnick L, Jager C, Zhang Y, Mille M-L, Rogers MW. Preparation of anticipatory postural adjustments prior to stepping. *J Neurophysiol* 97: 4368–4379, 2007. First published April 25, 2007; doi:10.1152/jn.01136.2006. Step initiation involves anticipatory postural adjustments (APAs) that propel the body mass forward and laterally before the first step. This study used a startle-like acoustic stimulus (SAS) and transcranial magnetic stimulation (TMS) to examine the preparation of APAs before forward stepping. After an instructed delay period, subjects initiated forward steps in reaction to a visual "go" cue. TMS or SAS was delivered before (−1,400 or −100 ms), on (0 ms), or after (+100 ms for TMS, +200 ms for SAS) the imperative "go" cue. Ground reaction forces and electromyographic activity were recorded. In control trials, the mean reaction time was 217 ± 38 ms. In contrast, the SAS evoked APAs that had an average onset of 110 ± 54 ms, whereas the incidence, magnitude, and duration of the APA increased as the stimulus timing approached the "go" cue. A facilitation of motor-evoked potentials in the initial agonist muscle was observed only when TMS was applied at +100 ms. These findings indicate that there was an initial phase of movement preparation during which the APA-stepping sequence was progressively assembled, and that this early preparation did not involve the corticomotor pathways activated by TMS. The subsequent increase in corticomotor excitability between the imperative stimulus and onset of the APA suggests that corticospinal pathways contribute to the voluntary initiation of the prepared APA-stepping sequence. These findings are consistent with a feedforward mode of neural control whereby the motor sequence, including the associated postural adjustments, is prepared before voluntary movement.

INTRODUCTION

The execution of purposeful movement typically requires that postural components are effectively coordinated with the intended action. Anticipatory postural adjustments (APAs), constituting a general form of postural accompaniment, act to stabilize posture and equilibrium before the initiation of a voluntary movement (Massion 1992). A well-studied example of APAs occurs during the transition from stationary standing to stepping forward to initiate gait (Brunt et al. 1991; Carlsoo 1966; Crenna and Frigo 1991; Jian et al. 1993; Mann et al. 1979; Rogers et al. 2001). These APAs involve a sequence of muscle activations and changes in the ground reaction forces (GRFs) that move the net center of pressure (CoP) beneath the feet backward and toward the initial swing limb. This sequence of activity produces the forces and moments necessary to propel the body forward and toward the single-stance limb for the regulation of whole body balance and posture before and during initiation of the voluntary step.

A variety of studies in cats and humans have investigated the neural mechanisms contributing to the generation of the APAs that precede and accompany voluntary movement (e.g., Brown and Frank 1987; de Wolf et al. 1998; Massion 1992; Schepens and Drew 2003, 2004). One perspective is that the putative regions involved in the control of posture and the intended movement are organized separately (Brown and Frank 1987; Massion 1992; Schepens and Drew 2003, 2004). Based on the findings of lesion studies, Massion (1992) proposed that the neural networks responsible for the control of APAs are organized subcortically. This idea is supported by studies in quadrupeds showing that the pontomedullary reticular formation is involved in the control of posture through brain stem–spinal pathways that may be engaged through motor corticofugal connections (Drew et al. 2004; Kably and Drew 1998; Matsuyama et al. 2004; Prentice and Drew 2001; Schepens and Drew 2003, 2004). Thus the motor cortical command for a goal-directed movement might include feedforward signals for generating the postural adjustments that anticipate and accompany movements (Drew et al. 2004; Massion 1992; Massion et al. 1999, 2004). In this manner, the timing of the postural responses is considered to be inextricably linked to the cortical command for the voluntary initiation of movement. However, the fact that the onset timing of APAs and the accompanying EMG activity can vary independently from the onset of the volitional movement suggests that these components of movement may be controlled, and possibly planned, separately (Brown and Frank 1987; Schepens and Drew 2003).

To date, studies in humans of the organization and planning of APAs have been restricted to the examination of changes in the timing of onset and magnitude of APAs after an imposed perturbation or an imperative cue to initiate a step. Yet, there is evidence that APAs may be planned in advance of the volitional cue to initiate movement. Valls-Solé et al. (1999) demonstrated that the APA sequence could be released with reaction times considerably shorter than a normal simple voluntary reaction when the imperative cue to initiate the movement was replaced by a loud acoustic stimulus sufficient in intensity to evoke a startle reaction. Most important, the rapid release of movement sequences by an acoustic startle occurs only when the task is known in advance, suggesting that the acoustic startle stimulus releases a planned movement (Carlsen et al. 2004a). The pathways contributing to the early release of the movement sequence by a loud acoustic stimulus are currently unknown, but it has been proposed that the prepared motor program is released from the same reticular structures that mediate the startle reflex (Valls-Solé et al. 1999).

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Irrespective of the pathways that mediate the release of movement sequences by a loud acoustic stimulus, the startle technique provides a probe to examine the planning of intended actions and possibly the APAs that precede or accompany these actions. Accordingly, we used a loud acoustic stimulus to probe the state of preparation of APAs at multiple time points before, on, and after the onset of the imperative stimulus to initiate stepping. We hypothesized that the presentation of the acoustic stimulus before or at the time of the imperative stimulus to initiate stepping would result in the early release of the APA-stepping sequence. In addition, we applied transcranial magnetic stimulation (TMS) to probe the state of excitability of corticomotor projections to muscles involved in the APA. If the preparation and release of APAs are time-locked to the motor cortical command to initiate movement (Massion et al. 2004) then changes in the excitability of pathways involving APA muscles should be closely linked to changes in corticomotor excitability mediating the goal-intended movement. Accordingly, we also hypothesized that the excitability of corticomotor projections to the target muscles that initiate APAs would increase before the imperative stimulus.

METHODS

Subjects

Ten subjects ranging in age from 20 to 40 yr (seven female, three male) participated in these experiments. All subjects were neurologically healthy, had no musculoskeletal disorders affecting the lower limbs, and were right-leg dominant by self-report. Written informed consent was obtained before inclusion in the study. The procedures were approved by the Institutional Review Board at Northwestern University.

Transcranial magnetic stimulation (TMS)

The excitability of corticomotor projections from the contralateral primary motor cortex to muscles of the stepping leg was investigated using single-pulse TMS. Stimulation was delivered using a Magstim 200 stimulator (Magstim, Whitland, Carmarthenshire, UK) with a figure-eight coil (7 cm outer diameter of wings). The coil was positioned over the midline about 2 cm posterior of the vertex of the scalp and oriented with the handle of the coil directed posteriorly. This position and orientation were adjusted to find the optimal site for eliciting motor-evoked potentials (MEPs) in the TA muscle. The optimal site was established using a stimulus intensity sufficient to evoke the MEP at rest and finding the scalp surface site that produced the largest MEP and shortest latency response at that intensity. The optimal site and coil orientation for stimulation was marked on the scalp with a wax marker and the coil was held in place by an experimenter who gently pushed downward on the coil to maintain scalp contact but not impede step initiation. Stimulation intensity was set to 120% of resting motor threshold (RMT), defined as the intensity required to evoke a MEP of >50 μV in five of ten trials in the relaxed right TA muscle while sitting.

Startle-like acoustic stimulus (SAS)

A computer-generated analog tone (1 kHz, 40 ms) was used to create the acoustic stimulus. The tone was amplified and presented by loudspeaker placed 15 cm behind the head of the subject. The peak intensity of the tone near the subject's ears was approximately 115 dB (tested using a Brüel & Kjær Impulse Precision Sound Level Meter type 2204). This intensity was sufficient to reliably cause an early release of the planned movement but did not reliably evoke a startle response in the neck muscles (see RESULTS). For this reason, we used the term "startle-like acoustic stimulus" (SAS) to describe the loud acoustic tone.

Experimental design and task

Subjects stood on two separate force platforms with their feet placed a natural and comfortable distance apart. An outline of each foot was drawn on the floor to ensure foot placement was the same across trials. A visually cued delayed-response paradigm was used to examine the transition from a stationary standing posture to the rapid initiation of forward stepping. Task instruction stimuli were presented to the subject using a horizontal bank of LED lights placed at eye level 3 m in front of the subject (Fig. 1*A*). A precue light was presented for 100 ms that provided one of three instructions: right-light precue = get ready to step forward with your right leg; left-light precue = get ready to step forward with your left leg; center-light precue = no stepping leg instruction provided. The imperative "go" cue light was presented 3.5 s later using the same bank of lights and consisted of the illumination of the either the right or left light for 100 ms. Right- or left-step precue instruction was always paired with the corresponding "go" cue. Thus the right- or left-precue lights allowed subjects to prepare in advance to step with a specific leg, whereas the center light acted only as a warning stimulus for a forthcoming choice reaction time task. Subjects were instructed to initiate walking "as quickly as possible" in response to the "go" cue but not to initiate the step before the cue. Subjects took a minimum of two steps forward in response to the "go" cue. Data were inspected on-line for each trial to ensure that the subject did not begin to lean forward after presentation of the precue. Trials in which subjects leaned forward or stepped with the incorrect leg were discarded and repeated.

Subjects performed a minimum of 272 trials of step initiation (Fig. 1*B*). In 104 (38%) of the stepping trials no SAS or TMS stimulus was applied (control step trials). In 104 (38%) of the stepping trials TMS was applied at one of four time points: $-1{,}400$, -100, 0, or $+100$ ms relative to the "go" cue (Fig. 1*A*). The TMS stimulus applied 100 ms after the "go" cue was designed to test for changes in corticospinal excitability in the approximately 10- to 150-ms interval immediately

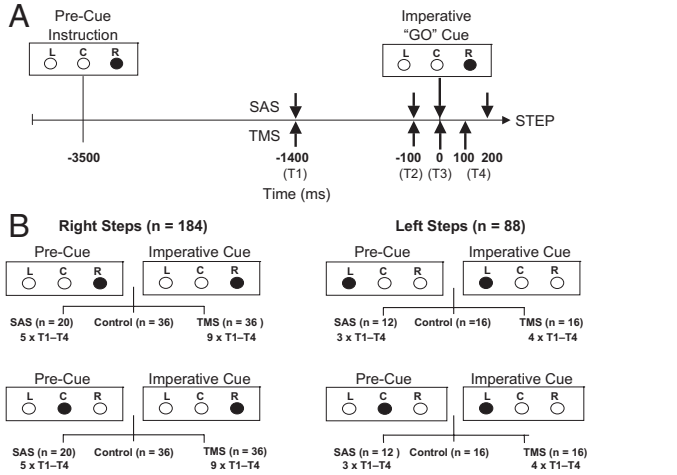

FIG. 1. *A*: schematic diagram of the timing of the precue and imperative cue lights and the delivery of either the acoustic stimulus ($\downarrow$) or TMS ($\uparrow$). The precue consisted of a left (L, left step), center (C, no instruction), or right (R, right step) light presented for 100 ms. The imperative "go" cue light was presented 3.5 s after the precue and consisted of either a left or right light shown for 100 ms. The startle tone was presented either 1,400 (T1), 100 (T2), or 0 ms (T3) before the "go" tone or 200 ms (T4) after the "go" tone. TMS was delivered either 1,400 (T1), 100 (T2), or 0 ms (T3) before the "go" tone or 100 ms (T4) after the "go" tone. *B*: summary diagram of the different experimental conditions and number of trials.

before the onset of the TA EMG burst that initiates the APA (Chen et al. 1998; MacKinnon and Rothwell 2000). In 64 (24%) of the stepping trials a SAS was applied at −1,400, −100, 0, or +200 ms relative to the "go" cue. The SAS was applied 200 ms after the "go" cue (rather than at 100 ms) to approximately coincide with the onset of the APA preceding the voluntary step. In this manner, we could observe whether EMG activity or ground reaction forces evoked by the SAS interfered with, or augmented, the APA preceding the voluntary step. The number of trials with a SAS was kept at 24% of all trials to ensure that subjects did not habituate to the stimulus (Carlsen et al. 2003a; Siegmund et al. 2001). In addition, control trials were also collected in which either TMS (9 trials) or a SAS (5 trials) was delivered without the presentation of a precue instruction or imperative cue. These served as control TMS and control SAS trials.

The order of presentation of control, TMS, and startle trials was partly randomized with the exception that a startle trial was not presented during the first five trials and no more than two startle trials were presented in a row. We planned to restrict our analysis to the assessment of APAs preceding right steps only. For this reason, the number of trials with left steps was reduced to ensure that subjects did not fatigue over the duration of the experiment. This design resulted in a minimum of nine trials of TMS and five acoustic startle trials at each time point for the right-step conditions (see Fig. 1*B*).

Data collection

Ground reaction forces (GRFs) and moments were collected from two force platforms (AMTI, Watertown, MA) placed beneath the right and left feet. The center of pressure (CoP) beneath each foot and the net CoP response were derived from the forces and moments. GRFs were collected at 100 Hz.

Bipolar surface electromyogram (EMG) signals were recorded over the motor points of the right tibialis anterior (TA), soleus (SOL), and sternocleidomastoid (SCM) muscles. EMG signals were preamplified at the skin surface (gain = 35) then further amplified and filtered (20–250 Hz) and sampled at 500 Hz using a National Instruments data acquisition board and custom data collection software (LabView 6.0, National Instruments, Austin, TX). All data were collected from 4 s before to 4 s after the presentation of the "go" cue.

Data analysis

Data analysis was performed using customized software written in MatLab 6.0 (The MathWorks, Natick, MA). For right steps, an APA was considered to be present if the following components of the pattern were recorded (Brunt et al. 1991; Rogers et al. 2001): *1*) an early EMG burst in TA before the onset of the voluntary step, *2*) an initial increase in the right foot vertical GRF, *3*) an initial decrease in the left foot vertical GRF, *4*) an initial rightward shift in the CoP, and *5*) an initial posterior shift in the CoP (Fig. 2). For left steps, the vertical GRF and right-left CoP shifts were opposite to those for right steps. Note that because EMG was recorded only on the right side, EMG for right steps corresponded to the activity of the initial stepping leg and EMG for left steps corresponded to the activity of the initial stance leg. We only analyzed stepping trials that had all five components of an APA. In most subjects a clear suppression of SOL activity was observed during control trial APAs, but, because this was not a consistent feature in all subjects, a suppression of SOL activity was not considered to be a requirement for the identification of an APA. The following parameters were measured for each accepted right-step trial: onset and offset times of EMG activity in TA and SCM relative to the "go" or acoustic startle stimuli, onset of the posterior and rightward excursion of the CoP (leftward for left steps), onset of the increase in the right vertical GRF and decrease in left vertical GRF (opposite for left steps), average magnitude of the rectified EMG signal during the TA and SCM bursts, peak posterior and rightward displacements (leftward for left steps) of the CoP, and the peak increase and decrease in the right and left GRFs, respectively. Onset times of EMG, CoP, and GRF changes were calculated based on changes of >3 SDs from the mean signal recorded before the "go" or acoustic startle stimuli and were verified by visual inspection.

Motor-evoked potentials (MEPs) elicited by TMS were measured in the right TA and SOL muscles. The peak-to-peak amplitude of the MEP was measured for each trial then averaged across trials for each stimulation time point. The amplitude of the MEPs evoked during stepping trials was normalized to the average amplitude to the MEPs evoked during quiet standing control TMS trials. In addition, the peak-to-peak amplitude of the EMG signal over the 50-ms time interval immediately before application of the TMS pulse was measured for each trial. The amplitude of this signal was used as an estimate of the amount of the EMG activity present before TMS.

Statistical analyses

Data were analyzed using a repeated-measures ANOVA to examine the effects of instruction (center-right vs. right-right) and timing of stimuli. Repeated contrasts were used to test for a priori comparisons between control trials and the stimulus timing conditions. Post hoc comparisons of interaction effects were tested using the Student's *t*-test with Bonferroni correction for multiple comparisons. Differences between conditions were considered to be significant at the $P < 0.05$ level.

RESULTS

Effects of SAS on right-step APAs

Examples of the APAs observed in an individual during right-step control trials and trials with a SAS applied at different time intervals are shown in Fig. 3. Control trials (Fig. 3*A*) were characterized by an initial burst in TA beginning about 220 ms after the "go" cue, followed by directionally

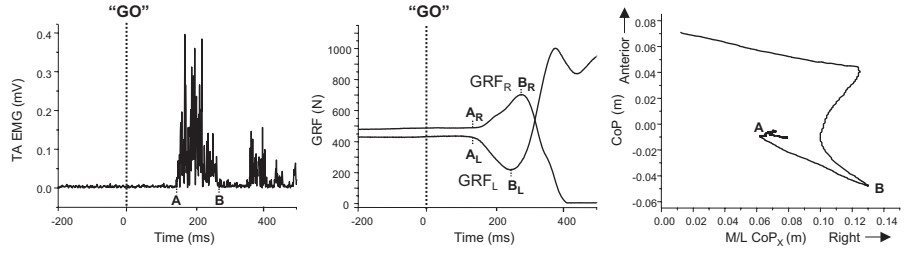

FIG. 2. Examples of the anticipatory postural adjustment (APA) parameters measured during right-step initiation. Onset, duration, and magnitude of the tibialis anterior (TA) electromyogram (EMG) burst was quantified from the start (*A*) to the end (*B*) of the initial burst. Onset, time-to-peak change, and magnitude of change in the right and left vertical ground reaction force (GRF) were measured from the start to the peak of the loading (A_R to B_R) and unloading (A_L to B_L) of the right and left legs, respectively. Onset, time-to-peak change, and magnitude of change in the medial/lateral (M/L) and anterior/posterior (A/P) net center of pressure (CoP) were measured from the start (*A*) to the peak (*B*) of the posterior and rightward excursions of the CoP.

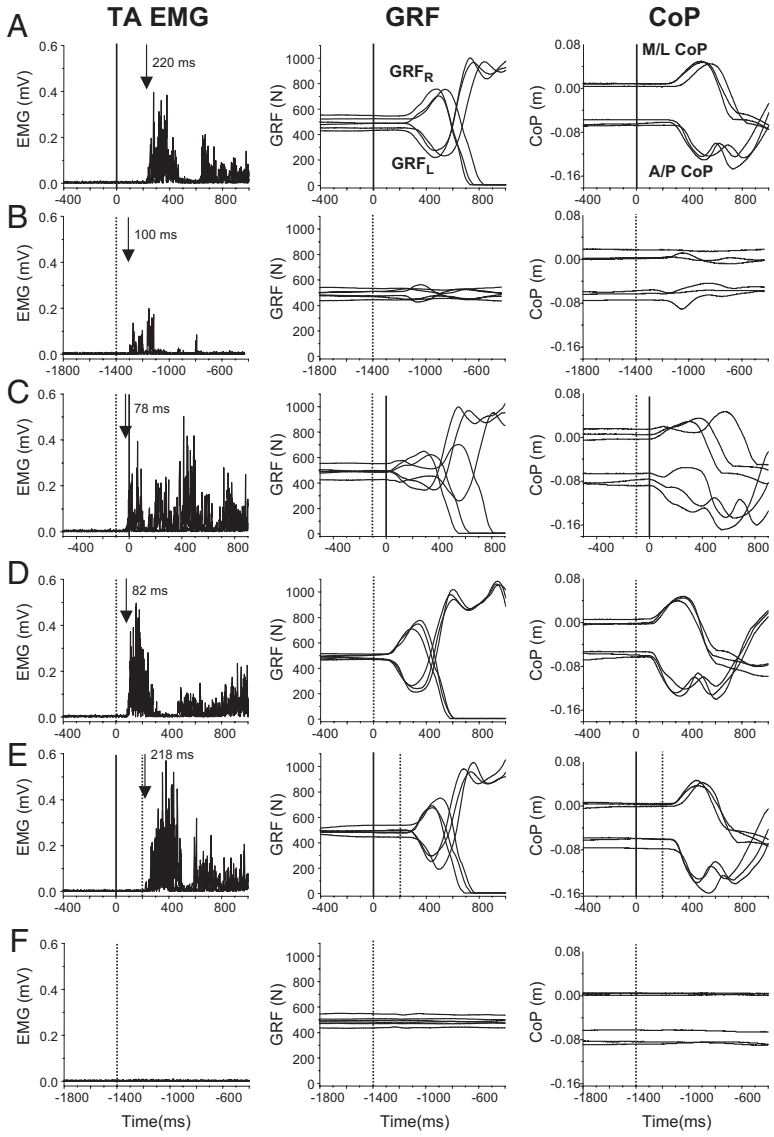

FIG. 3. Examples of APAs across conditions in an individual subject for right-step initiation. Three trials are superimposed on each plot. *Left column*: rectified EMG recorded in TA. *Center column*: right (GRF_R) and left leg (GRF_L) vertical ground reaction forces (GRFs). *Right column*: A/P (posterior is negative) and medial/lateral (M/L) (right is positive) net center of pressure (CoP) excursions. *A–E*: a right-right precue go-cue combination. *A*: control step trials. Note that the onset of the TA burst occurred at about 220 ms (down arrow) after the "go" cue (solid vertical line). *B–D*: APAs during trials when a startle-like acoustic stimulus (SAS) (dotted vertical line) was presented 1,400 (*B*), 100 (*C*), and 0 ms before the "go" cue (*D*). Note that the TA activity was initiated between 78 and 100 ms after the SAS and that the incidence, duration, and magnitude of the APAs increased as the stimulus timing approached step initiation. *E*: APAs during trials when the SAS was applied 200 ms after the "go" cue. *F*: example of activity produced when SAS was applied during normal quiet standing (control startle trial).

appropriate changes in the right and left GRF and CoP. APAs that included all components of the movement sequence were present in 98% of the control step trials across subjects. Presentation of a SAS during stepping trials was associated with the early release of the APA sequence (Fig. 3, *B–D*). When the SAS was delivered before or simultaneously with the "go" cue, onset of the TA burst occurred approximately 80 ms after the tone. Stimulation at −1,400 ms was associated with a small-amplitude EMG burst in TA and directionally appropriate changes in the GRFs and CoP, but no step was induced (Fig. 3*B*). As the timing of the SAS approached the "go" cue, the amplitude and duration of the TA activity, CoP excursions, and GRFs increased and a forward step was taken (Fig. 3, *C* and *D*). When the SAS was delivered 200 ms after the "go" cue, the stimulus timing coincided closely with the reaction time associated with control trials; thus no early release of the APA could be observed (compare Fig. 3, *A* and *E*). However, presentation of the SAS at +200 ms resulted in average increases in the magnitude of the initial TA burst, GRFs, and CoP excursions of 32, 10, and 5%, respectively, relative to control trials, and a reduction in the duration of these APA components by 26, 23, and 16%, respectively. During quiet standing (between stepping trials), the SAS did not induce any consistent sequence of muscle activation patterns in the postural muscles or ground reaction forces (Fig. 3*F*). Also, this subject did not show a consistent short-latency burst in the SCM muscle in response to the SAS (present in <20% of trials).

Similar findings were obtained in all subjects. A summary of the statistical analyses across the dependent variables is shown in Table 1. The incidence of short-latency APAs after the SAS varied depending on the timing of stimulation and precue instruction condition. Short-latency APA trials were defined as trials with a TA burst onset of <3 SDs from the mean control step reaction time. When a right-step precue was paired with a right-step "go" signal, short-latency right-step APAs were identified in 40, 64, and 88% of trials when the SAS was applied at −1,400, −100, and 0 ms, respectively. When a center-light precue was paired with a right-step "go" cue, short-latency right-step APAs were identified in 37, 60, and 90% of trials when the SAS was applied at −1,400, −100, and 0 ms, respectively.

TABLE 1. *Summary of the statistical analysis of the APA data for right steps*

Dependent Variable	Main Effects		SAS Timing × Instruction Interactions
	SAS Timing	Instruction	
TA Onset, ms	$F_{(4)} = 32.5$, $P < 0.001$	NS	NS
TA Duration, ms	$F_{(4)} = 21.3$, $P < 0.001$	NS	$F_{(1,4)} = 3.3$, $P = 0.030$
TA Burst Magnitude, mV	$F_{(4)} = 13.0$, $P < 0.001$	NS	NS
Posterior CoP, Excursion Onset, ms	$F_{(4)} = 115.4$, $P < 0.001$	NS	NS
Posterior CoP, Time-to-Peak Excursion, ms	$F_{(4)} = 52.8$, $P < 0.001$	NS	NS
Posterior CoP, Peak Excursion, cm	$F_{(4)} = 82.1$, $P < 0.001$	NS	NS
Rightward CoP Excursion Onset, ms	$F_{(4)} = 54.2$, $P < 0.001$	NS	NS
Rightward CoP Time-to-Peak Excursion, ms	$F_{(4)} = 5.5$, $P = 0.001$	NS	NS
Rightward CoP Peak Excursion, cm	$F_{(4)} = 35.9$, $P < 0.001$	NS	NS
Right GRF, Onset, ms	$F_{(4)} = 18.7$, $P < 0.001$	$F_{(1)} = 10.5$, $P = 0.010$	NS
Right GRF, Time-to-Peak Force, ms	$F_{(4)} = 18.2$, $P < 0.001$	NS	NS
Right GRF, Peak Force, %BW	$F_{(4)} = 16.9$, $P = 0.001$	NS	NS
Left GRF, Onset, ms	$F_{(4)} = 15.5$, $P < 0.001$	$F_{(1)} = 5.3$, $P = 0.047$	NS
Left GRF, Time-to-Peak Force, ms	$F_{(4)} = 15.1$, $P < 0.001$	NS	NS
Left GRF, Peak Force, %BW	$F_{(4)} = 57.2$, $P < 0.001$	NS	NS
Timing of onset of right toe-off, ms	$F_{(4)} = 1{,}365.9$, $P < 0.001$	NS	NS
Duration of first step, ms	NS	NS	NS

TA, tibialis anterior muscle; CoP, center of pressure; GRF, ground reaction force; SAS, startle-like acoustic stimulus; %BW, percentage of body weight; NS, not significant at the $P < 0.05$ level.

During right-step trials the average onset time of the TA burst (reaction time) was reduced by an average of 105 ms relative to control trials when the SAS was applied at −1,400, −100, and 0 ms (Table 2 and Fig. 4*A*). Comparable results were obtained for trials with right-right and center-right cue combinations. Note that these average onset latencies reflect the latencies of all stepping trials irrespective of whether the APA was initiated at short- or long latency. Thus the higher averages and variance for the trials with stimulation at −1,400 and −100 ms reflect the fact that the incidence of short-latency reaction times was lower for these conditions than for trials with stimulation at 0 ms. The average onset of the TA burst when the SAS was delivered 200 ms after the "go" cue was −17 ± 28 ms. This mirrors the fact that the voluntary reaction to the "go" cue typically coincided closely with the onset of the SAS. For this reason, APA onset timings for the trials with a SAS at +200 ms were analyzed relative to the onset of the "go" cue rather than the timing of the SAS. Analysis across conditions showed that the timing of the SAS had a significant main effect on the timing of onset of the TA burst. A priori contrasts showed that TA EMG onset times occurred earlier than control trials when the SAS was presented at −1,400, −100, and 0 ms ($P < 0.002$). The reaction times for trials with a right-step precue were shorter than trials with a center-light precue by an average of 12 ms, but this difference was just outside our significance cutoff ($P = 0.051$). There was no significant instruction × time interaction effect.

The timing of the SAS also had a significant main effect on the duration and magnitude of the TA burst in the initial step leg (see Table 1 and Fig. 4). The duration of the TA burst was significantly shorter than that of control trials when SAS was applied ($P < 0.016$). Similarly, the magnitude of the TA burst was significantly affected by the timing of the SAS and increased as stimulation timing approached step initiation (Fig. 4*B*). The magnitude of the TA burst when the SAS was applied at −1,400 ms was significantly lower than the burst for control step trials and trials with SAS at −100, 0, and +200 ms ($P < 0.005$). When stimulation was applied at −100 ms, the TA burst was significantly reduced relative to trials with stimulation at 0 and +200 ms ($P < 0.021$). When SAS was applied at +200 ms the TA burst tended to be larger than that of control trials (23% on average) (see Fig. 3*D*), but this difference did

TABLE 2. *Summary of the latencies of the dependent variables for right steps*

	Right-Right					Center-Right				
		SAS					SAS			
Dependent Variable	Control*	−1,400†	−100†	0†	+200*	Control*	−1,400†	−100†	0†	+200*
TA Onset, ms	214 ± 41	111 ± 25	105 ± 50	91 ± 14	221 ± 31	220 ± 36	149 ± 117	114 ± 50	100 ± 17	217 ± 29
Right GRF, Onset, ms	238 ± 45	148 ± 65	160 ± 75	129 ± 40	250 ± 40	260 ± 35	163 ± 76	196 ± 67	151 ± 27	249 ± 30
Left GRF, Onset, ms	249 ± 44	167 ± 64	176 ± 75	142 ± 36	258 ± 35	261 ± 35	159 ± 85	200 ± 72	162 ± 26	272 ± 49
Posterior CoP, Excursion Onset, ms	226 ± 42	108 ± 25	117 ± 38	106 ± 15	230 ± 33	234 ± 30	115 ± 22	113 ± 13	116 ± 17	230 ± 39
Rightward CoP Excursion Onset, ms	253 ± 47	134 ± 53	161 ± 74	129 ± 30	247 ± 39	274 ± 29	151 ± 77	178 ± 31	166 ± 25	250 ± 32
Timing of Onset of Right Toe-off, ms	679 ± 24	2,035 ± 13	703 ± 42	585 ± 33	636 ± 28	713 ± 23	2,035 ± 19	740 ± 28	600 ± 26	645 ± 25

Values are means ± 1 SD. *Timing expressed relative to imperative "go" cue. †Timing expressed relative to SAS. Right-Right, right-step instruction followed by a right-step imperative "go" cue; Center-Right, center-light instruction (no precue of stepping leg) followed by a right-step imperative "go" cue; SAS, startle-like acoustic stimulus; TA, tibialis anterior, GRF, ground reaction force; CoP, center of pressure.

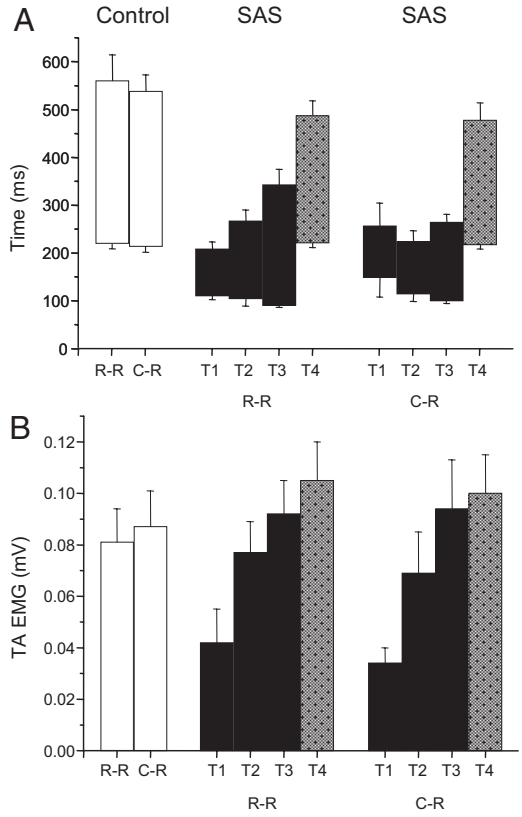

FIG. 4. Changes in the timing and magnitude of the right TA EMG burst for right steps. *A*, *top*: average timing (+1 SE) and duration of the onset and offset of the TA burst across conditions. A startle-like acoustic stimulus (SAS) was delivered at −1,400 (T1), −100 (T2), 0 (T3), and +200 ms (T4) relative to the "go" cue. Timing for the control trials (white bars) and trials with SAS applied at +200 ms (gray speckled bars) are plotted relative to the "go" cue. Trials when the SAS was delivered at −1,400, −100, and 0 ms are plotted relative to the timing of the SAS (black bars). TA burst was initiated an average of 107 ms earlier when the SAS was presented before or on the "go" cue. As the SAS approached step initiation, the duration of the burst approached the duration of the control step trials. *B*, *bottom*: average magnitude (+1 SE) of the TA EMG burst across conditions. Note the increasing magnitude of the burst as the SAS timing approached step initiation. R-R, right-step precue, right-step "go" cue; C-R, center-light precue, right-step "go" cue.

not reach significance ($P = 0.106$). There was no significant main effect of instruction or an instruction × time interaction effect on the TA burst duration or magnitude.

Similar findings were obtained for the APA kinetic variables (Fig. 5). There was a significant main effect of stimulation timing on the timing of onset of both the posterior and rightward excursions of the CoP (see Table 1 and Fig. 5, *A* and *B*). CoP excursions in both directions began significantly earlier than in control step trials when the SAS was applied at −1,400, −100, and 0 ms ($P < 0.009$). The time to the peak posterior and rightward excursions was significantly reduced relative to that of control trials for all SAS timing conditions, including stimulation at +200 ms ($P < 0.023$). The magnitude of the CoP excursion was also significantly decreased when stimulation was applied before or on the "go" cue ($P < 0.01$). The magnitude of the medial/lateral (M/L) CoP excursion tended to be larger than that of control trials (16% on average) when SAS was applied at +200 ms, but this difference did not reach significance ($P > 0.088$). There was no significant effect of instruction or instruction × time interaction on the CoP.

The timing of the SAS also had significant main effects on the onset timing, duration and magnitude of vertical GRFs (see Table 1 and Fig. 5, *C* and *D*). The onset of the increase in the right-leg GRF and decrease in left-leg GRF was significantly shorter when the stimulus was delivered before or on the "go" tone. The time-to-peak change in GRF was significantly shorter than that of control step trials for all SAS timing conditions, including stimulation at +200 ms ($P < 0.002$). In a similar manner, the peak magnitude of the right GRF increased and the left GRF decreased as the stimulus timing approached step initiation. Peak changes in the right and left GRFs for trials with stimulation at −1,400 and −100 ms were significantly different from those of the control step trials. In

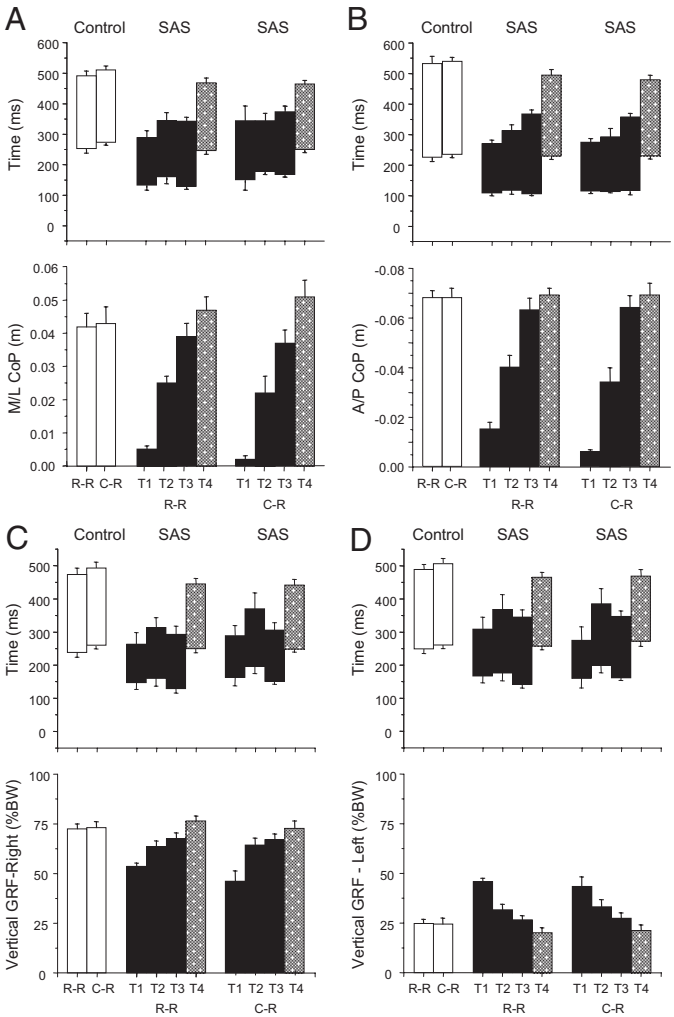

FIG. 5. *A* and *B*: changes in the timing and magnitude of the net center of pressure (CoP) excursion. Format of this figure is the same as that used for Fig. 4. *A*, *top plot*: average (+1 SE) onset timing and time to peak of the M/L CoP. *Bottom plot*: average (+1 SE) magnitude of the rightward CoP excursion. *B*, *top plot*: average (+1 SE) onset timing and time to peak of the A/P CoP. *Bottom plot*: average (+1 SE) magnitude of the posterior CoP excursion. *C* and *D*: changes in the timing and magnitude of the vertical GRFs. *C*, *top plot*: average (+1 SE) onset timing and time to peak for the increase in the right GRF. *Bottom plot*: average (+1 SE) magnitude of the peak increase in the right GRF. *D*, *top plot*: average (+1 SE) onset timing and time to peak for the decrease in the left GRF. *Bottom plot*: average (+1 SE) magnitude of the peak decrease in the left GRF. R-R, right-step precue, right-step "go" cue; C-R, center-light precue, right-step "go" cue. T1, SAS at −1,400 ms; T2, SAS at −100 ms; T3, SAS at 0 ms; T4, SAS at +200 ms.

addition, the magnitude of the left GRF was significantly reduced relative to that of control trials ($P = 0.034$) by an average of 16% when the SAS was applied at +200 ms. There was also a significant main effect of instruction on both the right GRF and the left GRF onset times. GRF onset times were shorter with a right-right cue combination than a center-right combination by an average of 22 and 16 ms for the right and left GRFs, respectively.

The timing of presentation of the SAS had a significant main effect on the timing of toe-off of the stepping leg [right leg for right-step precue, right-step "go" cue (R-R) and center-light precue, right-step "go" cue (C-R) trials], irrespective of precue instruction (Table 2). When the SAS was presented at −1,400 ms, the small APA that was evoked (see Fig. 3*B*) was rarely followed by a step before the "go" signal. Instead, subjects normally terminated this initial APA early and then initiated a new APA sequence in response to the "go" signal. This was reflected in a mean toe-off time of 642 ± 41 ms relative to the "go" signal. In contrast, stimulation at −100 ms evoked a short-latency APA that was immediately followed by a step such that the timing of toe-off relative to the SAS was not significantly different from that of control trials. Stimulation at 0 or +200 ms was associated with a significantly earlier toe-off time relative to that of control trials [$F_{(1)} > 13.6$, $P < 0.006$]. The time taken to complete the first step (right toe-off time to left toe-off time) was not significantly affected by the SAS.

Effects of SAS before left steps

Figure 6*A* (*left plots*) shows an example from a single subject of the average GRF and CoP profiles observed when a left-step or center-light precue was followed by a SAS applied 100 or 0 ms before a left-step "go" cue. For trials with a left-step precue and left-step "go" cue condition, the SAS evoked a pattern of GRFs and CoP shifts consistent with a left-step APA. Short-latency left-step APAs were identified in 48, 74, and 96% of trials when the SAS was applied at −1,400, −100, and 0 ms, respectively, after a left-step precue. These SAS-evoked left APAs were associated with significantly earlier activation of the right TA muscle (initial stance leg for left steps) [$F_{(4)} = 10.1$, $P < 0.001$] relative to control trials when stimulation was applied at −1,400, −100, or 0 ms ($P < 0.003$) (Fig. 6*B*). The duration and magnitude of the TA burst were significantly attenuated with stimulation at −1,400 ms ($P < 0.016$) and gradually increased as the stimulation timing approached the "go" signal. These findings were comparable to those observed with a right-step precue and right-step "go" cue.

SAS applied in the interval between a center-light precue and a left-step "go" cue usually evoked an initial pattern consistent with a right APA (Fig. 6*A*, *right plots*). This initial pattern was rapidly terminated after the presentation of the left-step "go" cue and followed by an appropriate reversal to a left APA and step. Note that the initiation of an inappropriate right APA resulted in a marked delay in unloading of the left leg and initiation of the left step. As a result, the left toe-off time was significantly delayed [$F_{(1,4)} = 14.3$, $P < 0.005$] for the center-light precue condition relative to the left-step precue condition (Fig. 6*C*). A priori contrasts showed that toe-off occurred significantly later for the center-light precue trials relative to left-step precue trials when SAS was applied at −1,400, −100, and 0 ms ($P < 0.05$). The SAS evoked short-latency right APAs in 33, 61, and 44% of trials when a center-light precue was followed by stimulation applied 1,400,

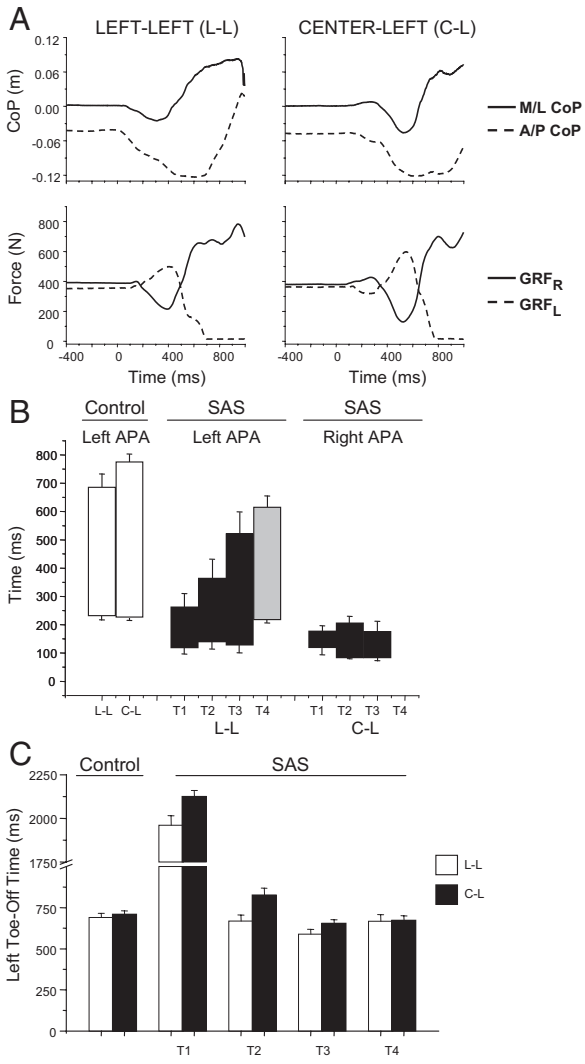

FIG. 6. Comparison of response characteristics for trials with left-left (L-L) and center-left (C-L) precue go-cue instruction combinations in a representative subject. *A*: examples of CoP (*top plots*) and vertical GRFs (*bottom plots*) observed with L-L (*left plots*) or C-L (*right plots*) instruction combinations. Trials with a SAS presented at either −100 or 0 ms have been temporally aligned to the SAS and averaged across all trials. Presentation of SAS during trials with the L-L instruction combination was associated with a pattern of CoP and GRF changes consistent with a left-step APA. In contrast, SAS trials with the C-L instruction combination were often associated with an initial kinetic pattern consistent with a right-step APA that was quickly reversed to a left-step APA. *B*: average timing (+1 SE) and duration of the onset and offset of the right TA burst across conditions. Format is the same as that in Fig. 4, with the exception that stimulation during the L-L condition evoked a left APA, whereas stimulation in the C-L condition normally evoked a right APA. Note that, for left APAs, right TA activity corresponded to activity of the initial stance leg and for right APAs corresponded to activity in the stepping leg. TA burst was initiated an average of 114 ms earlier when the SAS was presented before or on the "go" cue, but TA activation for right APAs was rapidly terminated. No data are shown at T4 in the C-L condition because a right APA was never observed. *C*: average (+1 SE) time of left toe-off for the L-L and C-L conditions. Toe-off timing is expressed relative to the imperative "go" cue for the control conditions and for SAS at T4 (+200 ms), and relative to the SAS for the T1 (−1,400 ms), T2 (−100 ms), and T3 (0 ms) timing conditions. Toe-off was significantly delayed for the C-L relative to the L-L condition when the SAS was applied at −1,400, −100, and 0 ms ($P < 0.05$).

100, and 0 ms before a left step "go" cue, respectively. The 44% incidence of right APAs with SAS at 0 ms contrasts with the 88 and 90% incidence of right APAs when a right-step or center-light precue preceded a right-step "go" cue, suggesting that inappropriate responses could sometimes be suppressed when the SAS was presented at the same time as the imperative stimulus. Short-latency left APAs were very rare when stimulation was applied after a center precue light (only four trials across all subjects). The burst of TA EMG activity associated with these initial right-step APAs occurred significantly earlier than that in control trials [$F_{(4)} = 35.994$, $P < 0.001$], although the duration of the burst was markedly shortened, particularly with SAS at −100 and 0 ms (Fig. 6*B*). This shortened duration of the stance leg TA activity reflects an early termination of the right-step APA because this initial pattern was inappropriate for a left forward step.

Activation of SCM during stepping

Control stepping trials were accompanied by a burst of EMG activity in the SCM muscle that began after the onset of TA activity (average SCM burst onset latency values were 311 ± 98 and 332 ± 85 ms for the right-right and center-right conditions, respectively). The average duration of the burst was 375 ± 61 ms. SAS applied during stepping trials resulted in an earlier average onset of the SCM burst (213 ± 149 and 236 ± 153 ms for the right-right and center-right conditions). The large variance in the onset time was the result of a bimodal distribution of onset latencies. Three subjects showed short-latency responses in SCM (<60 ms) in over half of the SAS trials. Latencies of these responses were similar to latencies observed during control startle trials (50 ± 10 ms) in the same subjects. Short-latency SCM responses were rare during both control startle and stepping trials in the other subjects.

Transcranial magnetic stimulation

In contrast to the startle-evoked APAs, changes in the amplitude of MEPs evoked in TA by TMS did not occur until after the "go" cue (Fig. 7). An example of the average MEPs evoked in TA and SOL for each TMS timing condition in a single subject is shown in Fig. 7*A*. In the TA muscle, the magnitude of the MEP was not different from that in control trials (TMS alone, no stepping) when TMS was applied at −1,400, −100, and 0 ms. A marked increase in the TA MEP was observed when TMS was applied at +100 ms. In contrast, there was no change in the SOL MEP amplitude relative to that in control trials at any stimulation time point. A significant main effect of stimulus timing was obtained for the amplitude of the TA MEP [$F_{(1,4)} = 13.8$, $P < 0.001$]. Post hoc analysis showed that the MEPs evoked by TMS applied 100 ms after the "go" cue were significantly larger ($P < 0.007$) than the MEPs evoked by stimulation at −1,400, −100, and 0 ms and control trial MEPs. The changes in MEP amplitude were selective to TA because no significant change in the SOL MEPs was found at any stimulation time point. The increase in TA MEP could not be accounted for by a change in background EMG activity because these values were not significantly different from those in control conditions.

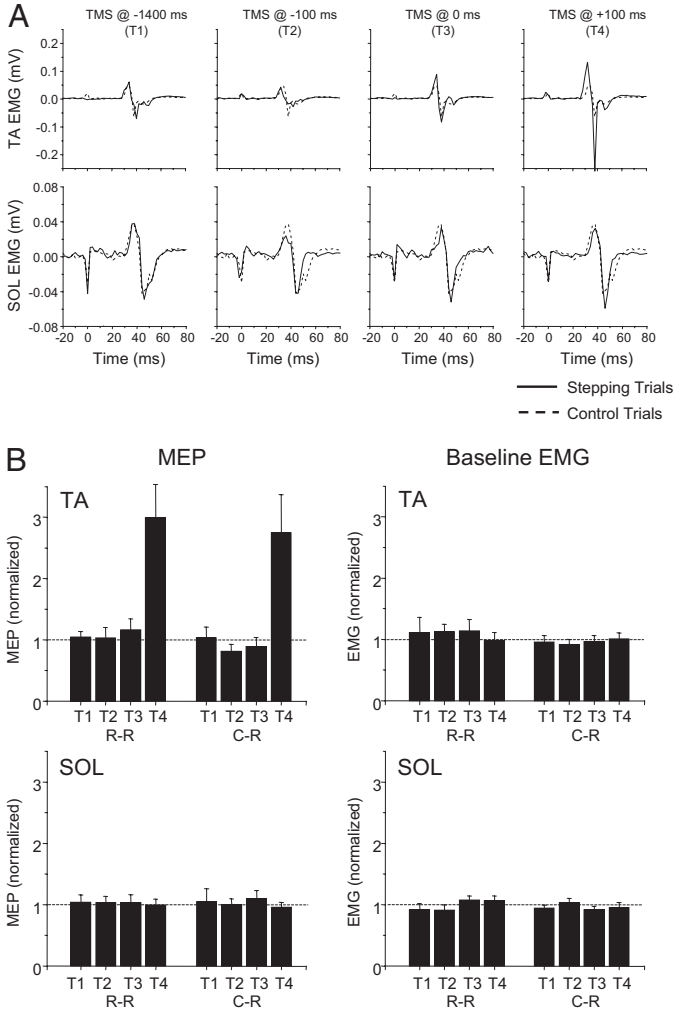

FIG. 7. *A*: example of the average motor-evoked potentials (MEPs) recorded in tibialis anterior (TA) and soleus (SOL) when transcranial magnetic stimulation (TMS) was applied at −1,400 (T1), −100 (T2), 0 (T3), and +100 ms (T4) during stepping trials (solid line) and quiet standing control trials (dashed lined) in a single subject. Note that increased MEPs were observed only in TA and only with stimulation at +100 ms. *B*, *right plots*: average normalized amplitude of the MEPs recorded in TA (*top plot*) and SOL (*bottom plot*) when TMS was applied at −1,400 ms (T1), −100 ms (T2), 0 ms (T3), and +100 ms (T4). MEP amplitudes are expressed relative to the MEP evoked during normal quiet standing (control TMS trials). Note that increases in MEPs were selective to TA and were present only when TMS was applied 100 ms after the "go" cue (~10 ms before the initiation of the TA burst). *Left plots*: average baseline amplitude of EMG activity in the time period immediately before the TMS pulse. R-R, right-step precue, right-step "go" cue; C-R, center-light precue, right-step "go" cue.

DISCUSSION

There are two main findings from this study. First, a SAS presented in the preparatory period between the precue and onset of the imperative "go" stimulus resulted in the rapid release of the APA sequence when subjects had foreknowledge about the required stepping leg. Moreover, the incidence, duration, and magnitude of the SAS-evoked APA progressively increased toward control trial values as the timing of the stimulus approached the "go" cue, suggesting that the spatial and temporal characteristics of the APA were progressively developed over this time period. The absence of facilitation of MEPs in the lower limbs when TMS was applied during the

instructed delay period suggests that the early preparation of the APA occurred independently of the corticomotor pathways tested with TMS. Second, there was a marked and spatially selective increase in the excitability of corticomotor projections to the initial agonist muscle (TA) in the reaction time period between the imperative stimulus and onset of the APA-stepping sequence. Application of the SAS at a time that closely coincided with initiation of the voluntary APA did not interfere with the voluntary APA-stepping sequence and instead resulted in a modest increase in APA amplitude and an earlier step onset. Taken together, these findings show that corticomotor pathways are engaged during the reaction time period and likely contribute to the initiation of the voluntary APA-step sequence, possibly through the release of the prepared APA and initiation of the goal-directed movement.

Early preparation and rapid release of the APA by a SAS

Our findings are consistent with previous studies that have shown that an acoustic startle stimulus, presented at the time of the imperative "go" cue, evokes a movement pattern that includes all of the spatial and temporal components that characterized movement when the subjects normally reacted to the "go" cue (Carlsen et al. 2003a,b, 2004a,b; Siegmund et al. 2001; Valls-Solé et al. 1995, 1999). Moreover, these patterns were initiated considerably faster than normal voluntary reactions. A key feature of this early response is that it is produced only when a voluntary motor sequence has been preinstructed and thus subjects can prepare the motor response in advance (Carlsen et al. 2004a). This is illustrated by the absence of APAs during control trials in which the SAS was presented alone (Fig. 3*F*). For this reason, it has been proposed that the acoustic stimulus results in the early release of the prepared and intended motor sequence. This phenomenon has been demonstrated for a variety of movements including ballistic movements of the head (Siegmund et al. 2001), wrist (Carlsen et al. 2003a,b, 2004a,b; Valls-Solé et al. 1999), rising onto the toes during standing (Valls-Solé et al. 1999), and now for APAs preceding step initiation.

Furthermore, we found that APAs can be evoked when a SAS is presented as much as 1,400 ms in advance of the imperative "go" cue. There is also evidence that movement sequences of the upper limb can also be prepared in advance of the imperative stimulus (Cressman et al. 2006; Valls-Solé 2004). Our findings further show that the incidence, magnitude, and duration of the APA were significantly affected by the timing of the SAS. Stimulation applied 1.4 s before the "go" cue was frequently associated with a small-amplitude, short-duration movement sequence that contained all five components we used to define an APA. These small APA sequences were evoked in only about 40% of trials and were not usually associated with an early step. Presentation of the SAS at 100 ms before the "go" cue resulted in a sequence that was closer in magnitude and duration to the control step APAs and was immediately followed by a step. When the SAS was presented at 0 ms, the APA was equal in amplitude to that of control trials, but the duration was shorter and toe-off occurred earlier. Stimulation at +200 ms coincided closely with the average reaction time to the imperative stimulus and was associated with an increase in APA amplitude and shortening of the APA duration. Comparable findings have been reported for ballistic upper limb movements by Kumru and Valls-Solé (2005), who found that the amplitude of the SAS-evoked movement was enhanced throughout the reaction time period between the imperative stimulus and the onset of movement. These findings suggest that the amplitude and duration of the response are progressively prepared in anticipation of the imperative stimulus and are maintained in a state of increased excitability until the voluntary initiation of movement.

Mechanisms of release of the APA by a SAS

The pathways by which a SAS evokes a prepared motor sequence are currently unknown. The generalized auditory startle reflex is evoked by an unexpected and strong acoustic stimulus and is characterized by a short-latency, typically bilateral, burst of activity EMG in cranial, axial, and distal muscles (Brown et al. 1991). In animals, this reflex is mediated by input from auditory nerves to the cochlear nuclear complex, then by mono- or disynaptic connections to nuclei in the reticular formation, and descending reticulospinal projections to the spinal cord (Yeomans and Frankland 1996). Based on conduction time estimates and the fact that the early release of a movement sequence occurred when the acoustic stimulus was of sufficient intensity to evoke a startle, it was proposed that the prepared sequences were released from similar brain stem pathways (Valls-Solé et al. 1995, 1999). It was argued that it was unlikely that these responses were initiated by the motor cortex because the onset latency of agonist EMG activity (77 ± 7 ms for wrist muscles) was considered to be too short compared with estimates of the synaptic and conduction delays associated with a transcortical pathway. Yet, an early release of movement from fast conducting pathways to the cortex cannot be ruled out. Transcortical pathways are considered to mediate, in part, the long-latency EMG responses evoked by imposed displacements of the distal upper and lower limbs (Christensen et al. 2000; Matthews 1991). The onset of these EMG responses (~5 ms for the wrist flexors) is less than the SAS-evoked latencies reported in the literature. Under specific task instruction conditions, the imposed displacement can trigger the early release of the planned movement irrespective of the direction of the imposed displacement (Crago et al. 1976; Koshland and Hasan 2000; Rothwell et al. 1980). In the present study, the average onset of the TA burst was 102 ± 33 ms. This timing was slightly faster than the onset of the SAS-evoked TA burst accompanying APAs associated with rising onto the toes (mean onset = 115 ms) reported by Valls-Solé et al. (1999) and close to the average latency of the long latency (M3) EMG response (95 ± 9 ms) evoked by imposed stretch of the TA muscle (Christensen et al. 2000). Thus a SAS can result in the release of a planned movement with onsets close to transcortical conduction times, but considerably faster than normal voluntary reaction times (Rogers et al. 2001).

There is accumulating evidence that the pathways mediating the EMG responses associated with the generalized startle reflex and the early release of the planned movement by a SAS may be separate. Valls-Solé et al. (2005) previously showed that prepulse inhibition, a salient feature of the generalized startle reflex, does not affect the timing or magnitude of the SAS-evoked movement sequence, but does result in a suppression of the startle response in the neck musculature. In addition, an early release of movement can be observed in the

absence of short-latency EMG bursts in eye and neck muscles (Carlsen et al. 2003a). However, movement sequences are released an average of 19 ms earlier when the response is accompanied by a startle-evoked burst of activity in the SCM muscle compared with responses without an early SCM burst (Carlsen et al. 2003a). Taken together, these findings suggest that preparatory or attentional processes that reduce the threshold excitability of the startle reflex have a parallel effect on the pathway mediating the SAS-evoked release of movement. The sensorimotor responses associated with the generalized startle reflex are thought to be mediated by input to giant neurons in the nucleus reticularis pontis caudalis (NRPc) (Koch 1999). Preparatory movement-related neurons have been recorded in the region of the NRPc as well as the nucleus reticularis gigantocellularis (NRGc) and magnocellularis (NRMc) (Buford et al. 2004; Schepens and Drew 2004). Thus there is some overlap in the regions that mediate both startle- and movement-related activity in the pontomedullary reticular formation, but it is also plausible that different regions of the reticular formation mediate the generalized startle reflex (e.g., NRPc) and SAS-mediated release of prepared movement (e.g., NRGc). However, as noted in the preceding paragraph, a transcortical route cannot be ruled out.

Activation of the sternocleidomastoid muscle by a SAS

We observed a consistent short-latency (<100 ms) SCM burst in only three of the subjects tested. In the subjects that did not show a SAS-evoked SCM burst during stepping trials, this burst was also absent or highly variable even for the control startle condition. Because of the low number of trials with an early SCM burst, we did not analyze trials with and without a SCM burst separately. However, we observed that some subjects that did not have an early burst in SCM had mean reaction times comparable to those of subjects that did have an early burst. For example, the average TA burst onset time in the subject who showed the most consistent early SCM burst was 81 ms when SAS was applied at 0 ms, whereas a subject who did not show any short-latency SCM activity had a average TA onset time of 84 ms for the same condition. The low incidence of early SCM bursts may have arisen from the slightly lower stimulus intensity used in the present study (115 dB) compared with that of previous studies (>120 dB). Nonetheless, all subjects demonstrated an early release of the APA when the SAS was applied during stepping trials, particularly with stimulation at −100 or 0 ms. The average onset latency of the TA burst relative to the SAS (111 ms) was comparable to the onset of the TA burst accompanying APAs associated with rising onto the toes (mean onset = 114 ms) reported by Valls-Solé et al. (1999) who used a markedly louder stimulus (130 dB). Thus the timing of onset of the APAs in the present study was consistent with a startle-evoked release of the planned movement.

APAs evoked by a SAS during choice reaction time conditions

An unexpected result was the observation that right-step APAs were frequently evoked before the "go" stimulus, even when the precue provided no information about the required stepping leg (center-light precue condition). We had expected that these trials would show a generalized startle response, suggesting a lack of movement preparation, or that a mixture of left or right APAs would be evoked, suggesting that subjects guessed the stepping leg. Instead, the incidence, onset time, magnitude, and duration of the APAs evoked by the SAS after a center-light precue, but before a *right*-step "go" cue, were remarkably similar to the APAs observed after a right-step precue. When stimulation was applied after a center-light precue, but before a *left*-step "go" cue, an early burst of TA activity was evoked and a small-amplitude right APA was commonly observed; however, this pattern was rapidly terminated and quickly followed by an appropriate left APA. This initial, incorrect right APA resulted in a significant delay in the initiation of the left forward step. In the case of SAS applied at 0 ms (simultaneously with the left-step "go" cue), the incidence of an inappropriate right APA was greatly reduced (from 90% for the center-right instruction condition to 44% for the center-left condition), suggesting that, under certain conditions, the early release of the planned right-step APA could be rapidly suppressed. Early responses that resembled a left-step APA after a center-light precue were very rare (a total of four trials observed across all subjects). In contrast, early left-step APAs were consistently evoked by SAS when a left-step precue was provided. These findings suggest that a default right-step APA was often prepared after the presentation of a neutral precue.

When subjects were presented with a center-light precue, the task was equivalent to a choice reaction time paradigm. Two previous studies that examined startle-evoked movements of the upper limb using a choice reaction time task also reported that response errors were substantially more common in the dominant right limb (Kumru et al. 2006; Valls-Solé 2004). This finding was interpreted to suggest that subjects may prefer to preplan a movement sequence in advance, even if that plan is incorrect (Kumru et al. 2006). Yet, one other choice reaction time study did not show a response side bias (Carlsen et al. 2004a). Methodological differences between studies may explain these different observations. In the present study, all subjects were right-leg dominant by self-report. Thus it is possible that the default preparation of a right step is an innate strategy used in right-leg–dominant individuals. However, we cannot exclude the possibility that the right-step bias we observed was caused by the experimental design. A greater number of right-step trials were presented than left-step trials (184 vs. 88). In addition, EMG electrodes and joint markers were placed only on the right side. For these reasons, it is conceivable that our subjects were aware of the fact that the experiment was focused on the collection and analysis of right steps and thus prepared a right-step response when the precue did not specify the stepping leg.

Changes in corticomotor excitability

In contrast to the APAs evoked after a SAS, changes in the excitability of the corticomotor projections to the lower limb muscles, as tested by TMS, were observed only when TMS was applied 100 ms after the "go" cue. This modulatory effect occurred before the voluntary increase in TA EMG activity and could not be attributed to changes in background EMG activity immediately before the arrival of the TMS-induced corticospinal volley. Moreover, the changes in MEP amplitude were

selective to TA, despite the fact that the optimal sites for evoking MEPs in TA and SOL are essentially the same. The timing of this increase in excitability is consistent with previous studies that examined activity preceding voluntary hand movements (Chen et al. 1998). These findings show that, during the instructed delay period between the onset of the precue and the imperative stimulus, the excitability of corticomotor pathways remained unchanged from excitability levels during quiet standing. Subsequently, in the reaction time period between the imperative "go" stimulus and onset of the APA, there was a marked increase in the excitability of corticomotor projections to TA. Because we probed for changes only at +100 ms, we could not determine the time course of the change in excitability or whether the increase was time-locked to the "go" cue or initiation of the APA. Nonetheless, these data provide evidence that corticomotor projections from the primary motor cortex likely contribute to the initiation of the APA that precedes and accompanies the voluntary step.

The absence of an early change in corticomotor excitability in the preparatory period preceding the imperative "go" cue should not be interpreted to suggest that the cortex was not involved in the motor preparation for this task. On the contrary, it is likely that early motor and premotor cortical activity contributes to the planning and preparation of APAs, but these changes in excitability occur independently of the corticomotoneuronal pathway tested with TMS. This pathway includes the corticospinal neurons projecting from the primary motor cortex, the local intrinsic interneurons that mediate the I-waves, and the segmental motoneurons that generate the MEP. Our experimental design used a fixed time interval (3.5 s) between the presentation of the precue cue and the imperative "go" cue. This design is associated with a slowly rising premovement potential that begins >1.5 s before movement onset and is maximal in amplitude over premotor regions of the frontal cortex (Cunnington et al. 1995). These premotor regions have extensive descending projections to reticular nuclei involved in sensorimotor coordination (Kably and Drew 1998; Keizer and Kuypers 1989; Kuypers and Lawrence 1967; Matsuyama and Drew 1997). Recent studies in behaving monkeys have shown that a subset of neurons in the medial pontomedullary reticular formation show movement-related preparatory activity during an instructed delay task (Buford and Davidson 2004). Thus the early buildup of preparatory premotor cortical activity could conceivably be involved in the organization and preparation of the APA at the level of the brain stem. Alternatively, it is also conceivable that the early preparation of the APAs, and subsequent release by the SAS, occurred at the segmental level. Premotor regions of the frontal cortex also send extensive projections to the intermediate zone of the spinal cord (He et al. 1993, 1995). A subset of segmental interneurons shows changes in activity during movement preparation similar to those observed in the premotor cortex and reticular formation (Prut and Fetz 1999). Similarly, preparatory changes in monosynaptic spinal reflexes occur before the onset of the imperative stimulus during reaction time tasks (Brunia 1993). Thus it is possible that the APA sequence is somehow stored and released by a network of segmental interneurons and motoneurons. However, for this to happen, the preparatory changes in the excitability of this network would have had to occur independently of the motoneurons innervated by the fast-conducting corticospinal system tested with TMS.

Preparation and control of APAs that precede and accompany voluntary movement

Studies in both humans and cats have provided compelling evidence that the systems responsible for the organization and execution of APAs are separate from those that control the initiation of the goal-directed voluntary movement (Brown and Frank 1987; de Wolf et al. 1998; Massion 1992; Schepens and Drew 2003, 2004). Based on lesion studies, Massion (1992) proposed that subcortical regions were responsible for the control of APAs. In keeping with this idea, Schepens and Drew (2004) showed that a population of reticulospinal neurons in the pontomedullary reticular formation of the cat had discharge patterns that were temporally coupled to both the imperative cue and onset of the APA, but not with the onset of the voluntary reach. They also identified other reticulospinal neurons that had activity that was temporally coupled to both the APA and the onset of the voluntary reach. Based on these findings, Schepens and Drew (2004) concluded that independent pathways from the pontomedullary reticular formation contribute to the control of APAs that precede and accompany voluntary movement.

If indeed there are separate subcortical and cortical pathways that control the initiation of the APA and the goal-directed movement, respectively, then an important question that remains is how these control systems are integrated into a coordinated postural-movement sequence. Based on studies of patients with different neuropathological conditions and an extensive body of experimental findings from healthy individuals, Massion and collaborators (Massion 1992; Massion et al. 1999) proposed that the control command for goal-directed movement provides a timing signal for the release of the APA from subcortical regions. In this scheme, the supplementary motor area together with other premotor areas select the neural networks responsible for the phasic postural adjustments that precede the goal-directed movement, whereas the motor cortex contralateral to the voluntary movement controls both initiation of the goal-directed movement and the preselected subcortical networks responsible for the control of APAs that accompany that movement (Massion et al. 1992). In general, the results of our experiments appear to support this model. Our findings suggest that premotor networks, separate from the corticomotor pathways that are activated by TMS over the primary motor cortex, are engaged during the early stages of movement preparation and are responsible for the progressive elaboration of the spatial and temporal components of the APA. This early preparation of the APA likely extends into the reaction time period, at least, ≤100 ms before movement onset (Kumru and Valls-Solé 2005). Corticomotor pathways projecting to the initial agonist muscle become engaged in the reaction time period and are facilitated 100 ms after the imperative "go" stimulus. The timing of this change in corticomotor excitability is similar to that reported for voluntary movements of the hand (Chen et al. 1998). Thus it is plausible that corticomotor commands contribute to the timing of release of the prepared APA, as proposed by Massion (1992). The fact that a SAS applied near the voluntary onset of the APA (+200 ms) did not interfere with the APA-stepping sequence and only modestly facilitated the APA magnitude and step onset timing suggests that the prepared movement sequence had already been released by corticomotor pathways. These findings are consistent

with a feedforward mode of neural control whereby the motor sequence, including the associated postural adjustments, is prepared prior to voluntary movement.

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REFERENCES

Brown JE, Frank JS. Influence of event anticipation on postural actions accompanying voluntary movement. *Exp Brain Res* 67: 645–650, 1987.

Brown P, Rothwell JC, Thompson PD, Britton TC, Day BL, Marsden CD. New observations on the normal auditory startle reflex in man. *Brain* 114: 1891–1902, 1991.

Brunia CH. Waiting in readiness: gating in attention and motor preparation. *Psychophysiology* 30: 327–339, 1993.

Brunt D, Lafferty MJ, McKeon A, Goode B, Mulhausen C, Polk P. Invariant characteristics of gait initiation. *Am J Phys Med Rehab* 70: 206–212, 1991.

Buford JA, Davidson AG. Movement-related and preparatory activity in the reticulospinal system of the monkey. *Exp Brain Res* 159: 284–300, 2004.

Carlsen AN, Chua R, Inglis JT, Sanderson DJ, Franks IM. Startle response is dishabituated during a reaction time task. *Exp Brain Res* 152: 510–518, 2003a.

Carlsen AN, Chua R, Inglis JT, Sanderson DJ, Franks IM. Can prepared responses be stored subcortically? *Exp Brain Res* 159: 301–309, 2004a.

Carlsen AN, Chua R, Inglis JT, Sanderson DJ, Franks IM. Prepared movements are elicited early by startle. *J Motor Behav* 36: 253–264, 2004b.

Carlsen AN, Hunt MA, Inglis JT, Sanderson DJ, Chua R. Altered triggering of a prepared movement by a startling stimulus. *J Neurophysiol* 89: 1857–1863, 2003b.

Carlsoo S. The initiation of walking. *Acta Anat* 65: 1–9, 1966.

Chen R, Yaseen Z, Cohen LG, Hallett M. Time course of corticospinal excitability in reaction time and self-paced movements. *Ann Neurol* 44: 317–325, 1998.

Christensen LOD, Petersen N, Andersen JB, Sinkjaer T, Nielsen JB. Evidence for transcortical reflex pathways in the lower limb of man. *Prog Neurobiol* 62: 251–272, 2000.

Crago JE, Houk JC, Hasan Z. Regulatory actions of human stretch reflex. *J Neurophysiol* 39: 925–935, 1976.

Crenna P, Frigo C. A motor programme for the initiation of forward-oriented movements in humans. *J Physiol* 437: 635–653, 1991.

Cressman EK, Carlsen AN, Chua R, Franks IM. Temporal uncertainty does not affect response latencies of movements produced during startle reactions. *Exp Brain Res* 171: 278–282, 2006.

Cunnington R, Iansek R, Bradshaw JL, Phillips JG. Movement-related potentials in Parkinson's disease. Presence and predictability of temporal and spatial cues. *Brain* 118: 935–950, 1995.

De Wolf S, Slijper H, Latash ML. Anticipatory postural adjustments during self-paced and reaction-time movements. *Exp Brain Res* 121: 7–19, 1998.

Drew T, Prentice S, Schepens B. Cortical and brainstem control of locomotion. *Prog Brain Res* 143: 251–261, 2004.

He S-Q, Dum RP, Strick PL. Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral surface of the hemisphere. *J Neurosci* 13: 952–980, 1993.

He S-Q, Dum RP, Strick PL. Topographic organization of corticospinal projections from the frontal lobe: motor areas on the medial surface of the hemisphere. *J Neurosci* 15: 3284–3306, 1995.

Jian Y, Winter DA, Ishac MG, Gilchrist L. Trajectory of the body COG and COP during initiation and termination of gait. *Gait Posture* 1: 9–22, 1993.

Kably B, Drew T. Corticoreticular pathways in the cat. I. Projection patterns and collaterization. *J Neurophysiol* 80: 389–405, 1998.

Keizer K, Kuypers HGJM. Distribution of corticospinal neurons with collaterals to the lower brain stem reticular formation in monkey (*Macaca fascicularis*). *Exp Brain Res* 74: 311–318, 1989.

Koch M. The neurobiology of startle. *Prog Neurobiol* 59: 107–128, 1999.

Koshland GF, Hasan Z. Electromyographic responses to a mechanical perturbation applied during impending arm movements in different directions: one-joint and two-joint conditions. *Exp Brain Res* 132: 485–499, 2000.

Kumru H, Urra X, Compta Y, Castellote JM, Turbau J, Valls-Solé J. Excitability of subcortical motor circuits in Go/noGo and forced choice reaction time tasks. *Neurosci Lett* 406: 66–70, 2006.

Kumru H, Valls-Solé J. Excitability of the pathways mediating the startle reaction before execution of a voluntary movement. *Exp Brain Res* 169: 427–432, 2006.

Kuypers HGJM, Lawrence DG. Cortical projections to the red nucleus and the brain stem in the Rhesus monkey. *Brain Res* 4: 151–188, 1967.

MacKinnon CD, Rothwell JC. Time-varying changes in corticospinal excitability accompanying the triphasic EMG pattern in humans. *J Physiol* 528: 633–645, 2000.

Mann RA, Hagy JL, White V, Liddell D. The initiation of gait. *J Bone Joint Surg Am* 61: 232–239, 1979.

Massion J. Movement, posture and equilibrium: interaction and coordination. *Prog Neurobiol* 38: 35–56, 1992.

Massion J, Alexandrov A, Frolov A. Why and how are posture and movement coordinated? *Prog Brain Res* 143: 13–27, 2004.

Massion J, Ioffe M, Schmitz C, Viallet F, Gantcheva R. Acquisition of anticipatory postural adjustments in a bimanual load-lifting task: normal and pathological aspects. *Exp Brain Res* 128: 229–235, 1999.

Matsuyama K, Drew T. Organization of the projections from the pericruciate cortex to the pontomedullary brainstem of the cat: a study using the anterograde tracer Phaseolus vulgaris-leucoagglutinin. *J Comp Neurol* 389: 617–641, 1997.

Matsuyama K, Mori F, Nakajima K, Drew T, Aoki M, Mori S. Locomotor role of the corticoreticular-reticulospinal-spinal interneuronal system. *Prog Brain Res* 143: 239–249, 2004.

Matthews PBC. The human stretch reflex and the motor cortex. *Trends Neurosci* 14: 87–91, 1991.

Prentice SD, Drew T. Contributions of the reticulospinal system to the postural adjustments occurring during voluntary gait modifications. *J Neurophysiol* 85: 679–698, 2001.

Prut Y, Fetz EE. Primate spinal interneurons show pre-movement instructed delay activity. *Nature* 401: 590–594, 1999.

Rogers MW, Kukulka CG, Brunt D, Cain TD, Hanke TA. The influence of stimulus cue on the initiation of stepping in young and older adults. *Arch Phys Med Rehab* 82: 619–624, 2001.

Rothwell JC, MacKinnon CD, Valls-Solé J. Role of brainstem-spinal projections in voluntary movement. *Mov Disord* 17, *Suppl.* 2: S27–S29, 2002.

Rothwell JC, Traub MM, Marsden CD. Influence of voluntary intent on the human long-latency stretch reflex. *Nature* 286: 496–498, 1980.

Schepens B, Drew T. Strategies for the integration of posture and movement during reaching in the cat. *J Neurophysiol* 90: 3066–3086, 2003.

Schepens B, Drew T. Independent and convergent signals from the pontomedullary reticular formation contribute to the control of posture and movement during reaching in the cat. *J Neurophysiol* 92: 2217–2238, 2004.

Siegmund GP, Inglis JT, Sanderson DJ. Startle response of human neck muscles sculpted by readiness to perform ballistic head movements. *J Physiol* 535: 289–300, 2001.

Valls-Solé J. Contribution of subcortical motor pathways to the execution of ballistic movements. *Suppl Clin Neurophysiol* 57: 554–562, 2004.

Valls-Solé J, Kofler M, Kumru H, Castellote JM, Sanegre MT. Startle-induced reaction time shortening is not modified by prepulse inhibition. *Exp Brain Res* 165: 541–548, 2005.

Valls-Solé J, Rothwell JC, Goulart F, Cossu G, Munoz E. Patterned ballistic movements triggered by a startle in healthy humans. *J Physiol* 516: 931–938, 1999.

Valls-Solé J, Solé A, Valldeoriola F, Munoz E, Gonzalez LE, Tolosa ES. Reaction time and acoustic startle in normal human subjects. *Neurosci Lett* 195: 97–100, 1995.

Yeomans JS, Frankland PW. The acoustic startle reflex: neurons and connections. *Brain Res Rev* 21: 301–314, 1995.