

RESEARCH ARTICLE

Control of Movement

Forelimb movements contribute to hindlimb cutaneous reflexes during locomotion in cats

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Abstract

During quadrupedal locomotion, interactions between spinal and supraspinal circuits and somatosensory feedback coordinate forelimb and hindlimb movements. How this is achieved is not clear. To determine whether forelimb movements modulate hindlimb cutaneous reflexes involved in responding to an external perturbation, we stimulated the superficial peroneal nerve in six intact cats during quadrupedal locomotion and during hindlimb-only locomotion (with forelimbs standing on stationary platform) and in two cats with a low spinal transection (T12–T13) during hindlimb-only locomotion. We compared cutaneous reflexes evoked in six ipsilateral and four contralateral hindlimb muscles. Results showed similar occurrence and phase-dependent modulation of short-latency inhibitory and excitatory responses during quadrupedal and hindlimb-only locomotion in intact cats. However, the depth of modulation was reduced in the ipsilateral semitendinosus during hindlimb-only locomotion. Additionally, longer-latency responses occurred less frequently in extensor muscles bilaterally during hindlimb-only locomotion, whereas short-latency inhibitory and longer-latency excitatory responses occurred more frequently in the ipsilateral and contralateral sartorius anterior, respectively. After spinal transection, short-latency inhibitory and excitatory responses were similar to both intact conditions, whereas mid- or longer-latency excitatory responses were reduced or abolished. Our results in intact cats and the comparison with spinal-transected cats suggest that the absence of forelimb movements suppresses inputs from supraspinal structures and/or cervical cord that normally contribute to longer-latency reflex responses in hindlimb extensor muscles.

NEW & NOTEWORTHY During quadrupedal locomotion, the coordination of forelimb and hindlimb movements involves central circuits and somatosensory feedback. To demonstrate how forelimb movement affects hindlimb cutaneous reflexes during locomotion, we stimulated the superficial peroneal nerve in intact cats during quadrupedal and hindlimb-only locomotion as well as in spinal-transected cats during hindlimb-only locomotion. We show that forelimb movement influences the modulation of hindlimb cutaneous reflexes, particularly the occurrence of long-latency reflex responses.

cutaneous reflexes; interlimb coordination; locomotion; spinal cord injury

INTRODUCTION

Proper integration of cutaneous feedback within central neural circuits is required to execute specific motor tasks and perform necessary movement corrections. For instance, during

locomotion when the foot dorsum contacts an obstacle during the swing phase, a coordinated reflex response, termed the stumbling corrective reaction, allows the perturbed limb to step away from and over the obstacle to avoid falling, as shown in cats and humans (1–11). Electrically stimulating cutaneous

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Submitted 15 March 2024 / Revised 15 April 2024 / Accepted 25 April 2024



nerves of the foot also elicits a coordinated reflex response, consistent with the stumbling corrective reaction in cats and humans (1, 4, 7, 8, 10–18). In cats, during the swing phase, short-latency (P1; ~8–12 ms) and mid-latency (P2; ~20–25 ms) excitatory responses in ipsilateral hindlimb (i.e., the limb stimulated) muscles that flex the knee and ankle joints are observed after electrical stimulation, resulting in limb flexion. To maintain balance, the activity of extensor muscles of the contralateral hindlimb (i.e., the limb opposite stimulation) is reinforced via mid-latency (P2) excitatory responses. The same stimulus applied during stance generates a short-latency inhibitory response (N1; latency of ~8–12 ms) in ipsilateral extensor muscles to briefly pause stance, referred to as the stumbling preventive reaction, which is followed by mid-latency (P2) or long-latency (P3; ~35 ms) responses to then assist with forward propulsion (6, 15). Phase-dependent modulation ensures that cutaneous reflexes are functionally relevant (19). Reflex responses, mainly at mid- and long latencies, evoked by stimulating nerves of the foot are also observed in arm/forelimb muscles in cats and humans (4, 20–23). These responses are also modulated with phase. Thus, cutaneous reflexes of each foot are distributed to the four limbs and are part of a whole body response to a perturbation (6).

During quadrupedal locomotion in terrestrial mammals and bipedal locomotion in humans, all four limbs contribute to dynamic stability and proper coordination. A bidirectional influence between the arms and legs has been demonstrated in humans by showing that the position or rhythmic movement of the arms or legs can influence the activity of the legs or arms, respectively (4, 24–37). For instance, in humans, stretch or tendon tap reflexes in leg muscles are modified by the position of the arm (25). Rhythmic arm cycling reduced soleus H-reflex amplitude in humans when the legs were in a static position (28). Passive shoulder flexion-extension movements facilitated the soleus H reflex (30), whereas active arm swinging movements reduced its amplitude (29). Using a transverse split-belt treadmill (unequal speed of the fore- and hindlimbs), studies found that movement of the forelimbs influenced the activity of the hindlimbs and vice versa in decerebrate cats (38) as well as in intact adult cats and kittens (39–41). Interestingly, these bidirectional influences appear asymmetric, with a greater influence of the forelimbs/arms on the hindlimbs/legs in cats (20, 38, 41) and humans (42). How forelimb movements contribute to reflex modulation in the hindlimbs of quadrupeds is not known.

We recently compared the hindlimb pattern of intact cats during quadrupedal treadmill locomotion and during hindlimb-only locomotion where the forelimbs were placed on a stationary platform as well as cats after spinal transection (i.e., spinal cats) during hindlimb-only locomotion (43). We found that the spatiotemporal pattern as well as the activity of hindlimb muscles during hindlimb-only locomotion in spinal cats more closely resembled the pattern during hindlimb-only locomotion of intact cats. Thus, in intact cats, forelimb movements shape the hindlimb locomotor pattern. The goal of the present study was to investigate the contribution of forelimb movements to hindlimb cutaneous reflexes in intact cats by comparing responses during quadrupedal locomotion and hindlimb-only locomotion. In the second part of this study, we compared these data with hindlimb cutaneous reflexes obtained after a complete

spinal transection. We hypothesized that forelimb movements contribute to the modulation of hindlimb cutaneous reflexes during locomotion.

MATERIALS AND METHODS

Animals and Ethical Information

All procedures were approved by the Animal Care Committee of the Université de Sherbrooke and were in accordance with policies and directives of the Canadian Council on Animal Care (Protocol 442-18). Six adult cats, four males and two females, weighing between 3.7 and 6.5 kg (5.0 ± 1.0) were used in the present study. We followed ARRIVE guidelines for animal studies (44). Before and after experiments, cats were housed and fed in a dedicated room within the animal care facility of the Faculty of Medicine and Health Sciences at the Université de Sherbrooke. As part of our effort to maximize the output of each animal, cats were used in other studies to answer different scientific questions, many of which are in preparation.

Surgical Procedures, Electrode Implantation, and Spinal Transection

Surgeries were performed under aseptic conditions with sterilized instruments in an operating room. Before surgery, cats first received an intramuscular injection containing ketamine (0.05 mL/kg), butorphanol (0.4 mL/kg), and dexmedetomidine (0.06 mL/kg). Once sedation was visible, the cat was placed on a heating mat and covered with a blanket. A long-lasting Optixcare eye gel (hyaluronic acid) was administered. The bladder was emptied by manual pressure, and vital signs were monitored, including heart rate (Masimo SET Radical-7 pulse Co-Oximeter), respiratory rate (Datex Ohmeda Capnomac Ultima), mucous membrane color, and body temperature (PetMap graphic II), as well as depth of sedation. In the surgical room, cats were then anesthetized with isoflurane (1.5–3%) and O₂ (1 L/min) with a mask and then intubated with a flexible endotracheal tube. Cats received a continuous infusion of lactated Ringer solution (3 mL/kg/h) during the surgery through a catheter placed in a cephalic vein. Anesthesia was maintained by adjusting isoflurane concentration as needed and by monitoring cardiac and respiratory rates. Body temperature was monitored with a rectal thermometer and maintained within physiological range ($38 \pm 0.5^\circ\text{C}$) with a water-filled heating pad placed under the animal and an infrared lamp ~50 cm over it. The depth of anesthesia was confirmed by applying pressure to a paw (to detect limb withdrawal) and by assessing the size and reactivity of pupils. Before surgery, the animal's skin was cleaned with chlorhexidine soap and meloxicam (0.2 mg/kg), an anti-inflammatory agent, was injected subcutaneously along with an anesthetic block at the incision sites (2:1 bupivacaine 0.25%-lidocaine 2.0%). After surgery, we taped a transdermal fentanyl patch (25 µg/h) to the back of the animal 2–3 cm rostral to the base of the tail for prolonged analgesia (removed after 5–7 days). Cats were placed in an incubator until they regained consciousness. Meloxicam and chloramphenicol (Chlor Palm), an anti-inflammatory and an antibiotic, were given orally for 4 and 5 consecutive days, respectively. At the conclusion of the experiments, cats

received a lethal dose (120 mg/kg) of pentobarbital sodium through the cephalic vein.

Electrode implantation.

To record the electrical activity of muscles [electromyography (EMG)], a skin incision was made at the midline of the skull and muscles and fascia were retracted laterally. Pairs of Teflon-insulated multistrain fine wires (AS633; Cooner Wire, Chatsworth, CA) were directed subcutaneously from two head-mounted 34-pin connectors (Omnetics Connector, Minneapolis, MN). Skin and fascial incisions were made to expose selected hindlimb muscles, and electrodes were sewn into the belly for bipolar recordings, with 1–2 mm of insulation stripped from each wire. The head connector was secured to the skull with six screws and dental acrylic. Electrode placement was verified during surgery by electrically stimulating each muscle through the appropriate head connector channel. The present dataset includes EMG from the following muscles bilaterally: soleus (SOL, ankle extensor), vastus lateralis (VL, knee extensor), biceps femoris anterior (BFA, hip extensor), anterior sartorius (SRT, hip flexor and adductor/knee extensor), semitendinosus (ST, knee flexor/hip extensor), and tibialis anterior (TA, ankle flexor). For nerve stimulation, pairs of Teflon-insulated multistrain fine wires (AS633; Cooner Wire) were passed through a silicon tubing. A horizontal slit was made in the tubing, and wires within the tubing were stripped of their insulation. The ends protruding through the cuff were knotted to hold the wires in place and glued. The ends of the wires away from the cuff were inserted into four-pin connectors (Hirose or Samtec) for bipolar nerve stimulation. Cuff electrodes were directed subcutaneously from head-mounted connectors and placed around the left and right superficial peroneal (SP) nerves at ankle level. At this level, the SP nerve is purely cutaneous (45).

Spinal transection.

The skin was incised over the last thoracic vertebrae, and after muscle and connective tissue were carefully set aside, a small dorsal laminectomy was made. After exposure of the spinal cord, xylocaine (lidocaine hydrochloride, 2%) was applied topically, followed by two or three intraspinal injections. The spinal cord was then completely transected with surgical scissors between the 12th and 13th thoracic vertebrae. We cleaned the 0.5-cm gap between the two cut ends of the spinal cord and stopped any residual bleeding. We verified that no spinal cord tissue remained connecting rostral and caudal ends, which we later confirmed histologically. A hemostatic agent (Spongostan) was placed within the gap, and muscles and skin were sewn back to close the opening in anatomic layers. After spinal transection, we manually expressed the cat's bladder and large intestine one or two times daily, or as needed.

Data Collection and Analysis

The main goal was to assess the modulation of cutaneous hindlimb reflexes during quadrupedal and hindlimb-only locomotion in the intact state. In two cats, we also assessed the modulation of hindlimb cutaneous reflexes during hindlimb-only locomotion 6 wk after spinal transection. During experiments, cats performed tied-belt (equal left-right

speeds) locomotion in quadrupedal and hindlimb-only conditions on a treadmill with two independently controlled running surfaces 120 cm long and 30 cm wide (Bertec) at a treadmill speed of 0.4 m/s. In the hindlimb-only condition, the forelimbs were placed on a stationary platform. In the spinal state, we collected data with perineal stimulation. For perineal stimulation, an experimenter manually pinched the skin under the tail with the index finger and thumb. We did not provide weight support, although an experimenter gently held the tail to provide equilibrium. Videos of the left and right sides were obtained with two cameras (Basler AcA640-100g) at 60 frames/s with a spatial resolution of 640×480 pixels. A custom-made program (LabVIEW) acquired the images and synchronized them with EMG data. EMG signals were preamplified ($10\times$, custom-made system), band-pass filtered (30–1,000 Hz), and amplified ($100\text{--}5,000\times$) with a 16-channel amplifier (A-M Systems model 3500). EMG data were digitized (5,000 Hz) with a National Instruments card (NI 6032E), acquired with custom-made acquisition software, and stored on a computer. We electrically stimulated the SP nerve with a Grass S88 Stimulator at an intensity of 1.2 times the motor threshold, defined as the voltage required to elicit a small, consistent short-latency (~ 10 ms) excitatory EMG response in an ipsilateral flexor, such as semitendinosus (ST). We determined the motor threshold during quadrupedal locomotion and applied the same stimulation intensity during hindlimb-only locomotion. This stimulation intensity mainly activates large-diameter A β afferents (8, 15, 46–48). Each stimulation consisted of a train of three 0.2-ms pulse durations at 300-Hz frequency. During a trial, ~ 120 stimuli were delivered pseudorandomly every two to four locomotor cycles at varying delays relative to an ipsilateral hindlimb extensor burst onset to evoke responses at different times during the step cycle. We obtained a minimum of five stimuli per bin in each condition for all cats. Each locomotor condition tested lasted 6–9 min. For a given nerve stimulation, we collected data for both locomotor conditions within a single session and at the same stimulation intensity.

Kinematic data were analyzed as described previously (43, 49, 50). By visual detection, we determined hindlimb contact as the first frame where the paw made visible contact with the treadmill surface and limb liftoff as the most caudal displacement of the toe. Cycle duration corresponded to the interval of time between consecutive paw contacts of the same limb. Stance duration was defined as the interval of time from initial contact to liftoff of the same limb. Swing duration was calculated as the difference between cycle duration and stance duration. We measured the proportion of the stance and swing phases as a percentage of cycle duration. We measured the temporal and spatial symmetry indexes because left-right symmetry has been shown to influence reflex modulation (21, 51). We measured the temporal phase interval by dividing the interval of time between stance onsets of the right and left hindlimbs by right hindlimb cycle duration. For the gap interval, we first measured step length as the distance between the leading and trailing limb at stance onset of the leading limb and stride length as the horizontal distance traveled from stance offset to onset plus the distance traveled by the contralateral treadmill belt during the swing phase. Then, we divided right hindlimb

step length by right hindlimb stride length. For the temporal symmetry index, we measured the absolute deviation of the temporal phase interval from a perfect symmetry of 0.5. For the spatial symmetry index, we measured the absolute deviation of the gap interval from a perfect symmetry of 0.5. Symmetry indexes were then multiplied by 100 and expressed as a percentage.

Reflex data were analyzed as described previously (20, 51, 52). We removed sections from analysis where the cat stepped irregularly or removed its forelimbs from the platform in the hindlimb-only condition based on EMG and video data. The step cycle was synchronized to the soleus (SOL) extensor burst onset in the stimulated limb (the ipsilateral limb). Cycles were tagged as stimulated (i.e., cycles with stimulation) or control (i.e., cycles without stimulation) and divided into 10 subphases (bins) of equal duration. Control cycles were averaged, rectified, and separated into 10 subphases to provide a baseline locomotor EMG (bEMG) in each bin. Stimulated cycles were averaged, rectified, and separated in each bin according to the time when stimulation occurred within the cycle. To quantify reflex responses, a window of 80 ms around the stimulation was opened and the bEMG was superimposed on the EMG averaged from stimulated cycles for the 10 bins. The same experimenter (J.H.) determined onset and offset responses by visual inspection, defined as prominent positive or negative deflections away from the bEMG, using previous studies as guidelines (20, 48, 51–55) and 97.5% confidence intervals. Short-latency excitatory (P1) and inhibitory (N1) responses in ipsilateral and contralateral limbs started at 9–18 ms after stimulation. Mid-latency (20–25 ms) excitatory and inhibitory responses are termed P2 and N2 responses, respectively. Long-latency (35 ms) excitatory and inhibitory responses are termed P3 and N3 responses, respectively. We found N2 and N3 responses inconsistently and excluded them from further analysis.

The EMGs of reflex responses were integrated and then subtracted from the integrated bEMG in the same time window to provide a net reflex value. This net reflex value was then divided by the integrated bEMG value to determine whether reflex modulation is independent from bEMG activity (20, 51, 52, 56–59). Phase and condition (quadrupedal and hindlimb-only locomotion) modulation was illustrated by normalizing reflex responses in each muscle to the maximal value (expressed as a percentage) obtained in one of the two locomotor conditions. To determine the effect of the locomotor condition on the phase-dependent modulation, we obtained a reflex index by measuring the difference between the largest and smallest responses out of the 10 bins for each locomotor condition (20, 51, 52).

To characterize EMG bursts, we selected 15–30 control cycles. The onset and offset of bursts in the selected muscles were identified through visual examination (J.H.) using a custom-made program applied to the raw EMG waveforms. For muscles with a double burst in one or both conditions, such as ST and TA, we measured only a single burst. In ST and TA, we measured the burst occurring at the stance-to-swing transition and during swing, respectively. The burst durations were then expressed as a percentage of the cycle duration. Mean EMG amplitude was measured by integrating the full-wave rectified EMG burst from onset to offset

and dividing it by its burst duration. Subsequently, we normalized this value by the maximal value obtained in one of the two locomotor conditions.

Statistical Analysis

We quantified reflex responses in the ipsilateral and contralateral SOL, VL, BFA, SRT, ST, and TA. In five of six intact cats, both the left and right SP nerves evoked responses. We treated responses evoked by left and right nerves separately and pooled them for statistical analysis ($n = 11$ nerves stimulated in 6 cats). To compare the occurrence of responses in the two locomotor conditions, we performed a chi-square test on the presence or absence of reflex responses across bins. To assess phase-dependent modulation, we performed a nonparametric one-factor (bins) Friedman test on short-, mid-, and longer-latency responses (N1, P1, P2/P3) for pooled data. In muscles that showed P2 and P3 responses, we pooled them because we believe they are mediated by the same pathway and depend on N1 duration. We used the Wilcoxon signed-rank test and determined the effect size (r value) on pooled data to assess the effect of locomotor condition on the proportion of stance and swing durations, EMG burst durations and amplitudes, as well as symmetry indexes and reflex modulation indexes. Statistical significance for all tests was set at $P < 0.05$.

RESULTS

Modulation of Hindlimb Muscle Activity during Quadrupedal and Hindlimb-Only Locomotion

In the present study, the main focus was to investigate cutaneous reflexes during quadrupedal and hindlimb-only locomotion in intact cats at a treadmill speed of 0.4 m/s. We recently published a study characterizing the locomotor pattern, including electromyography (EMG) in selected hindlimb muscles, in the two locomotor conditions over a range of treadmill speeds (43), which revealed significant differences in spatiotemporal parameters, such as reduced cycle and stance durations, as well as shortened step and stride lengths during hindlimb-only locomotion compared with quadrupedal locomotion. Here, we present findings that can influence cutaneous reflexes, such as changes in EMG activity. During hindlimb-only locomotion, we observed changes in EMG activity, such as a longer semitendinosus (ST) burst and double bursts in the iliopsoas and anterior sartorius (SRT) (Fig. 1A). Note the shorter cycle and stance durations as well as double bursts in the SRT and tibialis anterior (TA) during hindlimb-only locomotion. However, the proportions of stance ($P = 0.249$; $r = 0.470$) and swing ($P = 0.249$; $r = 0.470$) durations as a function of cycle duration were similar between hindlimb-only and quadrupedal locomotion (Fig. 1B). The normalized burst durations of vastus lateralis (VL) ($P = 0.046$; $r = 0.727$; +7%) and biceps femoris anterior (BFA) ($P = 0.046$; $r = 0.813$; +8%) were significantly longer during hindlimb-only locomotion, whereas the normalized durations of the swing-related burst of SRT ($P = 0.028$; $r = 0.898$; –10%) and TA ($P = 0.028$; $r = 0.898$; –7%) muscles were significantly shorter compared with quadrupedal locomotion (Fig. 1C). We found a significant decrease in EMG burst amplitude for soleus (SOL) ($P = 0.028$; $r = 0.898$; –10%) and

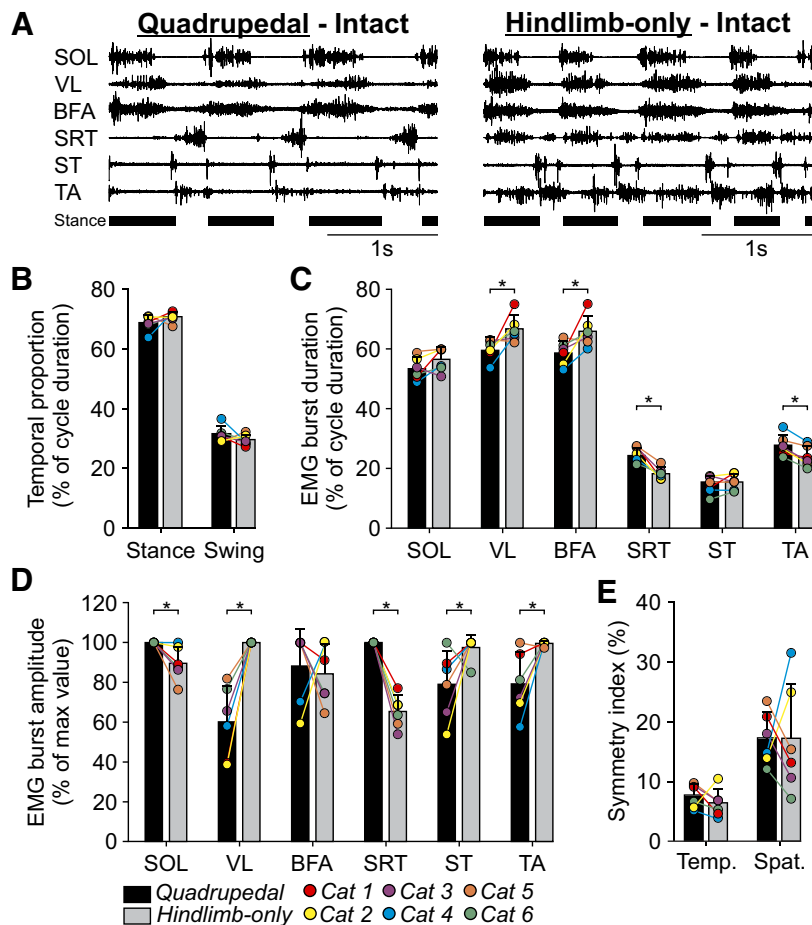


Figure 1. Electromyographic (EMG) activity and kinematic characteristics of quadrupedal and hindlimb-only locomotion in intact cats at 0.4 m/s. **A:** EMG activity from 6 hindlimb muscles along with stance phases (horizontal thick lines) of the right (R) hindlimb in 1 intact cat during quadrupedal and hindlimb-only locomotion. Data are from cat 4. BFA, biceps femoris anterior; SOL, soleus; SRT, anterior sartorius; ST, semitendinosus; TA, tibialis anterior; VL, vastus lateralis. **B:** stance and swing durations expressed as a percentage of cycle duration. **C:** EMG burst duration expressed as a percentage of cycle duration. Note that for SRT and TA we only measured the flexor/swing-related burst. **D:** EMG burst amplitudes expressed as a percentage of the maximum value obtained in 1 of the 2 conditions. **E:** temporal (Temp.) and spatial (Spat.) symmetry indexes, calculated by measuring the relative deviation of the phase and gap intervals from a perfect out-of-phase interval of 0.5, expressed as a percentage. Each bar is the mean $\pm$ SD for the group ($n = 6$ cats). Individual data are shown with different colored circles. Significant effect of condition (Wilcoxon): * $P < 0.05$.

SRT ($P = 0.028$; $r = 0.898$; -34%) muscles during hindlimb-only locomotion compared with quadrupedal locomotion, whereas a significant increase was observed for VL ($P = 0.028$; $r = 0.898$; $+39\%$), ST ($P = 0.046$; $r = 0.641$; $+18\%$), and TA ($P = 0.046$; $r = 0.813$; $+20\%$), with no significant difference for BFA ($P = 0.753$; $r = 0.042$) (Fig. 1D). We also measured temporal and spatial symmetry indexes during quadrupedal and hindlimb-only locomotion to assess the consistency of left-right coordination on a step-by-step basis between the two tasks and found no significant differences for temporal ($P = 0.345$; $r = 0.384$) or spatial ($P = 0.917$; $r = 0.042$) indexes (Fig. 1E).

Cutaneous Reflex Responses in Ipsilateral Hindlimb Muscles

Table 1 shows the occurrence of ipsilateral and contralateral responses for each muscle with SP nerve stimulation. For a given muscle, the number of nerves stimulated ($n = 11$ in 6 cats) where N1, P1, or P2/P3 responses were observed is represented as a fraction of the total number of nerves stimulated, with the percentage indicated in parentheses. We also calculated the number of bins where responses were observed as a fraction of the total number of bins, with the percentage indicated in parentheses. We used chi-square tests to determine whether reflex response occurrence differed between locomotor conditions by comparing the number of bins with a response. To investigate the phase- and

condition-dependent modulation of cutaneous reflexes, we stimulated the SP nerve at different times during the step cycle and recorded responses in ipsilateral and contralateral muscles in both tasks.

To facilitate comparisons between conditions and muscles, we organized Figs. 2–4 in the same way. In these figures, the two leftmost panels show reflex responses evoked in the 10 bins during quadrupedal and hindlimb-only locomotion for a single cat. To illustrate the deviation of the responses from the normal EMG pattern, the EMG from cycles that received stimulation (black traces) was averaged and superimposed on the averaged EMG from cycles without stimulation (the bEMG in gray). On the right of each panel, we show the average rectified EMG activity of the muscle from control cycles and the stance phase normalized to ipsilateral SOL onset. For each nerve stimulation, we normalized responses in each bin to the largest response obtained out of both conditions. Thus, responses in some bins are less visible because of this scaling, but they were still analyzed. The scatterplots to the right of the individual cat data show normalized reflex response amplitudes for pooled data. Periods of muscle activity in both locomotor conditions are shown above the scatterplots in the two conditions.

We first describe cutaneous reflexes in the ipsilateral SOL (Fig. 2), a uniarticular ankle extensor (plantar flexor) that activated before hindlimb contact and remained active for $\sim 80\%$ of the stance period. When SOL was active, stimulating the SP

Table 1. Occurrence of responses elicited by SP nerve stimulation in ipsilateral and contralateral hindlimb muscles during quadrupedal and hindlimb-only locomotion in intact cats

	iSOL	iVL	iBFA	iSRT	iST	iTA	cSOL	cVL	cBFA	cSRT
N1										
Quad										
# of n.	11/11	9/9	11/11	4/8		3/10				3/8
% of n.	(100%)	(100%)	(100%)	(50%)		(30%)				(38%)
# of bins	64/110	59/90	66/110	27/40		12/30				8/30
% of bins	(58%)	(66%)	(60%)	(68%)		(40%)				(27%)
Hind-only										
# of n.	11/11	9/9	8/11	8/8		3/10				6/8
% of n.	(100%)	(100%)	(73%)	(100%)		(30%)				(75%)
# of bins	65/110	63/90	52/80	59/80		13/30				13/60
% of bins	(59%)	(70%)	(65%)	(74%)		(43%)				(22%)
P value	0.891	0.587	0.058	1.0^{e-4}		0.686				0.251
Direction of change	=	=	=	↑		=				=
P1										
Quad										
# of n.	4/11		1/11		10/10	5/10				
% of n.	(36%)		(9%)		(100%)	(50%)				
# of bins	15/40		4/10		55/100	36/50				
% of bins	(38%)		(40%)		(55%)	(72%)				
Hind-only										
# of n.	3/11		1/11		10/10	5/10				
% of n.	(27%)		(9%)		(100%)	(50%)				
# of bins	10/30		3/10		50/100	24/50				
% of bins	(33%)		(30%)		(50%)	(48%)				
P value	0.288		0.701		0.500	0.098				
Direction of change	=		=		=	=				
P2/P3										
Quad										
# of n.	8/11	6/9	10/11	2/8	9/10	8/10	9/11	5/9	5/11	5/8
% of n.	(73%)	(67%)	(91%)	(25%)	(90%)	(80%)	(82%)	(56%)	(45%)	(63%)
# of bins	49/80	25/60	55/100	7/20	41/90	43/80	58/90	37/50	37/50	22/50
% of bins	(61%)	(42%)	(55%)	(35%)	(46%)	(54%)	(64%)	(74%)	(74%)	(44%)
Hind-only										
# of n.	3/11	5/9	2/11	4/8	8/10	9/10	6/11	4/9	1/11	8/8
% of n.	(27%)	(56%)	(18%)	(50%)	(80%)	(90%)	(55%)	(44%)	(9%)	(100%)
# of bins	10/30	19/50	7/20	15/40	29/80	52/90	43/60	27/40	5/10	38/80
% of bins	(33%)	(38%)	(35%)	(38%)	(36%)	(58%)	(72%)	(68%)	(50%)	(48%)
P value	2.93^{e-9}	0.312	6.32^{e-13}	0.072	0.113	0.221	0.042	0.138	4.03^{e-8}	0.015
Direction of change	↓	=	↓	=	=	=	↓	=	↓	↑

For each muscle, the number of nerves stimulated (# of n.) where N1, P1, and P2/P3 responses were observed is represented as a fraction of the total number of nerves stimulated, with the percentage indicated in parentheses (% of n.). We also calculated the number of bins (# of bins) where responses were observed as a fraction of the total number of bins, with the percentage indicated in parentheses (% of bins). Chi-square test was performed between conditions on the number of bins where responses occurred. Values in bold indicate significant differences in reflex response occurrence between the two conditions. Up and down arrows indicate a significant increase or decrease, respectively, in reflex response occurrence during hindlimb-only locomotion compared with quadrupedal locomotion. The equal sign represents no significant changes. BFA, biceps femoris anterior; c, contralateral; i, ipsilateral; SOL, soleus; SP, superficial peroneal; SRT, anterior sartorius; ST, semitendinosus; TA, tibialis anterior; VL, vastus lateralis.

nerve during quadrupedal locomotion evoked short-latency inhibition, or N1 responses, in 100% of nerves stimulated ($n = 11/11$, Table 1) and in 58% of bins in the cycle ($n = 64/110$). N1 responses are only visible when a muscle is active. These results are consistent with previous findings from intact cats during quadrupedal locomotion (1, 2, 20, 48, 51, 53, 55–57, 60, 61). During hindlimb-only locomotion, N1 responses were also present in all nerves stimulated ($n = 11/11$, 100%) and in 59% of bins in the cycle ($n = 65/110$). We found no significant differences in N1 response occurrence between locomotor conditions ($P = 0.891$). The N1 responses were significantly modulated by phase in both locomotor conditions (quadrupedal, $P = 1.03^{e-12}$; hindlimb-only, $P = 1.61^{e-12}$). Studies have shown that N1 responses are followed by mid-latency (P2) or long-latency (P3) excitatory responses (53, 55, 56, 58, 62). Considering that the duration of the inhibitory response influences the appearance of the excitatory response, we combined

the mid- and long-latency responses (P2/P3) together. In this study, we observed P2/P3 responses in the ipsilateral SOL with 73% of nerves stimulated ($n = 8/11$) during quadrupedal locomotion but only in 27% of nerves stimulated during hindlimb-only locomotion ($n = 3/11$). We also found significantly more P2/P3 responses during quadrupedal locomotion ($n = 49/80$ bins or 61%) compared with hindlimb-only locomotion ($n = 10/30$ bins or 33%) ($P = 2.93^{e-9}$). P2/P3 responses in the ipsilateral SOL were significantly modulated by phase for pooled data during quadrupedal locomotion ($P = 0.007$) but not during hindlimb-only locomotion ($P = 0.161$). During quadrupedal locomotion, we also observed short-latency excitation, or P1 responses, in 36% of nerves stimulated ($n = 4/11$) and 38% of bins in the cycle ($n = 15/40$), mainly when the ipsilateral SOL was inactive, consistent with previous findings (56). During hindlimb-only locomotion, P1 responses were present in 27% of nerves stimulated ($n = 3/11$) and 33% of bins in the cycle

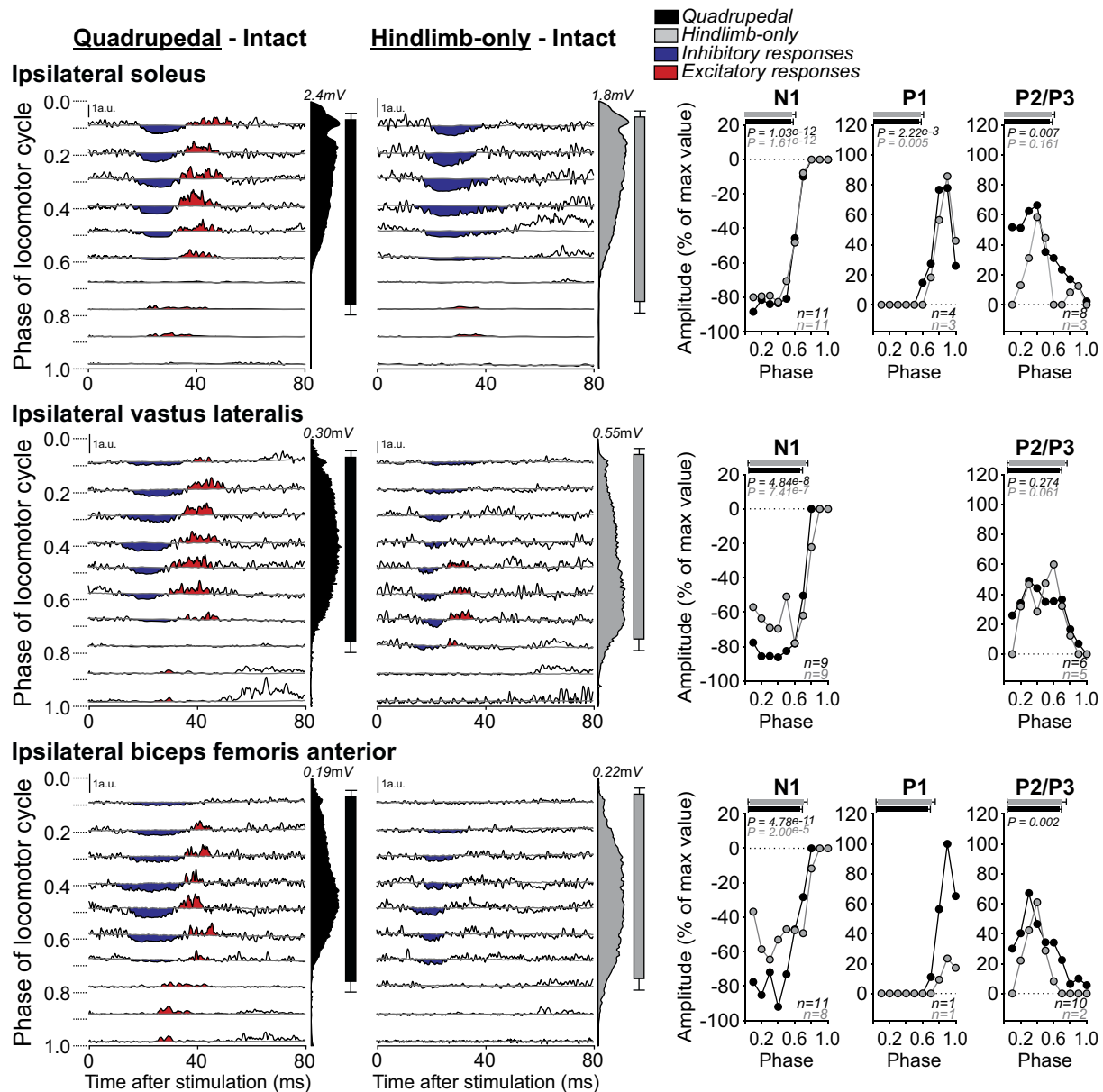


Figure 2. Phase- and condition-dependent modulation of cutaneous reflexes in ipsilateral extensor muscles of intact cats. Superficial peroneal (SP) nerve stimulation evoked responses in the ipsilateral soleus ($n = 11$ nerves in 6 cats), vastus lateral ($n = 9$ nerves in 6 cats), and biceps femoris anterior ($n = 11$ nerves in 6 cats) during quadrupedal and hindlimb-only locomotion. Each panel represents averaged waveforms in the 10 bins of the cycle with a poststimulation window of 80 ms in a single cat (*cat 3* for all 3 muscles). Black traces represent averaged cycles that received a stimulation (i.e., reflex responses, $n = 7$ –18 stimuli per bin). Gray traces represent averaged cycles with no stimuli. Scale bars are shown in arbitrary units (a.u.) for both locomotor conditions. Blue and red areas represent inhibitory and excitatory responses, respectively. Aligned vertically is the average rectified electromyogram (EMG) and the stance phase aligned to ipsilateral soleus muscle onset. Scatterplots represent reflex amplitudes (mean) in each bin of the cycle expressed as a percentage of the maximal response found in 1 of the bins for each nerve stimulated during quadrupedal (black circles) and hindlimb-only (gray circles) locomotion. Short-latency (P1, N1) and mid-/longer-latency (P2/P3) responses are shown separately. Horizontal bars at top of the scatterplots represent the period of muscle activity for each locomotor condition ($n = 7$ –15 control cycles) for pooled data. P values at top of the scatterplots show the result of Friedman's test to assess phase modulation.

($n = 10/30$), with no significant differences between locomotor conditions ($P = 0.288$). The P1 responses evoked by ipsilateral SP nerve stimulation were significantly modulated by phase for pooled data in both locomotor tasks (quadrupedal, $P = 2.22 \times 10^{-3}$; hindlimb-only, $P = 0.005$).

The ipsilateral VL and BFA (Fig. 2), knee and hip extensor muscles, respectively, became active around foot contact and throughout the majority of the stance phase. As for the

ipsilateral SOL, when VL and BFA are active, stimulating cutaneous or mixed nerves of the foot during quadrupedal locomotion in intact cats evokes N1 responses followed by P2/P3 responses, as shown previously (53, 55, 61, 62). In the present study, N1 responses were present in 100% of nerves stimulated for the ipsilateral VL in both locomotor conditions (quadrupedal, $n = 9/9$; hindlimb-only, $n = 9/9$; Table 1). For the ipsilateral BFA, N1 responses were present in more nerves

stimulated during quadrupedal locomotion ($n = 11/11$) compared with hindlimb-only locomotion ($n = 8/11$). For both muscles, we observed a similar number of bins with N1 responses during quadrupedal locomotion (VL, $n = 59/90$ bins or 66%; BFA, $n = 66/110$ bins or 60%) and hindlimb-only locomotion (VL, $n = 63/90$ bins or 70%; BFA, $n = 52/80$ bins or 65%), with no significant difference in reflex occurrence between locomotor conditions (VL, $P = 0.587$; BFA, $P = 0.058$). In both tasks, N1 responses were significantly modulated by phase for VL (quadrupedal, $P = 4.84 \times 10^{-8}$; hindlimb-only, $P = 7.41 \times 10^{-7}$) and BFA (quadrupedal, $P = 4.78 \times 10^{-11}$; hindlimb-only, $P = 2.00 \times 10^{-5}$). For the ipsilateral VL, we observed P2/P3 responses in 67% of nerves stimulated ($n = 6/9$) during quadrupedal locomotion and in 56% of nerves stimulated during hindlimb-only locomotion ($n = 5/9$). The number of bins in the cycle with P2/P3 responses was similar during quadrupedal locomotion ($n = 25/60$ bins or 42%) and hindlimb-only locomotion ($n = 19/50$ bins or 38%), with no significant differences between locomotor conditions ($P = 0.312$). The P2/P3 responses in the ipsilateral VL were not significantly modulated by phase for pooled data (quadrupedal, $P = 0.274$; hindlimb-only, $P = 0.061$). For the ipsilateral BFA, we observed P2/P3 responses in 91% of nerves stimulated ($n = 10/11$) during quadrupedal locomotion but only in 18% of nerves stimulated during hindlimb-only locomotion ($n = 2/11$). P2/P3 responses were more frequent during quadrupedal locomotion ($n = 55/100$ bins or 55%) compared with hindlimb-only locomotion ($n = 7/20$ bins or 35%) ($P = 6.32 \times 10^{-13}$). During quadrupedal locomotion, P2/P3 responses in the ipsilateral BFA were significantly modulated by phase for pooled data ($P = 0.002$). During the inactive phase, we found P1 responses in a single cat in both locomotor conditions.

Next, we evaluated the modulation of cutaneous reflexes in ipsilateral muscles that flex the hip (SRT), knee (ST), and ankle (TA) joints, although the SRT and ST also extend the knee and hip, respectively (Fig. 3). The SRT burst covered most of the hindlimb swing phase during quadrupedal locomotion in intact cats. During hindlimb-only locomotion the SRT also displayed a prominent second burst during the stance phase, as shown previously (43, 63, 64). In the literature, stimulating the SP nerve during quadrupedal locomotion in intact cats evokes N1 responses followed by P2 responses when SRT is active and mainly P2 responses when it is inactive (20). Here, we observed N1 responses in 50% of nerves stimulated ($n = 4/8$, Table 1) and in 68% of bins ($n = 27/40$) during quadrupedal locomotion in SRT. Interestingly, N1 responses were present in 100% of nerves stimulated ($n = 8/8$) during hindlimb-only locomotion and in 74% of bins ($n = 59/80$ bins), a significant increase in response occurrence compared with quadrupedal locomotion ($P = 1.0 \times 10^{-4}$). N1 responses in the ipsilateral SRT were not significantly modulated by phase for pooled data during quadrupedal locomotion ($P = 0.146$) and hindlimb-only locomotion ($P = 0.116$). We also observed P2 responses in both locomotor conditions. During quadrupedal locomotion, we found P2 responses in 25% of nerves stimulated ($n = 2/8$) and in 35% of bins ($n = 7/20$). These excitatory responses appeared at the time of liftoff and just before the onset of muscle activation. During hindlimb-only locomotion, we found P2 responses in 50% of nerves stimulated ($n = 4/8$) and in 38% of bins ($n = 15/40$), with no significant differences in

occurrence between conditions ($P = 0.072$). P2 responses evoked during hindlimb-only locomotion were not significantly modulated by phase for pooled data ($P = 0.056$), and we did not have sufficient data ($n = 2$) for quadrupedal locomotion.

The ST is a knee flexor and hip extensor muscle that displayed a short burst at swing onset in both locomotor conditions and a prominent second burst during early stance in hindlimb-only locomotion at the same speed (43). In the present study, we observed P1 responses in the ipsilateral ST in 100% of nerves stimulated ($n = 10/10$, Table 1) and in 55% of bins ($n = 55/100$) during quadrupedal locomotion. During hindlimb-only locomotion, we also found P1 responses in 100% of nerves stimulated ($n = 10/10$) and in 50% of bins ($n = 50/100$), with no significant difference in occurrence between locomotor conditions ($P = 0.500$). In both tasks, P1 responses were significantly modulated by phase (quadrupedal, $P = 1.17 \times 10^{-11}$; hindlimb-only, $P = 7.09 \times 10^{-12}$), with prominent responses during the activity of the ipsilateral ST and throughout swing, whereas they were generally suppressed during stance. P1 responses were generally followed by P2 responses, as shown by several studies during quadrupedal locomotion in intact cats (20, 48, 52, 56, 60). Here, we observed P2 responses in the ipsilateral ST in 90% of nerves stimulated ($n = 9/10$) and in 46% of bins ($n = 41/90$) during quadrupedal locomotion. During hindlimb-only locomotion, we found P2 responses in 80% of nerves stimulated ($n = 8/10$) and in 45% of bins ($n = 36/80$), with no significant difference in occurrence between locomotor conditions ($P = 0.113$). In both tasks, P2 responses were significantly modulated by phase (quadrupedal, $P = 4.67 \times 10^{-7}$; hindlimb-only, $P = 1.14 \times 10^{-9}$).

The TA is a uniarticular ankle flexor, with a main burst covering most of the swing phase during quadrupedal locomotion. However, in two cats a second burst was also observed during the stance phase. We found the same pattern of muscle activation during hindlimb-only locomotion, with an additional cat displaying a second burst during stance. Here, stimulating the SP nerve evoked responses in the ipsilateral TA that differed based on cats and nerves stimulated (Fig. 3). We found N1 responses at the beginning and end of the cycle in 30% of nerves stimulated in both locomotor conditions ($n = 3/10$, Table 1). We observed no significant difference in N1 response occurrence between locomotor conditions ($P = 0.686$). N1 responses were not significantly modulated by phase (quadrupedal, $P = 0.161$; hindlimb-only, $P = 0.196$). We also found P1 responses in 50% of nerves stimulated ($n = 5/10$) and in 72% of bins ($n = 36/50$) during quadrupedal locomotion and in 50% of nerves stimulated ($n = 5/10$) and in 48% of bins ($n = 24/50$) during hindlimb-only locomotion, with no significant difference in response occurrence between locomotor conditions ($P = 0.098$). In both tasks, P1 responses were not significantly modulated by phase (quadrupedal, $P = 0.110$; hindlimb-only, $P = 0.192$). We also found P2 responses in 80% of nerves stimulated ($n = 8/10$) during quadrupedal locomotion and in 54% of bins ($n = 43/80$), mainly during swing. During hindlimb-only locomotion, we observed P2 responses in 90% of nerves stimulated ($n = 9/10$) and in 58% of bins ($n = 52/90$), mainly during stance, with no significant difference in P2 response occurrence between locomotor conditions ($P = 0.221$). In both tasks, P2 responses were significantly

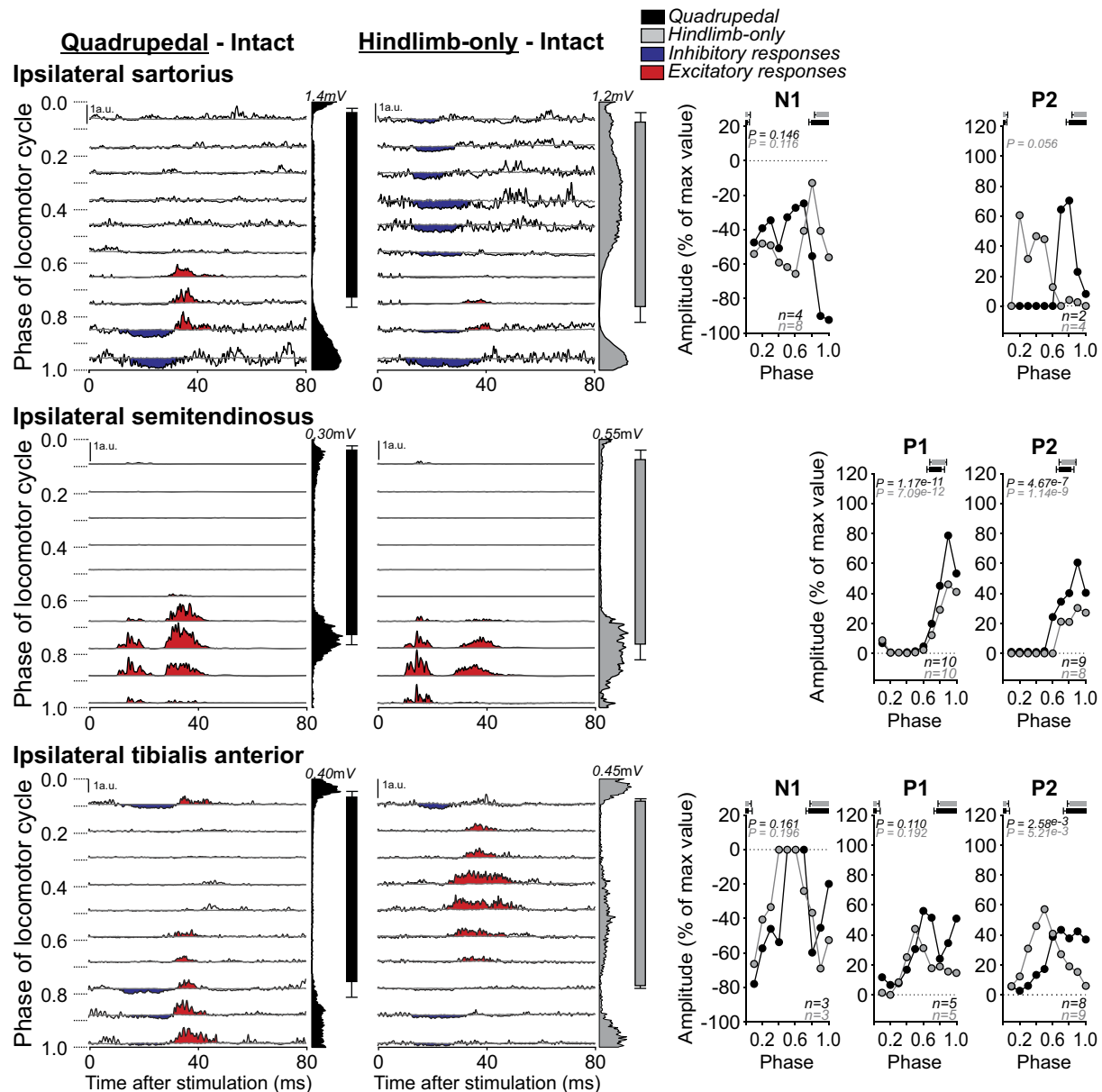


Figure 3. Phase- and condition-dependent modulation of cutaneous reflexes in ipsilateral muscles that flex the hip, knee, and ankle joints in intact cats. Superficial peroneal (SP) nerve stimulation evoked responses in the ipsilateral sartorius ($n = 8$ nerves in 6 cats), semitendinosus ($n = 10$ nerves in 6 cats), and tibialis anterior ($n = 10$ nerves in 6 cats) during quadrupedal and hindlimb-only locomotion. Each panel represents averaged waveforms in the 10 bins of the cycle with a poststimulation window of 80 ms in a single cat (sartorius and semitendinosus, cat 1; tibialis anterior, cat 3). Black traces represent averaged cycles that received a stimulation (i.e., reflex responses, $n = 4$ –22 stimuli per bin). Gray traces represent averaged cycles with no stimuli. Scale bars are shown in arbitrary units (a.u.) for both locomotor conditions. Aligned vertically is the average rectified electromyogram (EMG) and the stance phase aligned to ipsilateral soleus muscle onset. Scatterplots represent reflex amplitudes (mean) in each bin of the cycle expressed as a percentage of the maximal response found in 1 of the bins for each nerve stimulated during quadrupedal (black circles) and hindlimb-only (gray circles) locomotion. Short-latency (P1, N1) and mid-latency (P2) responses are shown separately. Horizontal bars at top of the scatterplots represent the period of muscle activity for each locomotor condition ($n = 9$ –14 control cycles) for pooled data. P values at top of the scatterplots show the result of Friedman's test to assess phase modulation.

modulated by phase (quadrupedal, $P = 2.58 \times 10^{-3}$; hindlimb-only, $P = 5.21 \times 10^{-3}$).

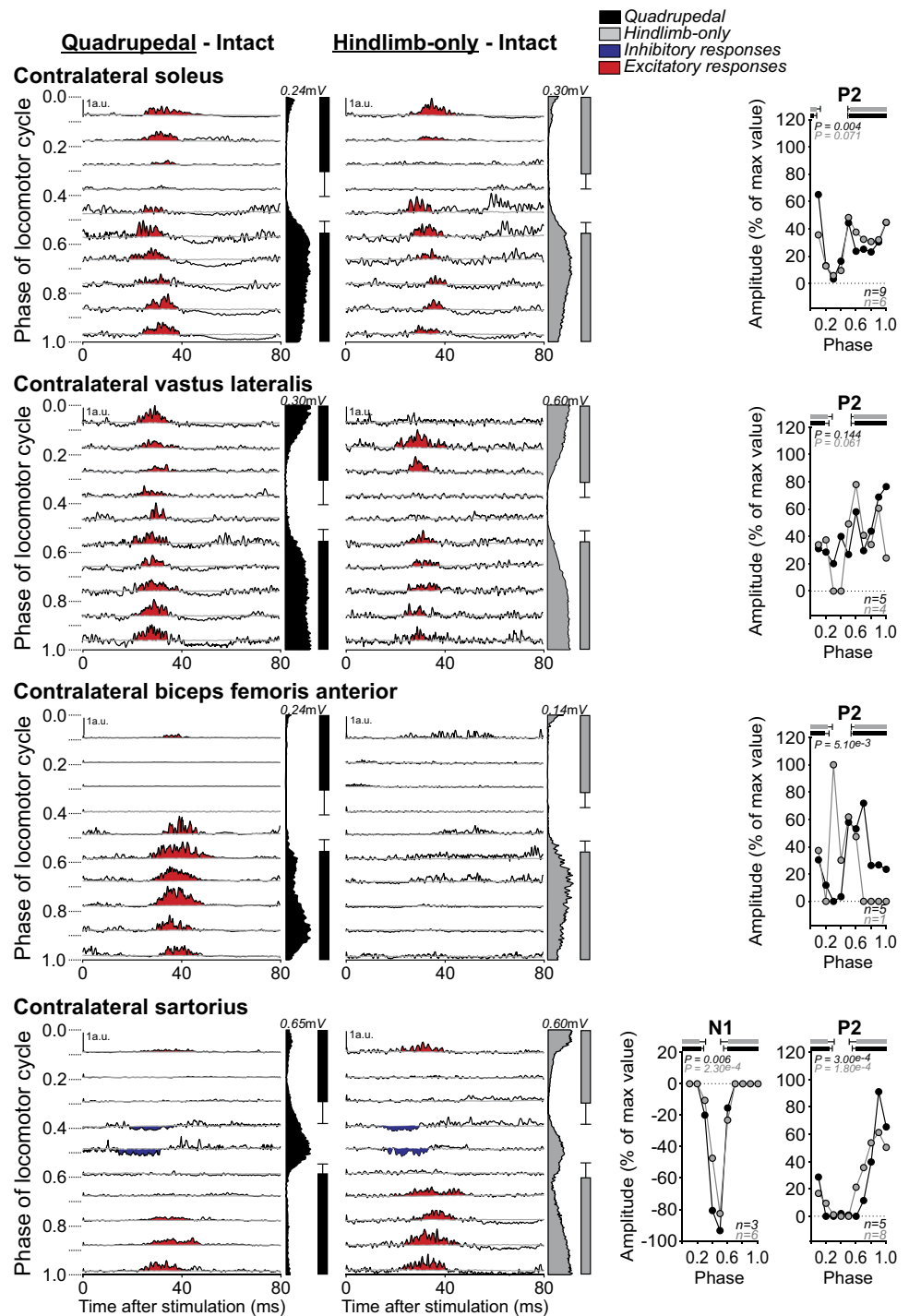
Cutaneous Reflex Responses in Contralateral Muscles

Responses evoked by electrical stimuli of the foot dorsum in ipsilateral limb muscles lift the limb away from and over the simulated contact of the foot dorsum during swing, whereas responses in contralateral limb muscles mainly

reinforce weight support (1, 2, 7, 12, 20, 51–53). Here, we investigated cutaneous reflexes in four muscles (SOL, VL, BFA, and SRT) contralateral to the stimulation (Fig. 4 and Table 1). We did not include responses in contralateral ST and TA because we only found them in one cat.

In the contralateral SOL (Fig. 4), we observed P2 responses in 82% of nerves stimulated ($n = 9/11$) and in 64% of bins ($n = 58/90$) during quadrupedal locomotion. During

Figure 4. Phase- and condition-dependent modulation of cutaneous reflexes in contralateral muscles of intact cats. Superficial peroneal (SP) nerve stimulation evoked responses in the contralateral soleus ($n = 10$ nerves in 6 cats), vastus lateral ($n = 6$ nerves in 5 cats), biceps femoris anterior ($n = 8$ nerves in 6 cats) during quadrupedal and hindlimb-only locomotion. Each panel represents averaged waveforms in the 10 bins of the cycle with a poststimulation window of 80 ms in a single cat (*cat 1* for the 4 muscles). Black traces represent averaged cycles that received a stimulation (i.e., reflex responses, $n = 4$ –22 stimuli per bin). Gray traces represent averaged cycles with no stimuli. Scale bars are shown in arbitrary units (a.u.) for both locomotor conditions. Aligned vertically is the average rectified electromyogram (EMG) and the stance phase aligned to ipsilateral soleus muscle onset. Scatterplots represent reflex amplitudes (mean) in each bin of the cycle expressed as a percentage of the maximal response found in 1 of the bins for each nerve stimulated during quadrupedal (black circles) and hindlimb-only (gray circles) locomotion. Horizontal bars at top of the scatterplots represent the period of muscle activity for each locomotor condition ($n = 9$ –15 control cycles) for pooled data. P values at top of the scatterplots show the result of Friedman's test to assess phase modulation.



hindlimb-only locomotion, we observed P2 responses in 55% of nerves stimulated ($n = 6/11$) and in 72% of bins ($n = 43/60$), a significant reduction in response occurrence compared with quadrupedal locomotion ($P = 0.042$). Although we observed the same overall profile of phase-dependent modulation, P2 responses were significantly modulated by phase for pooled data during quadrupedal ($P = 0.004$) but not hindlimb-only ($P = 0.071$) locomotion.

In the contralateral VL and BFA (Fig. 4), we observed P2 responses during the muscles' active periods in both

locomotor conditions. For contralateral VL, we found P2 responses in 56% of nerves stimulated ($n = 5/9$, Table 1) and in 74% of bins ($n = 37/50$) during quadrupedal locomotion and in 44% of nerves stimulated ($n = 4/9$) and in 68% of bins ($n = 27/40$) during hindlimb-only locomotion, with no significant difference in P2 response occurrence between conditions ($P = 0.138$). In both tasks, P2 responses in the contralateral VL were not significantly modulated by phase (quadrupedal, $P = 0.144$; hindlimb-only, $P = 0.061$). For the contralateral BFA, we found P2 responses in 45% of nerves

stimulated ($n = 5/11$) and in 74% of bins ($n = 37/50$) during quadrupedal locomotion, but during hindlimb-only locomotion we observed P2 responses in only 9% of nerves stimulated ($n = 1/11$) and in 50% of bins in the cycle ($n = 5/10$), a significant reduction in response occurrence compared with quadrupedal locomotion ($P = 4.03 \times 10^{-8}$). During quadrupedal locomotion, P2 responses were significantly modulated by phase ($P = 5.10 \times 10^{-3}$).

In the contralateral SRT (Fig. 4), we observed N1 responses during the swing phase in 38% of nerves stimulated ($n = 3/8$; Table 1) and in 27% of bins ($n = 8/30$) during quadrupedal locomotion and in 75% of nerves stimulated ($n = 6/8$) and in 22% of bins ($n = 13/60$) during hindlimb-only locomotion, with no significant difference in response occurrence between conditions ($P = 0.251$). In both tasks, N1 responses were significantly modulated by phase (quadrupedal, $P = 0.006$; hindlimb-only, $P = 2.30 \times 10^{-4}$). Stimulating the SP nerve evoked P2 responses in 63% of nerves stimulated ($n = 5/8$) and in 44% of bins ($n = 22/50$) during quadrupedal locomotion and in 100% of nerves stimulated ($n = 8/8$) and in 48% of bins ($n = 38/80$) during hindlimb-only locomotion, a significant increase in response occurrence compared with quadrupedal locomotion ($P = 0.015$). In both locomotor conditions, P2 responses were significantly phase modulated (quadrupedal, $P = 3.00 \times 10^{-4}$; hindlimb-only, $P = 1.80 \times 10^{-4}$).

The symbols (arrows and equal signs) in Table 1 summarize response occurrence during quadrupedal and hindlimb-only locomotion for the different ipsilateral and contralateral muscles. We can summarize response occurrence as follows. N1 and P1 response occurrences were similar in both conditions, with the exception of the ipsilateral SRT, where it was greater during hindlimb-only locomotion. P2/P3 response occurrences were reduced during hindlimb-only locomotion in ipsilateral SOL, ipsilateral BFA, contralateral SOL, and contralateral BFA but increased in contralateral SRT. In the ipsilateral VL, SRT, ST, and TA as well as contralateral VL, P2/P3 response occurrences were similar in both conditions.

Hindlimb-Only Locomotion Reduces Cutaneous Reflex Modulation in the Ipsilateral Semitendinosus

To compare reflex responses evoked in the two conditions, we measured a modulation index by subtracting the smallest response from the largest response observed in the 10 bins (Fig. 5) (20, 51). In the ipsilateral ST muscle, we observed a significantly smaller reflex modulation during hindlimb-only locomotion compared with quadrupedal locomotion for P1 ($P = 0.022$; $r = 0.661$; 41% difference) and P2 ($P = 0.017$; $r = 0.841$; 55% difference) responses. In the other muscles where we had a sufficient number of excitatory reflex responses for statistical analysis, the modulation index did not significantly differ between the two locomotor conditions. This was the case for P1 ($P = 0.686$; $r = 0.181$) and P2 ($P = 0.779$; $r = 0.098$) responses in the ipsilateral TA, P2 responses in the contralateral SOL ($P = 0.249$; $r = 0.471$), and P2 responses in the contralateral SRT ($P = 0.080$; $r = 0.783$).

Cutaneous Reflex Responses in Intact and Spinal Cats

In previous sections, we described cutaneous reflexes during quadrupedal and hindlimb-only locomotion in intact

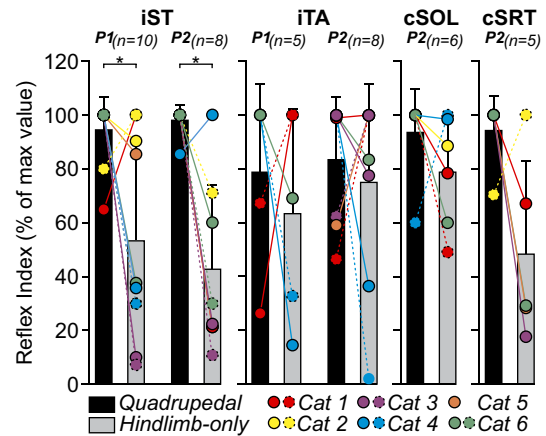


Figure 5. Cutaneous reflex modulation across locomotor conditions for pooled data in intact cats. Each bar represents the mean reflex index $\pm$ SD for pooled data of responses evoked in the ipsilateral semitendinosus (iST) and tibialis anterior (iTA) as well as the contralateral soleus (cSOL) and sartorius (cSRT). The colored circles represent the nerves stimulated in the 6 cats. The solid and dotted lines of the circles are for the right and left SP nerves, respectively. Significant differences between conditions (Wilcoxon): * $P < 0.05$.

cats. In this section, we compare cutaneous reflexes evoked in two cats ($n = 4$ nerves stimulated) in the two intact conditions and during hindlimb-only locomotion after spinal transection. We did this to determine whether changes in cutaneous reflex response in spinal cats are due to the lesion or to the absence of forelimb movements. Figure 6 shows cutaneous reflex responses in three ipsilateral muscles at their periods of mid-activity and mid-inactivity.

In the ipsilateral SOL, we observed N1 responses in 100% of nerves stimulated ($n = 4/4$) in both intact locomotor conditions and during hindlimb-only locomotion in the spinal state. In all three conditions, N1 responses were significantly modulated by phase (quadrupedal, $P = 2.28 \times 10^{-3}$; hindlimb-only, $P = 4.12 \times 10^{-3}$; spinal hindlimb-only, $P = 2.56 \times 10^{-3}$). Whereas mid- or longer-latency excitatory responses (P2/P3) following the N1 responses were found in 75% of nerves stimulated during intact quadrupedal locomotion ($n = 3/4$), P2/P3 responses were only present in 25% of nerves stimulated during intact hindlimb-only locomotion ($n = 1/4$) and not at all in the spinal state ($n = 0/4$). During the SOL's inactive phase, P1 responses were observed in 50% ($n = 2/4$), 0% ($n = 0/4$), and 25% ($n = 1/4$) of nerves stimulated during quadrupedal and hindlimb-only locomotion in the intact state and during hindlimb-only locomotion in the spinal state, respectively.

During the active period of the ipsilateral BFA, we observed N1 responses in 100% of nerves stimulated ($n = 4/4$) in both intact conditions and during hindlimb-only locomotion in the spinal state. In all three conditions, N1 responses were significantly modulated by phase (quadrupedal, $P = 0.001$; hindlimb-only, $P = 0.002$; spinal hindlimb-only, $P = 0.002$). We observed longer-latency excitatory responses (P3) in 100% of nerves stimulated during quadrupedal locomotion ($n = 4/4$) but in only 25% of nerves stimulated during intact hindlimb-only locomotion ($n = 1/4$) and not at all in the spinal state ($n = 0/4$). During the ipsilateral BFA's inactive phase, we did not observe P1 or P2 responses in all three locomotor conditions.

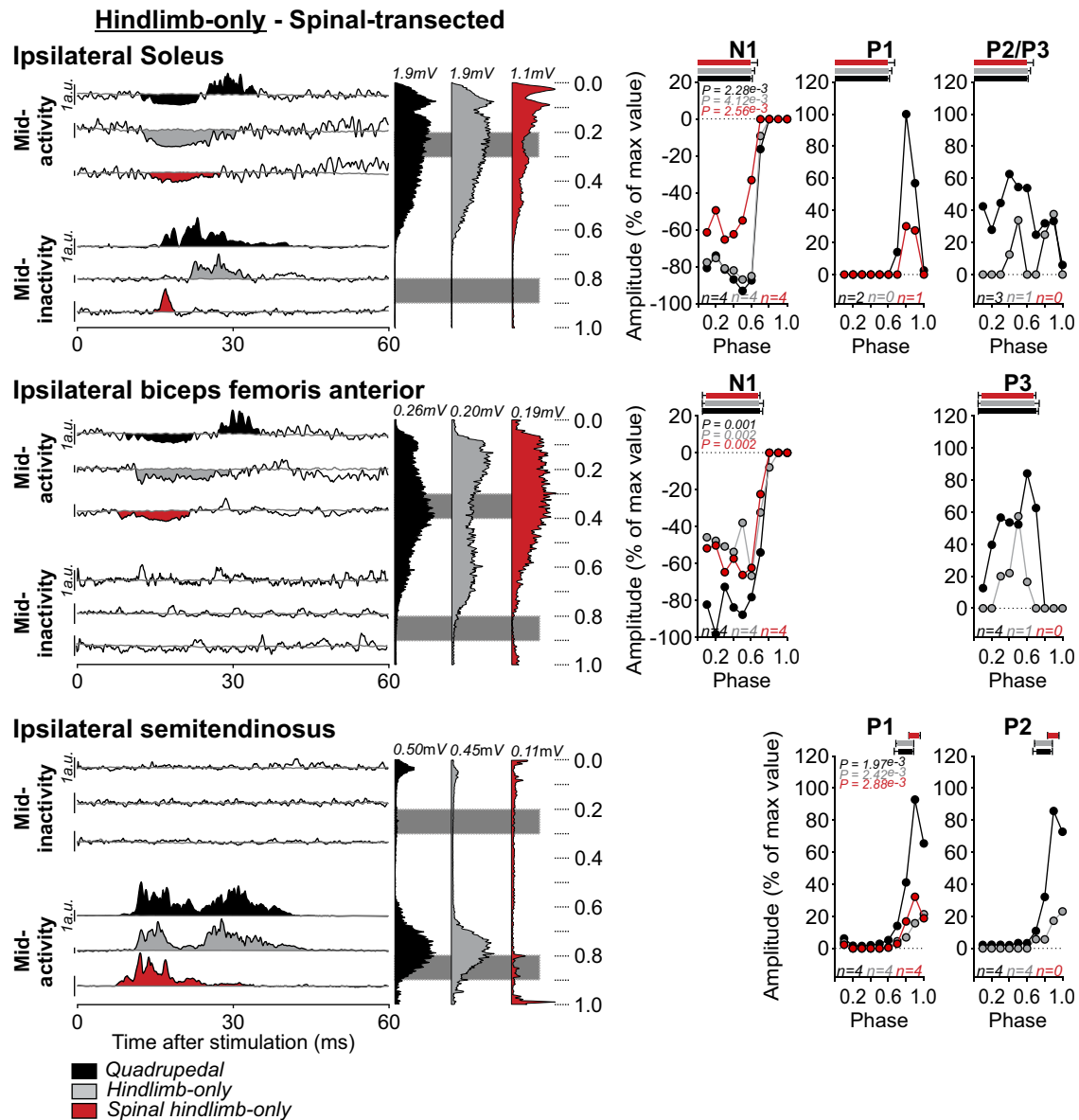


Figure 6. Phase- and condition-dependent modulation of cutaneous reflexes in intact and spinal cats. Superficial peroneal (SP) nerve stimulation evoked responses in the ipsilateral soleus ($n = 4$ nerves in 2 cats), biceps femoris anterior ($n = 3$ nerves in 2 cats), and semitendinosus ($n = 4$ nerves in 2 cats) in the intact state during quadrupedal and hindlimb-only locomotion and after spinal transection in the hindlimb-only condition. Each panel represents averaged waveforms at mid-activity and mid-inactivity of the muscle's main burst with a poststimulation window of 80 ms from a single cat (soleus, cat 3; biceps femoris anterior, cat 6; semitendinosus, cat 3). Black traces represent averaged cycles that received a stimulation (i.e., reflex responses, $n = 7$ –18 stimuli per bin). Gray traces represent averaged cycles with no stimuli. Scale bars are shown in arbitrary units (a.u.) for both conditions of locomotion. Aligned vertically is the average rectified electromyogram (EMG) aligned to ipsilateral soleus muscle onset. The gray areas correspond to the bins taken in periods of mid-activity and mid-inactivity of the muscle. Scatterplots represent reflex amplitudes (mean) in each bin of the cycle expressed as a percentage of the maximal response found in 1 of the bins for each nerve stimulated between quadrupedal (black circles) and hindlimb-only (gray circles) locomotion in the intact state and hindlimb-only condition in the spinal state (red circles). Short-latency (P1, N1) and mid-/longer-latency (P2/P3) responses are shown separately. Horizontal bars at top of the scatterplots represent the period of muscle activity for each locomotor condition ($n = 7$ –15 control cycles) for pooled data. P values at top of the scatterplots show the result of Friedman's test to assess phase modulation.

During the active period of the ipsilateral ST, we observed P1 responses in 100% of nerves stimulated ($n = 4/4$) in both intact conditions and during hindlimb-only locomotion in the spinal state. In all three conditions, P1 responses were significantly modulated by phase (quadrupedal, $P = 1.97 \times 10^{-3}$; hindlimb-only, $P = 2.42 \times 10^{-3}$; Spinal hindlimb-only, $P = 2.88 \times 10^{-3}$). Whereas the P2 responses following the P1 responses were observed in 100% of nerves stimulated ($n = 4/4$) in both

intact conditions, we found no P2 responses in the spinal state.

DISCUSSION

In the present study, we investigated task-dependent modulation of hindlimb cutaneous reflexes by comparing responses evoked during quadrupedal and hindlimb-only

locomotion to determine the contribution of forelimb movements to the modulation of hindlimb reflex pathways. Consistent with our hypothesis, we found differences in reflex responses between the two conditions. This is not surprising because forelimb and hindlimb movements must be coordinated during quadrupedal locomotion. Depending on the muscle, not all nerve stimulations elicited a response during quadrupedal or hindlimb-only locomotion. We found that the occurrence of short-latency inhibitory or excitatory responses was similar in most muscles in both conditions, with the exception of the ipsilateral SRT, a hip flexor/knee extensor, where short-latency inhibitory responses were more frequent during hindlimb-only locomotion. Thus, the absence of forelimb movements has little effect on short-latency reflex pathways. On the other hand, the occurrence of longer-latency excitatory responses decreased in some extensor muscles bilaterally (SOL and BFA) during hindlimb-only locomotion, with the exception of the contralateral SRT, where it increased. These results and those in spinal cats suggest that the absence of forelimb movements in intact cats suppresses spino-bulbo-spinal pathways (65) that contribute to longer-latency excitatory responses in extensor muscles (i.e., those mostly active during stance) while having little to no effect on short-latency responses. In the next sections, we discuss the contribution of forelimb movements in the modulation of hindlimb reflex responses/pathways and its functional significance for locomotor control.

Factors Influencing Reflex Responses during Quadrupedal and Hindlimb-Only Locomotion

Several factors can influence reflex responses. One is the background level of EMG, or automatic gain compensation (59, 66, 67). In general, larger and/or more frequent responses are evoked when a muscle is active because more motoneurons and premotor interneurons are firing or close to threshold when the incoming sensory inputs arrive and negative/inhibitory responses require a certain baseline level of EMG activity to be visible and measured. Thus, if a muscle stays active longer, it increases the probability of evoking or observing a response, particularly an inhibitory response, and, conversely, fewer responses are observed if EMG activity is weaker or shorter, particularly excitatory responses. A greater occurrence of N1 responses in the ipsilateral SRT and P2/P3 responses in the contralateral SRT during hindlimb-only locomotion can be explained by the prominent second burst in this muscle during the stance phase (Fig. 1A). The stance-related activity of the SRT muscle during hindlimb-only locomotion was shown by others (43, 68). However, the reduction of P2/P3 responses in SOL and BFA, ipsilateral and contralateral to the stimulation, during hindlimb-only locomotion cannot be explained by shorter periods of activity because the stance-related BFA burst occupied a greater proportion of the cycle compared with quadrupedal locomotion in intact cats whereas there was no difference in SOL (Fig. 1C). The mean amplitude of SOL was significantly reduced during hindlimb-only locomotion, however (Fig. 1D). In hindlimb-only locomotion, the forelimbs are slightly elevated on a stationary platform. This likely produces a caudal shift of the body's center of mass, potentially increasing loading on the hindlimbs and the amplitude of EMG activity

of extensor muscles. We observed a significant increase in the EMG amplitude of VL, a knee extensor, during hindlimb-only locomotion compared with quadrupedal locomotion but a decrease in SOL, an ankle extensor, and no difference in BFA, a hip extensor (Fig. 1). The occurrence of short-latency inhibitory reflex responses in the ipsilateral SOL, VL, and BFA was similar in quadrupedal and hindlimb-only locomotion. Thus, the background level of EMG did not necessarily translate into greater or fewer reflex responses, as shown by others in cats (8, 11, 20, 21, 51) or humans (11).

Another potential factor affecting cutaneous reflex responses during locomotion is the symmetry between the left and right sides, particularly its spatial component, as shown in intact and spinal cats (21, 51, 52). However, this is unlikely to be a factor in reflex modulation/response occurrence in the present study because we found no significant differences in temporal or spatial left-right asymmetry between quadrupedal and hindlimb-only locomotion in intact cats (Fig. 1E). This is not surprising because tied-belt locomotion at 0.4 m/s is inherently symmetric, with a strict out-of-phase alternation of the hindlimbs (69).

Functional Considerations

Electrically stimulating the superficial peroneal nerve mimics a mechanical contact to the dorsum of the hind paw, generating a stumbling corrective reaction during swing and a stumbling preventive reaction during stance. In cats, the main muscles responsible for the stumbling corrective reaction are muscles that flex the knee and extend the hip, such as ST and posterior biceps femoris, which move the limb away and vertically from the contact point during swing (2, 12). In the present study, we found a significant reduction in the depth of reflex modulation of short- and mid-latency excitatory responses in the ipsilateral ST muscle during hindlimb-only locomotion compared with quadrupedal locomotion (Fig. 5). This would reduce knee flexion/hip extension during the swing phase following contact of the foot dorsum during hindlimb-only locomotion. We also observed fewer longer-latency excitatory responses in extensor muscles (SOL and BFA) of the contralateral hindlimb during the swing phase of hindlimb-only locomotion. The less frequent responses in contralateral muscles might be because the ipsilateral hindlimb flexes less (smaller excitatory responses in the ipsilateral ST), requiring less contralateral support, and because the body is in a more stable position during hindlimb-only locomotion. In quadrupedal locomotion at 0.4 m/s on a treadmill, the pattern consists of alternating periods of double and triple support, where two and three limbs are contacting the surface, respectively, with some brief and infrequent periods of quadruple support, where four limbs contact the surface, which replace diagonal support periods (70). In hindlimb-only locomotion, as the forelimbs always contact the surface, the pattern alternates between periods of triple and quadruple support, which offers greater stability.

With stimulation during ipsilateral stance (i.e., the stumbling preventive reaction), we found more frequent inhibitory responses in the ipsilateral SRT, a hip flexor/knee extensor, and fewer longer-latency excitatory responses in some ipsilateral extensor muscles (SOL and BFA) during

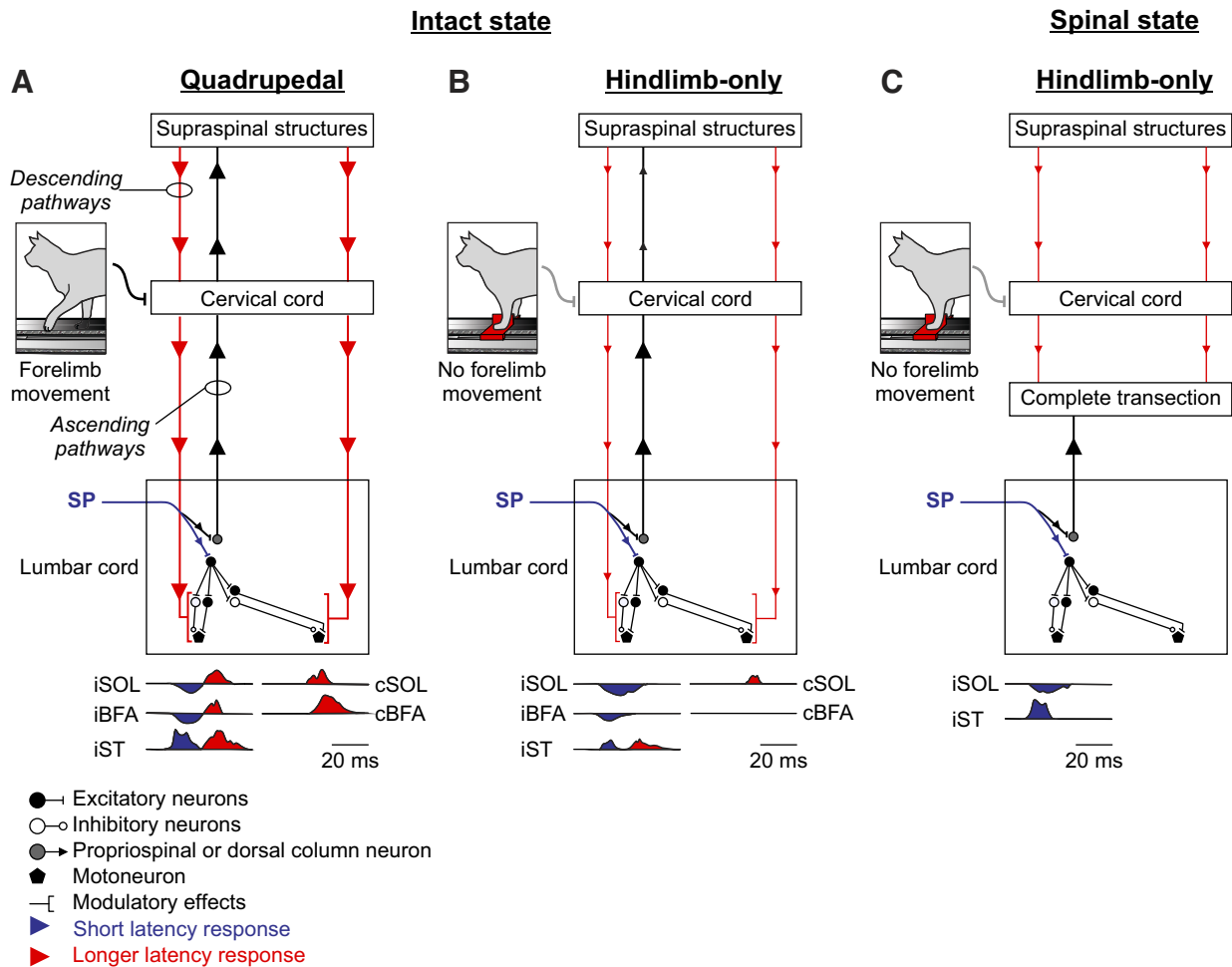


Figure 7. Spinal and supraspinal contributions to short- and longer-latency reflex responses during quadrupedal and hindlimb-only locomotion. The figure shows possible spinal and supraspinal contributions to reflex responses from superficial peroneal (SP) afferents in the intact and spinal states during quadrupedal and hindlimb-only locomotion. **A:** during intact quadrupedal locomotion, SP afferents activate lumbar circuits that elicit short-latency reflex responses in the ipsilateral hindlimb and in the contralateral hindlimb via crossed projections. Afferent inputs also activate neurons that project to circuits of the cervical cord and brain, which then project back to lumbar circuits to evoke longer-latency responses. **B:** during hindlimb-only locomotion in the intact state, SP afferents make the same synaptic contacts, but the absence of forelimb movements suppresses descending signals from the brain and cervical cord that contribute to longer-latency responses. **C:** during hindlimb-only locomotion in the spinal state, SP afferents make synaptic contacts with lumbar circuits to evoke short-latency reflexes, but ascending and descending pathways to and from the brain/cervical cord are abolished and no longer contribute to longer-latency responses. BFA, biceps femoris anterior; c, contralateral; i, ipsilateral; SOL, soleus; ST, semitendinosus.

hindlimb-only locomotion. As the SRT shows a prominent burst in the stance phase of hindlimb-only locomotion and because it flexes the hip, it makes functional sense that it receives inhibition during stance to prevent the limb from flexing and potentially destabilizing the pattern. Fewer excitatory responses in extensor muscles might be because the animal is in a more stable position and the longer-latency responses, which are likely mediated by supraspinal loops/pathways, are not required. Longer-latency reflex responses are thought to allow supraspinal structures to integrate somatosensory information and modify limb trajectory and posture according to phase and state (71).

Spinal vs. Supraspinal Contributions

One way to investigate the relative contribution of supraspinal versus spinal mechanisms in evoking and modulating hindlimb reflexes is to compare responses before and after spinal transection. After thoracic spinal transection, all

communication between the brain/cervical cord and lumbosacral circuits controlling hindlimb movements is permanently disrupted. Although the stumbling corrective reaction is observed in spinal cats and stimulating the SP nerve evokes phase-dependent reflex responses, mid- and long-latency responses in hindlimb muscles are generally reduced or abolished (Fig. 6), consistent with an important supraspinal contribution to these responses (2, 47, 51, 52, 61, 72). In the present study, we showed that the absence of forelimb movements has little effect on short-latency responses but that longer-latency responses are suppressed in intact cats. This reinforces the notion that short-latency reflexes are mainly mediated and modulated by spinal circuits/mechanisms whereas longer-latency responses require contributions from supraspinal structures and/or sensorimotor circuits controlling the forelimbs in the cervical cord. As the forelimbs do not need to be coordinated with the hindlimbs during hindlimb-only locomotion, the descending pathways from the

brain and cervical cord that contribute to longer-latency responses in hindlimb muscles are suppressed.

We currently do not know where longer-latency reflex pathways are gated. Figure 7 provides a plausible scenario that remains speculative. In quadrupedal locomotion, primary afferents from the SP nerve make contact with spinal interneurons that generate short-latency inhibitory or excitatory responses in ipsilateral muscles as well as with interneurons with crossed projections that generate longer-latency excitatory responses in contralateral muscles (Fig. 7A). Afferent inputs from the SP nerve also contact neurons that ascend via propriospinal or dorsal column tracts to the cervical cord and supraspinal structures. These ascending inputs make contact with neurons in the brain stem that then send descending inputs to the lumbar cord and contribute to longer-latency reflex responses in ipsilateral and/or contralateral muscles (73). The absence of forelimb movements during hindlimb-only locomotion suppresses ascending inputs to supraspinal structures and/or their descending influences (Fig. 7B). These responses can be gated at lumbar, cervical, and/or supraspinal levels. After thoracic spinal transection, ascending inputs no longer reach the cervical cord and supraspinal structures no longer contribute to longer-latency reflex responses (Fig. 7C).

Concluding Remarks

In conclusion, our results suggest that forelimb movements have a strong influence on hindlimb cutaneous reflexes and their modulation during quadrupedal locomotion. Additionally, some changes in reflex responses observed in spinal cats can be explained by the absence of forelimb movements and are not just because of removing supraspinal/cervical contributions. Humans are thought to have conserved a quadrupedal-like coordination of their arms and legs during walking (74), and making use of this interlimb circuitry could benefit rehabilitation approaches in various neurological conditions, such as stroke, Parkinson's disease, and spinal cord injury (75–77). Animal models such as the cat provide a substrate to better understand the neural pathways and mechanical properties that help coordinate the limbs during locomotion and shape patterns of activity (20, 78, 79).

DATA AVAILABILITY

The datasets generated during and/or analyzed during the present study are available from the corresponding author on reasonable request.

ACKNOWLEDGMENTS

We thank Philippe Drapeau (Université de Montréal) from the Rossignol and Drew labs for developing the data collection and analysis software.

GRANTS

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada Grant (NSERC RGPIN-2016–03790 to A.F.) and the National Institutes of Health (Grant R01 NS110550 to A.F., I.A.R., and B.I.P.). A.F. and C.I.-M. are Senior and Junior Research Scholars, respectively, supported by the Fonds de recherche du Québec–Santé (FRQS). J.H., J.A., and O.E. received doctoral scholarships from the FRQS. P.J. received

a postdoctoral fellowship from the Centre de Recherche du CHUS.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.H., B.I.P., I.A.R., and A.F. conceived and designed research; J.H., R.A., S.M., S.Y., O.E., P.J., J.A., and C.L. performed experiments; J.H. and R.A. analyzed data; J.H. and A.F. interpreted results of experiments; J.H. and R.A. prepared figures; J.H. and A.F. drafted manuscript; J.H., R.A., S.M., S.Y., O.E., P.J., J.A., C.L., C.I.-M., B.I.P., I.A.R., and A.F. edited and revised manuscript; J.H., R.A., S.M., S.Y., O.E., P.J., J.A., C.L., C.I.-M., B.I.P., I.A.R., and A.F. approved final version of manuscript.

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