The effect of crossed reflex responses on dynamic stability during locomotion

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The effect of crossed reflex responses on dynamic stability during locomotion. J Neurophysiol 114: 1034–1040, 2015. First published June 10, 2015; doi:10.1152/jn.00178.2015.—In recent studies, we demonstrated that a neural pathway within the human spinal cord allows direct communication between muscles located in the opposing limb. Short-latency crossed responses (SLCRs) are elicited in the contralateral triceps surae at an onset of 40–69 ms following electrical stimulation of the ipsilateral tibial nerve (iTN). The SLCRs are significantly affected by lesions of the central nervous system where the patients are unable to attain normal walking symmetry. The aim of this study was to elucidate the functionality of SLCRs by investigating their effects on the center of pressure (CoP) and pressure distribution. SLCRs were elicited by iTN stimulation at the end of the ipsilateral swing phase while the participants (n = 8) walked on a treadmill. CoP location and pressure distribution on the sole of the contralateral foot were recorded using instrumented insoles inserted bilaterally in the participant’s shoes. The SLCR induced a significant displacement of the CoP toward the medial and anterior direction, associated with a significant increase in pressure at the level of the first metatarsal head. The SLCR contributed to dynamic stability, accelerating the propulsion phase of the contralateral leg and thus preparing for a faster step in the event that the ipsilateral leg is not able to support body weight. The results presented here provide new insight into the functionality of SLCRs, introducing the perspective that training these reflexes, as shown successfully for other reflex pathways, would increase dynamic stability in patients with impaired locomotion.

human locomotion; interlimb reflexes; functionality; interlimb communication

THE ACTIVITY FROM LEG and thigh muscles is of primary importance in maintaining balance control when an external perturbation occurs (Tang et al. 1998). Interlimb reflexes are known to be relevant for postural stability (Dietz 2002). For instance, during walking, bilateral leg muscle responses are elicited by unilateral rotational hip or knee joint perturbations, and these may serve to restore the physiological movement trajectory (Dietz et al. 2004). Bilateral responses elicited by unilateral treadmill acceleration or deceleration or electrical stimulation of the tibial nerve appear to be specific for the type of perturbation and dependent on the phase of the gait cycle in which the perturbation occurs (Berger et al. 1984). Similar functional requirements likely govern the responses elicited by these various perturbation types. Thus the early ipsilateral responses tend to reposition the displaced leg, whereas the early contralateral and late ipsilateral responses compensate for body displacement (Berger et al. 1984). Bilateral responses are more prominent when a perturbation is delivered during phase transitions (Berger et al. 1984; Dietz et al. 2004; Stevenson et al. 2013), suggesting that at these times the need for interlimb coordination to compensate for external perturbations is increased.

The functionality of interlimb reflexes is often inferred based on electromyogram (EMG) recordings. However, none of the previous studies investigated the effects of interlimb reflexes on behavioral measures, as for example the center of pressure (CoP). Such measurements are commonly used to evaluate balance and postural control (Hof et al. 2005; Palmieri et al. 2002). Postural sway during quiet standing is commonly characterized by quantifying the displacement of the CoP over time. CoP measurements have also been adopted during walking, showing for instance that CoP displacement and velocity during gait initiation can be used to discriminate between elderly fallers and nonfallers (Uemura et al. 2011). The quantification of CoP displacement produced by the interlimb reflexes would allow to describe the strategy, such as for instance increasing the base of support, adopted to maintain balance and therefore provide direct evidence for the functional significance of these responses.

Recent studies demonstrate that short-latency crossed responses (SLCRs) are elicited in the contralateral triceps surae following ipsilateral tibial nerve (iTN) stimulation; an inhibitory response is elicited in the contralateral soleus (cSOL; Stubbs and Mrachacz-Kersting 2009; Stubbs et al. 2011a,b) ~40 ms after stimulation, whereas an excitatory response is elicited in the contralateral gastrocnemius lateralis (cGL; Gervasio et al. 2013) ~69 ms following iTN stimulation. These responses are likely functional since they exhibit a phase-dependent modulation during locomotion with the most prominent responses occurring during the swing-to-stance transition of the stimulated leg (Gervasio et al. 2013; Stubbs et al. 2011b). Moreover, the interlimb pathway between soleus (SOL) muscles is significantly affected in patients following stroke, and this impairment may contribute to abnormal symmetry between the movement of the legs (Stubbs et al. 2012). The functional significance of the SLCR is further supported by the observation that the crossed facilitation elicited in cGL is reverted to an inhibition when an opposite reaction is required (Gervasio et al. 2013).

The purpose of the present study was to confirm the functionality of SLCRs by investigating the effects of SLCR on the pressure distribution under the contralateral foot. It was hypothesized that, following an ipsilateral perturbation, the SLCR could be part of a strategy to stop the progression of the contralateral leg and keep the projection of the center of mass...
to the ground within the margin of stability by inducing a flexion of the knee (Duyssens et al. 1991; Gervasio et al. 2013). Accordingly, it was expected that the SLCR elicited by iTN stimulation would induce a shift of the CoP of the contralateral foot backward compared with the unperturbed trajectory.

MATERIALS AND METHODS

Participants. Eight participants (5 men and 3 women, age 20–22 yr) took part in the experiments. None of the participants was affected by any central or peripheral movement disorders. The participants provided their written, informed consent to the protocol approved by the Scientific Ethics Committee of North Jutland (approval no. N-20110040) as required by the standards of the Declaration of Helsinki.

Experimental protocol. Participants walked on a treadmill (WOODWAY, Weil am Rhein, Germany) at their self-selected walking speed to ensure an enhanced stability and adaptability of the gait cycle (Jordán et al. 2007). The mean gait cycle duration was estimated from the 1st 30 steps as the average of the stride times, defined as the time between 2 consecutive foot contacts of the leg ipsilateral to the stimulation.

SLCRs were elicited by applying single electrical stimuli to the iTN (Gervasio et al. 2013). Stimuli were delivered at 80% of the ipsilateral gait cycle as at this time the most prominent short-latency facilitation (an increase in the EMG signal) in the gastrocnemius has been observed (Gervasio et al. 2013; S. Gervasio, M. Voigt, U. G. Kersting, D. Farina, T. Sinkjær, and N. Mrachacz-Kersting, unpublished observations). However, due to the intraindividual variability of stride time, the effective stimulation time is reported in RESULTS.

Electrical stimulation. Electrical stimulation of the iTN (1-ms pulse width) was delivered using an isolated stimulator (Noxitest IES 230, Aalborg, Denmark). The stimulating electrodes were placed while standing, the cathode (model no. 879100, PALS Platinum Round Electrode; Axelgaard Manufacturing, Lystrup, Denmark) over the popliteal fossa (Fig. 1) and the anode (model no. 895240, PALS Platinum Rectangular Electrode; Axelgaard Manufacturing) at the anterior aspect of the knee joint, 1 cm above the patella. To find the optimal electrode placement, stimuli were delivered every 6 s and the position of the cathode was adjusted until the location was found where the stimulation intensities that elicited an H-reflex and an M-wave in the ipsilateral soleus (iSOL) were the lowest. The participant was then asked to walk on the treadmill. The maximum M-wave peak-to-peak amplitude (M-max) was determined by adjusting the stimulation intensity, starting at 0 mA and progressively increasing this by 5 mA every three stimuli. The intensity was then decreased in 1-mA steps to identify the stimulation intensity that elicited 85% M-max. This value was used for all subsequent parts of the experiment.

Data acquisition. Surface EMG (sEMG) was recorded using a single differential derivation from the iSOL and from the cGL (Fig. 1). Disposable surface electrodes (Neuroline 720 silver/silver-chloride; Ambu) were placed following appropriate skin preparation and in accordance with the recommendations of Cram et al. (1998) for iSOL and with the SENIAM recommendations for cGL (Hermens et al. 1999). The reference electrodes were placed over the tibial bone. sEMG signals were preamplified, sampled at 2 kHz, and stored using a custom-made LabVIEW software (Mr. Kick II 2.3; Aalborg University).

Appropriately sized pressure-sensitive insoles (pedar-x; novel) were inserted between the participants’ socks and shoes. The pedar-x system provided a signal for the ipsilateral foot contact, which allowed the determination of the stride time and the synchronization of the pressure and sEMG signals. A trigger level was used to identify the ipsilateral foot contact. The subject was asked to walk on the treadmill, and the trigger level was then adjusted individually for each participant until an obvious foot contact, defined as initial contact

Fig. 1. Experimental setup: sketch of a participant walking on a treadmill. Tibial nerve stimulation was delivered to the ipsilateral leg, and electromyogram (EMG) signals from the ipsilateral soleus (iSOL) and the contralateral gastrocnemius lateralis (cGL) were recorded. Pressure-sensitive insoles inserted between the participants’ socks and soles of the shoes were used to record changes in pressure distribution and the center of pressure (CoP) displacement caused by the short-latency crossed response (SLCR). iTN, ipsilateral tibial nerve.
ent timings: before stimulation (75% of the gait cycle), immediately after stimulation (85% of the gait cycle), when the maximal displacement in CoP location was observed, and when the last displacement in CoP location was observed.

Statistics. Significant responses in the cGL sEMG were identified using a paired-sample t-test comparing the RMS in the defined time window between stimulation and control condition. Significant displacements in CoP location were established using a two-way repeated-measures ANOVA with factors the condition and the percentage of the gait cycle. The CoP may, however, fall on a point that is not actually loaded but intermediate between the real points beneath the foot where forces are applied (Rogers 1988). Pressure distribution was therefore also quantified to provide the specific location of pressure changes as they occur on the sole of the foot. Differences in the pressure values were assessed through a three-way repeated-measures ANOVA using as factors the condition, the percentage of the gait cycle, and the sensor. In case of a significant interaction among the factors, a post hoc pairwise comparison with Bonferroni correction was used to establish the location of the difference.

Stance and swing phase duration during the stimulated step were compared, respectively, with the stance and swing phase duration of the previous and following step using a one-way repeated-measures ANOVA. The same procedure was repeated for both legs.

Results were considered significant for P values <0.05. Results are expressed as means ± SD.

RESULTS

The participants chose a self-selected walking speed of 3.6 ± 0.3 km/h. The speed ranged within limits that ensured a comparison between participants. Stimulation was delivered at 80.7 ± 2.7% of the ipsilateral gait cycle and elicited a significant short-latency facilitation in the cGL ([\(t_6\) = 3.56, \(P = 0.01\)] in seven out of eight participants. The facilitation occurred on average at 57.6 ± 9.5 ms after the stimulation, lasted 30.4 ± 6.6 ms, and had an average magnitude of 146.8 ± 29.3% of the control signal. Figure 2, A and B, shows the iSOL and the cGL for one representative participant. A short-latency crossed facilitation was elicited in the cGL of this volunteer 50.5 ms following iTN stimulation. A later response was also observed ~120 ms following iTN stimulation. Because of its longer latency, this response might represent a voluntary reaction (Pierrot-Deseilligny and Burke 2005) and might thus be mediated by pathways different from the SLCR. Accordingly, this later response was not further investigated.

Alteration in pressure distribution and CoP location. Figure 3 shows the rectified cGL sEMG (Fig. 3A) and the CoP location for the contralateral foot (Fig. 3, B and C) during the averaged gait cycle for a representative participant. The cGL facilitation occurred 50.5 ms following stimulation, which was delivered on average at 79.4% of the gait cycle. The CoP displacement commenced at 90% of the gait cycle (126 ms following the stimulation). During the stimulated condition, the CoP deviated from the unperturbed trajectory for a maximum of 1.5 mm medially (Fig. 3B) and 3.1 mm frontally (Fig. 3C). The maximum displacement occurred at 96% of the ipsilateral gait cycle for both directions. Figure 4 shows the rectified cGL sEMG (Fig. 4A) and the CoP location (Fig. 4, B and C) during the averaged gait cycle for the only participant that did not show a short-latency crossed facilitation. No evident displacement in CoP trajectory was observed.

When observing the CoP trajectory in the medial-lateral direction for all participants, a significant interaction \([F_{16} = 7.22, P < 0.01\] was found between the factors (condition and the percentage of the gait cycle). The post hoc pairwise comparison revealed a significant displacement of the CoP location toward the medial direction from 92 to 100% of the ipsilateral gait cycle \((P < 0.03)\). The maximum deviation from the control condition occurred at 98% of the ipsilateral gait cycle and was of 1.0 ± 0.7% of the foot length (Fig. 5A).

A significant interaction \([F_{16} = 2.12, P = 0.01\] between the factors (condition and the percentage of the gait cycle) was observed for the contralateral foot CoP location in the anterior-posterior direction. The post hoc pairwise comparison revealed a significant displacement of the CoP location toward the anterior direction from 90 to 98% of the ipsilateral gait cycle \((P < 0.048)\). In this direction, the maximum deviation from the control condition occurred at 92% of the ipsilateral gait cycle and was of 3.7 ± 3.0 mm (Fig. 5B).

Based on the results above, 95% of the gait cycle was chosen as the average time when the maximum displacement occurred in both directions, and 100% of the gait cycle was chosen as the last time point before the CoP location regained its normal trajectory. The pressure distribution at these times was compared with those recorded before (75% of the gait cycle) and just after the stimulation (85% of the gait cycle). The statistical analysis revealed a significant interaction \([F_{294} = 1.22, P = 0.01\] among the factors (condition, percentage of the gait cycle, and sensor). Post hoc pairwise comparisons revealed significant differences between the pressure recorded during the stimulation and control condition from six sensor locations.

Fig. 2. Short-latency facilitation in the cGL after iTN stimulation. A: the iSOL surface EMG (sEMG) is shown for 1 representative participant when iTN stimulation was delivered; the M-wave elicited by electrical stimulation was used to define the stimulation intensity (85% maximum M-wave peak-to-peak amplitude). B: the rectified cGL sEMG for the same participant is shown. A short-latency crossed facilitation (magnitude 167.2% of the control signal) is elicited in this muscle 50.5 ms following iTN and lasted 33.5 ms. The traces in both A and B are the averages of 30 stimuli. The black trace is the averaged signal when a stimulation occurred, whereas the gray line is the average of the control signal (no stimulation). In the time axis, 0 corresponds to the onset of the stimulation, which is further indicated by the dashed vertical line.

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Alterations in step parameters. For the ipsilateral leg, the stance phase duration during the stimulated step was 0.80 ± 0.05 s, which was significantly different \( F(1.39) = 12.56, P = 0.005 \) in pressure in the stimulated condition are indicated in Fig. 5C in white. A significant decrease \( P < 0.05 \) in pressure is indicated in black (Fig. 5C).

Alterations in step parameters. For the ipsilateral leg, the stance phase duration during the stimulated step was 0.80 ± 0.05 s, which was significantly different \( F(1.39) = 12.56, P = 0.005 \) from that of the previous and successive steps (0.80 ± 0.05 and 0.78 ± 0.06 s, respectively). Post hoc pairwise comparison revealed that the stance phase of the step following the stimulation was significantly shorter from that of the stimulated and of the previous step \( P = 0.01 \). No differences

\((P = 0.58)\) between stance duration of the stimulated step and of the previous one were observed (Fig. 6A). No significant differences \( F(1.61) = 0.66, P = 0.51 \) were shown among the swing phase duration of the stimulated step \((0.46 \pm 0.03 \text{ s})\) and the step before \((0.45 \pm 0.03 \text{ s})\) and after \((0.46 \pm 0.03 \text{ s})\) the stimulated one (Fig. 6B).

For the contralateral leg, the duration of the stance phase was not significantly different \( F(1.57) = 1.97, P = 0.20 \) for the step before \((0.78 \pm 0.05 \text{ s})\), during \((0.79 \pm 0.05 \text{ s})\), and after \((0.78 \pm 0.05 \text{ s})\) the stimulation (Fig. 6C). Moreover, no significant difference \( F(1.25) = 0.92, P = 0.42 \) was observed in swing duration for the stimulated step \((0.45 \pm 0.03 \text{ s})\).

at 95% (all \( P < 0.05 \)) and from one location at 100% of the gait cycle \((P < 0.02)\). Sensors recording a significant increase \( P < 0.042 \) in pressure in the stimulated condition are indicated in Fig. 5C in white. A significant decrease \( P < 0.05 \) in pressure is indicated in black (Fig. 5C).

Fig. 4. sEMG and CoP for the participant that did not show a SLCR. A: the rectified sEMG cGL muscle is shown from the same participant as in Fig. 2. The black trace is the averaged signal when a stimulation occurred, whereas the gray line is the average of the control signal (no stimulation). B and C: the displacement of the CoP location for the contralateral foot is shown. Measurements are the average of 30 gait cycles (GCs) for each condition, stimulation (full dots) or control (empty dots). The maximum displacement of the location of the CoP occurred at 96% of the ipsilateral GC (iGC) for both medial-lateral \( (B; 1.5 \text{ mm medially}) \) and anterior-posterior \( (C; 3.1 \text{ mm frontally}) \) directions. The stimulation is indicated by the black dashed vertical line. The onset of the facilitation is indicated by the vertical dotted gray line. The displacement in CoP (vertical dashed gray line) occurred \( \sim 126 \text{ ms} \) after the stimulation.

Fig. 3. SLCR and displacement of CoP for a representative participant. A: the rectified sEMG cGL is shown from the same participant as in Fig. 2. The black trace is the averaged signal when a stimulation occurred, whereas the gray line is the average of the control signal (no stimulation). B and C: the displacement of the CoP location for the contralateral foot is shown. Measurements are the average of 30 gait cycles (GCs) for each condition, stimulation (full dots) or control (empty dots). The maximum displacement of the location of the CoP occurred at 96% of the ipsilateral GC (iGC) for both medial-lateral \( (B; 1.5 \text{ mm medially}) \) and anterior-posterior \( (C; 3.1 \text{ mm frontally}) \) directions. The stimulation is indicated by the black dashed vertical line. The onset of the facilitation is indicated by the vertical dotted gray line. The displacement in CoP (vertical dashed gray line) occurred \( \sim 126 \text{ ms} \) after the stimulation.
DISCUSSION

This study investigated the functional significance of the SLCRs elicited by iTN in the triceps surae muscles by analyzing the changes in the pressure distribution under the contralateral foot. The SLCR induced a displacement of the CoP toward the medial and anterior direction, associated with an increase in pressure at the level of the first metatarsal head. In addition, the ipsilateral leg showed a significantly shorter stance phase duration in the first step following the stimulation, which was delivered at the end of the swing phase.

The facilitation elicited by iTN in both heads of the contralateral gastrocnemius (Gervasio et al. 2013; S. Gervasio, M. Voigt, U. G. Kersting, D. Farina, T. Sinkjær, and N. Mrachacz-Kersting, unpublished observations) together with the crossed inhibition observed in the cSOL (Stubbs et al. 2011b) is more prominent when the stimulation is delivered at the end of the ipsilateral swing phase, when the ipsilateral foot is approaching heel strike while the contralateral leg is in terminal stance. It has been suggested that the short-latency inhibition observed in cSOL (Stubbs et al. 2011b) coupled with the facilitation in the knee flexor gastrocnemius might have the purpose of preventing the push off of the contralateral foot by maintaining the body weight on the contralateral leg (Gervasio et al. 2013).
Nonetheless, pressure measurements revealed a displacement of the contralateral foot CoP toward the medial and anterior direction following the SLCR. From a mechanical point of view, the following mechanisms seem to be responsible for dynamic stability after a perturbation: increasing in the base of support, counterrotating segments around the center of mass, and applying an external force such as grasping (Arampatzis et al. 2011; Bierbaum et al. 2013; Hof et al. 2005). At the time the perturbation was delivered, the projection of the center of mass to the ground was outside of the base of support or still inside but directed outside of the base of support. The CoP observed displacement could therefore reflect a mechanism for accelerating the propulsion phase, preparing the contralateral leg for a faster step and increasing the base of support if the ipsilateral leg, approaching heel strike, is not able to accept the body weight. During unperturbed walking, the CoP trajectory typically propagates from the heel along the midline of the foot and progresses medially at terminal stance so that by toe off the CoP lies under the first or second toe (Khoury et al. 2013; Rodgers 1988). Hence, the CoP displacement toward the medial and anterior direction likely indicates an acceleration of the natural progression of the CoP trajectory toward the propulsion phase. This hypothesis is supported by the observation that the ipsilateral leg exhibited a significantly shorter stance phase just after the stimulation was delivered.

The stimulation elicited opposite responses in cSOL and cGL, inhibition and facilitation, respectively. The functional role of these muscles work antagonistically (Neptune et al. 2001; Stewart et al. 2007). Whereas SOL reduces dorsiflexion after the foot is flat on the ground, gastrocnemius seems to promote tibial advance (Stewart et al. 2007). Moreover, the model-based study by Neptune et al. (2001) suggests that the energy produced from the SOL accelerates the trunk forward, whereas gastrocnemius delivers almost all of its energy to accelerate the leg into initiate swing (Neptune et al. 2001). It is thus possible that the inhibition in cSOL and the facilitation in cGL aim at inducing ankle dorsiflexion and knee flexion, accelerating the leg into initiating swing.

The iTN stimulation was delivered on average at 80.7% of the gait cycle, and the first significant displacement of CoP was observed at 90% of the gait cycle. Considering that the mean recorded gait cycle duration for all participants was 1.25 s, it took ~116 ms to observe any changes in pressure after stimulation. The average latency of the SLCR in the current study was 58 ms; considering the electromechanical delay associated with EMG onset and transmission of muscle force (~50 ms; (Zatsiorsky 2000), changes in pressure if produced by the SLCR should occur 108 ms after the stimulation. This shows a good correspondence with the observed onset of CoP displacement. Moreover, the only participant that did not show a SLCR had no apparent displacement of the position of the CoP compared with the unperturbed trajectory. It is thus reasonable to assume that indeed the SLCR was the main contributor to the CoP displacement reported in this study.

**Methodological considerations.** The crossed responses investigated in the current study were elicited by electrical stimulation of the iTN. The artificial synchronous afferent volley evoked by the electrical stimulation differs from the asynchronous volleys that would be produced by a physiological stretch of the plantar flexors. Indeed, ipsilateral spinal reflexes elicited by electrical stimulation have been shown to be processed differently from, for example, a muscle stretch induced by tendon tap (Morita et al. 1998). Nevertheless, responses similar to the one elicited by electrical stimulation in the current study have been observed following ipsilateral mechanical perturbation such as a holding impulse (Dietz et al. 1986) or a stretch of the plantar flexors induced by mechanical rotation of the ankle joint (Razdan et al. 2009). However, a mechanical perturbation might alter the position of both legs such that the observed responses may originate from the contralateral leg itself. By using electrical stimulation instead, it was possible to stimulate the iTN afferents specifically and therefore to identify better the afferents mediating the crossed response. Moreover, it cannot be excluded that naturally occurring perturbations would trigger a SLCR similar to the one observed following iTN stimulation. When stumbling over an obstacle during the swing phase of walking, short-latency responses in ankle and knee flexor and extensors occur (Schillings et al. 1999). These responses have been attributed to widespread muscle spindle activity caused by the collision with the obstacle (Pierrot-Deselligny and Burke 2005; Schillings et al. 1999). We previously showed that muscle afferents likely mediate the SLCR in cGL during normal walking, as stimulation of cutaneous afferents does not produce similar responses (Gervasio et al. 2013). Therefore, is it possible that the muscle spindle activation caused by encountering an obstacle at the end of the swing phase would activate the crossed spinal pathway mediating the SLCR. Moreover, although an
unexpected perturbation was used to elucidate the SLCR, it cannot be excluded that the interlimb pathway mediating this response might contribute to unperturbed locomotion.

**Conclusion.** The results of the current study support the functionality of SLCRs in the triceps surae muscles as a possible method to maintain dynamic stability following a perturbation. It has been previously shown that the amplitude of reflexes can be altered by following appropriate conditioning protocols and that downconditioning the SOL H-reflex in the most affected leg in patients with incomplete spinal cord injuries results in a faster and more symmetrical locomotion (Thompson et al. 2013). Based on this evidence, future research should evaluate the perspective that conditioning the SLCRs would increase dynamic stability in patients with impaired locomotion.

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**DISCLOSURES**

There is no conflict of interest regarding the material discussed in the manuscript.

**AUTHOR CONTRIBUTIONS**

S.G., U.G.K., D.F., and N.M.-K. conception and design of research; S.G. performed experiments; S.G. and N.M.-K. analyzed data; S.G., U.G.K., D.F., and N.M.-K. interpreted results of experiments; S.G. and N.M.-K. drafted manuscript; U.G.K., D.F., and N.M.-K. edited and revised manuscript; S.G., U.G.K., D.F., and N.M.-K. approved final version of manuscript.

**REFERENCES**


