Differential modulation of descending signals from the reticulospinal system during reaching and locomotion

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Dyson KS, Miron JP, Drew T. Differential modulation of descending signals from the reticulospinal system during reaching and locomotion. J Neurophysiol 112: 2505–2528, 2014. First published August 20, 2014; doi:10.1152/jn.00188.2014.—We tested the hypothesis that the same spinal interneuronal pathways are activated by the reticulospinal system during locomotion and reaching. If such were the case, we expected that microstimulation within the pontomedullary reticular formation (PMRF) would evoke qualitatively similar responses in muscles active during both behaviors. To test this, we stimulated in 47 sites within the PMRF during both tasks. Stimulation during locomotion always produced a strongly phase-dependent, bilateral pattern of activity in which activity in muscles was generally facilitated or suppressed during one phase of activity (swing or stance) and was unaffected in the other. During reaching, stimulation generally activated the same muscles as during locomotion, although the modulation of the magnitude of the evoked responses was less limb dependent than during locomotion. An exception was found for some forelimb flexor muscles that were strongly facilitated by stimulation during the swing phase of locomotion but were not influenced by stimulation during the transport phase of the reach. We suggest that during locomotion the activity in interneuronal pathways mediating signals from the reticulospinal system is subject to strong modulation by the central pattern generator for locomotion. During reach, we suggest that, for most muscles, the same spinal interneuronal pathways are used to modify muscle activity but are not as strongly gated according to limb use as during locomotion. Finally, we propose that the command for movement during discrete voluntary movements suppresses the influence of the reticulospinal system on selected forelimb flexor muscles, possibly to enhance fractionated control of movement.

cat; gating; locomotion; reaching; reticular formation

DESCENDING INPUTS from supraspinal structures to the spinal cord are subject to phase-dependent modulation during locomotion. In the case of reticulospinal inputs, this phase-dependent modulation is generally expressed as a facilitation of activity in ipsilateral flexor muscles when a reticulospinal volley is induced by microstimulation during the swing phase of locomotion, together with a site-specific mixture of facilitation or suppression in ipsilateral extensor muscles when stimulation is applied during ipsilateral stance (Drew 1991; Drew and Rossignol 1984; Orlovsky 1972). In the contralateral limbs, the responses are generally reciprocal, so that stimulation in ipsilateral swing produces facilitation in the contralateral extensor muscles and stimulation in ipsilateral stance produces facilitation of contralateral flexors. Similar effects are seen in both fore- and hindlimb muscles (Drew 1991; Drew and Rossignol 1984). We, and others, have suggested that this phase-dependent modulation is the result of changes in excitability in interneurons that either form part of or are influenced by the central pattern generator (CPG) for locomotion (Degtyarenko et al. 1993; Drew 1991; Drew and Rossignol 1984; Floeter et al. 1993; Orlovsky 1972; Perreault et al. 1994; Shefchyk and Jordan 1985). Figure 1A (modified from Drew et al. 2004) illustrates our conceptual representation of this process. The signal from the pontomedullary reticular formation (PMRF) is illustrated as influencing interneuronal networks in both the left and right limbs, with the CPG determining the phase-dependent, generally reciprocal pattern of activation in different muscle groups. Input from the cortex is illustrated with dashed lines in Fig. 1A to indicate that this input is probably facilitative during unobstructed locomotion (see Armstrong and Drew 1984a). Similar spinal mechanisms are suggested to ensure that changes in cell activity during voluntary gait modifications of one limb produce appropriate, phase-dependent, changes in muscle activity in the supporting limbs (Prentice and Drew 2001). In this latter case the cortical input becomes important as in our original illustration (Drew et al. 2004).

Recently, we have suggested that the same mechanisms that are responsible for coordinating activity in the limbs during locomotion may also be used to coordinate the postural responses that occur during reaching movements with the left and right forelimbs (see Schepens and Drew 2006). During reach, movement of one limb to a target is associated with postural responses in the contralateral, supporting, limb. These postural responses are organized such that one pattern of muscle activity is observed with movement of the left forelimb and the reciprocal pattern is observed for movement of the right forelimb (Schepens and Drew 2003). However, recordings of activity in reticulospinal neurons that we, and others, have suggested to be responsible for initiating and modulating anticipatory postural activity (Drew et al. 2004; Luccarini et al. 1990; Massion 1992; Sakamoto et al. 1991; Schepens and Drew 2004) show that many exhibit a generally similar, non-reciprocal, pattern of activity during voluntary movement of either forelimb (Schepens and Drew 2006). Clearly, the discharge activity in such cells cannot specify a reciprocal pattern of postural activity, and we have suggested that the activity in these cells signals primarily the timing and the magnitude of the postural activity. The expression of the postural responses is then dependent on the excitability of the interneurons onto which the descending signal impinges.
We suggest that the interneurons mediating the coordinated postural responses during reaching include those that equally mediate the coordinated responses observed to PMRF stimulation during locomotion. During voluntary movements, we suggest that the excitability of these interneurons is influenced by the descending signals responsible for the voluntary movement, including those transmitted by the corticospinal tract. In our schematic representation of this circuit (Fig. 1B, modified from Schepens and Drew 2006), we have kept the same representation of the spinal circuits as in Fig. 1A to emphasize the hypothesis that the interneuronal circuits innervated by the PMRF would maintain their intrinsic capacity to coordinate activity between the two limbs. The initial period of activity during ipsilateral swing (orange) is suggested to facilitate activity in the ipsilateral flexors and the contralateral extensors (also in orange). The subsequent period of activity (blue), occurring during ipsilateral stance, will modify the activity of the ipsilateral extensors and the contralateral flexors. During reaching, many reticulospinal cells discharge during both left and right reach, despite the reciprocal requirements for postural support in the 2 conditions. We have suggested that cortical inputs will gate spinal circuits to modify reticulospinal input in a manner similar to that observed during locomotion. Specifically, activity during left reach (orange) is suggested to modify ipsilateral flexor muscle and contralateral extensor muscle activity. Activity during right reach will produce reciprocal responses.

We therefore trained cats both to walk on a treadmill and to perform the same reaching task as used in our recent experiments (Schepens et al. 2008; Yakovenko et al. 2011; Yakovenko and Drew 2009). Stimulation in a given site was...
applied during locomotion as well as during reaching. The results support some aspects of our hypothesis while at the same time emphasizing important differences in the contribution of the PMRF to the regulation of locomotion and reaching. On the basis of these results, we modify our conceptual representations of the interactions between the PMRF and spinal interneuronal pathways to incorporate our new findings.

METHODS

Task

Two male cats (weights 3.9 and 5.8 kg, respectively) were trained in two tasks. First, they were trained to walk steadily on a treadmill for periods of 5–10 min at a speed of 0.4 m/s, a speed at which cats walk comfortably for relatively long periods. Second, the cats were trained to stand quietly and, when instructed, to reach forward and to press a lever with their left (ipsilateral) or right (contralateral) forelimb (Fig. 2A). This task has been used in several of our previous studies (Schepens et al. 2008; Yakovenko et al. 2011; Yakovenko and Drew 2009). In brief, a warning tone alerted the cat to the start of a trial, and 1.5 s later a tone instructed the cat to reach with either the left (frequency 400 Hz) or the right (4 kHz) forelimb. This instruction tone lasted a random period of 0.5–1.5 s, and at its cessation a shutter opened, giving the cat access to a lever that it was trained to press to obtain a food reward. Successful movements required the application of a force of 2 N applied through an angle of 5°.

Surgical Procedures

The cats were prepared for surgery in aseptic conditions and under general anesthesia with a mixture of oxygen and isoflurane (2–3%). A craniotomy was made over the occipital bone, and a rectangular stainless steel baseplate (internal diameter 8 × 6 mm) was positioned over the cerebellum to provide access to the left PMRF. A recording chamber was formed by building four walls around the baseplate with dental acrylic. Pairs of braided stainless steel wires were inserted into selected proximal and distal muscles of the four limbs of the cats and used to record electromyographic (EMG) activity. In one cat (RS27), we implanted 40 pairs of electrodes, 34 of which were both functional and verified postmortem to be inserted into the targeted muscles. In the second cat (RS28), electrodes were inserted into 24 muscles, 18 of which were included in the analysis. All surgical and experimental manipulations were approved by the local deontology committee at the Université de Montréal and followed the guidelines of the Canadian Council for the Protection of Animals.

![Fig. 2. Reaching task and stimulation protocol. A: schematic representation of the reaching task (see text for details) (adapted from Schepens et al. 2008 with permission). B: stimuli were applied at 5 different times during the movement, as defined in METHODS. Figure shows approximate time of application of stimuli 2–5 superimposed on an example reach trial with the left forelimb. Gray bar indicates occurrence of the anticipatory postural response (pAPA) that precedes movement. The onset of the transport phase of the reach is defined by the sharp onset of activity in the left brachialis (lBr, top). The onset of the lever press is indicated by the initial decrease in the lever trace (bottom). Data are aligned to the onset of the Go signal. C: schematic showing the time period during which control (open boxes, unstimulated) activity was collected for each of the 5 stimulus epochs, E1–E5 (shaded rectangles). The equivalent epochs for reach of the contralateral limb were E6–E10 (indicated by gray numerical values in B and C, top). Note that for E1 the stimulated period was 2,000 ms before the Go signal and is not illustrated to scale. Values on right indicate the time of the applied stimulus with respect to the events used to trigger the stimulator (see text). FV, vertical ground reaction force; l, left; r, right; TriL, lateral head of triceps.](http://jn.physiology.org/doi/10.1152/jn.00188.2014)
Protocol

In each experiment, an electrode held in a custom micromanipulator was driven manually through the cerebellum to the top of the brain stem while the cat was held lightly on the experimenter’s lap. The electrode was then advanced slowly, and trains of stimuli (cathodal current, 11 pulses at 330 Hz, pulse duration 0.2 ms) were applied at a frequency of 1/s at every 0.5 mm while the movements and the EMG activity evoked by the stimuli were examined. Stimulus intensity was maintained at 25 μA for all sites in both cats. As reported previously (Drew and Rossignol 1990a, 1990b), stimulation generally evoked face and neck movements in more dorsal locations and then produced ipsilateral flexion and contralateral extension of the forelimb as the electrode was lowered. Further advance of the electrode frequently evoked responses in both the forelimbs and the hindlimbs. For stimulation during locomotion, the electrode was positioned either at the depth at which fore- and hindlimb responses were evoked or, in the absence of hindlimb responses, at the depth at which the strongest responses in the forelimbs were evoked. All stimuli, in both cats, were applied on one side of the brain stem only. In both cats we stimulated the left brain stem, and reaches with the left limb, as well as all muscles recorded on the left side, are referred to as ipsilateral.

The cat was then placed on the treadmill, and stimulation was applied during locomotion. Stimuli were triggered on the onset of activity in the ipsilateral (left) brachialis (iBr) and were applied at different delays with respect to iBr onset in every third step cycle. Stimuli were applied in groups of 10–20 stimuli and in a fixed order at delays of approximately 100, 0, 200, 400, 600, 800, 150, 300, 500, 700, and 900 ms with respect to the onset of iBr activity. This order ensured that any long-term trends in the responses evoked by the stimulus would not bias the results. EMG activity was band-pass filtered at 100 Hz and 475 Hz, and a continuous record of the locomotion data was digitized to computer at 1 kHz. In addition, a period of 50 ms prior to the stimulus and 150 ms subsequent to each stimulus was digitized at 2 kHz and stored as a frame of data.

After this series of stimulation, the cat was transferred to the reaching apparatus and stimuli were applied in the same location and at the same strength (25 μA) at five different epochs of the reach for each limb (Fig. 2, B and C) in blocks of 8 (cat RS27) or 10 (cat RS28) stimuli. Stimuli were applied at epochs 1) 2,000 ms prior to the Go signal with the cat standing quietly before the onset of the instruction; 2) 300 ms prior to the Go signal, during the instructed delay period; 3) 130 ms after the Go signal, at the approximate time of the anticipatory postural adjustment preceding the movement (pAPA); 4) 70 ms after the vertical force dropped below 3 N during the transport phase of the reach; and 5) 50 ms after the cat depressed the lever. The corresponding stimuli for the contralateral limb are referred to as epochs 6–10 (Fig. 2, B and C). For one complete series of stimulation during reach we therefore have 10 epochs of stimulation. Each experimental series consisted of 90 trials in cat RS27 (8 stimuli in each epoch × 5 epochs/limb × 2 limbs + 10 unstimulated cycles) and 110 trials in cat RS28. The entire behavioral sequence of activity for the reach for 2,500 ms prior to the Go signal and 3,000 ms subsequent to it was digitized at computer at 1 kHz. EMGs were band-pass filtered as for locomotion, and force traces were low-pass filtered at 100 Hz. As for locomotion, we also digitized a frame of data at 2 kHz consisting, in this task, of the period of 50 ms prior to a stimulus and 200 ms subsequent to it.

In addition, for each trial, we also acquired additional frames of unstimulated data at 2 kHz corresponding in time to the five stimulation epochs in each limb. For example, for a stimulus applied during epoch 1, 2,000 ms prior to the Go signal (E1 in Fig. 2C), we also digitized a period of 50 ms before and 200 ms after the theoretical time of application of a stimulus in epochs 2–5, i.e., 300 ms before the Go signal (epoch 2), 150 ms after the Go signal (epoch 3), 70 ms after lift (epoch 4), and 50 ms after lever press (epoch 5). These periods are represented by the open rectangles in Fig. 2C. By combining these periods of unstimulated activity with the equivalent periods from the 10 unstimulated trials, we were able to average the background activity from >25 trials for each epoch.

Data Analysis

Data obtained during locomotion were analyzed as detailed previously (see, e.g., Bretzner and Drew 2005). In brief, because the time of the (online) application of the stimuli during the experiment was only approximate, we first calculated, off-line, the true time of each stimulus with respect to iBr onset. This value was converted to a phase (0–1.0) of the average duration of the unstimulated step cycle. The stimuli were then divided into 10 equal groups, with group 1 representing phases between 0.0 and 0.1 and group 10 those between 0.9 and 1.0 (see Fig. 4A). The EMG responses evoked by all of the stimuli in each group were averaged together and superimposed on the activity of the muscles at the same phase (average phase of all stimuli included in each group) but in the absence of any stimulus (see, e.g., Fig. 5A). Similarly, during reach, the responses evoked by the 8 (cat RS27) or 10 (cat RS28) stimuli applied in each epoch were averaged together and superimposed on the average of the EMG activity during the identical epoch from unstimulated trials (see, e.g., Fig. 7).

For both the reach and the locomotion data, we first manually identified the onset and offset of the averaged evoked responses evoked by the stimulation. We used the deviation of the stimulus traces from the interval of confidence (P = 0.01) of the standard error (SE) of the background traces as a guide to identify the response. To be included in the analysis, the latency of the onset of the response had to be >50 ms and the duration ≥5 ms (Bretzner and Drew 2005). In addition, if there were more than two responses to the stimulus that fulfilled these criteria, only the initial, primary, response was included. This analysis was applied to all EMGs (and forces for the behavioral data) for all 10 groups during locomotion and for all 10 behavioral epochs of the reach. For each cat, we then calculated the averaged time of the onset and the offset of these responses, using the data from all stimulated sites. These average onsets and offsets were then used to define a window, which was used for the majority of our quantitative analysis. For each trial, we calculated the integrated EMG activity (sum of each 0.5-ms bin within the region identified as a response) for both stimulated and unstimulated data. The average integrated value for the unstimulated data was then subtracted from each individual stimulated trace to provide a net value for the stimulation for each trial. The net values for each stimulated trial in a given phase or epoch were then averaged.

For the analysis of the force traces, illustrated in Fig. 10, we found that the variation in forces under the standing limbs prior to the stimulation was sufficiently large that it proved difficult to use this same method to quantify changes in vertical force. For the force data, we therefore used the results obtained by simply averaging the data for all stimuli in an epoch. We averaged the stimulated and the unstimulated traces and then displaced the stimulated traces so that the activity in the period before stimulus onset overlapped that in the unstimulated traces. The net change in activity was then calculated from these averaged traces as above (see Fig. 10 for additional details).

When averaging together the mean values from different tracks to obtain the overall mean activity in a given muscle (see, e.g., Fig. 11), we first removed any outliers as defined by Tukey’s method for determining the interquartile range (IQR). Outliers were defined as exceeding IQR × 1.5 of the upper and lower fences. This procedure was performed on a file containing all of the data values for each track and each group for the locomotion and each epoch for the reach.

Histology

At the end of the series of experiments, each cat was deeply anesthetized and perfused per cardia with formaldehyde. The brain

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stem was blocked and sectioned in the sagittal plane at 40 μm before being stained with cresyl violet. The location of the different electrode penetrations was determined on the basis of marking lesions (30–40 μA) placed in selected penetrations during the experiments. The calculated locations of the stimulus sites were transposed to sections of the brain stem based on standard sections from the atlas of Berman (1968).

**Terminology**

For stimuli during locomotion, we use the term “phase of stimulation” to refer to the general time of application of a stimulus (swing or stance) and the term “group” to refer to the specific moment of stimulus application in 1 of the 10 groups into which the step cycle was divided. During reach, we use the term “epoch” to refer to the specific moment during the behavioral trial at which stimulation was applied. We use the term “transport phase” (equivalent to epochs 4 and 9) to emphasize the major comparison that we wish to make between the swing phase of locomotion and the transport phase of the reach. Similarly, we use “phase dependent” in a general manner for both locomotion and reach.

**RESULTS**

**Database**

Stimulation was applied in 19 sites in cat RS27 and 28 sites in cat RS28. The locations of these 47 stimulus sites, all in the left PMRF, are illustrated in Fig. 3. All 47 sites were included in the PMRF, with the most rostral tracks being located in the nucleus reticularis pontis caudalis (NRpc) and the most caudal being located in the nucleus reticularis gigantocellularis (NRgc), rostral to the level of the inferior olive. All of these stimulation sites were located between 0.3 and 1.8 mm of the midline, with the eight most medial stimulation sites in RS28 lying in the MLF (Fig. 3).

**Behavioral Activity**

The behavior of the cats and the EMG activity recorded during the locomotion and reaching tasks were similar to those detailed in previous publications from this laboratory (Drew and Rossignol 1987; Schepens and Drew 2003; Yakovenko et al. 2011). The pattern of EMG activity for selected ipsilateral (i) and contralateral (co) forelimb and hindlimb muscles during unobstructed locomotion is illustrated in Fig. 4A (the anatomical location of these muscles is illustrated in Fig. 4D). In brief, most limb muscles exhibited a single period of activity during locomotion during either the swing (corresponding approximately to stimulation groups 1–4 for muscles of the ipsilateral forelimb) or the stance (corresponding approximately to stimulation groups 5–10 for the ipsilateral forelimb) phase of locomotion (Fig. 4A). Muscles active during ipsilateral forelimb swing included muscles acting around the shoulder [spino-deltoides (iSpD)], the elbow (iBr), and the wrist and digits [extensor digitorum communis (iEDC)]. Other recorded muscles that were active during the swing phase but that are not illustrated in Fig. 4A included the shoulder muscles teres major (iTrM) and latissimus dorsi (iLdD) and the elbow flexor and shoulder protractor cleidobrachialis (iCIB). The two shoulder muscles discharged at the onset and the end of the swing period (see, e.g., Drew and Rossignol 1987; Krouchev et al. 2006). Recorded extensor muscles active during ipsilateral forelimb stance included the lateral and the long heads of triceps (iTrIL and iTri, respectively), acting primarily around the elbow, the wrist plantarflexor palmaris longus (iPaL), and the shoulder muscle acromiotoracipzenus (iAcT); the latter also had a burst of activity in swing. The period of activity in the flexor and extensor muscles in the contralateral (right) forelimb was 0.5 out of phase with that of the ipsilateral forelimb (Fig. 4A).

EMG activity in the flexor muscles of the ipsilateral hindlimb, such as the ankle flexor tibialis anterior (iTA), was slightly phase-advanced with respect to the ipsilateral forelimb flexor iBr. Because of the cyclical nature of locomotion, the iTA activity is represented as occurring in groups 8–10 in the illustration of Fig. 4A (equivalent to phases of −0.2 to 0.0). Muscles in the following text are referred to as flexors or extensors based on their predominant period of activity during locomotion (see also Drew and Rossignol 1987; Rho et al. 1999).

During ipsilateral reach (Fig. 4B), most muscles, and particularly the extensors, were tonically active during quiet standing (see e.g., iTriL and iAcT). After the Go signal, the initial change was in the activity of the extensor muscles of the ipsilateral and contralateral forelimb, corresponding to the pAPAs that precede the reach (Schepens and Drew 2003). In Fig. 4B, this is most evident for the iTriL. As Fig. 4B is synchronized to the onset of the activity in the iBr, the pAPAs precede the synchronization event. After the pAPA, there was a brief, phasic increase in activity in all of the ipsilateral forelimb flexor muscles that resulted in the transport of the limb forward, toward the lever. Note that the iAcT, which was mostly active in stance during locomotion, shows a strong period of activity during the transport phase. There was also a more prolonged increase in activity in the contralateral forelimb extensor muscles that anticipated, and compensated for, the loss of support under the reaching limb. Ipsilateral forelimb extensor muscles were reactivated after the reach in order to depress the lever. In Fig. 4, B and C, this is most evident for the iPaL during the ipsilateral reach and the coTriL during the contralateral reach. The overall magnitude of the EMG activity was slightly greater than during unobstructed locomotion, especially for the flexor muscles. Changes in the hindlimb muscles were more tonic in nature.

The changes in the ground reaction forces under each paw (not illustrated here, but see Fig. 10) were similar to those previously detailed (Schepens and Drew 2003), with an increase in vertical force in the supporting forelimb during the reach. Smaller changes were observed in the hindlimbs, with the hindlimb diagonal to the supporting limb showing increased force and the other hindlimb decreased force.

Changes in EMG activity during contralateral forelimb reach (Fig. 4C) were reciprocal to those observed during ipsilateral forelimb reach. Note that forelimb flexor muscles were only phasically active during the transport phase of the reaching limb (see iBr and coBr) in much the same way that they were only active during the swing phase of the respective limb during locomotion (Fig. 4A).

**Responses Evoked by PMRF Stimulation During Locomotion**

The effects of stimulation of the PMRF on EMG activity during locomotion were similar to those previously described for a more limited number of muscles (Drew 1991) and are only briefly described here.
An example of the responses evoked during swing and stance in one track from cat RS27 is shown in Fig. 5A. Stimulation during ipsilateral swing (group 1) produced large, brief, short-latency twitch responses in the ipsilateral forelimb flexor muscles (iBr, iSpD) as well as an increase in the iAcT. There was also an activation of the wrist and digit extensor (but physiological flexor during locomotion), iEDC. There was a small facilitation of the iTriL and a suppression of the activity in the coTriL. In the hindlimb, there was a facilitation of the iTA and a suppression of the activity in the soleus (iSol). Note that prominent responses were observed not only in proximal muscles but also in those with a more distal action (e.g., iEDC and iTA). During ipsilateral stance (and contralateral swing) (group 6), responses were absent in most ipsilateral forelimb muscles, with the exception of a suppression of activity in the iAcT, but a prominent facilitation was observed in the coBr. There was a suppression of activity in the iSol.

The phase-dependent nature of the responses is clearly seen in the graphs of Fig. 5B, which illustrate data for the muscles illustrated in Fig. 5A as well as other selected muscles. In general, EMG activity in flexor muscles in each of the four limbs (Fig. 5B, i–iv) was facilitated during the period of activity of the muscles and was unresponsive out of phase with this activity. The responses in extensor muscles (Fig. 5B, v–x)
were more complex, consisting generally of either no response or a small facilitation during the swing phase. There was a mix of facilitation and suppression during their period of activity in stance. In the forelimb, both the ipsilateral and contralateral long head of triceps (iTri and coTri, Fig. 5B, v and vi) were facilitated during the period of activity of the muscle in stance while activity in the lateral head (iTriL and coTriL, also Fig. 5B, v and vi) was suppressed at the end of the period of activity but showed a small facilitation during swing. The iAcT and the ipsilateral spinotrapezius (iSpT) (Fig. 5Bix) were facilitated during swing and exhibited strong suppression during stance. Activity in the ipsilateral hindlimb extensors was depressed during stance for most recorded muscles but facilitated for the gluteus medius (iGlM, Fig. 5Bviii). Responses were facilitatory in the contralateral gastrocnemius, lateral head (coGl) (Fig. 5Bvilii), and there was mixed facilitation and suppression in the contralateral vastus lateralis (coVL) (not illustrated).

The results of stimulation at all sites examined within the PMRF of cats RS27 and RS28 are shown in Fig. 6, A and B, respectively, for selected muscles. The two major observations here are that 1) the pattern of evoked responses in any given muscle is very similar for all sites stimulated, albeit with some differences in the magnitude of the responses, and 2) responses are observed in both proximal and distal muscles, including the EDC. For example, in the iBr and the iSpD, evoked responses from all 19 stimulated sites in cat RS27 were maximal during the period of swing, when the muscles were active. Similarly, in the iEDC, the maximal responses were slightly later in the period of swing, when the muscles were active. Similarly, in the iTA, maximal responses were slightly later in the step cycle (group 3) corresponding to the delayed period of locomotor activity in the EDC compared with the iBr (Fig. 4A). Responses in the coBr and in the iTA were also maximal during the period of activity of the muscle as illustrated for the individual example in Fig. 5. Responses were more variable in some of the extensor muscles. For example, although stimulation most commonly elicited suppression of the activity in the iTriL, some sites produced facilitation or a mixture of facilitation and suppression. Similarly, in the coTriL, there were two periods in which facilitatory responses were produced, at the beginning and end of the period of activity, and these were separated by a small period in which there was either no response or a small...
Fig. 5. Responses evoked by PMRF stimulation during locomotion. A: averaged responses evoked in selected muscles by stimulation of the PMRF during ipsilateral swing (group 1, left) and stance (group 6, right). As indicated in Fig. 4, group 1 corresponds to phases of 0.0–0.1 and group 6 corresponds to phases of 0.5–0.6. Thick lines indicate responses during stimulation, and thin lines indicate activity in unstimulated cycles. EMG traces for each muscle are scaled to the same arbitrary units for swing and stance. Shaded rectangles indicate the approximate region from which we measured responses. B, i–x: for each muscle the responses evoked in each group are plotted as % of the maximum response (100%) evoked in that muscle. Traces above each plot show the averaged and normalized activity of the indicated muscle during locomotion (scaled to minimum and maximum levels) as calculated from the 118 steps illustrated in Fig. 4A. FL, forelimb; HL, hindlimb.
suppression. On the other hand, all sites produced profound suppression of the activity in the iSol throughout its period of activity during hindlimb stance. Qualitatively similar responses were observed in the muscles recorded in cat RS28 (Fig. 6B), although the suppression in the iTriL was more pronounced during stance than in cat RS27. Note that the responses evoked by stimulation of the reticulospinal axons within the MLF in cat RS28 (Fig. 6B, red traces) are intermingled with those evoked from more lateral sites.

Responses Evoked by PMRF Stimulation During Quiet Standing and Reach

The responses evoked in selected muscles by PMRF stimulation at different times of the reaching movement are shown in Fig. 7 for the same stimulation site as in Fig. 5. Figure 7, A–E, illustrate, respectively, the responses evoked in different muscles by stimulation at each of the five epochs of the ipsilateral reach, as illustrated in Fig. 2B. In general, the evoked responses showed several similarities during all five epochs, even though these included static support of the body (Fig. 7, A and B), the pAPA (Fig. 7C), the transport phase of the reach (primarily flexion, Fig. 7D), and lever press (dynamic extension, Fig. 7E).

For stimulation during quiet standing, 2,000 ms before the Go signal (Pre-Cue, Fig. 7A), the responses included a weak facilitation of the activity in the ipsilateral flexor muscles iBr and iEDC (but not the iSpD) together with a suppression of the iTriL (not visible at the scale illustrated). On the contralateral side, there was no response in the coBr, a suppression of the coTriL, and facilitation of the coTri. In the ipsilateral hindlimb,
there was an increase in the iTA and suppression of the iSol. A similar pattern was observed for stimulation following the cue (Fig. 7B) and during the pAPA (Fig. 7C) epochs, except for the appearance of a small response in the iSpD (Fig. 7C). The transport phase of the reach (Fig. 7D), which we consider the analog of the swing phase of locomotion, was marked by a complete abolition of the short-latency response in the iBr. This is in contrast to the robust evoked responses observed during the swing phase of locomotion (Figs. 5 and 6). There was also a marked increase in the size of the facilitation in the
iSpD. During the lever press (Fig. 7E), the responses in all muscles were similar to the basic pattern described for Fig. 7A. During the transport phase of the contralateral reach, the responses in the ipsilateral forelimb muscles were small, or absent. In those muscles of the contralateral forelimb and the ipsilateral hindlimb in which responses were clearly visible, both the sign and the magnitude of the responses were similar to those observed during the ipsilateral reach. Thus, surprisingly, a suppression of the coTriL and a facilitation of the coTri were observed regardless of whether the contralateral forelimb was the support limb, as in Fig. 7D, or the moving limb, as in Fig. 7F.

The most striking observation in these results is the complete lack of an evoked response in the iBr muscle during the transport phase of the ipsilateral forelimb (Fig. 7D), and this despite the existence of a large response in some other ipsilateral muscles active during flexion, such as the iSpD. That this task-related gating of the response was during the transport phase of the ipsilateral forelimb (Fig. 7D) was the support limb, as in Fig. 7E, or the moving limb, as in Fig. 7F. A similar gating of activity was observed during quiet standing equally evoked clear responses for three of the four sites. When stimulation was applied during the transport phase of the reach (Fig. 8A, right), however, no clear facilitatory responses were evoked. In contrast, the same stimulation, in the same four sites, produced strong, short-latency responses in the iSpD during both reaching and locomotion, although no responses during quiet standing (Fig. 8B). Responses in the coBr (Fig. 8C) showed a response pattern qualitatively similar to those in the iBr. Stimulation during contralateral swing evoked responses from all four of the illustrated sites. In contrast, the same stimulation during the transport phase of the contralateral reach evoked facilitatory responses in three of the four sites and, in three sites, evoked instead a clear suppression of the background activity. A similar gating of activity was observed in the CIB (Fig. 8D), which, unlike the Br, remains active throughout the transport phase of the reach (Schepens and Drew 2003). During locomotion, stimulation evoked facilitation of iCIB and coCIB during the swing phase of the respective limb. During the transport phase of the same, respective, limb, a small facilitatory response was evoked from one of the illustrated sites and in the other two sites there was either no response or a suppression of activity. In this situation it is important to note that the background muscle activity during the stimulated epoch of the reach is comparable to that observed during the stimulation during the swing phase of locomotion.

The summary of the responses for selected muscles shown in Fig. 9 serves to illustrate several general points concerning the relative organization of the responses evoked by PMRF stimulation during locomotion and reach. First, the lack of any short-latency increase in the iBr during the transport phase of the reach (epoch 4 in Fig. 9), illustrated for selected tracks in Figs. 7 and 8, is confirmed for many (13/19) of the stimulated sites for cat RS27 (Fig. 9A) and all 28 sites in cat RS28 (Fig. 9B). A few sites in RS27 (n = 6) did evoke facilitatory responses during the transport phase, but these responses were generally smaller than those observed during locomotion. Four of these sites were within the NRpc, while the other two were in the NRgc. In RS28, there were large responses when stimulation was applied in epoch 3 (pAPA) but not during the transport phase of the ipsilateral reach (epoch 4). Moreover, these responses were seen not only during the pAPA preceding the ipsilateral forelimb reach but also during the pAPA (epoch 8) preceding the contralateral forelimb reach. Responses were absent during the lever press period of each limb (epochs 5 and 10). Thus the effects in the iBr can be described as a generalized facilitation during standing and the pAPA, together with suppression during reach and lever press. In contrast to the results for the iBr, there were pronounced facilitatory responses in the iSpD and the iEDC during the transport phase of the reach and these were of a similar, or larger, magnitude to those observed during locomotion (see Figs. 5 and 8). As for the iBr, the responses in the coBr during the transport phase of the contralateral limb, in both cats RS27 and RS28, were either absent or much smaller than those observed in locomotion.

Responses in the iTriL exhibited a similar pattern of activation in both cats RS27 and RS28. In general, activity in this muscle was suppressed during all stages of the reach except for the transport phase, and that for both the ipsilateral (epoch 4) and the contralateral (epoch 9) limb. In cat RS27 stimulation produced facilitation of activity from many sites during the transport phase of the ipsilateral and contralateral reach, while in cat RS28 responses during this epoch were absent (magnitude = 0), i.e., the suppression was absent but was not replaced by facilitation. In both cats there was suppression of the coTriL during the transport phase of the ipsilateral reach (epoch 4) and weak facilitation in the transport phase of the contralateral reach (epoch 9). In the iTA, facilitatory responses were evoked at all epochs of the forelimb reaching movements. As during locomotion, stimulation during the reach consistently evoked suppression of activity in the iSol. Moreover, suppression was observed at all epochs.

Note again that responses evoked from the MLF in cat RS28 (Fig. 9B) were intermingled with those from more lateral sites.

Ground Reaction Forces to PMRF Stimulation During Reach

The behavioral expression of the stimulation on the forelimbs in the form of ground reaction forces in the vertical plane is illustrated in Fig. 10A for the same stimulation site as illustrated in Fig. 7, while the responses from all sites are illustrated in Fig. 10B. As already described, stimulation during quiet standing (Fig. 10A) produced suppression of the activity in the iTriL and the coTriL, together with facilitation of the iTri and coTri. Despite the reciprocal responses in the two heads of the triceps, stimulation evoked a decrease in the vertical force under the ipsilateral forelimb and an increase in vertical force under the contralateral forelimb. Evoked changes in vertical force under the hindlimbs were smaller and more variable (not illustrated).

Overall, stimulation at the majority of sites in cat RS27 (Fig. 10B) produced decreases in vertical force in the ipsilateral forelimb and increases in the contralateral forelimb to stimulation during all epochs. During the transport phase of the
ipsilateral reach (epoch 4), the changes in vertical force under the contralateral forelimb were significantly decreased with respect to quiet standing (ANOVA and t-test with Bonferroni correction) and in some cases reversed to a small suppression. Similarly, during the transport phase of the contralateral limb (epoch 9) the suppression of vertical force in the ipsilateral forelimb was significantly less than during quiet standing and in some cases was reversed to a facilitation. Similar changes were observed in cat RS28 during the transport and lever press periods.
Comparison of Responses During Locomotion and Reach:

Summary

To summarize these data we calculated the overall averaged activity of the responses for each phase or epoch of stimulation for each muscle, after removal of outliers for each phase (see METHODS). These averages are illustrated in Fig. 11 for four selected muscles and in Figs. 12 and 13 for most of the forelimb and hindlimb muscles, respectively, recorded in cat RS27. The overall average response in the iBr was largest at the onset of swing (group 1) and declined progressively throughout swing. In the contralateral flexor, coBr (Fig. 11B), the evoked responses were maximal in group 6, i.e., 50% out of phase with the ipsilateral response; again the evoked responses during stance were minimal. The responses in the iSpD (Fig. 11C) were slightly more heterogeneous but showed the same basic organization as for the iBr, with the largest responses occurring in group 1. The coTri (Fig. 11D) was facilitated throughout the period of the normal activity of the muscle, being effectively antiphase with the responses evoked in the coBr.

During the reach, the magnitude of the overall average of the evoked responses in the iBr (Fig. 11E) during the transport phase of the reach (epoch 4) was less than that observed during the swing phase of locomotion (reduced by 78%). This was equally true when only considering those few stimulation sites that produced facilitation during the reach (Fig. 11E, see also Fig. 9). Moreover, the responses evoked during the transport phase of the ipsilateral reach were only slightly larger than those observed during quiet standing (epoch 1) or during the contralateral transport phase (epoch 9). This is quite different from the strongly phase-dependent responses observed during locomotion. A similar result was observed for the coBr, with the responses during the transport phase of the contralateral reach being substantially smaller (reduced by 96%) than those observed during locomotion. In contrast, the averaged maximal response in the iSpD during the reach was substantially larger than that observed during locomotion (increased by 123%). In this muscle the evoked responses were also substantially larger than those evoked during quiet standing or during the contralateral reach. Finally, in the coTri, the average response during the transport phase of the ipsilateral reach, when the coTri is involved in weight support (see Fig. 4C), was facilitatory and was slightly larger (increased by 21%) than both the maximal response during locomotion (group 2, Fig. 11D) and the response evoked during quiet standing (epoch 1, Fig. 11H). The evoked response was maximal during the lever press.
reach and lever press (epochs 4 and 5) when the cat was exerting a downward force to depress the lever with the ipsilateral limb. Note, however, that facilitatory responses were also observed during the transport phase of the contralateral reach (epoch 9), when that limb is not supporting the body.

Figure 12 extends this summary by comparing the average responses during reach and locomotion for several of the forelimb muscles recorded from cat RS27. Note that in this figure we illustrate the overall average percent change, calculated as the average of the percent change observed in each group (locomotion), or epoch (reach), from all 19 sites in cat RS27. Plotting the average of the percent change allows us to plot the responses from different muscles on a single graph. In this representation, a muscle in which the largest response is always evoked in group 1 during locomotion will have an overall average of 100% (during group 1). In contrast, a muscle that shows its largest response at variable times during the gait cycle (in different experiments) will have a maximum value of <100%.

The average responses evoked in all of the ipsilateral forelimb flexor muscles during locomotion were very consistent (Fig. 12A). Facilitatory responses were maximal (and close to 100%) at the onset of swing (group 1) in most muscles and were smaller or absent during stance in the majority. In the EDC, responses were maximal in group 3, again with a magnitude of 100%. During the transport phase of the ipsilateral reach (epoch 4 in Fig. 12B), responses were absent for the iClB and the iBr (as illustrated in Fig. 11) but were larger than during locomotion for the iEDC, the iSpD, and the iLtD. The responses in the contralateral flexors coBr and coClB during locomotion were generally reciprocal to those observed in the ipsilateral flexor muscles, and similar to those illustrated for the coBr in Fig. 11. Similarly, responses were weak or absent during reach (not illustrated).

Evoked responses in the forelimb extensor muscles were more variable. Mixed responses (facilitation and suppression) were evoked in most muscles (Fig. 12C). Activity in the iAcT and iSpT (latter not illustrated) was suppressed throughout the stance phase of locomotion, while activity in the iTri (and the iPaL) was suppressed only at the end of stance. Facilitation was the major effect observed in the iTri. All of the recorded extensors, except for the iPaL, were weakly facilitated by stimulation during swing. During quiet standing (Fig. 12D), activity in all five of the recorded forelimb extensors, except for the iTri, was suppressed. In contrast, during the transport phase of the ipsilateral reach (epoch 4), most muscles were facilitated. During the lever press (epoch 5), when the extensor muscles were recruited, activity in all five recorded extensor muscles was again suppressed. During the transport phase of the contralateral reach (epoch 9), when the ipsilateral extensor muscles were active in a postural context to support the cat’s weight, the responses in the iAcT (and the iSpT and iPaL) was...
equally suppressed while activity in the iTri and iTriL was facilitated to approximately the same level as that evoked during the ipsilateral reach.

In the contralateral extensor muscles that we recorded (coTri and coTriL), responses analogous to those for the ipsilateral muscles were observed during locomotion (Fig. 12E). The coTri was, on average, facilitated during the period of activity of the muscle, and the coTriL was generally suppressed. During reach (Fig. 12F) the coTri was facilitated at all epochs in both limbs. Activity in the coTriL was generally suppressed, including during the transport phase of the ipsilateral limb, but was facilitated during the transport phase of the contralateral reach as well as during lever press.

Stimulation in the PMRF caused consistent phase-dependent facilitatory responses in hindlimb flexor muscles during their period of activity (Fig. 13A) similar to those evoked in the forelimb muscles. Phase-dependent, but muscle-specific, facilitatory and suppressive responses were observed in the ipsilateral extensor muscles during the stance phase of the hindlimb gait cycle (Fig. 13C, groups 2–8). During the reach, the hindlimb muscles show postural changes during the movements of the forelimbs (Fig. 4 and Schepens and Drew 2003). In the flexor muscles of the ipsilateral hindlimb, the magnitude of the responses evoked during standing and reaching (Fig. 13B) was substantially larger than that observed during locomotion (Fig. 13A). There was a supplementary increase in the level of activity during the transport phase of the reach (epoch 4) and during the lever press (epoch 5). A similar overall increase during reach was observed in the contralateral sartorius (coSrt) as well as in the ipsilateral sartorius (iSrt) and semitendinosus (iSt) in cat RS28 (not illustrated). The ipsilateral hindlimb extensor muscles generally showed smaller changes during reach (Fig. 13D) but of the
same sign as during locomotion (Fig. 13C) and showed no clear modulation according to the stimulation phase in either RS27 (Fig. 13D) or RS28 (not illustrated). Contralateral hindlimb extensors showed a similar, unmodulated response pattern during reach (not illustrated).

The responses evoked in cat RS28 were generally similar to those illustrated for cat RS27 as described in the following section.

Comparison of Responses During Locomotion and Reach: Synthesis

The relative magnitude of the PMRF-evoked responses in shoulder and more distal flexor muscles in the transport phase of reach compared with the swing phase of locomotion is illustrated in Fig. 14 for both the ipsilateral and contralateral forelimb and for both cats RS27 and RS28. For this analysis we used the maximum values obtained during the swing phase of locomotion and during the respective reach (epoch 4 or 9). For example, for the iBr the responses during epoch 4 were compared to those during group 1 during locomotion, for the iEDC responses in epoch 4 were compared to group 3, and for the coBr responses in epoch 9 were compared to group 6 (see Fig. 9). In some muscles, as shown in Fig. 12B, there was an increase in the relative magnitude of the response. This is clearest for the shoulder muscles (iSpT, iSpD, iLtD, iAcT) but was also observed in the distal muscle, the iEDC. Most pertinent, however, is the consistently smaller level of activity evoked in some of the other flexor muscles, particularly those involved in flexion of the elbow and retraction of the shoulder. In the Br, CIB, and TrM in both the ipsilateral and contralateral forelimb and in both cats RS27 and RS28, the amplitude of the evoked responses in eight of nine muscles during reach was <25% of the amplitude evoked during locomotion; the exception was the iTM (58.9%).

The variability in the magnitude of the responses evoked from different sites (Figs. 6 and 9) raises the question as to whether the magnitude of the responses during reach is correlated to the magnitude of the responses during locomotion. In essence, does the presence of a large facilitatory response in a given muscle from a given site during locomotion predict a large response during reach, and is this relationship graded and significant? Such is clearly not the case for the iBr in cat RS27 as can be seen in the scatterplot of Fig. 15A. As one might expect from the data illustrated in Figs. 6 and 9, response magnitude during reach was clearly independent of magnitude during locomotion and responses during reach were depressed from all sites, including those producing both large and small facilitatory responses during locomotion. As a result, all of the
and none of these correlations was significant (Fig. 15). 

In contrast, many other muscles had slopes of >0.5 that were significantly different from 0. For example, the coTri (Fig. 15C) showed a significant linear relationship ($R^2 = 0.71$, $P < 0.001$) between the responses evoked in the muscle during contralateral stance during locomotion and those evoked during the transport phase of the ipsilateral reach (when the contralateral limb is weight-supporting), with a slope close to unity (1.05). Similarly, all of the ipsilateral forelimb shoulder muscles in cat RS27 had slopes of >0.5 and many of these were significant, including the iAcD, iAcT, iLtD, and iSpT (Fig. 15D). A significant relationship was also observed for the iSpD in cat RS28. Significant slopes between the evoked responses in ipsilateral reach and ipsilateral swing (contralateral stance) were observed in both contralateral elbow extensor muscles (coTri, coTriL) in both cats (Fig. 15D). Note that the contralateral extensors are naturally active at both of these periods to support the weight of the cat. In addition, slopes for the relationships of the ipsilateral extensor muscles (iTri and iTriL in cat RS27 and iTri in cat RS28) for ipsilateral swing and ipsilateral reach were also significant (Fig. 15D). A relationship for the evoked responses of the ipsilateral extensors during ipsilateral stance and contralateral reach (muscles active during both periods) was not calculated because of the inhibitory responses observed during stance. Significant slopes >0.5 were also seen in the three ipsilateral hindlimb flexors (iSrt, iSt, iTA) recorded in cat RS28 and in the iSrt in cat RS27 (Fig. 15D). The slope of the activity in the ipsilateral biventer cervicis (iBvC), recorded only in cat RS28, was also close to unity (Fig. 15D).

Several other muscles showed nonsignificant relationships in which the evoked responses during reach were mostly above the line of equivalence. For example, evoked responses in the iSpD from cat RS27 (Fig. 15E) showed larger responses during the transport phase of reaching than during the swing phase of...
EFFECT OF PMRF STIMULATION DURING LOCOMOTION AND REACHING

The present results extend these previous studies during locomotion by recording from a larger range of muscles. Most importantly, the results illustrate that the evoked responses produced by stimulation of the PMRF during reaching maintain many of the attributes of those produced during locomotion. The responses evoked in most muscles during reach were of sign and magnitude similar to those evoked during locomotion. They were equally distributed to flexors and extensors of all four limbs, including both the moving limb and those acting as postural support. However, the results also show some differences in the organization of the responses. For example, in contrast to locomotion, during which responses during the swing phase of the ipsilateral and contralateral limbs were highly reciprocal, the magnitude of the evoked responses during reaching in many muscles showed much weaker modulation according to the limb that was used. Most significantly, while stimulation of the PMRF during the swing phase of locomotion evoked strong facilitatory responses in all flexor muscles, the same stimulation during the transport phase of the reach was much less effective in producing activity in a number of these flexor muscles. This strongly suggests some differential modulation of reticulospinal signals during reaching and locomotion.

DISCUSSION

We confirm the phase-dependent nature of the modulation of descending volleys from the PMRF during locomotion as reported previously (Drew 1991; Drew and Rossignol 1984). The present results extend these previous studies during locomotion by recording from a larger range of muscles. Most importantly, the results illustrate that the evoked responses produced by stimulation of the PMRF during reaching maintain many of the attributes of those produced during locomotion. The responses evoked in most muscles during reach were of sign and magnitude similar to those evoked during locomotion. They were equally distributed to flexors and extensors of all four limbs, including both the moving limb and those acting as postural support. However, the results also show some differences in the organization of the responses. For example, in contrast to locomotion, during which responses during the swing phase of the ipsilateral and contralateral limbs were highly reciprocal, the magnitude of the evoked responses during reaching in many muscles showed much weaker modulation according to the limb that was used. Most significantly, while stimulation of the PMRF during the swing phase of locomotion evoked strong facilitatory responses in all flexor muscles, the same stimulation during the transport phase of the reach was much less effective in producing activity in a number of these flexor muscles. This strongly suggests some differential modulation of reticulospinal signals during reaching and locomotion.

Modulation of Muscle Activity During Locomotion by stimulation of PMRF

The results obtained in this study confirm and extend those obtained in our previous publications. First, we show that the phase-dependent nature of the responses in the limb muscles is true for a wide variety of muscles acting around shoulder, elbow, and wrist in the forelimb and hip, knee, and ankle in the hindlimb. Second, we show that these responses are very stereotypical, being almost identical in form in any one muscle over the entire range of the brain stem examined in this study. As in our previous publications (Drew 1991; Drew and Rossignol 1984), we suggest that the phase-dependent modulation is determined more by the activity of the spinal circuits than by changes in excitability of reticulospinal neurons in the vicinity of the stimulating electrode. This suggestion is supported by
the finding that the responses evoked by stimulation of the MLF (comprised predominantly of reticulospinal axons) were similar to those evoked from within the PMRF itself (see also Drew 1991). Third, we show that stimulation of the PMRF evokes strong responses in two distal muscles, the wrist dorsiflexor extensor carpi radialis (ECR) and the wrist and digit dorsiflexor EDC. These distal muscles were not examined in our previous study (Drew 1991), and the present result both confirms the findings of Baker (Riddle et al. 2009; see also Davidson and Buford 2006) of a strong descending input from the PMRF to muscles acting on distal muscles of the forelimb and shows that the presence of such connections is not restricted to the primate. Although it is possible that some responses may have been produced, in part, by activation of corticospinal collaterals (see, e.g., Kably and Drew 1998; Matsuyama and Drew 1997), the fact that similar responses were observed from stimulation of the MLF would suggest that most of these responses are truly produced by the reticulospinal system. Moreover, the predominance of the large responses in proximal muscles would not be expected from stimulation of the corticospinal tract.

Sampling from a larger number of extensor muscles than in our previous study in the intact cat (Drew 1991) serves to emphasize the more complex pattern of activity in these muscles than in the flexor muscles. In particular, the results emphasize the mixed nature of the facilitatory and suppressive responses evoked not only in each limb, but also in each muscle. In the ipsilateral limb, stimulation evoked suppression of activity in the majority of muscles, especially toward the end of the stance phase. In the contralateral limb, we confirmed the presence of facilitatory responses in a coTri during its period of activity but were surprised to observe suppression in the coTriL. This difference may reflect the different biomechanical functions of these two muscles, with the coTri acting only around the elbow while the coTri acts also to retract the shoulder (see Drew and Rossignol 1987).

Finally, it is worth noting that the general suppressive effect of PMRF stimulation on ipsilateral forelimb extensor muscles is also observed in the hindlimb. As illustrated in Fig. 13, stimulation of the PMRF produced a suppression of activity in most of the hindlimb extensor muscles from which we recorded, including the iGL, the ipsilateral gastrocnemius, medial head (iGM), the iSol, and the iVL. However, as in the forelimb, facilitatory responses were observed in other muscles, including the GLM.

Overall, the results from this and the other studies strongly support the suggestion that the functional expression of an induced descending reticulospinal volley as a complex pattern of facilitatory and suppressive responses in different muscles is the result of the excitability of the spinal circuits and is almost certainly determined, in great part, by the activity of the CPG (Fig. 1A) as emphasized in several other studies (Degtyarenko et al. 1998; Drew 1991; Drew and Rossignol 1984; Floeter et al. 1993; Gossard et al. 1996; Orlovsky 1972; Perreault et al. 1994). The responses are generally evoked only in one phase or the other, swing or stance, of the behavior, with a pronounced difference in the magnitude of the responses in these two phases. However, the modulation is not homogeneous. Responses vary in different groups of muscles and even within one phase of locomotion (see, for example, the iTriL). This suggests that there exist multiple interneuronal pathways that are activated by the PMRF and that each of these pathways may be subject to a differential modulation.

Modulation Of Muscle Activity During Reach by Stimulation of PMRF

Forelimb muscles: similarities between locomotion and reach. Anatomically, it is evident that the interneuronal pathways available for transmitting commands for the reticulospinal system during reaching are the same as those available to transmit commands during locomotion. However, the question is whether these anatomical substrates are equally available during different behaviors or whether some are available, or used, only during one behavior or the other. In other words, are the anatomically available pathways differentially gated during the tasks? In our original hypothesis, as detailed in the introduction, we proposed that the same interneuronal pathways would be used in both behaviors. The responses evoked by stimulation of the PMRF during the transport phase of the ipsilateral reach should therefore be similar to those evoked by stimulation during the swing phase of the ipsilateral limb during locomotion. Similarly, those evoked during contralateral reach should be similar to those observed during contralateral swing (ipsilateral stance). Our results support this proposal for many muscle groups but contradict them for some others.

The factors supporting the hypothesis include the fact that responses of the same sign are observed in most muscles acting around the shoulder during locomotion and reach. Moreover, the magnitude of the averaged maximal responses evoked in many of the shoulder muscles during reach was proportional to that observed during locomotion. Some sites produced small responses in both behaviors, while others produced large responses in both behaviors (Fig. 15D). This suggests that the same pathways mediated transmission from the reticulospinal pathways to the shoulder motoneurons. However, for some shoulder muscles the magnitude of the responses was greater during reach than during locomotion, representative of increased facilitation of transmission produced by the stimulation during reaching.

Evoked responses in the extensor muscles were also similar during reach and locomotion. For example, most of the ipsilateral extensor muscles were suppressed during quiet standing and during the lever press, and they were facilitated (or disinhibited) during the transport phase of reach, in much the same way that some of these muscles were facilitated during the swing phase of locomotion. Although these responses were similar in both behaviors they are also, to some extent, incongruous with the behavioral requirements of the task. For example, both during ipsilateral stance and during the contralateral transport phase, activity in the ipsilateral muscles, which are weight-bearing at these times, was suppressed. The reason for this suppression is not clear, but it should be emphasized that the reticulospinal system has bilateral effects and that the effect from the contralateral brain stem in the ipsilateral muscles would be one of facilitation. It is possible that the overall expression of muscle activity in the ipsilateral extensors during load bearing is a balance of suppression from the ipsilateral brain stem and facilitation from the contralateral side.

The presence of facilitatory responses in the ipsilateral extensors during the transport phase of ipsilateral reach and during swing is equally incongruous with the expected behav-
ior of these muscles. However, it should be noted that mechanical perturbation of the limb during the swing phase of locomotion (Drew and Rossignol 1987) also produces out-of-phase responses in extensors. In this situation, the extensor muscle activity has a clear functional importance in bracing the forward movement of the limb to prevent it pushing against the obstruction. The facilitation of the ipsilateral extensors by the PMRF stimulation during swing and stance may reflect a contribution of the PMRF to this type of response, perhaps as part of a spino-bulbo-spinal pathway (Drew et al. 1996; Shimamura 1973; Shimamura and Kogure 1979; Stapley and Drew 2004). The facilitatory pathway to the ipsilateral extensors may be activated by removal of the limb from the ground. This is supported by the fact that the facilitatory response during the transport phase of the limb reverted to suppression upon contact of the paw with the lever.

Forelimb muscles: differences between locomotion and reach. The most important difference in the responses evoked during locomotion and reach was the almost complete absence (gating) of the responses evoked in some of the forelimb flexor muscles during the reach, namely the Br, CIB, and TrM. This was a robust finding observed for these flexor muscles in each limb and in both cats. We believe that the lack of response reflects an active gating of the input from the PMRF rather than being the result of differences in the background activity of these flexor muscles. For example, absence, or even suppression, of responses in the cIB was observed despite a level of background activity similar to that during locomotion (Fig. 8D). The fact that background activity during reach was less than the magnitude of the response observed by stimulation during locomotion suggests that occlusion was not a major factor. This indicates the presence of a strong suppressive gating of some of these pathways during the transport phase of the reach, perhaps by the command for movement transmitted by descending pathways, including the corticospinal tract. Such a targeted suppression might serve to ensure that the less specific control signals observed in reticulospinal neurons (broad discharge patterns throughout the transport phase) do not conflict with the more precise and fractionated discharge patterns observed in the motor cortex during the same task (Yakovenko et al. 2011).

Even in those forelimb muscles in which responses were evoked in both behaviors there were some important differences. Among these was the phase or limb dependence of the responses. During locomotion, the evoked responses in most muscles were strongly phase (and thus limb) dependent. Particularly in the flexor muscles, responses were maximal in the period of activity of a given muscle during the swing phase of the respective limb and mostly silent during the stance phase. As a correlate of this, there was no activity during the swing phase of the limb contralateral to the analyzed muscle. However, during reaching, the magnitude of the responses during the transport phase of either limb was not as modulated as during locomotion. This suggests that the interneuronal pathways are more strongly gated during the swing and stance phases of locomotion than during the different behavioral epochs of the reach.

Hindlimb muscles. While the forelimbs are actively displaced during both locomotion and reaching, the hindlimbs are used quite differently in the two tasks. During locomotion they are active rhythmically, while during the reach they provide only postural support. The responses evoked in hindlimb muscles by PMRF stimulation during locomotion and reach reflect this difference in function. While the evoked responses during locomotion were strongly phase dependent, those evoked during reach varied little throughout all 10 epochs of stimulation. This suggests that there is little phase-dependent or limb-dependent gating of the reticulospinal volley in the lumbo-sacral cord during reach. However, it should be noted that the sign of the responses, particularly in the hindlimb extensor muscles, is the same during both locomotion and reaching, suggesting that the same pathways are used during locomotion and during postural support. In some of the ipsilateral hindlimb flexor muscles, however, there was a greatly increased activity during the reach compared with locomotion. This suggests an active facilitation of these pathways over and above that observed during the swing phase of locomotion. The fact that the activity in the forelimb muscles is strongly modulated while that in the hindlimb shows little phase dependence again argues for a spinal rather than a brain stem origin for phase- and limb-dependent gating.

Similar Mechanisms for Locomotion and Reaching?

A synthesis of the points made in the previous paragraphs leads us to suggest that, to a large extent, the same interneuronal pathways are being used in both behaviors but that they are differentially modulated in the two tasks. We have therefore modified the schema that we introduced in Fig. 1 to better reflect the organization suggested by the present results. First, our results support the view that the major gating of the evoked responses during locomotion, expressed in the form of phase-dependent modulation of the descending volley, originates in the rhythmic activity produced by the CPG. However, we have modified our original conceptual model (Fig. 1A) by emphasizing the presence of reticulospinal inputs to the interneuronal groups that lie outside the CPG (Fig. 16A). Whether both rhythm- and pattern-generating parts of the CPG, as proposed by McCrea et al. (McCrea and Rybak 2007; Rybak et al. 2006), should be included in this representation of the CPG or whether our interneuronal pathways may indeed represent pattern-generating pathways is not known. We have maintained a projection to the CPG to reflect the fact that stimulation of the PMRF can produce changes in cycle timing and may also reset the step cycle (Drew and Rossignol 1984; Perreault et al. 1994). Although we collapse the complexity of the circuit into a single conceptual model, we emphasize that the gating signals from the CPG are differentially modulated according to muscle group. This is clear in the complexity of the phase-dependent modulation observed in some muscles, and particularly in the extensors. The input from the cortex is again illustrated with a dashed line indicating its nonessential contribution to unobstructed locomotion. Whether the cortex modifies the output from the PMRF during unobstructed locomotion is, however, unknown.

During reaching, the reticulospinal pathways project to the same interneuronal groups, which, in this task, are obviously not influenced by the rhythm generator. Instead, we propose that during reaching the gating of activity is the result of a modulation of interneurons by the descending command for movement, including the corticospinal tract. The fact that there is a specific suppression of activity in the forelimb flexor
muscles while other muscles, such as some shoulder muscles, are facilitated suggests that this gating is likely to be at the spinal level. In Fig. 16B, the gating of the flexor muscles is represented by an inhibitory interneuron. The differential effects observed in different groups of muscles also suggest modulation of activity in specific interneuronal pathways projecting to different groups of muscles. Nonetheless, the large variability in the responses of some of the shoulder muscles during reaching (Fig. 15, E–G), despite the relative invariance of the behavior, equally raises the possibility that there is some facilitation of the activity of reticulospinal neurons in the brain stem.

In contrast to the situation during locomotion, the results suggest that apart from the strong and specific inhibition of the pathways to the flexor motoneurons, the gating to most pathways is not as strong as during locomotion. This is implicit in the results showing that the magnitude of the responses is not as clearly dependent on the activity of a given limb as during locomotion. In addition to the gating from descending pathways, it is probable that feedback from the paws is also important in modulating activity as discussed above for the effects observed during locomotion. This activity might be particularly important in modifying the responses to the extensors depending on whether the limb is in contact, or not, with a support surface.

Comparison with Other Studies

There are few other studies that have examined gating of supraspinal activity during reaching, and only one other group that we are aware of has examined this in the reticulospinal system, and that in the primate. Davidson and Buford (2004, 2006) used stimulus-triggered averaging to examine the pattern of activity evoked in arm muscles by stimulation of the PMRF. They found two results that are pertinent to our study. First, they reported that stimulation at different stages of the movement, or when movements were made in different directions (Davidson and Buford 2004) or even with different limbs (Davidson and Buford 2006), made no difference to the responses that were evoked. This is different from what we report, although as we emphasize the relative difference in the pattern of the responses to stimulation at different periods is

Fig. 16. Conceptual models illustrating possible gating controls during locomotion and reach. A: modified version of the model illustrated in Fig. 1A emphasizing the existence of reticulospinal projections that influence muscle activity both via the CPG as well as through interneuronal pathways (contained within the rectangle) that are influenced by, but are not part of, the CPG. The interneuronal pathways include separate channels to arm flexor muscles and other shoulder muscles, but these are influenced similarly during locomotion. B: modified version of the model illustrated in Fig. 1B for the situation during reaching. This model contains only the interneuronal pathways that are outside the CPG. During reach, there is input from the RST to these interneuronal pathways as well as input from the CST. This latter tract is suggested to suppress activity in flexor interneurons influenced by the RST and to facilitate other interneurons influenced by the RST. In this schema, the CST initiates and regulates limb movement via a separate population of interneurons that is not influenced by the RST. See text for other explanations. Sh, shoulder.
much less than during locomotion. As pointed out by Davidson and Buford (2006), this may reflect the difference in postures and context, particularly the need for load bearing during both locomotion and our reaching task. However, it probably also reflects differences in the protocols in that we specifically designed these experiments to detail the responses at specific phases of the task. The second finding was that the weakest responses were in the forelimb flexor muscles that they studied. Very few sites produced facilitation of any of the ipsilateral arm flexor muscles studied, namely, the ipsilateral and contralateral biceps brachii and iBr, and none produced facilitation of the coBr (Davison and Buford 2006). This is very similar to what is reported in our study and supports our view that signals from the PMRF to the arm flexor muscles are suppressed during voluntary movements. In contrast, they reported strong responses in shoulder muscles, as we also found in our study. Together, these findings strongly support our contention that there is a specific suppression of the pathway from the PMRF to some of the arm flexor muscles during the reach.

The responses evoked by stimulation of the PMRF can be usefully contrasted with those observed to motor cortical stimulation. For example, Cheney (Park et al. 2004) showed that stimulus-triggered averaging of the responses produced by single stimuli to the primate monkey cortex during a complex reaching task produced strong and frequent facilitation of the Br. Similarly, we have found that stimulation of the motor cortex during the transport phase of the reach frequently evokes responses in the coBr (Drew et al., unpublished observations). Lemon (Lemon et al. 1995) showed that transcranial magnetic stimulation during different phases of a reach and grasp movement in humans also produced strong facilitation of arm and hand muscles, including the forearm flexor brachioradialis.

### Relationship to Cell Activity During Behavior

The discharge characteristics of reticulospinal neurons have been detailed both in intact cats during locomotion (Drew et al. 1986; Matsuyama and Drew 2000) and reaching tasks (Schepens et al. 2008; Schepens and Drew 2004, 2006) as well as during reaching in primates (Buford and Davidson 2004; Soteroopoulos et al. 2012). All of these studies have emphasized the complexity of the discharge patterns of reticulospinal neurons, and the results show that few cells discharge during the locomotor phases of the transport phase in the same way as motor cortical neurons (Drew 1993; Griffin et al. 2008; Krouchev and Drew 2013; Lavoie and Drew 2002; Yakovenko et al. 2011). Moreover, while most pyramidal tract neurons discharge only, or predominantly, during movement of a single limb, many reticulospinal neurons discharge during movements of multiple limbs (Drew et al. 1986; Prentice and Drew 2001; Shimamura and Kogure 1983).

The results from the present stimulation study are fully compatible with the conclusions drawn from our previous unit recording studies. Cells active during reach of both the ipsilateral and contralateral forelimbs are likely to influence muscle activity on both sides of the body, and the sign and magnitude of this interaction will vary with the limb moved and the epoch of the movement under study. A given cell active during reach of a given limb will therefore likely produce a differential and bilateral modification of the activity of a wide range of muscles. However, given the results obtained in this stimulation study, cell activity during reach may be prevented from influencing the activity of the muscles involved in flexing the arm. This would suggest that the primary effect of reticulospinal activity during a reach is on the more postural activity (including both joint stabilization and equilibrium) than on the muscles involved primarily in controlling the active movement.

We emphasize, however, that these results do not imply a complete lack of any specificity in the information content of reticulospinal cells. Stimulation in the PMRF is likely to activate a quite diverse population of cells, in contrast to stimulation in the motor cortex, where the stimulation will activate a more homogeneous population. In addition, the stimulation will also activate axons of passage, possibly leading to activation of cells distant from the site of the stimulation (see Davidson and Buford 2004 and Drew and Rossignol 1990a for a fuller discussion of this point). As such, we have to consider that the stimulation effects demonstrated in this study may, to some extent, reflect a combination of more specific and heterogeneous response effects or may mask more specific effects. For example, during both locomotion and reaching some reticulospinal neurons do have quite restricted discharge patterns and the termination patterns of reticulospinal axons are quite variable (see, e.g., Matsuyama et al. 1988, 1993, 1997). In this respect our spike-triggered averaging studies (Schepens and Drew 2006) are important in showing that activity in some cells does directly suppress activity in ipsilateral extensor muscles, consistent with the results from this study. Moreover, this suppression is observed only during quiet standing and contralateral reach and is not seen (i.e., is gated) during the ipsilateral reach, as are the stimulation effects. This latter observation would be compatible with the proposed role of peripheral afferent input in gating PMRF responses from suppression during standing to facilitation during reach.

### Summary and Conclusions

In this study we have compared the effects of transiently activating the reticulospinal system on the natural muscle activity underlying locomotion and reaching. The results emphasize the importance of the CPG in producing the strongly phase-dependent responses that are generally observed in flexors and extensors during swing and stance. These responses are strongly reciprocal, generally being present in one phase and largely absent in the other. In contrast, the responses during reaching, with the exception of some arm flexors, are not subject to the same rigorous modulation, with similar levels of activity frequently being observed during quiet standing and during transport of either limb. We suggest that this provides more flexibility for the PMRF to influence motor activity in different contexts during discrete voluntary movements than during locomotion, in which behavior the response is more strictly regulated by the CPG. At the same time, we suggest that the expression of the response is dependent to a certain extent on the excitability of the interneurons onto which the axons of the cells impinge, and we further suggest that this excitability is modified by activity in other descending pathways, including the corticospinal tract. In the case of some of the forelimb flexor muscles, the modulation of the interneuronal excitability is sufficient to completely suppress the influ-

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ence of the reticulospinal system during the transport phase of the limb. We suggest that this is a mechanism to ensure precise control over the targeted muscles while still permitting more generalized, postural responses in the muscles of the supporting limbs. It is to be noted that shoulder flexor muscles are unaffected or even facilitated by the PMRF during reaching, perhaps reflecting the relative lack of control over shoulder and axial muscles by the motor cortex in the cat (Armstrong and Drew 1984b, 1985; Yakovenko et al. 2011) and the relative importance of the PMRF for their control.

Finally, it is interesting to speculate that if reticulospinal activation of these forelimb muscles is suppressed by the corticospinal system, loss of this suppression, for example, by cortical infarct, should release this pathway to reticulospinal control. Such an effect is supported by the findings of Zaaimi et al. (2012) of increased reticulospinal efficacy following pyramidal tract lesion in the primate.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: K.S.D., J.-P.M., and T.D. analyzed data; K.S.D., J.-P.M., and T.D. prepared figures; K.S.D. drafted manuscript; K.S.D., J.-P.M., and T.D. approved final version of manuscript; J.-P.M. and T.D. performed experiments; T.D. conceived and designed research; T.D. edited and revised manuscript.

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